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Formulation and Inhibiting Activity of Paper Soap Yogurt Whey with Natural Dye of *Hylocereus polyrhizus* Skin Against *Staphylococcus aureus*

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Abstract

Background: Yogurt is a product of fermented cheese liquid waste, which is known to contain lactoferrin, which functions as an antimicrobial. Whey-based yogurt has not been used as an ingredient in making paper soap. This study aims to determine the formula and evaluation of paper soap with the addition of whey yogurt with natural dyes from *Hylocereus polyrhizus* skin. **Methods:** This study is an experimental study. The preparation of liquid soap from whey yogurt with natural dyes from red dragon fruit skin (*Hylocereus polyrhizus*) was made into four whey yogurt formulas with concentrations of 25%, 50%, 75%, and 100%. Product quality includes organoleptic values, pH values, foam height, and alkali antibacterial activity test against *Staphylococcus aureus* using the Disk Disk method. **Results:** The results showed that all formulas of Paper soap whey yogurt with dragon fruit (*Hylocereus polyrhizus*) skin dye showed good physicochemical characteristics, pH 9.0–11 (ASTM 9-11 requirements), foam content 41.7–52.5, and free alkali content 0.02–0.04. Paper soap whey yogurt formula with dragon fruit (*Hylocereus polyrhizus*) skin dye produced an inhibitory power of 11.27–29.6, including the medium and strong categories. **Conclusions:** The best formula for Paper soap whey yogurt with dragon fruit (*Hylocereus polyrhizus*) skin dye is soap with raw material whey yogurt of 75% whey 25% fresh cow's milk. The results of this study indicate that Paper soap whey yogurt with dragon fruit (*Hylocereus polyrhizus*) skin dye has the potential as a natural antiseptic soap.

Keywords: Antibacterial; Paper soap; *Staphylococcus aureus*; Whey; Yogurt

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Introduction

Hands are the body parts most frequently exposed to bacteria and microorganisms, making them a potential risk factor for infection. One effective way to maintain good health and hygiene is to wash your hands with soap. Soap is a product used to remove microorganisms or as an antiseptic. Antiseptic soaps available in the market include solid soap, liquid soap, and transparent soap. Among these antiseptic soaps, paper soap is currently in high demand (Verawaty, Dewi, and Wela 2020). Carrying a bar or liquid soap while traveling is considered impractical and takes up a lot of pocket space.

To maintain hand hygiene, people can use hand sanitizer as an antiseptic rather than solid or liquid soap, but it is less effective at killing microorganisms and germs than soap (Cordita, Soleha, and Mayangsari 2019). The use of alcohol-based hand sanitizers can cause hands to become dry because hand oil is reduced. Hence, the skin becomes dehydrated, thus providing easy accessibility for microbes to penetrate the skin layer

(Pradhan et al. 2020). So, there is a need for soap innovation, one of which is paper soap, which is an alternative to solid hand soap products in a small, practical, and easy-to-carry form. Paper soap has advantages, including its distinctive shape and characteristics of being lightweight, practical, and easy to carry. Paper soap is a water-soluble polymer component and a soap that comes in thin, paper-like sheets. Paper soap is a convenient soap, characterized by its water-soluble, flexible, stable, and hygienic characteristics. Paper soap is generally used for single-use. Paper soap is rarely found in Indonesia. Most paper soap is manufactured in China on an industrial scale (Eryani, Nurmalasari, and Fadilah 2023).

Many antiseptic soaps currently contain synthetic chemicals, such as triclosan, trichlorocarbanilide, and chloroxylenol, which have been linked to carcinogenic effects (Estikomah et al. 2023). Whey contains lactoferrin, which functions as a natural antiseptic (Perraudin and Valck 2020). Soaps sometimes include not only antibacterial agents, but also synthetic dyes, usually Tartrazine. (Putu et al. 2020). Dragon fruit (*Hylocereus polyrhizus*) peel contains antioxidants (Boyapati, Singh, and Ghosh 2022) and natural antibacterials (Puttaraju et al. 2021). Antibacterial agents can be replaced with natural resources, such as antibacterial agents from yogurt whey (Rum and Suherman 2021), and natural dyes can be made from dragon fruit peel. The combination of antimicrobial compounds and antioxidants in liquid cheese waste with natural dyes can be used as an agent to inhibit bacterial growth. The production of paper soap using yogurt whey from liquid cheese waste as an antiseptic has not been reported. This study aims to produce paper soap with antiseptic properties from yogurt whey, a byproduct of liquid cheese waste processing, by applying green chemistry principles to achieve a pollution-free environment.

