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The Effects of Lactic Acid Bacteria Growth Inhibitors of *Staphylococcus epidermidis* Supplementation on the Quality of Anti-Microbial Lotion

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ABSTRACT

Staphylococcus epidermidis infections of the skin can cause boils, itching and long-lasting sores. Currently, around 75% to 90% of S. epidermidis infection cases are resistant to the antibiotic methicillin. Thus, this becomes a concern in the health sector. This research aims to discover a new method to treat S. epidermidis infection by using lactic acid bacteria as its opponent, so that the use of antibiotics can be reduced. The physical test includes confirmation of Lactic Acid Bacteria (LAB), inhibition test of lab on S. epidermidis, lotion formulation, organoleptic and homogeneity test of lotion, lotion hedonic test, LAB total plate count test in lotion, and data analysis using Microsoft Excel, Statistical Program Service Solution (SPSS), and ANOVA test. Lactic acid bacteria isolates were obtained from previous research stocks and tested for their ability to inhibit S. epidermidis in vitro. These lactic acid bacteria were added to the lotion. The favorability level of consumers to the lotion was also tested. The results indicated that lactic acid bacteria were able to inhibit the growth of S. epidermidis and remained effective until the seventh day. In addition, respondents provided a positive response to the physical quality of the lotion that contained lactic acid bacteria.

Keywords: *Lactic Acid Bacteria, Lotion Formula, Pathogenic Bacteria, Probiotics, Skin Infection, Staphylococcus epidermidis*

INTRODUCTION

Skin infections caused by bacteria are commonly found in people with improper sanitation (Wulaisfan & Hasnawati, 2017). Bacterial infections, one of which may be caused by *Staphylococcus* (Chessa et al., 2015). *Staphylococcus* bacterial infections of the skin may present with the characteristic signs of abscess formation. *Staphylococcus* is often considered a normal microbiota of the skin and mucous membranes (Sabaté Brescó et al., 2017).

Staphylococcus epidermidis is a type of staphylococcus that causes various infections and may require medical treatment. The antibiotic used against this infection is methicillin. However, it is currently found that 75% and 90% of cases of skin diseases caused by *S. epidermidis* are resistant to methicillin antibiotics. Resistance can occur due to uncontrolled use of antibiotics or even many available antibiotics that can be purchased without a prescription (Chessa et al., 2015).

Lactic Acid Bacteria (LAB) is a type of gram-positive bacteria, spherical or rod-shaped, non-sporing and produces lactic acid as its main metabolite product in the process of carbohydrate metabolism. Lactic acid bacteria can be antimicrobial agents through the production of metabolites produced (Negara & Meilani, 2023). According to Fabbrocini et al. (2016), the use of lactic acid bacteria can help inhibit pathogenic bacteria and provide positive effects for the skin, both in oral and topical use. The type of probiotic that dominates in research on the use of probiotics in topical preparations is LAB of the genus Lactobacillus (Hill et al., 2018). The increasing demand for dosage formulations with probiotic content is increasing, while the addition of probiotics in topical preparations still requires further research (Pratiwi & Susanti, 2021). Therefore, this research aims to elucidate the potential of LAB as an antimicrobial that can inhibit the growth of *S. epidermidis* and study the durability of LAB in lotion preparations and their effect on the physical quality of lotions.

RESEARCH METHODOLOGY

Confirmation of LAB

Gram staining was obtained by dripping crystal violet on a microscope slide that had been smeared with isolated LAB for 24 hours, then rested for 1 minute and washed with distilled water. Then, one drop of iodine was added to the glass slide and washed with distilled water. The glass slide was washed with absolute alcohol and then washed with distilled water. One drop of safranin was added on the glass slide and washed with distilled water. The slides containing the bacteria were dried and observed with a microscope.

Inhibition Test of LAB towards *Staphylococcus epidermidis*

Suspensions of LAB isolates were made by comparing McFarland density 1.5. Lawn of S. epidermidis was prepared on MHA media using a sterile swab. Sterile paper discs were placed on the surface of the lawn and 20 microliters of LAB suspension was applied. Paper discs with sterile water served as negative control and 1 µg/µL Cefadroxil monohydrate served as positive control.

