

Antimicrobial Activity of Natural Solid Soap with a Combination of Liquid Cheese Waste, Turmeric (Curcuma longa), and Dragon Fruit (Hylocereuspolyrhizus) Peel

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ABSTRACT. Yoghurt whey is a product from fermented liquid cheese waste known to contain lactoferrin which functions as an antimicrobial. Yoghurt made from whey has not been widely used as a solid soap ingredient. This research focuses on producing solid natural soap made of yoghurt whey with added natural dye of turmeric and dragon fruit peel, soap evaluation characteristics, and analysis of the soap's antibacterial activity toward *Staphylococcus aureus*. The first stage of this research was the making of a yoghurt formula with the main ingredient of a varied concentration (25, 50, 75, and 100%) of whey added with natural dyes (turmeric and dragon fruit peel). The solid soap was processed through the saponification reaction between palm oil, coconut oil, olive oil, and canola oil with sodium hydroxide and variations of yoghurt whey. The characteristic test was conducted by determining free alkali, water content, pH, and foam height. The well diffusion method was applied to test the antibacterial activity. The results show that all formulas of solid soap from yoghurt whey indicated good physicochemical characteristics. The results indicate that the solid soap formula with 100% whey yogurt variant and added curcumin was found to be the best formula because it has good physicochemical characteristics and better antibacterial activity compared to other formulae.Soaps with yoghurt whey without addition and with additive dragon fruit peels and turmeric can potentially be used as antimicrobial soaps.

Keywords: Antimicrobial, dragon fruits, natural soap, tumeric

INTRODUCTION

The skin serves to protect the body against infections from environmental influences, such as microorganisms (Eisenstein, 2020). Staphylococcus aureus is a normal flora bacteria of the skin, but in large quantities, it can cause skin disease (lhuma et al., 2013). This generates the need for additional protection to the skin such as antibacterial soap. Current commercial antimicrobial soaps contains synthetic chemicals such as triclosan, triclorocarbanilide, and chloroxylenol, most of which are thought to be carcinogenic, mutagenic, and or generate allergic reactions (Ameh, et al., 2013). In this regard, to reduce the risk of health problems, natural antibacterial alternatives are needed in soap manufacturing.

Soap is the main product of the saponification process between triglyceride (fixed oil from seed) and dye solution (Atolani et al., 2016). Soaps are divided into two main categories based on its chemical components: natural and synthetic. The formulation of natural soapsare similar to that of traditional soaps. The primary components in most of these types of soaps, oils and fats originates from plants or animalswithout any additional components such as plasticizers, binders, preservatives, and parabens (Friedman, 2016). Synthetic soap is often mixed with antibacterial agents (Weatherly & Gosse, 2018) detergent products, such as triclosan and and sodium lauryl sulfate (SLS). These compounds have been recognized to induce various skin allergies and been classified as carcinogenic by the have International Agency for Cancer Research (Atolani et 2016). al., Soaps sometimesconsist not only antibacterial agent but also synthetic dyes, commonly Tartrazine(Putu et al., 2020). Turmeric (Curcuma longa) and dragon fruit peel ((Hylocereus polyrhizus) have many antioxidants (Rathore et al., 2020; Boyapati et al., 2022) and antibacterial agents (Boyapati et al, 2022; Puttaraju et al., 2021). The antibacterial agents can be replaced by natural such as an antibacterial agent from resources, yoghurt whey (Rum & Suherman, 2021) and pigments made from dragon fruit peel and turmeric.

Yoghurt whey is a product from fermented liquid cheese waste containing lactoferrin (Coimbra et al., 2013) that functions as an antimicrobial agent (Perraudin & Valck, 2020). Dragon fruit peel and turmeric are local species commonly used as ingredients in natural soaps (Purwanto et al., 2021; Wongthongdee & Inprakhon, 2013). Turmeric is a dry rhizome with a stark yellow color due to its curcuminoid content (Suryawanshi et al., 2017). Dragon fruit peel contains moderately high natural anthocyanin dyes. Anthocyanins are chemical compounds that could be used as red dyes has been suggested as an antimicrobial agent in inflammatory skin conditions such as atopic dermatitis caused by S. aureus. The combination of antimicrobial compounds and antioxidant content in liquid cheese waste with natural dyes that can be used as an agent to inhibit bacterial growth. The production of solid soap using yoghurt from liquid cheese waste as an antiseptichas not yet been reported. This present study aims to produce natural soaps with antiseptic properties from yoghurt made of liquid cheese waste and show the potential of non-food whey as a natural ingredient in skin care cosmetics by adapting the principle of green chemistry to attain a pollution-free environment.

