

Physicochemical and microbiological properties of yogurt made with microencapsulation probiotic starter during cold storage

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Abstract. Rossi E, Restuhadi F, Efendi R, Dewi YK. 2021. *Physicochemical and microbiological properties of yogurt made with microencapsulation probiotic starter during cold storage. Biodiversitas 22: 2012-2018.* This study's purposes were to characterize probiotic properties, identify strains of K12.1, produce a microencapsulated starter (MS) for yogurt, and evaluate yogurt's microbiological quality during storage refrigerated temperatures. This research was conducted experimentally using isolate was further identified based on 16S rRNA gene sequence analysis. The identified strain and *Streptococcus thermophilus* were microencapsulated using sago starch and whey isolate protein (1:1 w/w) for yogurt starter. The starter was added as much as 1, 3, 5, or 7% (v/v), then incubated for 18 hours at 37°C. The best yogurt was evaluated for its microbiological quality at refrigerated temperature for 0, 7, 14, 21, and 28 days. The results showed that K12.1 isolate with probiotic characteristics was *Lactobacillus plantarum* VP3.3. The microencapsulation starter concentration affected acidity, total acid, total Lactic Acid Bacteria (LAB), viscosity, and total solids. The use of 7% microencapsulation starter of *Lactobacillus plantarum* VP-3.3 and *Streptococcus thermophilus* gave the best yogurt, with a pH value 4.36, total lactic acid 0.99%, total LAB 10.00 log CFU mL⁻¹, viscosity 546.37 cP, and total solids 10.40%. The total amount of LAB in yogurt stored during cold temperature for 28 days still meets the probiotics standards (9.98 log CFU/mL).

Keyword: *Lactobacillus plantarum*, microencapsulation, sago starch, *Streptococcus thermophilus*, whey isolate protein

INTRODUCTION

One of the most popular functional foods today is probiotic drinks. This drink contains probiotics in live microbes that have beneficial effects on the host's health (Salminen and Wright 2011). Besides, probiotics can have a physiological impact on health, such as preventing various digestive tract problems (Howarth and Wang 2013), lowering triglycerides (Kassaian et al. 2019). Most probiotics are Lactic Acid Bacteria (LAB), also known as General Recognized As Safe (GRAS), which means microbes are not a health risk (Tadesse et al. 2005). Some of them have probiotic activity, such as LAB isolated from solid waste of soymilk production, K12.1 LAB isolate (Rossi 2018). Several previous studies have isolated LAB from curd (Hawaz 2014), okara (Aritonang et al. 2017), pastirma (Öz et al. 2017), and goat milk (Yelnetty et al. 2020).

Use of probiotics in food has been widely used such as synbiotic ice cream (Aritonang et al. 2019). However, extreme conditions in the digestive tract can reduce its resistance in the intestine so that its physiological function for the host will decrease. To survive in the gut, organisms must be tolerant of the low pH and bile toxicity prevalent in the small intestine. For colonization, probiotics should exhibit good surface hydrophobicity and aggregation properties (Del Re et al. 2000; Collado et al. 2007). Functionally, by inhibiting toxins' action or production (Hugo et al. 2008) by expressing bacteriocins and inhibiting mucosal surface pathogens' binding (Collado et

al. 2006), they can neutralize the effects of pathogens. It may also demonstrate antioxidant and immunomodulatory activities (Vitiñi et al. 2000).

Several studies have reported that probiotics are very sensitive to environmental conditions and have low survivability in the digestive tract (Shi et al. 2012). Factors that can affect probiotics' viability include pH and bile salt (Rossi et al. 2018). The method used to protect and maintain LAB resistance in the digestive tract is an encapsulation that forms a layer in a matrix. The inside is spherical in shape like a capsule wall, which covers the encapsulated material (Vidhyalakshmi et al. 2009). The advantage of encapsulation is that it has a semipermeable, round, and strong membrane so that the bacterial cell can survive in extreme conditions. The materials commonly used for encapsulation are various polysaccharides and proteins, such as starch, alginate, albumin, and casein.