Lotion Formulation

The lotion formulation was done in 3 phases: oil phase by mixing sunflower oil, stearic acid, dimethicone and seteraryl alcohol, water phase by mixing glycerin, TEA and distilled water and cooling phase by adding LAB isolate and milky vanilla fragrance (Arpiwi et al., 2020).

Organoleptic and Homogeneity Test of Lotion

The organoleptic test was carried out by observing the dosage form such as texture, aroma and color by the researcher, while the homogeneity test was carried out by placing the lotion on a glass plate and rubbing it and observing it (Saptarini & Hadisoebroto, 2020).

Lotion Hedonic Test

Panelists were randomly selected and filled in the prepared questionnaire. 15 panelists rated the texture, color and aroma of the lotion with LAB supplementation and the commercial lotion. The favorability scale (Hedonic) used ranged from 1-3, where (1) dislike, (2) like and (3) love it (Yanti et al., 2020).

LAB Total Plate Count Test in Lotion

Total plate count (TPC) testing of LAB in the lotion was carried out on the 1st, 3rd, 7th, 15th and 30th days. 1 mL of lotion sample was put into 9 mL of sterile distilled water and obtained a dilution level of 10-1. Dilution of each lotion is continued until the dilution level is obtained 10-2 to 10-5. A total of 1 mL of dilution 10-4 and 10-5 was put in a petri dish, sterile MRSA media was poured into the petri dish as much as 10 mL and moved slowly to form a figure 8 and allowed to solidify. Incubation was carried out for 48 hours at 37℃ anaerobically.

Data Analysis

Inhibition test data were averaged using Microsoft Excel. Meanwhile, ALT and hedonic lotion data were analyzed using Statistical Program Service Solution (SPSS) using ANOVA test.

RESULT AND DISCUSSION

Research Result

Confirmation Test Results of LAB and *Staphylococcus epidermidis*

Confirmation test was conducted by staining and catalase test. The catalase test of LAB indicated negative catalase (Figure 1), meanwhile, the results of the staining test indicated that LAB had a bassil form and belonged to Gram positive, and this was indicated by the purple LAB cells obtained from crystal violet staining which can be seen in Figure 2.

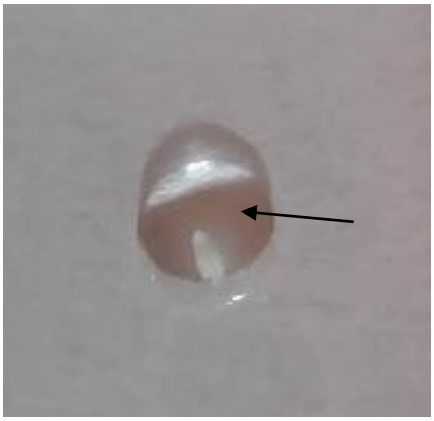

Figure 1. Negative Catalase Results (No Bubbles Formed)
Source: Observed Data by Researchers

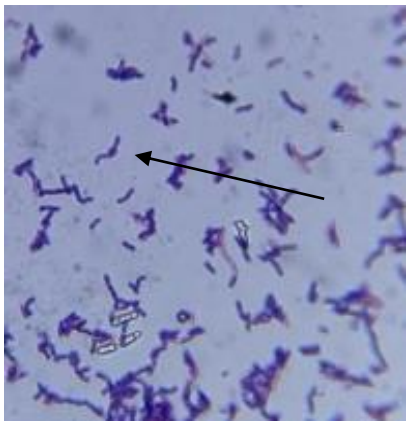

Figure 2. LAB Cells are Rod-shaped and Gram-positive
Source: Observed Data by Researchers

The catalase test on *S. epidermidis* indicated a positive catalase test (Figure 3). The staining test indicated that *S. epidermidis* has a coccus shape and is included in the Gram-positive bacteria group (Figure 4). This is indicated by the purple *S. epidermidis* cells obtained from crystal violet staining which can be seen in Figure 4.