EXPERIMENTAL SECTION

Materials and Reagents

The recycled products used as primary raw materials in the soap formulae used liquid cheese waste. Whey samples were acquired from Cangkringan, Yogyakarta, Indonesia. Dragon fruit peel, tumeric, palm oil, canola oil, and olive oil were purchased from the marketplace in Surakarta, Indonesia. Of all the chemical substances, such as NaOH and ethanol 95% were from E-Merck. Bacteria used in this research were S. aureus ATCC 25923, Nutrient Agar (NA, Oxoid), and antibiotics chloramphenicol 30µg/diskwere used the for antibacterial activity test.Reagents for antimicrobial tests, such as NA(Oxoid), and Mueller Hinton Agar (MHA), were obtained from the Integrated Laboratory Faculty of Health, Universitas Darussalam Gontor.

Organoleptic Test

The organoleptic test is conducted to describe the texture and color of the soaps (Sitompul, 2018).

Tumeric Extraction

Turmericextraction is made by applying some steps. Turmeric is washed thoroughly and then grated to maximize the extraction of turmeric extract. This test used 100 g of grated turmeric to get smooth turmeric. Next, the grated turmeric is added to 400 mL of water and filtered to obtain the extract (Mardiana et al., 2020).

Dragon Fruits Extraction

Dragon fruit extraction was made by firstly skinning the dragon fruit. Red dragon fruit skin is steamed for 15 minutes to remove its typical smell and reduce the amount of pectin contained in its skin. Next, 100 grams of dragon fruit peel is blended, 400 mL of water is added and then filtered to obtain the extract (w/v) (Mardiana et al., 2020)

Yoghurt Whey Production

Yoghurt whey was made from a combination of fullcream milk and whey with variations of 100, 75, 50, and 25% whey. The sample was added to extract the red dragon fruit peel and 2% ofturmeric. Fresh milk and whey were pasteurized for 20 to 30 minutes at 80°C, then cooled to an inoculation temperature of 40°C with starter culture of 2 to 3% (Yoghurmet consisting of Streptococcus thermophilus and Lactobacillus bulgaricus) for 24 hours.

Bar Soap Production

In making soap, the first stage was mixing 23,33% yoghurt whey (from W, A1, A2, A3, B, B1, B2, B3, C, C1, C2, and C3), to dissolve 10%NaOH. In making basic soap, whey yogurt is replaced by using 23.33% aquadest. The second stage is a mixture of 26,66% of Virgin coconut oil, 13,33% of palm oil, 13,33% of olive oil, and 13,33% of canola oil. In the third-stage, the first mixture was poured dropwise into the second mixture using a hand blender, and a trace occurred (a condition in which the Soap had formed with a sign of the thickening soap mass). After that, the mixture was stirred again until homogeneous, then added fragrance. The bar soap mass is poured into a mold and incubated for 24 hours until it hardness (Handayania et al., 2021)

Evaluation of physicochemical parameters of saponification.

pН

The pH was determined by using a pH meter that was first calibrated using standard neutral pH buffer solution (pH 7.01) and acidic pH buffer solution (pH 4.01) until it indicated a stable pH value. Then the electrode was washed with distilled water and dried with tissue paper. Samples were produced with a 1% concentration (1 gram preparation was weighed and dissolved in 100 mL distilled water). Then the electrode was dipped into the sample solution until the instrument showed a constant pH value. The number shown on the pH meter is the pH value of the preparation (Sitompul, 2018).

Water content

Five grams of the sample were weighed into a petri dish. That had been dried in the oven at 105 °C for 30 minutes. The sample was heated in an oven at 105 °C for 1 hour.After that, it was cooled in a desiccator and weighed. Measurements were made until a constant weight was obtained (Helsy et al., 2018)

Foam level

One gram of soap sample is put into 10 mL of distilled water and put into a test tube. The tube was shaken to mix soap with distilled waterfor one minute before being allowed to stand for five minutes. The foam's height was measured and documented (Idoko et al., 2018).