Methods

Tools

The tools that will be used are analytical scales (AND Brand), glass (Pyrex), test tubes (Pyrex Brand), test tube racks, Erlenmeyer flasks (Pyrex Brand), 100 ml beakers, 10 ml measuring cylinders, stirring rods, horn spoons, preparations, spatulas, autoclaves (EYELA Brand Model HL36AE), LAV (Andaru Persada Brand), incubators (Memment Brand), ovens, Bunsen, vortex (EYELA Brand), stirrers (DLAB MS-H280-PN), bottles, analytical scales (AND Brand), dropper pipettes (Pyrex Brand), petri dishes, aluminum foil, micropipettes, cotton swabs, loop needles, tweezers, microscopes, calipers, incubators, refrigerators, and cork borers.

Materials

The materials used in this study were *Staphylococcus aureus* bacteria, brand X paper soap, paper disks, and filter paper, cheese wastewater, dragon fruit peel, palm oil, virgin coconut oil, olive oil, 96% ethanol, KOH, *Streptococcus thermophillus* and *Lactobacillus bulgaricus* starter cultures, Mannitol Salt Agar (MSA), and 0.5% McFarland solution. The method used in this study was experimental, consisting of making paper soap preparations and testing the paper soap preparations' inhibitory power with six treatments and four repetitions on *Staphylococcus aureus* bacteria.

Procedure

Making Whey Yogurt

Whey yogurt is made from a combination of fresh milk and whey, with variations of 100%, 75%, 50%, and 50% whey (Estikomah et al. 2023). Fresh milk is pasteurized for 30 minutes at 83–85°C, then cooled to 40°C with a starter culture of Lactobacillus bulgaricus and Streptococcus thermophiles at 3%. Stir until homogeneous, then incubate at 45°C for 16–20 hours.

Making Paper Soap

This liquid soap is made using a modification of the research by Wahid et al (2024). Minyak kelapa sebnayak 36 g of coconut oil is stirred with a magnetic stirrer until homogeneous. HPMC was added as much as 1 g, which had been developed in hot distilled water, and glycerin (10 g), and stearic acid (1.5 g), then stirred until homogeneous. The next step was to add distilled water to a volume of 100 ml, red dragon fruit peel extract to taste, and oleum rosae as a fragrance. A 10% KOH solution was added little by little while heated at a temperature of 50°C until a soap paste was formed. Yogurt whey is mixed according to the concentration (25%, 50%, 75%, 100%), stir until homogeneous, then add natural dye from red dragon fruit peel (*Hylocereus polyrhizus*), so that the following formulations are obtained (F0) paper soap formulation without active substances, (F1) paper soap formulation with a whey and milk ratio of 25%: 75%, (F2) paper soap formulation with a whey and milk ratio of 50%: 50%, (F3) paper soap formulation with a whey and milk ratio of 75%: 25%, (F4) paper soap formulation with a whey and milk ratio of 0: 100%, (K+) commercial paper soap formulation, (K-) Aquadest Put the finished soap preparation in a container. Paper soap is made using water-soluble paper. The paper is dried in an oven at 50°C and cut into 3 × 3 cm squares uniformly.

pH Test

pH measurements were performed using a pH meter (Hanna). Before use, the pH meter was calibrated using a pH 4-9 buffer solution. The electrode was rinsed with distilled water and dried. The pH measurement was performed by weighing 1g of whey yogurt soap with natural dragon fruit dye and dissolving it in 10ml of distilled water. The electrode was dipped into the container and allowed to fluctuate until a constant value was reached. The reading indicated by the pH meter represents the pH of the paper soap preparation (Zannah et al. 2025).

Foam Height

The foam height test was performed by taking 1g of the sample into a measuring cylinder, adding 10 ml of distilled water, and shaking the test tube by inverting it. The resulting foam height was allowed to stand for 5 minutes (Solikah et al. 2024).

Water Content Test

The water content test is performed to determine the water content of the soap. A sample of one sheet of paper soap is placed in a cup and then dried in an oven for 2 hours at 105°C. After 2 hours, the sample is removed. The cup and sample are weighed after oven drying (Rohmah and Estikomah, 2018). The water content test can be determined using the following formula:

$$\frac{W1-W2}{W} X\ 100\%$$

W1 : Weight of sample + cup (grams)
W2 : Weight of sample after drying (grams)
W : Weight of sample (grams)

Free Alkali Test

The free alkali test is performed by weighing approximately one sheet of paper soap and placing it in a beaker. Next, 100 ml of 96% alcohol, a boiling stone, and a few drops of phenolphthalein indicator solution are added, then heated to a boil. If the resulting

solution is purple, it is titrated with 0.1 N HCl solution until the purple color disappears (Verawaty, Dewi, and Wela 2020).