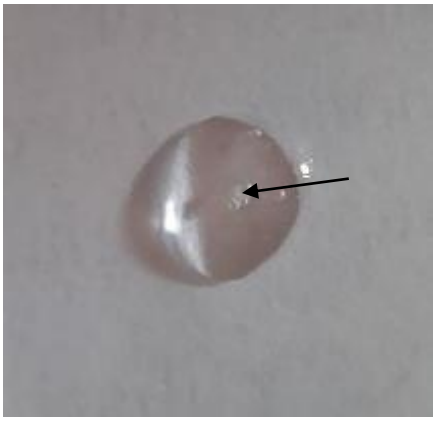

Figure 3. Bubbles Produced by *Staphylococcus epidermidis* When Breaking H_2 O_2
Source: Observed Data by Researchers

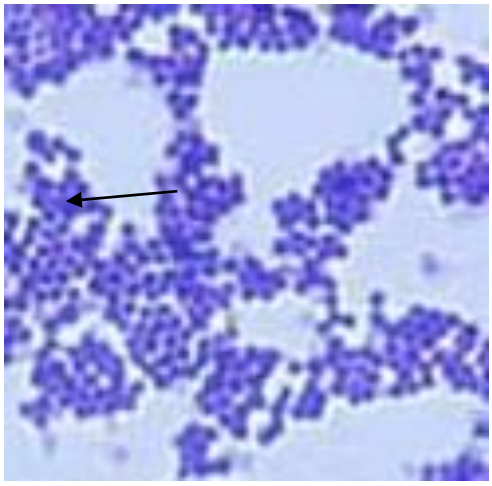

Figure 4. *Staphylococcus epidermidis* Cells are Coccus-shaped with Colonies Clustered as Grapes and Include Gram-positive
Source: Observed Data by Researchers

LAB Inhibition Test Results on *Staphylococcus epidermidis*

Based on the results of inhibition test, LAB has the potential to inhibit the growth of *Staphylococcus epidermidis*. This is indicated by the formation of a clear zone around the suspension of LAB isolate (Figure 5).

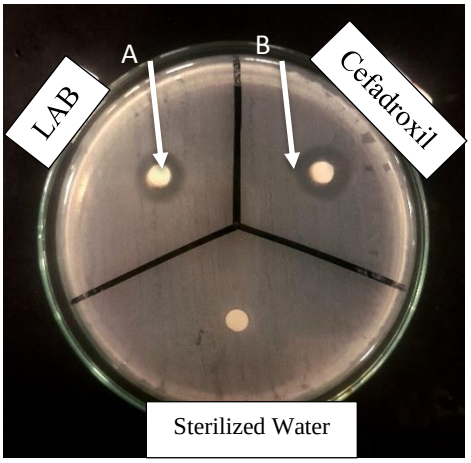

Figure 5. Inhibition Test Results of LAB against *Staphylococcus epidermidis*
Notes: A. Clear zone formed by LAB, B. Clear zone of control (Cefadroxil)
Source: Observed Data by Researchers

The diameter of the inhibition zone formed after 16 hours' incubation at 37℃, as in the following table.

Table 1. Inhibition Test Results of LAB towards *S. epidermidis*

No.	K+ Cefadroxil (mm)	LAB (mm)
1.	11	10
2.	15	11
3.	13	9
Average	13	10

Source: Processed Data by Researchers

It is evident from the results obtained that LAB isolates have antibacterial activity with the formation of inhibition zone diameter with an average of 10 mm. The positive control showed inhibition with an average of 13 mm, while the negative control showed no inhibition zone around the disc paper.

Lotion Organoleptic Test Results

Organoleptic tests were conducted to determine physical characteristics including visual observation of color, texture and aroma (Figure 6).

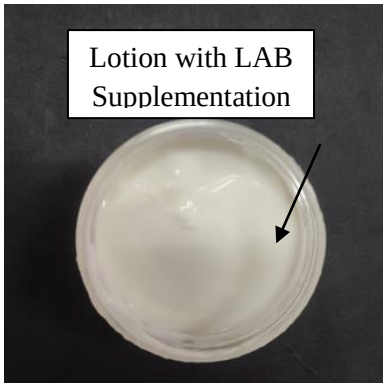

Figure 6. Lotion with LAB Supplementation
Source: Observed Data by Researchers

The results of the organoleptic test are presented in Table 2 below.