Antibacterial activity

Antibacterial activity was determined by measuring the diameter of the inhibition zone. The bacteria strain was grown on NA at 37 °C to rejuvenate the bacteria. Antibacterial activities were tested by agar well diffusion method for strains of S. aureus ATCC 25923. The relative antibacterial potential was measured by comparing the inhibition zone in the growth dish.The sample of solid soap was dissolved using aquadest (1:1) and sterilized using UV for 60 minutes. According to the spread plate method, a total of 20 mL of MHA plate medium was put in the petri dish. After the medium had been solidified, bacterial test suspension was leveled (swabbing) using a sterile cotton swab on the media, then wells with a diameter of 6 mm using eight cork borer wells in one Petri dish. Each well was filled with 50μ L with variants of yohurt soap preparations. Each petri was then incubated at 27 °C for 24 hours. The positive control was 30 μ g/disk chloramphenicol, and the negative control was distilled water only. At last, the diameter of the inhibition zone around the well diffusion was using callipers to determine the measured antibacterial activity (Aryani et al., 2022).

Data analysis

The quality tests for the soap bars were focused on analyzing the pH, free alkali content, water content, foam level, and antimicrobial activity. The solid soap quality test was performed based on the Indonesian National Standard (INS) (BSN, 2016) and Standard Guide for pH of Aueous Solution of Soap (ASTM-D 2002). The data organoleptic obtained were analyzed by description and quality test by analysis of variance (ANOVA) when there was a significant treatment effect; the differences between treatments were tested with Duncan.

RESULTS AND DISCUSSION

Organoleptic Evaluation of Natural Yoghurt Soap

The organoleptic was evaluated by observing the shape, colour, and texture. The bar soap variants produced in this study are shown in **Figure 1**. Yogurt whey soap is solid. The yogurt whey soap looks creamcolored while the basic soap appears light brown. The yogurt whey soap with turmeric appears yellow whie yogurt whey soap with peach-colored dragon fruit. Colour is a critical quality attribute in industries influencing consumers' choices and preferences.

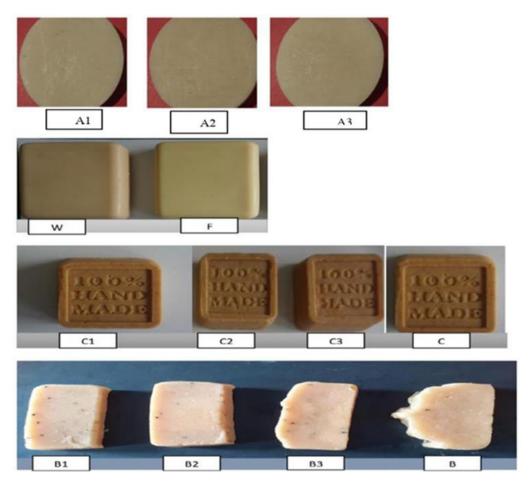


Figure 1. Liquid yoghurt waste soap (YW) with natural addactive. (note: W = YW 100%; A1 = YW75 %; A2 = YW 50%; A3 = YW 25%; B = YW100%+ red dragon fruit peels; B1 = YW 75% + red dragon fruit ;B2 = YW 50%+ + red dragon fruit peels; B3 = YW 25%+red dragon fruit peels; C = YW 100%+Tumeric;C1 = YW 75%+Tumeric; C2 = YW 50%+Tumeric;C3 = YW 25%+Tumeric; F=Basic soap)

Evaluation of Physicochemical Parameters of Soap Formulae.

The physicochemical parameters were evaluated to confirm the quality of prepared soap formula (Mahesar et al.,2019). The physicochemical parameters tests carried out in this research focus on analyzing pH value, water contains, free alkali and foam level (Atolaniet al., 2016; Habib et al., 2016; Pratama et al., 2021). The results of the characterization of solid soaps are summarized in **Table 1**.

