

# Characteristic and quality microbiology solid soap citronella oil with the addition of *Lactobacillus brevis*

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## Characteristic and quality microbiology solid soap *citronella* oil with the addition of *Lactobacillus brevis*

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**Abstract.** Lactic acid bacteria have the potential as probiotic candidates, where probiotics provide many benefits for humans and animals. The use of probiotics in the formulation of fragrant *Citronella oil* (*Citronella oil*) soap is expected to kill the pathogen *E. coli* and *S. aureus* commonly found on the skin's surface. This study aimed to determine the addition of lactic acid bacteria in the formulation of fragrant lemongrass soap. Besides, cow's milk in the formulation aims as a prebiotic for probiotic bacteria during the saponification of solid soap. The method used was a randomized block design with five treatments (A = 0ml, B = 2ml, C = 4ml, D = 6ml and E = 5ml) with each of the four treatments. The addition of *Lactobacillus brevis* NRC0138 in the formulation of fragrant *Citronella oil* soap affects the soap's pH value, moisture content, foam power, and antimicrobial activity. *L. brevis* can survive in an alkaline atmosphere with an initial pH of 14 and the saponification process for 14 days because strain-dependent to survive and adapt to environmental conditions.

### 1. Introduction

The addition of natural ingredients efficacious in soap is able to inhibit microbes' growth that irritates the skin and contamination. Also, the use of natural ingredients has few side effects compared to the use of synthetic chemicals. One thing that can be added in making soap is probiotics, where probiotics are here as antimicrobials. The study of [1] the addition of 6 ml of *Weissella paramesenteroides* ( $6 \times 10^6$  CFU / ml) gave the best results in inhibiting *Escherichia coli* O157. Besides, probiotics are also anti-aging (delay aging of the skin) as has been done [2] States that probiotics are increasingly being used mostly for skincare and health, prevention and treatment of skin diseases, anti-aging of the skin so that it represents a visible area for skin health. Also, several studies were conducted on the effects of probiotics on the skin, including those related to the skin in pediatrics [3] at some sections related to certain disorders such as atopic dermatitis [4] or a summary overview of the use of pre- and probiotics in clinical dermatology [5] ]. Also, there are several impacts regarding allergic disease therapy using probiotics [6], probiotics in aging skin [7], application of dermal probiotics [8].

#### 1.1. Purpose of research

The study aims to determine how adding LAB as a probiotic in the formulation of *Citronella oil* solid soap on the physical and microbiological properties.



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## 2. Research methods

### 2.1. Material of search

The study using lactic acid bacteria that are as probiotics isolated from fermentation fish the name *budu* from West Sumatra, *Citronella oil*, which results from distillation in the Sawah Lunto, West Sumatra.

### 2.2. Probiotic solid soap making

Make a solid soap [9] *Citronella oil* is put into a beaker, then heated on a hot plate at 40 °C. NaOH is weighed dissolved with cow's milk in another Erlenmeyer tube, stirring using a Magnetic Stirrer for ± 1 minute. To be mixed with homogeneous, thawed on a Hot Plate at a temperature of 60 °C for ± 5 minutes, after homogeneous NaOH solution with cow's milk is mixed while stirring. Stirring the mixture using a Magnetic Stirrer for ± 1 minute (Solution 1). Stearic acid is heated on a hot plate until it melts. Melted stearic acid was mixed with solution one while still hot into a soap solution at 50 °C homogenized let stand up to 40°C, Lactic acid bacteria from broth culture was put into soap solution bacteria *Lactobacillus brevis* (according to treatment A = 0ml, B = 2ml, C = 4ml, D = 6ml and E = 8ml) into a homogeneous soap formulation, the soap that has been added by LAB is poured into molds and allowed to stand at room temperature for 14 days the soap hardens and is allowed to stand at room temperature. The observations are made according to the observed variables.