Table 2. Organoleptic Test Results on Lotion Preparations with LAB Supplementation

Formulation	Color	Texture	Scent	Impressions on Skin
L1	White	Liquid	Milky vanilla scented	Gentle, not sticky, easy to apply on the skin
L2	White	Liquid	Milky vanilla scented	Gentle, not sticky, easy to apply on the skin
L3	White	Liquid	Milky vanilla scented	Gentle, not sticky, easy to apply on the skin
L4	White	Liquid	Milky vanilla scented	Gentle, not sticky, easy to apply on the skin
L5	White	Liquid	Milky vanilla scented	Gentle, not sticky, easy to apply on the skin

Notes:
L: Lotion

Source: Processed Data by Researchers

Based on the research data obtained, lotion formulations L1, L2, L3, L4 and L5 each contained different amounts of LAB supplementation. Lotion 1 produced lotion with a weak milky vanilla scent, due to the addition of 1 mL of LAB suspension. L1, L2, L3, L4 and L5 were white in color, with a liquid texture and had a gentle, non-sticky and easy to apply impression. This occurs due to the formulation process containing more water.

Results of pH and Homogeneity Test of Lotion with LAB Supplementation

The pH test results were conducted to determine whether the lotion was acidic or alkaline. The pH measurement was carried out using a digital pH meter. Meanwhile, the homogeneity test is conducted to determine whether the lotion formulation ingredients have been mixed perfectly. Homogeneity test is conducted by using object glass as in Figure 7.

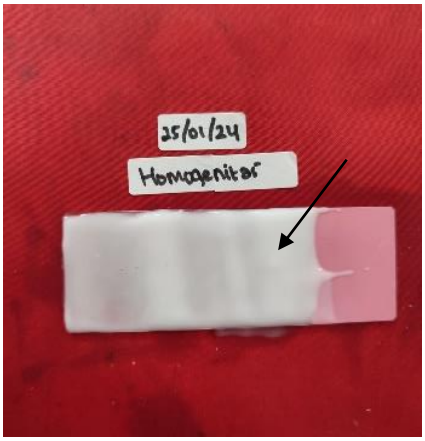

Figure 7. Lotion Homogeneity Test on Glass Object
Source: Observed Data by Researchers

The pH test results indicated an increase, where L1 had a pH of 6 while in lotion 2 (L2) to lotion 5 (L5) it increased to pH 6.1 (Table 4). These results meet the requirements of SNI 16-3499-1996 which is 4.5-8.0. The results of lotion homogeneity test showed that L1, L2, L3, L4 and L5 were homogeneous (Table 7), characterized by the absence of coarse particles on the glass object and no separation between the lotion base and LAB suspension. The homogeneity test is intended to ensure that the lotion ingredients can be mixed evenly and do not cause irritation when applied.

Table 3. pH and Homogeneity Test Results

Lotion	**pH Value**	**Homogeneity**
L1	6,0	Homogenous
L2	6,1	Homogenous
L3	6,1	Homogenous
L4	6,1	Homogenous
L5	6,1	Homogenous

Notes:
L: Lotion

Source: Processed Data by Researchers

Total Plate Count Test Result of LAB Resistance in Lotion

Total Plate Count (TPC) testing was conducted to calculate the total lactic acid bacteria that can survive in the lotion as shown in Figure 8.

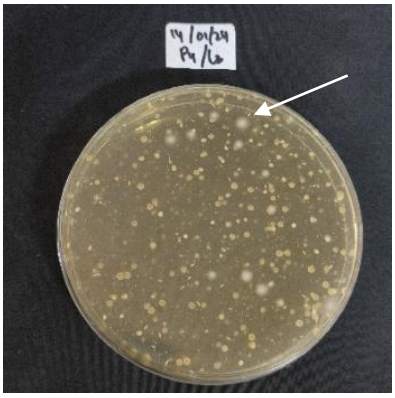

Figure 8. LAB Colonies in TPC Test LAB Resistance in the Lotion
Source: Observed Data by Researchers

Analysis of variance TPC resistance of LAB in lotion obtained the average LAB that could endure in lotion showed significantly different results in each lotion sample. The highest average growth was obtained on day 1.

