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The Utilization of Whey Cheese Fermented with Kefir Grains as An Antibacterial Solid Bar Soap

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Abstract

Staphylococcus aureus is a bacterium known for causing skin infections. Kefir whey cheese antibacterial soap emerges as a skin treatment and protector, leveraging compounds like lactoferrin with antibacterial properties. This study aimed to evaluate the characteristics of kefir whey cheese solid soap formulations and assess their effectiveness in inhibiting Staphylococcus aureus growth. The experiment involved creating five soap formulations with kefir whey cheese concentrations (P1: 25%, P2: 50%, P3: 75%, P4: 100% kefir, and P5: 100% whey cheese). Physicochemical characteristics, including free alkali, pH, and foam value, were analyzed to ensure soap quality. The bacterial inhibitory power was tested using the well diffusion method. Data analysis employed SPSS. Results indicated that all kefir whey solid soap formulations exhibited favorable physicochemical traits, with pH ranging from 10.22 to 11.55 (meeting ASTM requirements of 9-11), water content between 5.9% to 11% (below the SNI requirement of 15%), and free alkali within the range of 0.0055% to 0.024% (well below the SNI maximum of 2.5%). The study concluded that all formulations complied with SNI 3532:2016 and ASTM-D 2002 standards. The most effective formula was found to be 100% whey. This research presents kefir whey cheese solid soap as a potential alternative for antiseptic bar soap production, showcasing promising prospects in skin care and hygiene.

Keywords: Antibacterial, Natural, Skin, *Staphylococcus aureus*, Infections

1. Introduction

Introduction, *Staphylococcus aureus* bacteria are normal flora found on human skin, a type of pathogenic bacteria that can cause infections in the skin, entering the human body through the digestive system, respiratory system, urinary tract and skin(McFarland, 2000). Soap is an alternative that can be used to prevent infection. Soaps can be made naturally or synthesized. Soap synthesis is a bar of soap that is its manufactured using ingredients such as antibiotic that even have been shown to irritate the skin in some people. The use of natural ingredients in soap making is expected to improve the quality of the resulting soap (Handayania et al., 2021). One of the natural ingredients that can be used in the process of making soap is kefir whey ((I Helsy et al., 2018)

Whey as a major by-product of cheese production is the subject of surveys in the past decade because of its nutritional value. Characteristics of fermented whey using kefir grains have been investigated by different authors (Londero et al., 2011, 2012; Balabanova and Panayotov, 2011; Megalhães et al., 2011; Shukla and Kushwaha, 2017) Whey contains lactoferrin which functions as antioxidants (J.S. dos Reis Coimbra and J.A. Teixeira, 2013). Ingredients containing antioxidants can be used to reduce oxidative stress which triggers acne growth (Sarici et al., 2010; Batubara & Mitsunaga, 2013). Kefir can be considered a carrier of *probiotics* and various bioactive compounds, including peptides, polysaccharides, and organic acids that may play a functional role in skin care(Chen et al., 2006).

kefir has the potential to inhibit bacterial growth, investigated by different Levkov et al., (2021). as a potential ingredient of beauty and Kefir is used as an object of research in soap making By I Helsy et al (2018) and Marya et al.,(2023). Nevertheless, kefir whey has not been fully utilized either as a potential ingredient of natural soap antiseptic. This study tries to investigate kefir whey potential as a beneficial local product in the form of an organic antibacterial and evaluation characteristics that meets the qualification of the Indonesian National Standard (INS).

2. Materials and methods

2.1 Materials

The ingredients used in this study were whey from Cangkringan Yogyakarta, starter kefir, solid coconut oil, olive oil, coconut oil, *Staphylococcus aureus* bacteria, MHA (Mueller Hinton Agar) media, NaOH, aquadest, starter kefir.

2.2 Data collection procedures

2.2.1 The kefir manufacturing procedure

The kefir manufacturing procedure refers to the Indonesian Milk Kefir Association (2016) which has been modified. In the first stage, fresh milk is pasteurized at 85°C for 15 seconds. Milk is lowered in temperature until it reaches room temperature. At the next stage, the addition of whey is carried out. *The whey* used is a by-product of the cheese processing process. This *whey* results from the curd separation process. The concentration of *whey* used is 25% (A), 50%(B), 75%(C), and 100%(D). It was then inoculated with 5% *kefir grains* per 200 mL, muffled at room temperature (25-30°C) for 24 hours. Kefir harvesting is carried out using a stainless sieve which aims to separate kefir *grains* from harvested whey kefir. Then kefir *whey* storage is stored at room temperature less than 4°C for 1 day.