### 2.3. Lactic acid bacteria colony test

Method [10] All equipment is sterilized by autoclaving at 15 lbs at 121°C for 15 minutes. Dilution using de Mann Rogosa Sharpe (MRS) broth (Merck) as much as 0.93 grams (In one liter of aqua dest for 52.2 g of MRS Broth). Then homogenized and sterilized (15 minutes, 121°C, and pressure 15 lbs). Preparation for MRS Agar Media (Merck) (the general preparation is one liter of aqua dest for 68.2g MRS Agar). Then homogenized with a magnetic stirrer, with a temperature of 100 ° C, then autoclaved, after being slightly cool (± 55 ° C) then poured into each petri dish as much as ± 8 ml. Lemongrass oil solid soap that has been added with LAB is taken using 1 g of a sterile spoon, then dissolved in a test tube containing 9 ml of Merck MRS Broth solution, then vortex until homogeneous. This result is called a 10-1 dilution. The dilution results were taken 100 µL into an Eppendorf tube containing 900 µL of the Merck Broth MRS solution, then vortexed until homogeneous. The result of this dilution is called a 10-2 dilution, and so on until a 10-7 dilution. Dilution 10-7 was then taken 100 µl of the sample and planted by smearing it on a Petri dish containing MRS media. Then flatten with hockey stick that was previously sterilized with alcohol. This work is done in laminar flow and behind Bunsen. The inoculum was stored in an anaerobic chamber and incubated for 48 hours at 37 ° C. After 48 hours, count the number of colonies growing LAB using the Quebec colonies. The result of colony LAB calculation is multiplied by 10.

### 2.4. Moisture content

[11] Petri disk is dried and then put in an oven at 105°C for 30 minutes (W0). Samples were weighed and put in dried Petri dishes (W1). Preheated for one hour in an oven at 105°C. Let it cool in a desiccator to room temperature and then weigh (W2).

### 2.5. Foam power testing

Method of [12] foam power testing, namely a sample of 1 ml of solid soap pipette and dissolved in 9 ml of distilled water. The mixture of soap and aqua dest samples is put into a test tube with a measuring scale to facilitate the measurement of the foam formed later. Stirring is done with the help of a vortex tool for 2 minutes. The foam formed is observed, measured, and recorded the height of the foam formed. Foam height is measured again after 1 hour (high-end foam).

### 2.6. Antimicrobial activity

Modification [13] Solid soap weighed 1 g, homogeneous with 1 ml of sterile aqua dest in Eppendorf. Centrifuged to get a supernatant at a speed of 10,000 rpm for 5 minutes at 4°C, the supernatant was filtered with a 0.22 µm membrane, so that a cell-free supernatant was obtained, then prepare pathogenic microbes, pathogenic bacteria carried out rejuvenation (enrichment) namely *Staphylococcus aureus* ATCC25923

and *Escherichia coli* O157 at a temperature of the cell 37°C for 24 hours, pathogenic microbes were added as much as 0.2% into 20 ml Muller Hinton Agar (MHA), which had been cooled to a temperature of 50°C and allowed to stand until it solidified. Made wells on compacted MHA media 4 mm in diameter using cork borer. The base of the well that has been taken is repurposed with another MHA so as not to overflow the supernatant at the bottom of the Petri disk. LAB supernatant is inserted 50 mL supernatant (Stages 1) into the well on media that already contains pathogenic bacteria, allowed to stand for 1 hour at room temperature, measure the diameter of the clear zone formed around the well and antibiotic dish using calipers so that the zone area is obtained clear or antimicrobial inhibition that inhibits the growth of pathogenic bacteria.

5

### 3. Results and discussion

#### 3.1. Total lactic acid bacteria solid soap probiotic

The total LAB test at the beginning of the saponification period turned out to add probiotics in the formulation of *Citronella oil* solid soap still found LAB, let's look at the Table 2 below.