The quality of all soaps manufactured using whey yoghurt with natural additives meets the INS for moisture content, and free alkali. The table shows that different treatments affect the quality of the soap, such as the pH.The pH value of yoghurt solid whey soap tends to decrease along with the increasing whey concentration. Whey concentration is acidic with a pH range of 4-6 (Zandona, et al., 2021), so adding yoghurt whey can lower the basic pH of the soap. Basic soap has the highest pH caused by the basic ingredients of soap. The basic ingredients of soap made from coconut oil have a pH between 9 and 10. Whey yoghurt soap with turmeric extract has a lower pH level than basic soap. This is in line with the research conducted by Fatimah (2018) which states that adding turmeric extract can lower the pH value of the soap base. The pH of soap containing turmeric extract is lower than that of soap withoutturmeric extract, therefore so adding turmeric extract can lower the basic pH of the soap. The pH of this turmeric whey yoghurt soap is in line with Wongthongdee & Inprakhon's (2013) research on manufacturing solid turmeric soap, which produces a pH of 9. Whey yoghurt soap with red dragon fruit peel extract had has a lower pH than basic soap. The nutritional content found in the skin of dragon fruit (carbohydrates, fat, ash, protein, vitamin C, vitamin E, vitamin A, alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolic, carotene, and phyloalbumin), especially carbohydrates in the form of fructose sugar, can be a source of energy for LAB. The increase in the energy sources accompanied by the longer incubation time can stimulate the growth of LAB in producing lactic acid, affecting the pH value (Dianasariet al., 2020). pH is an important parameter in cosmetic products because the pH value can affect skin absorption (Warraet al., 2011).

pH value determines the soap's suitability as a solid soap. The resulting yoghurt solid soap is at a pH of 10-11. According to ASTM D 1172-95, the pH value of a good soap ranges from 9-11, so solid yoghurt soaps are safe for the skin (Pratama et al., 2021; Widyasanti et al., 2018). The pH value in soap indicates the absence of free caustic alkali (Gyedu-Akoto et al., 2015). Several studies on soap bars generally havesuggest a pH ranging from 9.01 to 10.00 (Tarun et al., 2014). Commercial solid soaps have a pH range of 9-10. For example; Lux has a pH of 10.23; Dettol has a pH of 10.17; And Imperial Leather has a pH of 10.12 (Idoko, et al., 2018); Lifebuoy has a pH of 9.71(Habib et al., 2016).

Water content is a parameter used to assess a product's shelf life. The results of ANOVA with a 95% confidence level showed that the variations in yoghurt significantly affected the water content of the solid soap produced. Further analysis using DMRT revealed that solid soap with yoghurt significantly. Water content measurement needs to be done because it will affect the quality of the soap. The value of the water content in solid bath soap according to Indonesian National Standard (INS) 3532: 2016. The water content in the quality requirements is a maximum of 15% (BSN, 2016), so it can be said that the research results is following INS.

Whey Yoghurt	pH value	Water contains (%)	Free alkali (%)	Foam level
Soap				
Α	9.51±0.01°	9.80±0.10 ^g	0.0320 ± 0.001 ^{cd}	12.5±0.25°
A1	9.59 ± 0.03^{b}	9.60±0.10 ^g	0.031 ± 0.001^{bc}	12.66±0.76°
A2	9.64±0.02°	8.93±0.15 ^f	0.030±0.001°	12.75±0.25°
A3	9.64±0.15°	9.90±0.10 ^f	0.029 ± 0.001^{ab}	13.25±0.25°
В	9.74±0.01 ^d	8.80 ± 0.10^{f}	0.028±0.001°	12.75±0.76°
B1	9.83±0.01°	6.50 ± 0.30^{b}	0.029±0.001 ^{ab}	12.83±0.01°
B2	9.86±0.01 ^f	6.68 ± 0.02^{bc}	0.030±0.001°	13.00±0.25°
B3	9.88 ± 0.02^{f}	5.90±0.02°	0.032 ± 0.001 ^{cd}	13.25±0.25°
С	9.90±0.03 ^f	7.90±0.05 ^e	0.029±0.001 ^{ab}	12.75±0.25°
C1	9.91 ± 0.02^{f}	7.70±0.01 ^{de}	0.031 ± 0.001 bcd	12.83±0.76°
C2	9.95 ± 0.02^{h}	7.53 ± 0.05^{d}	0.031 ± 0.002^{bcd}	13.08±0.25°
C3	9.96 ± 0.03^{h}	6.70 ± 0.02^{bc}	0.031 ± 0.001 bcd	13.23±0.25°
Basic	10.03 ± 0.04^{i}	11±0.11 ^h	0.033 ± 0.001^{d}	12.66±0.52°
INS	-	15	2.5	NF

 Table 1. Evaluation of physicochemical parameters of soap formulations

Note: NF=not found. W=cheese whey soap; A=YW 100%; A1=YW 75%; A2=YW 5; 0%; A3=YW 25%; B=YW 100% + red dragon fruit peels; B1=YW 75%+red dragon fruit; B2=YW 50%+red dragon fruit peels; C=YW 100%+Turmeric; C1=YW 75%+Tumeric; C2=YW 50%+Tumeric; C3=YW 25%+Tumeric. The notation followed by the same letter shows no difference.