Table 4. Results of Analysis of Variance of ALT Test for LAB Resistance in Lotion

Treatment	Average LAB CFU/mL		
	Day 1	**Day 3**	**Day 7**
L1	$43,8\pm3,3^{e}$ (10^{6})	$26,1\pm2,7^{e}$ (10^{6})	$8\pm0,5^{d}$ (10^{6})
L2	$34,6\pm3,3^{d}$ (10^{6})	$13,5\pm2,5^{d}$ (10^{6})	$4\pm0,5^{d}$ (10^{6})
L3	$27,7\pm2,3^{c}$ (10^{6})	$11\pm1,2^{c}$ (10^{6})	$1,7\pm0,2^{c}$ (10^{6})
L4	$17,7\pm2,3^{b}$ (10^{6})	$5\pm0,7^{b}$ (10^{6})	$0,6\pm1,8^{b}$ (10^{6})
L5	$37,2\pm0,7^{a}$ (10^{6})	$1,9\pm0,3^{a}$ (10^{6})	$0,2\pm1,5^{a}$ (10^{6})

***The values in Table 4 ± standard deviation are the mean of 5 repetitions. Values followed by unequal letters are means that are significantly different ($p<0.05$) based on Duncan's multiple range test, after analysis of variance (ANOVA).*

Source: Processed Data by Researchers

LAB resistance TPC test was conducted 5 times repetition of lotion samples with different amounts of bacterial supplementation. The TPC results in Table 4 indicate that on day 1 the number of L1 colonies was 43.8 x 106 CFU/mL then decreased on day 3 where the number of colonies was 26.1 x 106 CFU/mL and on day 7 to 8 x 106 CFU/mL. It also applies to L2, L3, L4 and L5. The result of LAB resistance is evident from the trend of decreasing the number of lactic acid bacteria colonies with the longer storage of lotion. In addition, lactic acid bacteria indicated the potential to survive in the lotion until day 7.

Hedonic Test Results

The lotion hedonic test was conducted to determine the level of respondents' preference for the lotion formulation produced, presented in Table 5. Lotion hedonic test with LAB supplementation on color, texture and scent indicated significantly different results. The most preferred lotion formulations by panelists were L5 and commercial lotion. Lotion L1 had the lowest score.

Table 5. Hedonic Test Result Data of Lotion with LAB Supplementation

Treatment	Average		
	Color	Texture	Scent
L1	1,6±0,63[a]	1,47±0,51[a]	1,73±0,7[a]
L2	1,93±0,56[ab]	1,47±0,51[a]	1,8±0,67[ab]
L3	1,93±0,45[ab]	1,73±0,6 [ab]	2,13±0,51[abc]
L4	2,07±0,25[b]	1,93±0,45[bc]	2,27±0,7[bc]
L5	2,20±0,56[b]	2,2±0,56[c]	2,40±0,63[cd]
Commercial Lotion	2,93±0,25[c]	2,73±0,45[d]	2,67±0,61[d]

*** The values in Table 5 ± standard deviation are the mean of 5 repetitions. Values followed by unequal letters are means that are significantly different ($p<0.05$) based on Duncan's multiple range test, after analysis of variance (ANOVA).*

Source: Processed Data by Researchers

Research Discussion

The results of the confirmation test indicated that lactic acid bacteria in the in vitro culture on MRSA media had a round colony shape with white to yellowish white edges. Sandi et al. (2014) also described that the characteristics of LAB colonies on MRSA are round, 1-2 mm in diameter and white in color. Other characteristics possessed by LAB are anaerobic and do not produce catalase enzyme which can break down hydrogen peroxide (Suhaeni & Syakur, 2016). Aerobic bacteria produce the enzyme catalase to protect themselves from the lethal effects of H_2O_2 that accumulates as the final product of aerobic carbohydrate metabolism. Therefore, as anaerobic bacteria, LAB exhibited a negative reaction in the catalase test (Reiner, 2016). The results obtained in this research (Figure 4), confirmed that the isolates used were confirmed as lactic acid bacteria.