**Table 1.** Total LAB in solid soap with probiotic

Sample	Total LAB (CFU/g)
A	0,00 <sup>a</sup>
B	152 x 10 <sup>7</sup> <sup>b</sup>
C	152 x 10 <sup>7</sup> <sup>b</sup>
D	205 x 10 <sup>7</sup> <sup>c</sup>
E	226 x 10 <sup>7</sup> <sup>d</sup>

In this study, the soap form's initial pH before adding BAL was 13-14, then added BAL and stored at room temperature for 14 days. After the saponification period of 14 days, the total LAB calculation was performed, BAL colonies were still found in the MRS Agar media. caused by the ability to grow in these bacterial isolates that are strain-dependent, where the viability depends on each species' ability even at the strain level of the bacteria itself. This is likely due to differences in the cytoplasmic membrane of each bacterium. [14] who said that the bacteria that have the best growth ability in acid conditions are lactic acid bacteria.

Report [15] that BAL species' differences in acid tolerance are related to relative permeability to protons (H<sup>+</sup>). One of the permeability of cells to H<sup>+</sup> is determined by the active outflow of protons activated by the membrane ATPase for translating H<sup>+</sup>. The optimum pH difference between the ATPase enzymes of each bacterium will determine the permeability of H<sup>+</sup>. The lower the optimum pH of the enzyme, the better the tolerance of bacteria 3 acids.

According to [16], the most critical factor determining the diversity of alkali tolerance in *Lactobacillus* is the alkali tolerance of the glycolysis strain reaction. Also, the pH formed in an alkaline environment, perhaps due to the potential of Donnan and antiporter Na (K) / H, which depends on specific energy, contributes to the tolerance of alkaline 3. Although various mechanisms help the survival of LAB in an acid environment, *Lactobacillus* seems to have poor evolutionary preparation for growth in an alkaline environment. It has been reported that previous exposure to weak environmental stresses dramatically increases tolerance to the corresponding stresses in various BAL strains [17][18]. Potential alkaline- conditioned 3 adaptations, such as those of *E. faecalis* ATCC 19433T [19], need to be examined at *Lactobacillus* for a more comprehensive understanding of the mechanism of alkali tolerance.

#### 3.2. pH value of solid probiotic soap

The pH value of the soap affects the net power and foam power of the soap. The pH value in this study lets look in table 2.



**Table 2.** The pH value of the solid soap with probiotic

Sample	pH
A	12,50 <sup>a</sup>
B	11,75 <sup>b</sup>
C	11,50 <sup>b</sup>
D	9,75 <sup>c</sup>
E	9,75 <sup>c</sup>

According to [20], the pH value of soap with a range of 9-11 can be said to be relatively safe to use. Soap with a basic pH value can help the skin in open pores during cleaning so that the foam on the soap will bind the dirt on the surface of the skin in [21]. The pH value should not be too acidic because it can cause skin irritation and should not be too alkaline because it can cause scaly skin [22]. According to [23], solid soap's pH value is in the range of 8-11. According to [24], soap with a fairly alkaline pH when used will increase the skin's pH. However, the skin can restore the skin's pH to its original state immediately after rinsing for 15-30 minutes. This buffer effect is due to the amino acid content found in the components of the skin. In addition to research [1], the addition of *Weissella paramesenteroides* lactic acid bacteria in making liquid soap with tallow base ingredients can also decrease the pH value of soap.

### 3.3. The water content of solid probiotic soap

From the research results that have been carried out, the water content of fragrant *Citronella oil* soap was obtained with the addition of LAB as follows.

**Table 3.** The water content of solid soap with probiotic

Sample	Water Content (%)
A	13,62 <sup>a</sup>
B	13,95 <sup>b</sup>
C	14,40 <sup>c</sup>
D	16,70 <sup>d</sup>
E	18,22 <sup>e</sup>

The results of this study when compared with [11] which states that the maximum water content in solid soap is 15%, meaning that only treatments A, B, and C meet the SNI criteria for solid soap while treatment D and E do not meet the criteria according to SNI because it has a moisture content above the maximum limit. According to [25] to get soap with good water content, soap is stored for 3-4 weeks. Soap with high water content can be stored in a dry place with the aim of water evaporating into the air. The water in the soap evaporates so that the water (humidity) can be minimized [26].