2.2.2 Solid Soap Making

Prepared coconut oil 13,34% grams, olive oil 13,34% grams, 26,66% coconut oil, and canola oil 13,34% grams. Mixed the oil ingredients into a container and stirred until homogeneous. Then pour 23,33% kefir whey (from A, B, C, D) to dissolve 10% NaOH. slowly put it into the oil mixture and stir, the fragrance is added and then mixed until homogeneous. The thickened dough is poured into a soap mold. It is allowed to stand at room temperature for 3-4 weeks so that the alkali can be neutralized (Handayania et al., 2021).

2.2.3 Evaluation of physicochemical parameters of saponification

A. Organoleptic Test

The organoleptic test is a method employed to assess the sensory attributes, namely color, odor, and appearance, of a particular preparation. This evaluation is conducted through direct observation utilizing the five human senses (Sitompul, 2018). The term "organoleptic" is derived from the Greek words "organon," meaning sense organ, and "leptos," signifying capable of perceiving. In essence, this test allows for a qualitative analysis of the product's physical characteristics that can be perceived by sight, smell, and touch. Researchers often rely on organoleptic testing to gain valuable insights into the overall sensory appeal and quality of a given substance or formulation, providing a holistic perspective on its aesthetic and perceptible qualities.

B. pH test

The pH test serves as a crucial parameter for assessing the suitability of soap for use. An optimal soap typically falls within the pH range of 9 to 11, ensuring its safety for skin application. The relatively elevated pH levels in soap can enhance skin absorption, but they also carry the potential risk of causing skin irritation, manifesting as issues such as peeling, dry skin, wounds, and itching. The process of soap pH testing involves taking 1 gram of mashed soap, placing it in a beaker, and adding 10 ml of distilled water while stirring until fully dissolved. Subsequently, pH measurement is conducted using a calibrated digital pH meter, calibrated with pH solutions of 4, 7, and 9. The pH meter is then allowed to stabilize until a consistent pH reading is obtained (Sitompul, 2018). This meticulous testing procedure ensures a thorough evaluation of the soap's pH, a critical factor in determining its safety and potential impact on skin health.

C. The foam height test value

The foam height test is a method employed to assess the foaming characteristics of a soap sample. The procedure involves taking a 1g sample of soap, which is then placed into a test tube containing 10 ml of distilled water (aquadest). Following this, the mixture is homogenized using a vortex, a device that imparts vigorous agitation to ensure thorough mixing. The resulting foam is then measured for its height, a quantitative indicator of the soap's foaming ability. This measurement is taken from the initial height of the foam to its final height. The foam height test provides valuable insights into the soap's ability to produce and sustain a stable and effective lather, a quality often associated with its cleansing efficacy and user experience (Idoko et al., 2018).

D. Inhibitory Power Test of Staphylococcus aureus Bacterial Solid Soap Preparations

The bacterial suspension test results were obtained by inoculating a volume of 0.1 mL onto Mueller-Hinton Agar (MHA) media, which was then evenly spread using a triangular tool. The inoculated plates were incubated in a controlled environment for 24 hours at a temperature of 37°C. Subsequently, wells were created using the agar media perforator. For the kefir whey cheese solid soap, a 5-gram portion was dissolved in 10 ml of distilled water (aquadest). A test sample of 4 μL from this diluted solution was placed into the well and incubated again for 24 hours at 37°C. The observation focused on the appearance of a clear zone surrounding the well. This methodology was employed to assess the antimicrobial activity of the kefir whey cheese solid soap against bacterial strains. The formation of a clear zone around the well indicates the inhibitory effect of the soap on bacterial growth. The process is crucial for evaluating the soap's efficacy in combating microbial activity and can provide valuable insights into its potential applications in hygiene and health-related contexts.

2.3 Data analysis

The quality assessment of the soap bars centered on the examination of various key parameters, including pH, free alkali content, water content, foam level, and antimicrobial activity. The evaluation of solid soap adhered to the guidelines set by the Indonesian National Standard (INS) as outlined by the National Standardization Agency (BSN) in 2016, along with the Standard Guide for pH of Aqueous Solution of Soap (ASTM-D 2002).