Microscopic morphology of LAB isolates can be observed after Gram staining. The results of Gram staining of LAB isolates indicated that LAB isolates and *S. epidermidis* isolates have Gram-positive characteristics. This is indicated by bacterial cells that are purple in microscopic observation of LAB cells (Figure 5) and *S. epidermidis* cells (Figure 7). Similar results were also reported by Putri et al. (2020) who performed Gram staining on LAB isolated from Apis mellifera

honey. Meanwhile, the results of Gram staining of *S. epidermidis* are also in accordance with those reported by Aroza et al. (2017), which showed Gram positive. Gram staining is a staining technique used to distinguish organisms based on the characteristics of their cell walls in binding dyes and providing an overview of morphological forms (Paray et al., 2023). Gram staining classifies bacteria into Gram negative bacteria and Gram positive bacteria based on their ability to retain the dye. Gram positive bacteria have a cell wall structure with a thick peptidoglycan content, while Gram negative bacteria have a cell wall structure with a high lipid content (Nurhidayati et al., 2015). Gram-positive bacteria cannot release the color of crystal violet thus the cells remain purple, while Gram-negative bacteria cells bind safranin and can release crystal violet resulting in red (Fitrah et al., 2017).

Gram staining results indicated that the LAB isolate cells had a rod shape and were of the genus Lactobacillus, while the *S. epidermidis* isolate had a coccus shape. This is in accordance with the characteristics of LAB reported by Mathialagan et al. (2018) who isolated LAB from various types of bees which were later identified as Lactobacillus. Isolates of *S. epidermidis* bacteria have a round shape clustered like grapes and purple in color.

LAB isolates in this research indicated antagonistic activity against *S. epidermidis* with the formation of a clear zone around the LAB isolates which had an average diameter of 10 mm, while the K+ inhibition zone had an average of 13 mm. According to Rahayu et al. (2021), inhibition zone activity is categorized into four categories: weak (<5 mm), moderate (5-10 mm), strong (>10-20 mm) and very strong (>20-30 mm). The results obtained can be categorized as moderate to strong inhibition zone activity. Several previous studies have suggested that LAB isolates exhibit antagonism towards *S. epidermidis*. Mohamed et al. (2020) conducted inhibition tests using Lactobacillus and Bifidobacterium bacteria as antagonism against 13 isolates of *Staphylococcus epidermidis* and 4 isolates of *Staphylococcus aureus* bacteria, and the results indicated that Lactobacillus showed antibacterial activity against *S. epidermidis* and *S. aureus*. The characteristics of antagonism shown by the formation of clear zones around LAB isolates are caused by the diffusion of LAB metabolites that are anti-bacterial in culture media (Dejene et al., 2021). Antagonism activity can be caused by bacteriocins and organic acids produced by LAB (Gaspar et al., 2018).

Bacteriocins are antimicrobial compounds produced by various bacterial species including LAB that have bactericidal or bacteriostatic activity (Kusmarwati et al., 2014). The compound is often used as a natural preservative in food because it can inhibit the growth of pathogenic bacteria (Kumariya et al., 2019). The compound inhibits pathogen growth by forming pores in the pathogen cell wall causing the cell to lose the ability to control the transport of material into or out of the cell (Vazquez-Munoz & Dongari-Bagtzoglou, 2021). In addition, bacteriocins may cause interference with cell wall formation, nucleotide bases and

protein synthesis (Reuben & Torres, 2024). Such disruption is probably responsible for the inhibition of *Staphylococcus epidermidis* growth, which in this research appeared as a zone of inhibition. This suggests LAB can be considered as an antagonistic agent of *Staphylococcus epidermidis* that could potentially be implemented in the prevention of *Staphylococcus epidermidis* bacterial infections.