Bacterial Inhibitory Test

A bacterial inhibitory test was conducted using the disc diffusion method. A bacterial suspension with the same turbidity as McFarland 0.5 was inoculated onto the surface of MSA agar. Paper soap was first dissolved in 1 ml of distilled water, and a single sample was taken from a glass beaker. The paper disc was placed on a sterile petri dish, and 0.1 ml of the paper soap solution was added using a micropipette, then soaked for 15 minutes. The paper disc containing the paper soap was placed on the medium containing the bacterial suspension and incubated for 2 x 24 hours at 37°C.

Data Analysis

The quality test of paper soap made from yogurt whey with natural dye from dragon fruit (*Hylocereus polyrhizus*) peel focused on pH, free alkali content, water content, foam content, and antimicrobial activity. The quality of paper soap made from yogurt whey and natural dragon fruit peel (*Hylocereus polyrhizus*) dye was tested based on the Indonesian National Standard (SNI). Data quality testing was performed using analysis of variance (ANOVA) to determine the significant effect of treatment when differences between treatments were tested using the Duncan test.

Result and Discussion

This study created paper soap from whey yogurt with natural dye from dragon fruit peel (*Hylocereus polyrhizus*). Four formulations were used based on whey and milk concentrations: F1 (25% whey: 75% milk), F2 (50% whey: 50% milk), F3 (75% whey: 25% milk), and F4 (100% whey). This study aimed to evaluate the quality of paper soap and its antibacterial properties. The paper soap manufacturing process used yogurt whey as the active ingredient, dragon fruit peel as a natural dye with antibacterial properties, and coconut oil as a cleaning agent and foaming agent.

pH Test

The pH test of paper soap made from whey yogurt with natural dye from dragon fruit peel (*Hylocereus polyrhizus*) aimed to determine the pH of the preparation. This study shows the pH test results of paper soap made from whey yogurt with natural dye from red dragon fruit peel (*Hylocereus polyrhizus*).

Table 1. pH Test

Formulation	pH Score	condition SNI 2588;2017
F0	11,0^{c}	
F1	10,1^{b}	
F2	9,9^{b}	4-11
F3	9,0^{a}	
F4	9,0^{a}	

The pH test on paper soap aims to ensure its quality and safety for the skin, according to the SNI 2588; 2017 standard, with a safe pH range of 4–11. A pH value below 4 causes skin irritation. Soap with a pH above 10 will cause scaly skin (Eryani, Nurmalasari, and Fadilah 2023). The pH test results for all yogurt whey paper soap formulas meet the requirements. Based on these results, it can be stated that the preparation is good and safe for use on the hands. All formulations meet the standard of 9–11. Based on these results, it can be stated that the preparation is good and safe for use on the hands.

Water Content

This study shows the results of water content testing of paper, soap, and yogurt whey with natural dye from red dragon fruit peel (*Hylocereus polyrhizus*). The water content test results are shown in Table 2 below:

Table 2. Water Content Test

Formulation	Moisture Content (%)	Standard Water Content SNI 06-4085-1996 (%)
F0	36,2[a]	
F1	45,4[b]	
F2	48,3[bc]	Max 60
F3	48,8[bc]	
F4	51,6[c]	

The water content test on paper soap aims to ensure the quality and suitability of the product as a cleaning soap. Based on the SNI standard, the maximum water content allowed is 60%. The test results showed that all formulations met the standard: F0 (6.2%), F1 (45.4%), F2 (48.3%), F3 (48.8%), and F4 (51.6%). The higher the concentration of cheese whey in the formulation, the higher the water content, with F4 (100% whey) having the highest water content. The base (F0), which does not contain whey, has the lowest water content, resulting in a thicker texture than the other formulations. The addition of cheese whey, which includes a highwater content (93.42%), caused a gradual increase in water content in F1, F2, and F3. Statistical analysis showed a significant difference between the formulations ($p<0.05$), confirming that cheese whey yogurt affects the water content of paper soap.

Foam Height

This study shows the foam height test results for yogurt whey paper soap with natural red dragon fruit (*Hylocereus polyrhizus*) peel dye.