The materials used for encapsulation need to be considered because each material has different characters and does not necessarily match the core material to be encapsulated (Desmond et al. 2002). Proteins and polysaccharides are widely used for encapsulating bioactive materials (Maleki et al. 2020). Sago starch (SS) is used as an encapsulation material because of its ability to form a gel when heated (Vamadevan et al. 2013). Sago starch can also be used as a prebiotic ingredient (Zhu 2019). Whey isolate protein (WIP) is also the most widely used encapsulation material. The advantages of WIP as an encapsulation material are that it can form a matrix that can trap LAB to be protected from extreme environmental

conditions (Maleki et al. 2020). To increase the protective effect on bioactive against external conditions and under simulated gastrointestinal conditions, two valuable nutrients, LAB and anthocyanins from black beans, were microencapsulated using WPI (Vasile et al. 2020). Besides, until the end of the product's shelf life, probiotic bacteria should remain viable (Tamime et al. 2005; Donkor et al. 2007). The purposes of this study were to characterize probiotic properties and identify K12.1 isolate using 16S-rRNA, obtain the best level of microencapsulated starter for producing yogurt, and evaluate the microbiological quality of yogurt during storage at 4°C.

MATERIAL AND METHOD

Material culture

Lactic Acid Bacteria isolate (K 12.1) obtained from soybean epidermis was a solid waste making soy milk from the home industry in Tampan District, Pekanbaru, Indonesia. This isolate was chosen because it has the best antimicrobial activity of the other 24 isolates. These isolates were evaluated for their probiotic characteristics. Pathogenic bacteria used were *Escherichia coli* O157: H7, *Listeria monocytogenes*, and *Staphylococcus aureus* ATCC 25923.

Probiotics properties of isolate LAB

Antimicrobial activity of Lactic Acid Bacteria isolate

Modification of the well diffusion test (Yang et al. 2012) was used to evaluate antibacterial activity against pathogens (*E. coli* O157: H7, *L. monocytogenes*, *S. aureus* ATCC 25923). The cell-free supernatant was grown in MRS broth for 24 hours at 37 °C, under anaerobic conditions, centrifuged at 10,000 rpm for 5 min at 4 °C. Fifty mL of cells and cell-free supernatant each were placed in a well where pathogenic bacteria on MHA medium (Mueller Hinton Agar, Merck) grew. The size of the zone of inhibition showing the antibacterial activity of the isolates was measured after 24 hours.

Viability of Lactic Acid Bacteria isolate to acids and bile salt

A low pH (pH 2 and 3) resistance of LAB was carried out according to the method described by Shi et al. (2013). Five mL of MRS Broth solution without and with pH adjustment of 2 and 3 using HCl in a test tube was inoculated with 1% LAB working culture, then shaken until blended. Furthermore, the LAB resistance was observed after the culture was incubated at 37 °C for 90 and 180 min.

By inoculating 1 mL of LAB isolate into MRS broth containing 0.3 and 0.5 % bile salts (Sigma), The culture's absorbance at 620 nm was measured after 0 and 5 h of incubation at 37°C. Acid and bile resistance tests were replicated three times each with duplicate analysis.

Hydrophobicity of Lactic Acid Bacteria isolate by in vitro

The K12.1 LAB isolates that had the potential as probiotics were observed for their adhesion properties on

the intestinal mucosa in vitro (Sánchez-Ortiz and Luna-González 2015). LAB cultures in MRS broth were incubated anaerobically at 37 °C for 18-22 hours, then centrifuged at 10,700 rpm for 5 min. LAB suspension was added 1 mL xylene in a cuvette and this suspension was left for 60 seconds. The suspension was then incubated for 2 hours at 37°C. There was an aqueous phase at the bottom of the cuvette, which is then taken and measured at OD 600 nm (A1). P-xylene was used because the cell surface's hydrophobic or hydrophilic nature was reflected by bacterial adherence to this solvent.

Identification K12.1 Lactic Acid Bacteria isolates using 16S rRNA.