According to [27], analysis of soap water content in the range of 10-20% is recommended so that the product is not overgrown by destructive microbes. The high implication of the water content in soap will cause a reaction to free fatty acids and glycerol in the saponification processor known as the hydrolysis process on soap during the storage period [28]. The water content obtained from this study is lower when compared to the study of [29], where the addition of white tea extract in the manufacture of transparent solid soap made from VCO at a concentration of 1.5% with a water content value of 21.28% is due to saponin content in white tea which is a group of glycosides so that it is hygroscopic which can absorb water vapor from the surrounding environment.

### 3.4. Probiotic solid soap foam power

Determination of foam power on a soap is one of the parameters that must be considered because to see the clean power of soap on an object. The foaming power in this research can be seen in the following table.

**Table 4.** Power foam solid soap probiotic

Sample	Power Foam (cm)
A	3,03 <sup>a</sup>
B	3,10 <sup>a</sup>
C	3,30 <sup>b</sup>
D	3,93 <sup>c</sup>
E	4,15 <sup>c</sup>

According to [30], an examination of high or low foam power on a soap is one factor for controlling a detergent or surfactant product to produce preparations that can produce foam power. The increased capacity of the liquid soap foam due to the increase in the addition of *Weissella paramesenteroides* probiotics is caused by an increase in the stability of the foam formed if the pH of the soap near seven will form a more stable foam. This is because soap bubbles will form optimally if the pH is not too acidic or too alkaline.

### 3.5. Probiotic solid soap antimicrobial activity

The addition of LAB in the manufacture of fragrant oil lemongrass soap is expected to increase the ability of fragrant *Citronella oil* soap in inhibiting and or killing pathogenic bacteria found in the skin.

**Table 5.** Antibacterial activity of solid soap with probiotic

Sample	Clear Zone (mm)	
	<i>S. aureus</i>	<i>E. coli</i>
A	2,78 <sup>a</sup>	2,03 <sup>a</sup>
B	3,28 <sup>a</sup>	2,10 <sup>a</sup>
C	4,10 <sup>b</sup>	2,50 <sup>a</sup>
D	8,58 <sup>c</sup>	3,53 <sup>b</sup>
E	14,49 <sup>d</sup>	4,48 <sup>c</sup>

The area of the clear zone obtained from the study can be said to belong to the *S. aureus* test bacteria's strong class. Because it is by the opinion of [31] stated the antimicrobial activity of the test with the classification criteria of intermediate class (6-9 mm), strong (10-14 mm), and very strong (15-18 mm). The addition of LAB in the cleansing of perfumed lemongrass soaps and being antimicrobial also protects and treats the skin. This is due to the LAB as a probiotic, where normal skin microflora is likely to be involved in excluding pathogens, a function that might be enhanced by probiotics [32]. Certain probiotics can contribute to modulating microflora skin sequences, lipid barriers, and the skin's immune system, leading to skin homeostasis preservation [7]. *Citronella oil* can inhibit bacteria with inhibition zones of 8 mm against the growth of *E. coli* and 13 mm to *Staphylococcus aureus*' growth at a concentration of 25% w/v [33]. The inhibition obtained in this study is different when compared to the citronella essential oil research reported [34] 3.0-11 mm for *E. coli* bacteria and 3.0-12 mm for *S. aureus* bacteria at concentrations of 25, 50, 75, and 100 ppm. The difference in inhibition obtained is caused by several factors, including the level of concentration, type of plant, and plant growth. In his study reported that essential oils of fragrant lemongrass leaves from Tawangmangu were able to produce inhibitory zones against *S. aureus* and *E. Coli* [35]. The results showed that essential oils' antibacterial activity from citronella leaves was greater against *S. aureus* bacteria.

## 4. Conclusion

The addition of LAB as much as 6 ml showed the best results with a total LAB colony of  $205 \times 10^7$  CFU/g, a pH value of 9.75, a foam power of 3,93 cm, antimicrobial activity in *S. aureus* 8,58 mm and *E. coli* 3,53 mm, because it has a good density. The higher LAB addition causes the soap water content to increase so that later it will affect the characteristics of solid soap.

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