



Effect of Addition of Jelly and Storage Time on the Number of Lactic Acid Bacteria in Yoghurt Processed Products

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Abstract

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BACKGROUND: Yoghurt is a milk fermentation product that is one of the highly nutritious food materials involving bacteria. Yoghurt is formed from lactic acid bacteria (LAB), namely, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Streptococcus thermophilus*. The bacteria are beneficial for health because it creates a balance of the intestinal microflora. Moreover, yoghurt has a distinctive freshness, aroma and taste, namely, sour. In the yoghurt fermentation process, LAB needed a substrate for their growth. The content of natural sugar in milk is minimal; therefore, jelly needs to be added, which the microorganism can directly use for its growth.

AIM: This study aims to determine the effect of adding jelly with various concentrations and storage time on the number of LAB in processed yoghurt products.

METHODS: This research used the measurement method of total LAB with plate count method using completely randomized design factorial pattern 1 × 3 with three repetitions. The factor I is the jelly concentration (0.5%, 0.75% and 1%) and factor II is storage time (7 days, 14 days, 21 days at a temperature of 2–8°C) with data processing using statistical tests, namely, two-way analysis of variance test followed by Duncan's Multiple Range Test.

RESULTS: The results showed significant differences in the storage time of 7 days, 14 days, and 21 days on agar with concentrations of 0.5%, 0.75%, and 1%. The highest LAB amount was on the 7th day with a concentration of 1% with 2.51×10^9 . While on the 21st day, with a concentration of 1%, it had the lowest LAB amount of 3.2×10^8 . However, the amount of LAB was still above the Indonesian National Standard, namely $>10^7$ CFU/mL.

CONCLUSION: This study concluded that the highest number of LAB was found in the addition of 1% jelly with a storage time of 7 days with a total LAB yield of 2.51×10^9 CFU/mL. Storage time on day 7, day 14 and day 21 and jelly concentrations of 0.5%, 0.75%, and 1% added to processed yoghurt had a significant effect ($p < 0.05$) on the total number of LAB ($p < 0.05$) LAB).

Introduction

Public awareness of the importance of health and nutritional adequacy in consuming foodstuffs makes them aware of drinking milk. Milk is one of the highly nutritious food ingredients because it contains complete and balanced food substances such as protein, fat, carbohydrates, minerals, and Vitamins that humans need. However, with the many benefits obtained from milk, there is a weakness in milk, which is easily damaged in a short time, so that milk becomes unfit for consumption if not appropriately handled. The development of modern technologies, so that by processing milk, you can get diverse and resistant to storage, one of which is yoghurt products [1].

Yoghurt is a fermented product that involves the services of microorganisms, namely bacteria.

Yoghurt is formed from lactic acid bacteria (LAB), namely *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Streptococcus thermophilus* are beneficial for health because they create a balance of intestinal microflora. The acidity produced can inhibit disease-causing bacteria, which are generally not resistant to acid. Fermentation is one of the technologies for preserving and processing milk; organic acids will be formed during fermentation, giving a distinctive taste to yoghurt. In addition, yoghurt has a unique freshness, aroma and flavour, namely sour and sweet [2].

LAB can produce antimicrobial compounds antagonistic to pathogenic bacteria, especially bacteriocins [3]. LAB are divided into two groups, namely homofermentative bacteria and heterofermentative bacteria. Homofermentative bacteria are bacteria capable of fermenting lactose and producing 85% lactic acid. Heterofermentative bacteria are bacteria capable

of fermenting lactose and producing 40% lactic acid and 60% acetic acid [4].

In the yoghurt fermentation process, LAB require a substrate for their growth. The natural sugar content of milk is minimal, so it is necessary to add other substrates, including jelly, which microorganisms can directly utilize for their growth [5]. Jelly is made with a hydrocolloid material, which, when mixed with water, will produce a chewy texture as a characteristic of jelly [6]. This study aims to determine the effect of adding jelly with various concentrations and storage time on the number of LAB in processed yoghurt products.