Based on 16S rRNA gene sequence analysis, the LAB species were further identified. Using kit *Presto™ Mini gDNA Bacteria*. The genomic DNA from the K12.1 isolate was extracted. The DNA gene was amplified by universal primers 27 F (5'-GAGTTTGATCCTGGCCTAG-3'), 1525 R (5'-AGAAAGGAGGTGATCCAGCC-3'). using PCR. The PCR amplification conditions were as follows: initial denaturation for 2 min at 95°C, 40 denaturation cycles for 45 s at 94°C, annealing for 1 min at 56°C, extension for 1 min and 40 sec at 72°C, and final extension for 10 min at 72°C. The reaction mixtures were subsequently cooled to 4°C, and agarose gel electrophoresis with 1 percent agarose was used to analyze the PCR products. The gel was placed in a container plus TBE until submerged. The gel, then seen under a UV lamp. The resulting sequence of isolates was analyzed with BLASTn on the NCBI website. The phylogenetic tree was generated using the Kimura-2-parameter model.

Yogurt production using microencapsulation inoculum

Full cream milk was pasteurized at 85°C for 30 min and cooled rapidly at 45°C (Donkor et al. 2006). The microencapsulated starter of *Lactobacillus plantarum* strain VP-3.3 and *Streptococcus thermophilus* (ratio 1: 1) was added as much as 1, 3, 5, or 7% (v/v), then incubated for 18 hours at 37°C. All the treatments were applied with three replications. The best yogurt made using a microencapsulation starter was evaluated for its microbiological quality at refrigerator temperature for 0, 7, 14, 21, and 28 days.

Encapsulation Lactobacillus plantarum strain VP-3.3

Harvested *L. plantarum* strain VP-3.3 and *S. thermophilus* (ratio 1: 1) were microencapsulated with sago starch (SS) and whey protein isolate (WPI). The encapsulation of bacteria was carried out with certain modifications using the method presented by Rajam et al. (2012). Preparation of 1: 1.5 core-to-wall ratio, which walls were sago starch and WPI powder in a ratio of 1: 3. A total of 37.5 g of SS and WPI were mixed with sterile water to a volume of 150 mL in a beaker. The solution was heated at 70°C and stirred slowly for 15 min to make it homogeneous. Probiotic cells (25 g) were added slowly to make uniform cell mixing. The final volume was produced with distilled water up to 210 mL and further mixed for 5 min. The probiotic bacterial suspension was mixed with the

wall material solution (1:1 and 1:1.5). The suspension of probiotic bacteria with a wall solution material (1: 1,5) was frozen for 48 hours at -60°C , then subjected to freeze-drying for 20 hours. The freeze-dried powder was stored at 4°C .

Analysis Physicochemical and Microbiological characteristics of Yoghurt

The yogurt pH was measured using a pH meter, calibrated with a buffer of pH4 and pH 7 (AOAC 2012). The total LAB was measured based on Roberts and Greenwood (2003). Titrable acidity (total lactic acid) was measured by mixing 10 gr of yogurt sample with 30 mL of distilled water. It was calculated with 0.1 N NaOH using phenolphthalein indicator until it showed pink color (Matela et al. 2019). The total solid content was measured by Official Methods of Analysis AOAC (2012). Total LAB was expressed in log CFU/mL.

Data Analysis

Probiotics Properties of Isolate LAB data which consisted of three replications were analyzed descriptively. Mean data of yogurt production and storage at refrigerator temperature were analyzed using analysis of variance. Data with significant influence ($P < 0.05$) continued with the Duncan's Multiple Range Test using SPSS software statistic 19.

RESULT AND DISCUSSION

Probiotics properties of isolate LAB

Characterization of K12.1 Lactic Acid Bacteria isolate as a probiotic

The results of LAB's characterization as a probiotic candidate and a potential bio-preservation producer can be seen in the data recapitulation in Table 1.