The high-water content in the soap will cause the excess water to react with the unsaponifiable fat to produce free fatty acids and glycerol in a process called soap hydrolysis on storage. The water content is highest in treatment (making soap without adding extra natural dyes) because whey contains 90% consisting of water (Purwanto et al., 2021). The higher the water content in the soap, the softer the soap will be and easily dissolve in water. Then, it will run out quickly when used. Soap with a high-water content will shrink faster when used. Meanwhile, soap that contains a little water can last its shelf life. However, the duration of soap storage can affect the hardness of the soap because the water content in the soap evaporates over time (Sari, & Nofita, 2015).

The free alkaline test aims to determine the suitability of the free alkaline content that exists against the quality standard of solid soap. The results of ANOVA with a 95% confidence level showed that the variations in yoghurt significantly affected the free alkali content of the solid soap produced. Further analysis using DMRT revealed that solid soap with yoghurt significantly. The high free alkali is caused by the ratio of water to whey as raw material (Idoko et al., 2018). The high-water content in the soap ingredients causes a high concentration of free alkalis. The free alkali residue shows a value that meets the INS, suggesting that the composition of oil and base used in the soap-making process is appropriate. According to INS 2016, the remaining free alkali must not be more than 2.5%. The free alkaline residue soap liquid yoghurt waste cheese shows the value that meets the INS, suggesting that the composition of oil and base used in the soap-making process is appropriate (Gyedu-Akoto et al., 2015).

Foam is defined as a dispersion system consisting of gas bubbles covered with a layerof liquid. The results of ANOVA with a 95% confidence level showed that the variations in yoghurt not significantly affect thefoam. The height of solid soap foam is influenced by the fatty acid content of the material. The best produce foam is yoghurt soap from varians yoghurt 75% milk 25% whey (soap ingredients used 23.33% yogurt whey, with varian yogurt 75% milk 25% whey) because soap with 75% milk contains more lauric acid, myristic acid, linoleic acid, and oleic acid. Cheese whey has a lower fatty acid content than milk. In cheese whey the fat has been used in cheese production. The quality of foam for soap products is not determined in INS, because the height of foam is not related to the ability of a soap product in the cleaning process, but it is related to consumer perception and aesthetics (Iriany et al., 2020). The foam height of the turmeric whey yoghurt soap foamed higher because it was influenced by the presence of foam produced from saponin compounds owned by turmeric. Saponin compounds have been known to form foam due to the combination of the structure of their constituent compounds, namely the nonpolar saponin chain and the polar side chain

which dissolves in water. Saponin has an important role as foam producer in soap (Warraet al., 2011).The content of saponins functions as a washing agent that has soap-like properties and acts as a surfactant. Saponins from a colloidal solution in water form a stable foam when shaken and do not disappear with the addition of acid (Hidayah et al., 2022).

Antimicrobial Activity

The antimicrobial activity was tested using well diffusion method shown in Figure 2. Solid soap from liquid yoghurt waste cheese has growth inhibition activity against S. aureus. This bacterium can cause skin infections (Chessa et al., 2016). This study uses different concentrations to determine the inhibition zone because antibiotic treatment is concentrationdependent (Greulich et al., 2017). Based on Ozer et al(2002), some conditions can affect the inhibitory, including concentration, sample dilution, and duration of contact with organisms. All soap exhibited resistant inhibition activity toward Gram-positive bacteria. The positive control used was chloramphenicol. This is based on the presence indications for these antibiotics used as a good antibacterial for gram-positive and gram-bacteria-negative. In addition, the antibiotic can be used as a topical preparation to treat skin infections. The positive control (chloramphenicol) can inhibit microbe growth (Handayani et al.,2018).The value of the inhibition zone chloramphenicol is 21 mm.The microbes Microbes do not grow in the negative control (distilled water). The overall results show an inhibited growth of S. aureus on every soap sample in this study, which is shown in **Figure 2**.