Methods

This research was conducted using an experimental method with descriptive narrative data analysis. The plate count method and a completely randomized design with a factorial pattern measure the total LAB. Factor I is the jelly concentration (0.5%, 0.75% and 1%), and factor II is storage time (7 days, 14 days, 21 days at 2–8°C). The combination of treatments from this study was $3 \times 3 = 9$ using three replications so that 27 trials were obtained. The research was carried out in April-June 2021 at the Microbiology Laboratory of the Health Polytechnic of the Ministry of Health, Jakarta III. The yoghurt used in this study has undergone an organoleptic test using the guide for making mother yoghurt by Widhyasih (2020) [7]. Yougermet® commercial starter bacteria used contains three bacteria, namely *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus*. *L. bulgaricus* and *S. thermophilus* is a combination of bacteria with symbiotic properties in milk medium.

Data analysis used univariate and bivariate analysis to analyze each variable including the amount of LAB at each jelly concentration and the length of storage days. Test the amount of LAB before doing the bivariate analysis; first, the data normality test was

carried out, namely the Shapiro Wilk test. The test results for the number of BAL data are normal, then a two-way analysis of variance (ANOVA) test is carried out, followed by Duncan's Multiple Range Test.

Results and Discussion

Yoghurt bacteria isolation

Yoghurt processed products with the addition of jelly of various concentrations and stored at a temperature of 2–8°C for 7 days, 14 days and 21 days, then isolated on de Man, Rogosa Sharpe (MRS Agar) media, incubates 2 x24 h at 37°C. The characteristics of the isolates obtained from processed yogurt can be seen in Table 1.

Total LAB test

Based on the study results, the number of LAB from mother yoghurt is shown in Table 2.

Table 2: The population of preliminary cultures of lactic acid bacteria from Mother Yoghurt

Starter	Pre-population CFU/mL
Yougermet® is a mixture of bacteria <i>Streptococcus thermophilus</i> , <i>Lactobacillus bulgaricus</i> and <i>Lactobacillus acidophilus</i>	5.9×10^8

In Table 2, the LAB test results on starter mother yoghurt are 10^8 CFU/mL. It indicates that the starter used has met the minimum quantity requirement of SNI 2981-2009 of 10^7 CFU/ml [8]. Processed yoghurt in this study was then given the addition of variations in jelly concentrations of 0.5%, 0.75%, and 1% and stored for 7 days, 14 days and 21 days at a temperature of 2–8°C. The results of the analysis of the amount of LAB in yoghurt with the addition of jelly based on the length of storage days can be seen in Table 3.

Table 3: The number of LAB in yoghurt with various jelly concentrations and storage days

Storage time (day)	Jelly concentration (%)	Number of LAB (CFU/mL)
7	Yoghurt (without jelly as control)	5.9×10^8
7	Yoghurt with 0.5% jelly addition	1.25×10^9
7	Yoghurt with 0.75% jelly addition	2.16×10^9
7	Yoghurt with 1% jelly addition	2.51×10^9
14	Yoghurt with 0.5% jelly addition	1.09×10^9
14	Yoghurt with 0.75% jelly addition	1.82×10^9
14	Yoghurt with 1% jelly addition	2.10×10^9
21	Yoghurt with 0.5% jelly addition	3.2×10^8
21	Yoghurt with 0.75% jelly addition	1.01×10^9
21	Yoghurt with 1% jelly addition	1.46×10^9

LAB: Lactic acid bacteria.

Based on the study results, the number of LAB in each treatment gave results that met the

Table 1: Characteristics of isolates obtained from processed yoghurt

Bacteria	Bacteria test	Isolate characteristics
<i>Streptococcus thermophilus</i>	Macroscopic	Transparent, milky white, round, flat and convex edges
	Macroscopic	Coccus chain
	Gram	Positive
<i>Lactobacillus acidophilus</i>	Macroscopic	Transparent, beige, irregular round, small and flat
	Macroscopic	Basil chain
	Gram	Positive
<i>Lactobacillus bulgaricus</i>	Macroscopic	Transparent, beige, irregular round, small and flat
	Macroscopic	Basil chain
	Gram	Positive

requirements of SNI 2981:2009, namely a minimum of 10^7 CFU/ml. Table 3 and Figure 1 show the average number of bacteria based on jelly concentration and length of storage days; the highest number was on day 7 with a concentration of 1% with a total of 2.51×10^9 , while on day 21 with a concentration of 0.5% has the least number of LAB, which is 3.2×10^8 .