Table 3. Foam Height Test

Formulation	Foam Height Test Value (mm)	Foam Testing Standard SNI 06-4085-1996 (mm)
F0	52,5	
F1	48,5	
F2	47,2	13-220
F3	46	
F4	41,7	

Foam tests were conducted to assess the stability and attractiveness of paper soap. The Indonesian National Standard (SNI) stipulates a good foam height in the range of 13–220 mm. The primary function of foam is to help remove fat or oil from the skin, but excessive foam can cause skin dryness or irritation. The test results showed that all formulations met the standards: F0 (46 mm), F1 (48.5 mm), F2 (47.2 mm), F3 (52.5 mm), and F4 (41.7 mm). Foam height did not show significant differences among the formulations. Factors that influence foam formation are the ratio of fatty acids (oil) and base (KOH) in soap making. Although foam height does not always reflect cleaning ability, consumers often associate foam with product aesthetics. Statistical analysis using the Kruskal-Wallis method showed significant results ($p=0.017$), but variations in yogurt whey formulations did not significantly affect foam height.

Free Alkali Test

This study shows the alkali test results of paper, soap, and yogurt whey with the natural dye red dragon fruit peel (*Hylocereus polyrhizus*).

Table 4. Free Alkali Test

Formulation	Free Alkali Test Value (%)	Standard Free Alkali Test SNI 06-4085-1996
F0	0,02	
F1	0,02	
F2	0,03	Max 0,14%
F3	0,04	
F4	0,04	

The content of unsaponified alkali metals during the saponification process. The low free alkali content indicates good soap quality, influenced by the heating process that allows KOH to react completely with fat (VCO). The test results showed free alkali levels: F0 (0.02%), F1 (0.02%), F2 (0.03%), F3 (0.04%), and F4 (0.04%). All formulations meet SNI standards with a maximum limit of 0.14%, indicating that the product is safe and of good quality. The low free alkali content is caused by the complete reaction between KOH and VCO during heating. Statistical analysis using the Kruskal-Wallis method showed insignificant results ($p = 0.065$), indicating that variations in the concentration of cheese whey and milk do not affect the free alkali content in paper soap.

Staphylococcus aureus Antibacterial Test

The inhibition zones formed from the antibacterial test results against *Staphylococcus aureus* are shown in Table 7.

Table 5. Average Diameter of Inhibition Zone (mm)

Formulation	Inhibition (*mm*)	Category
F0	-	-
F1	$11,27^{b}$	Medium
F2	$13,23^{b}$	Medium
F3	$29,6^{c}$	Strong
F4	$13,62^{b}$	Medium
K+	$15,35^{b}$	Medium

This study shows that paper soap whey yogurt with dragon fruit peel (*Hylocereus polyrhizus*) dye produces a clear zone around the paper disk, indicating the ability to inhibit the growth of *Staphylococcus aureus* bacteria. The inhibition zone was measured using a caliper, with the results showing that the concentration of cheese whey and milk in yogurt affects the inhibition power. Cheese whey, as a nutrient-rich waste, contains lactoferrin and bacteriocin, which function as antibacterials. This study used a positive control (commercial soap), a negative control (aquadest), and a base without yogurt whey (F0), with variations in the concentration of cheese whey and milk (25%:75%, 50%:50%, 75%:25%, 100%:0). The results showed a moderate inhibition zone (6–10 mm) to strong (11–20 mm). F3 (75% whey:25% milk) had the best inhibition zone, an average of 29.6 mm, superior to F4 (100% whey, an average of 9.6 mm). The combination of whey and milk increased antibacterial effectiveness. Statistical tests showed a significant difference ($p<0.05$) between treatments, with F3 having equivalent effectiveness to the positive control (commercial paper soap). This demonstrates the potential of cheese whey as an antibacterial ingredient in paper soap.

Conclusions

The results of the study showed that all formulas of Paper soap whey yogurt with dragon fruit (*Hylocereus polyrhizus*) skin dye showed good physicochemical characteristics, pH 9.0–11 (ASTM 9-11 requirements), foam content 41.7–52.5, and free alkali content 0.02–0.04. The Paper soap whey yogurt formula with dragon fruit (*Hylocereus polyrhizus*) skin dye produced an inhibitory power of 11.27–29.6, including the medium and strong categories. The best formula for Paper soap whey yogurt with

dragon fruit (*Hylocereus polyrhizus*) skin dye is soap with raw materials of yogurt whey of 75% whey 25% fresh cow's milk. The results of this study indicate that Paper soap whey yogurt with dragon fruit (*Hylocereus polyrhizus*) skin dye has the potential to be a natural antiseptic soap..

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Declaration statement

The authors reported no potential conflict of interest.

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