One of the important factors for survival in the digestive tract is the ability to compete with pathogenic bacteria in the intestine and is expressed by producing various antimicrobial compounds in the host (Salminen et al. 1998). Cell-free supernatant from K12.1 has inhibitory activity against the growth of *E. coli* O157: H7, *L. monocytogenes*, *S. aureus* ATCC 25923. Cell-free supernatants had different abilities to inhibit the growth of pathogenic bacteria. The highest inhibition of this antimicrobial was seen in *Listeria monocytogenes* CFSAN004330. Pan et al. (2009) stated that a high level of antimicrobial activity was detected if the diameter of the zone of inhibition was more than 6 mm. Several *L. plantarum* had been known to produce bacteriocins, called plantaricin, which have antimicrobial activity against pathogenic bacteria, such as *L. plantarum* LR/14 (Tiwari and Srivastava 2008) and *L. plantarum* A-1 isolated from tortillas (Hata et al. 2010).

One of the conditions for obtaining the benefits of LAB probiotics was that they could survive in the digestive tract, including stomach acid and bile salts. The observations of the viability of the K12.1 LAB isolate grown on MRS-B media with the addition of HCl (pH 2 and 3) for 90 min were 96.74 and 99.10%, respectively. The viability of LAB isolates in this study was greater than 90%. This shows that LAB isolates can survive after incubation for 90 min at pH 2 and 3 and are potential candidates for probiotics, which are expected to survive until the large intestine, following the opinion of Cheow and Hadinoto (2013), which explains that one of the criteria for LAB as a probiotic was that LAB must be able to withstand acidic conditions, with estimated viability of equal to or more than 50%.

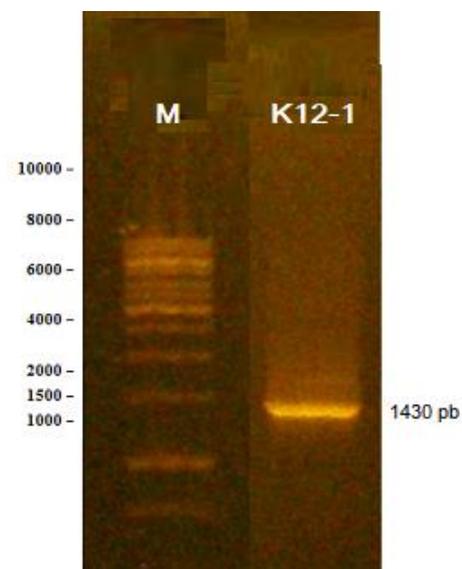


Figure 1. PCR electrophoresis product of K12.1 Lactic Acid Bacteria isolate

Table 1. Probiotics characteristic of K12.1 Lactic Acid Bacteria isolate

Probiotic characteristics	Value
Antimicrobial against (inhibition zone mm)	
<i>E. coli</i> O157:H7	7.48±0.07
<i>S. aureus</i> ATCC 25923	11.73±0.15
<i>L. monocytogenes</i> CFSAN004330	19.51±0.05
Viability in acidic conditions (pH 2) 90 min (%)	96.74±0.06
Viability in acidic conditions (pH 3) 90 min (%)	99.10±0.11
Viability in bile salt conditions 0.3% 5 hours (%)	98.20±0.09
Viability in bile salt conditions 0.5% 5 hours (%)	96.22±0.10
Hydropobicity (%)	51.93±0.04

Note: The values were expressed as the mean \pm standard deviation (n=4)