LAB resistance in the lotion was planned to be tested for 30 days, however, on the 15th day the total LAB did not grow (0). Based on the ALT test results (Table 4), lactic acid bacteria have the ability to survive in the lotion until day 7. The decrease in the number of LAB that can survive is related to the length of storage period, insufficient nutrients, moisture, or water activity (Fidyasari et al., 2022). Physiological conditions are essential for bacteria. According to Quinto et al. (2014), the physiological condition of bacteria contained during formulation in a product is an important factor in the durability of a probiotic. In addition to the above factors, other factors such as storage temperature also affect the durability of LAB in lotions. According to Noviardi et al. (2020) the optimum temperature of LAB is 37℃. This is also explained by Pangestu et al. (2021) which states that lactic acid bacteria have an optimal growth temperature of 30-37℃ at pH 5.4-6.4.

The physical characteristics of the lotions included stability evaluation, including organoleptic tests for aroma, color, and texture. The results showed lotions L1-L3 were slightly cloudy with a white color, while L4-L5 were bone white, with a liquid texture and milky vanilla aroma. L1 had a slight milky vanilla aroma, while L2-L5 had a stronger milky vanilla aroma. The scent of lotion depends on the ingredients used. All lotions were white in color due to their formulation, and had a liquid texture due to the addition of LAB. Lotion 1 was more liquid than L2-L3 due to the addition of more LAB. The texture of lotions is influenced by the variety of added ingredients (Karmilah & Rusli, 2018). According to Iskandar et al. (2021), the advantages of lotion include easy spreading and evenly applied. The liquid texture of the lotion is due to the higher water content compared to other topical preparations.

The pH test results indicated a value of 6 for L1 and between 6.1 for L2-L5, which meets the pH standard of SNI 16-4399-1996, which is a pH range of 4.5-8.0. Products in this research are in accordance with the standard pH as determined by BPOM. pH that is too acidic can cause skin irritation, while pH that is too alkaline can cause dry skin (Pratimasari et al., 2015). The homogeneity of the product made also meets the requirements set by BPOM. According to SNI standard 16-4399-1996, skin moisturizer preparations must be homogeneous without coarse grains. The study by Sapiun et al (2022) showed that the homogeneity test is needed to ensure that the ingredients in the lotion preparation are evenly mixed. This research showed that the addition of LAB did not affect the homogeneity of the lotion. The LAB suspension dissolved in the lotion formulation, so no coarse particles were formed during mixing, and the resulting lotion was homogeneous.

Hedonic test results of lotion with color, texture, and aroma parameters showed significant differences in the level of panelists' preferences for lotion color. Lactic acid bacteria (LAB) supplementation made the lotion color slightly pale. The addition of the washed LAB suspension produced a yellowish-white color, causing a change in the color of the lotion. According to Kurnia, Amir and Handayani (2020), LAB colonies are white to yellowish white. Lotion with LAB supplementation showed variation in the level of liking, where formulas L4 and L5 were most favored by panelists. The texture of LAB-supplemented lotions was slightly more liquid than commercial lotions due to the addition of LAB suspension containing 0.9% NaCl. However, the aroma showed no significant difference between formulations L3 and L5 with commercial lotion, indicating panelists' preference for formulations L3 to L5. A synthetic aroma can create a distinctive and dominant aroma in a product, thereby increasing consumer preferences or tastes (Antara & Wartini, 2014).

CONCLUSION AND SUGGESTION

Conclusion

Based on the results of the research, it can be concluded that: (1) lactic acid bacteria (LAB) have the ability to attack *Staphylococcus epidermidis*. This ability can be identified from the clear zone around the LAB isolate that indicates inhibition against *Staphylococcus epidermidis*; (2) lactic acid bacteria grew well in the lotion from the first day to the seventh day, but did not grow on the fifteenth day; (3) lotion containing LAB was liked by the panelists. Most respondents liked the lotion that contained LAB.

Suggestion

The secondary metabolites produced by lactic acid bacteria in the lotion need to be examined for further testing, and there is also an in vivo test to evaluate the antibacterial effectiveness of lactic acid bacteria in treating certain skin problems.

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