The antimicrobial activity test was performed according to the inhibition growth, as shown in Figure 2. Data analysis of ANOVA with a 95% confidence level showed that the variations in yoghurt significantly affected thediameter of inhabitation zone. Based on the Duncan Multiple range test, it can be concluded that the best concentration is at soap C (YW100% with tumeric). Whey turmeric yoghurt activity of the soap was higher than the activity of whey dragon fruit peel yogurt. This is probably due to turmeric has more than one antibacterial substance, namely curcuminoids, essential oils, flavonoids, alkaloids (Sitompul, 2018), phenols, steroids, saponins, and glucosides (Niamsa & Sittiwet, 2009). The value of the inhibition zone yoghurt whey soap is between 8.8-14.7 mm. Based on the Standard Clinical and Laboratory Standards Institute (CLSI) (Cavalieri et al., 2005).

The datain this study are distributed into three criteria, i.e., susceptible (≥ 21 mm), moderate (17-20 mm), and resistant (≤ 16 mm). All treatments showed an increase in the inhibition zone when whey concentration was added. Whey has inhibitory power because it contains lactoferrin (Dos Reis Coimbra & Teixeira, 2016). Lactoferrin functions as an antimicrobial (Perraudin & Valck, 2020), so the more added whey content, the greater the inhibition. By measuring the zone of inhibition at different, we can

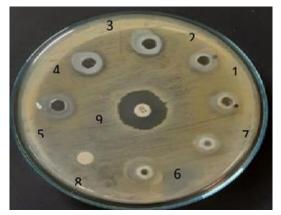


Figure 2. The area of clear zones caused by yogurt liquid waste cheese (YW) soap (mm) against E Staphylococcus aureus (Description: 1 = YW soap: 2 = YW 75%; 3 = YW 50%; 4 = YW 25%; 5 = YW 100%; 6 = YW 25% + red dragon fruit; 7 = YW soap 50% + red dragon fruit peels; 8 = negative control; 9 = positive control

Table 2. The Inhibitation zone yoghurt whey soap

Whey Yoghurt Soap	The Inhibitation zone (MM)		
Α	8.8 ± 0.1°		
Al	10.2 ± 0.2^{ab}		
A2	10.6 ± 0.1^{ab}		
A3	10.6 ± 0.2^{ab}		
В	10.9 ± 0.58^{ab}		
B1	11.11 ± 1.0^{bc}		
B2	12.43 ± 1.0^{cd}		
B3	12.46 ± 1.0^{cd}		
С	12.6 ± 1.5^{cd}		
C1	14.22 ± 1.1^{de}		
C2	14.28 ± 2.1^{de}		
C3	14.7 ± 1.5^{de}		
Basic	1.13		

W = cheese whey soap; A= YW100% A1 = YW75 %; A2 = YW` 50%; A3 = YW 25%; B = YW100%+ red dragon fruit peels; B1 = YW 75% + red dragon fruit ; B2 = YW 50% + red dragon fruit pells; B3 = YW25%+ red dragon fruit peels; C = YW100%+Tumeric; C1 = YW 75%+Tumeric; C2 = YW 50%+Tumeric; C3 = YW 25%+Tumeric. SD = Standard Deviasi. The notation followed by the same letter shows no difference.

see whether the antimicrobial is bacteriostatic or bactericidal. It is shown in this study that almost all treatments show a decrease in the diameter of the inhibition zone when its concentration is added. This indicates that the antimicrobial in this study is bacteriostatic.Soaps with yoghurt whey without addition and with additive dragon fruit peels and tumeric can potentially be used as antimicrobial soaps.

CONCLUSIONS

All formulae of solid soap from yoghurt whey showed good physicochemical characteristics, pH 9.51 – 10.03 (ASTM requirement 9-11), water content 5.9–11 % (SNI requirement less than 15%), free alkali 0.029 – 0.033% (SNI requirement maximum 2.5%), so it cat be concluded that all formulas meet the requirements established by SNI 3532: 2016 and ASTM-D 2002. The formula of solid soap variant yoghurt 100% whey with curcuminwas found to be a more promising formula as it shows good physicochemical characteristics and better antibacterial activity than other formulae. Soaps with yoghurt whey without addition and with additive dragon fruit peels and tumeric can potentially be used as antimicrobial soaps. These soaps can further be used as a biopharmaceutical product in a treatment of bacterial skin infections.

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