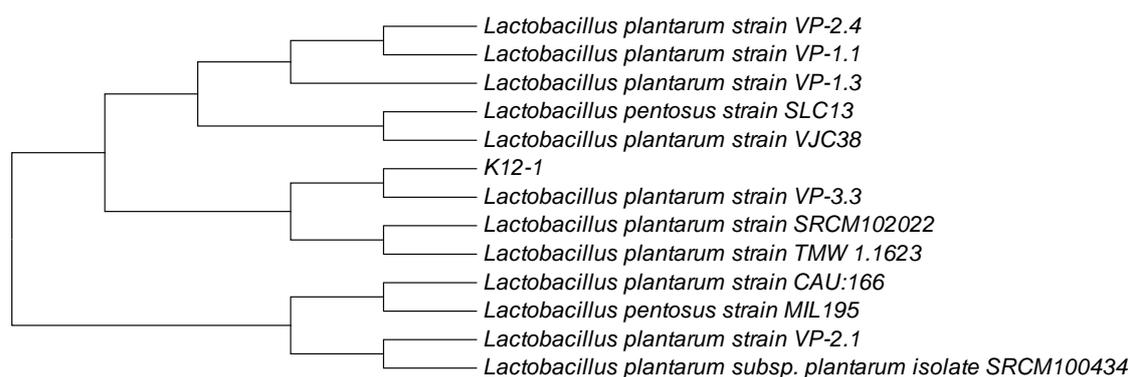


Figure 2. Phylogenetic K12.1 isolate of Lactic Acid Bacteria

To ensure optimum functionality and expression of health-promoting physiological functions by probiotics, survivability and colonization in the digestive tract were considered critical. From Table 1, it could be seen that the high viability of LAB was at 0.3 and 0.5% oxgall. This data showed only a slight decrease in the amount of LAB in the medium containing oxgall. This decrease in viability was since bile was an active compound, which can react with the cell membrane's lipolytic surface. The active nature of bile causes the lipolytic enzymes to react with the fat in the cell membrane. This condition affects the permeability of the cell membrane and will eventually cause cell lysis. According to Majeed et al. (2019) LAB, resistance to bile salts was related to the bile salt hydrolase enzyme, which helps hydrolyze de-conjugated bile salts, reduces toxic effects on bacterial cells, and can lower cholesterol. Based on the results of previous research Tokatli et al. (2015) and Rossi et al. (2018) showed that probiotics were LAB that was resistant to stomach acid and bile salts.

Identification K12.1 Lactic Acid Bacteria isolates using 16S rRNA.

In Figure 1, it can be seen that the amplification of the area of the 16S rRNA gene isolates Lactic Acid Bacteria from solid waste of making soy milk. It can be seen by the appearance of PCR fragment of size 1430 pb using the universal primers 27 F (5'-GAGTTTGATCCTGGCCTAG-3'), 1525 R (5'-AGAAAGGAGGTGATCCAGCC-3'). Phylogenetic trees based on 16S rRNA gene sequence analysis can be seen in Figure 2. Sequencing results of K12.1 isolates compared to Gene Bank data using the BLAST program on the NCBI website showed a similarity rate of 99.9% with *Lactobacillus plantarum* VP-3.3.

Yogurt production using microencapsulation inoculum Physicochemical and microbiology properties of yogurt

Table 2 shows that the pH of yogurt in treatment P4 (7% inoculum) was significantly different ($P < 0.05$) compared to the pH in treatment P1, P2, and P3 were inoculum concentrations of 1, 3 and 5%, respectively. The

P3 and P4 yogurt samples' pH complied with the Food and Drug Administration (FDA) specifications, stating that yogurt must have a maximum pH of 4.5 (Weerathilake et al. 2014). In treatment P1 and P2, the pH of yogurt did not meet the FDA requirements. This was due to the 1 and 3% of inoculum used for treatment P1 and P2, respectively. This small amount of inoculum causes the lactase enzyme produced to be insufficient to ferment all available lactate into lactic acid. Similar results were found El-Kholy et al. (2020), which determines the pH of low-fat yogurt containing inulin for 1, 7, and 14 day storage periods, respectively, were 4.54, 4.38, and 4.22. The pH value describes the amount of lactic acid produced from lactose fermentation by LAB (Oladipo et al. 2014). The results showed that the average pH value of yogurt was comparable to that stated by Ahmad et al. (2020), who studied the effect of fortification of apple skin polyphenol extract on probiotic characteristics yogurt with the highest pH of yogurt was 4.56 on the first day of storage. The lowest pH of yogurt was 4.02 on the 21st day of storage. The pH of yogurt is also in line with ul Haq et al. (2019) research on the development and evaluation of yogurt with lentil flour where its pH was decreased from 4.50, 4.33, 4.27, 4.15, and 4.05 for 1, 7, 14, 21, and 28 yogurt samples respectively.