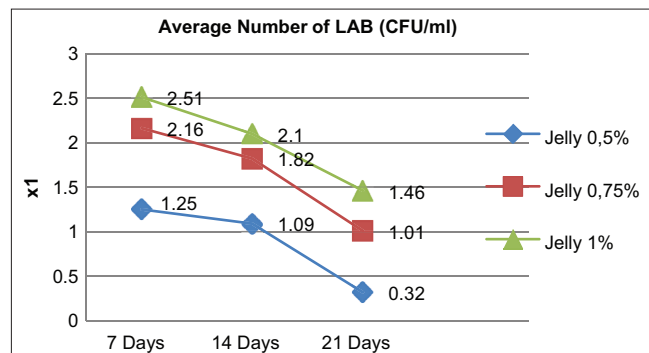


Figure 1: The number of lactic acid bacteria in yoghurt with various jelly concentrations and storage days

Before carrying out the two-way ANOVA test, the data must be tested for normality of data distribution and homogeneity of data variation. Both tests show a significance value >0.00 , then proceed to the two way ANOVA test, as shown in Table 4.

Table 4: Two-way ANOVA test results on jelly concentration and storage time

Intervention	p-value
Jelly concentration	0.00
Storage time	0.00

ANOVA: Analysis of variance.

Table 4 shows the results of the two way ANOVA test with a significant level (95%). It is known that the results of significance (Sig), namely, the storage time and jelly concentration are 0.00 (<0.05). It can be stated that there is a significant effect on each treatment, namely by adding jelly concentration and storage time to the amount of LAB. Then continued Duncan's Multiple Range Test to determine the difference in total LAB for each treatment.

Table 5 shows the results of Duncan's Multiple Range Test. It can be seen that the difference in storage time for yoghurt on day 7, day 14 and day 21 shows that the total LAB on day 7 is significantly different from day 14 and day 21, the longer it takes. Storage had a significant effect on the decrease in total LAB because the total LAB was highest with a storage time of 7 days. The decrease in LAB was due to the formation of metabolites and nutrients in the media utilized.

Table 5: Differences in the number of lactic acid bacteria based on storage days

Intervention	p-value
Day 7	100.78 ^a
Day 14	70.94 ^b
Day 21	36.67 ^c

DMRT test rate 5%.

In Table 6 shows the results of Duncan's Multiple Range Test. It can be seen that the difference

in yoghurt with jelly concentrations of 0.5%, 0.75%, and 1% from the results of Duncan's Multiple Range Test shows that the total LAB at a jelly concentration of 0.5% is not different significantly different from the jelly concentration of 0.75%. In comparison, it was significantly different at 1% jelly concentration with the jelly concentration of 0.5% and 0.75 jelly.

Table 6: Differences in the number of lactic acid bacteria based on the concentration of jelly

Intervention	p-value
Jelly 0,5%	47.83 ^a
Jelly 0,75%	58.17 ^a
Jelly 1%	102.39 ^b

Values followed by the same letter show no significant difference in the 5% DMRT test.

It is in line with research from Pranayanti and Sutrisno (2015), which states that the decrease in the number of LAB is caused by a long fermentation time but is not balanced by the availability of sufficient nutrients. Some gram-positive, require intake of essential amino acids and B vitamins in addition to carbohydrates [9].

Research results of Wirawati and Purwani (2019) explains that the highest total LAB with a storage time of 7 days, the decrease in LAB is due to the formation of metabolites and nutrients utilized in the media [10]. Another study conducted by Harjantini and Rustanti (2015) stated that at concentrations $>0.5\%$, the amount of jelly available in the ingredients could be sufficient for LAB to function optimally [5]. Several factors can cause the increase and decrease in the number of LAB, one of which is the fermentation factor, namely incubation, storage time, and the temperature used is $2-8^{\circ}\text{C}$ [10].

Conclusion

This study concluded that the highest number of LAB was found in the addition of 1% jelly with a storage time of 7 days with a total LAB yield of 2.51×10^9 CFU/mL. Storage time on day 7, day 14 and day 21 and jelly concentrations of 0.5%, 0.75%, and 1% added to processed yoghurt had a significant effect ($p < 0.05$) on the total number of LAB ($p < 0.05$ LAB).

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