Titrateable acidity was expressed as a percentage of total lactic acid formed during lactose fermentation by LAB. The average total value of yogurt lactic acid can be seen in Table 2. The percentage of titrateable acidity was 0.63, 0.74, 0.85, and 0.99%, respectively, for P1, P2, P3, and P4. Compared to other samples, samples P3 and P4 had extremely high titrateable acidity, which may be attributed to the greater availability of microbes used for fermentation. Tamime and Robinson (2007) stated that LAB's ability to produce acid varies depending on the species, the level of the inoculum, the state, and the composition of the growth media. Only P4 complied with the FDA specification among these four treatments, which specifies that yoghurt should have a minimum titrateable acidity of 0.9 percent (Weerathilake et al. 2014).

Table 2. Physicochemical and microbiology properties of yoghurt

Yogurt characteristics	SNI* 2981:2009 yoghurt	Microencapsulation starter (v/v)			
		P ₁ =1%	P ₂ =3%	P ₃ =5%	P ₄ =7%
pH		4.59 ^d ±0.013	4.48 ^c ±0.010	4.42 ^b ±0.014	4.36 ^a ±0.047
Titrate acidity (%)	0.5-2.0	0.63 ^a ±0.073	0.74 ^b ±0.086	0.85 ^c ±0.052	0.99 ^d ±0.073
Total Solid (%)	Min. 8.2	9.63 ^a ±0.170	9.82 ^a ±0.464	9.97 ^{ab} ±0.334	10.40 ^b ±0.227
Viscosity (cP)		423.22 ^a ±1.965	438.35 ^a ±1.380	533.47 ^b ±2.012	546.37 ^b ±1.694
Total Lactic Acid Bacteria (LogCFU/mL)	Min 7/10 ⁷ (col/g)	9.69 ^a ±0.056	9.83 ^a ±0.062	9.99 ^b ±0.051	10.08 ^b ±0.037

Note: Values followed by different superscripts in the same row were significantly different ($p < 0.05$). The value was expressed in mean ± standard deviation (n=4). *SNI: *Standar Nasional Indonesia* (Indonesian National Standard)

Tabel 3. pH, total Lactic Acid Bacteria count, and viability of Lactic Acid Bacteria

Storage time (days)	pH	Total LAB (log CFU/mL)	LAB Viability (%)
0	4.36 ^a ±0.047	10.08 ^c ±0.037	100.00±0.000
7	4.33 ^{ab} ±0.033	10.05 ^{ac} ±0.039	99.66±0.388
14	4.29 ^b ±0.021	10.03 ^{abc} ±0.041	99.51±0.407
21	4.27 ^b ±0.013	10.00 ^{ab} ±0.035	99.17±0.351
28	4.20 ^c ±0.016	9.98 ^a ±0.041	99.04±0.405

Note: Values followed by different superscripts in the same row were significantly different ($p < 0.05$). Value was expressed in mean ± standard deviation (n=4)

The use of different inoculum levels for yogurt production had a significant impact ($P < 0.05$) on the total solids of yogurt (Table 2). The highest total solid was 10.40% at P4 treatment, using 7% inoculum. The use of 7% inoculum as a starter yogurt causes the fermentation process to run well, characterized by decreased pH. A decrease in pH value indicates an increase in lactic acid production and total LAB, as seen in the P4 treatment with the highest total solid content. This total solid content affects the viscosity of yogurt. In this study, the highest total solids and viscosity were obtained in the P4 treatment, respectively 10.40% and 546.37 cP. Yogurt's viscosity is influenced by the total solid content of milk, heat treatment, and incubation temperature (Lee and Lucey 2010). Viscosity was also influenced by pH, where low pH causes milk protein (casein) to coagulate.

The LAB bacteria of the yogurt sample ranged from 9.69-10.00 log CFU/mL. Table 2 showed that using different amounts of starters in making yogurt had a significant effect ($P < 0.05$) on the total LAB content. Total LAB in treatment P3 and P4 was significantly different ($P < 0.05$) with P1 and P2 treatment. The high LAB in P3 and P4 was due to the two treatments using more starter than P1 and P2. This illustrates that the nutrient content contained in the medium is still sufficient for the growth of LAB in P3 and P4 using a starter of 5% to 7%. The total number of LAB in all treatments fulfilled the number of LAB requirements for probiotics (10^6 – 10^7 CFU/mL that reached the colon (Castro et al. 2015).

Change of pH and total Lactic Acid Bacteria count and their viability during storage at 4°C for 28 days

The pH of yogurt changed during the incubation and storage period due to biochemistry and microbial growth

changes. Statistical data showed that the pH of yogurt samples at 28 days of storage was significantly different ($p < 0.05$) from the pH of yogurt samples at other storage times (Table 3). During yogurt storage, the decrease in pH was due to the increased activity of *L. plantarum strain VP-3.3* and *S. thermophilus*, the inoculum for yogurt production. The fermentation was still running at 4°C, which was indicated by a decrease in pH to 4.2. This study's results were in line with Melia et al. (2020). There was a decrease in the pH of fermented goat milk from 4.48 to 4.28 using *Pediococcus acidilactici* was stored for up to 28 d at refrigerator temperature. Decreasing this pH showed that LAB's lactic acid was still released during storage time that fermented milk lactose. LAB fermented lactose into glucose and galactose, then converted glucose into lactic acid (Tamime and Robinson 2007). All of the yogurt samples had pH levels within FDA guidelines, stating that yogurt should have a maximum pH of 4.5 (FDA 2013). The decrease in yogurt's pH also occurred during storage for 28 days at 4°C (Sarwar et al. 2019). Calcium can be converted to its ionic form in yogurt due to its low pH, rendering it highly bioavailable for intestinal absorption. The low pH value of yogurt will also minimize dietary phytic acid's inhibitory effect on calcium bioavailability (Adolfsson et al. 2004).

During the storage period, the total LAB varied from 9.98 to 10.08 log CFU/mL. Both LAB fermented lactose into lactic acid. The total amount of LAB decreased significantly after 14 days of cold storage at 5°C. This decreasing total LAB was due to the limited nutrition in the yogurt because most of the nutrients had been used during the fermentation and storage processes. Another cause might be that secondary metabolic substances had begun to be produced, such as bacteriocins, which have

antimicrobial activity. This result was also in line with the research of (Melia et al. 2020), which produced fermented goat milk and the total LAB decreased at storage for up to 28 days (9,106 log CFU/mL). Karami (2018) reported that the amount of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in both ewe and cow milk yogurt decreased significantly after 15 days of cold storage 5°C. Terpou et al. (2017) also reported that a decrease in bacterial cells occurred after 30 days of storage.

The viability of LAB during storage decreased not significantly ($P>0.05$). The viability of LAB at each observation time ranges from 99-100%. If the viability of probiotic bacteria was at a high level, health benefits were achieved. The amount of LAB as a probiotic is usually 10^7 - 10^8 CFU/g. A decrease in the CFU amount below the range (107-108 CFU/g) decreases the desired pharmacological activity (Patil et al. 2019). This relatively high viability was due to one of the cultures used was *L. plantarum strain VP-3.3*, which was selected as LAB as probiotic characteristics (Table 1). Besides, sago starch and WIP through microencapsulation could protect LAB during fermentation and storage at refrigerated temperatures. These results were also confirmed in the LAB microencapsulation study by (De Prisco et al. 2017) that starter culture microencapsulation in making yogurt can increase LAB's viability, thereby improving its quality yogurt.

In conclusion, based on the molecular identification results with 16S rRNA, the K12.1 isolate with probiotic characteristics was *L. plantarum strain VP-3.3*. The microencapsulation starter concentration affects acidity, total acid, total LAB, viscosity, and total solids. The use of 7% microencapsulation starter of *L. plantarum strain VP-3.3* and *S. thermophilus* gave the best yogurt, with a pH value of 4.36, total lactic acid 0.99%, total LAB 10.00 log CFU/mL, viscosity 546.37 cP, and total solids 10.40%. Yogurt storage at cold temperatures for 28 days can still sustain the amount of LAB meeting the probiotic requirement of 9.7 log CFU/mL with a yogurt pH of 4.20 that still complies with the Indonesian National Standard of yogurt (No. 2981: 2009). The use of sago starch and whey protein isolate as microencapsulation materials maintained LAB viability in yogurt during storage for up to 28 days at 4°C above 99%.

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