To analyze the organoleptic data obtained, a descriptive approach was employed, supplemented by a quality test utilizing analysis of variance (ANOVA). In instances where a significant treatment effect was observed, the differences between the treatments were subjected to further examination using the Duncan test. This comprehensive testing methodology ensured a thorough and rigorous evaluation of the soap bars, encompassing both physical and chemical attributes, ultimately contributing to a comprehensive understanding of their quality and performance.

3. Results and discussion

3.1 Results and discussion

Table 1. Organoleptic Test Results

Formulation	**Parameter**		
	Texture	**Color**	**Aroma**
P-1	Dense	Old Beige	Pink Ocean andtypical milk
P-2	Dense	Brownish OldBeige	Pink Ocean and distinctive milk
P-3	Dense	Brownish OldBeige	Pink Ocean and distinctive milk
P-4	Dense	Young Beige	Pink Ocean and distinctive milk

P-5	Dense	Young Beige	Pink Ocean
K(-)	Dense	White Beige	Pink Ocean and Sting

Note: P-1 : Formulation of solid soap (kefir *whey* cheese 25%), P-2 :Formulation of solid soap (kefir *whey* cheese 50%), P-3 : Formulation of solid soap (kefir *whey* cheese 75%)P-4 : Formulation of solid soap (kefir 100%), P-5 : Formulation of solid soap (kefir *whey* 100%)K (-) : Soap base.

The solid soap preparation kefir *whey* cheese formulation P-1 showed a dark beige color because it gives less *whey* cheese concentration of 25%. The P-2 and P-3 formulations were dark brownish cream due to the administration of more *whey* cheese, which was 75%. As for the P-4 and P-5 formulations, they gave a light beige color, which came from the original color of kefir, which is yellowish white, allegedly due to the presence of beta-carotin, fat, and casein P-4.[125] And P-5 comes from the *whey* color of cheese, which was greenish-yellow in the form of a clear liquid.[126] The preparation of the K-soap base produced a white beige color due to the saponification reaction that occurs and the use of equates to replace kefir *whey cheese.*

2. pH Value

pH measurements are used to express the acidity or alkalinity of soap preparations and determine the viability of soap as a body wash. The pH measurement process uses a pH meter. Generally, soaps have a pH of about 9-11 safe for the skin.

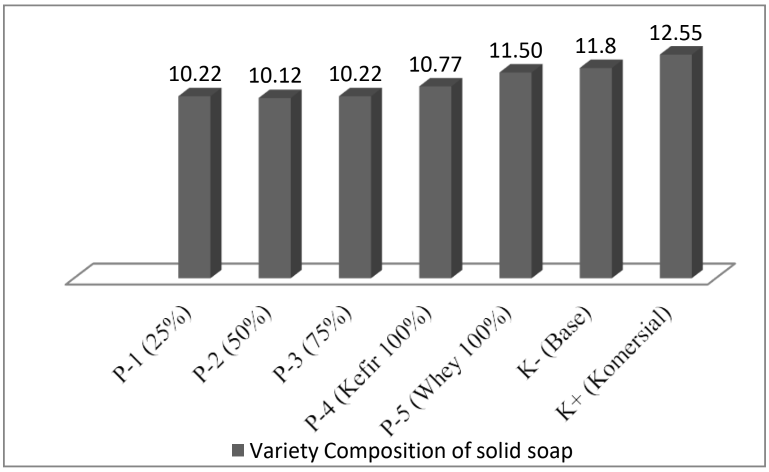

Figure 1. The test results of pH values for solid soap with various compositions

The test results of solid soap preparations kefir *whey* cheese P-1, P-2, P-3, and P-4 were included in the pH range of solid soap according to (ASTM requirement 9-11), which was 9-11,. The P-5 formulation had the highest pH due to the concentration of *whey* cheese as much as 100%. Judging from the chemical characteristics of *whey* cheese the pH value was 4.60 acids which could affect the pH results of solid soap kefir *whey* cheese to be more alkaline. P-4 was lower in pH than P-5 due to the administration of a kefir concentration of 100%. It was known that the pHof kefir is more acidic than *whey* cheese, which was 3.90 which can affect the pH yield of solid soap kefir *whey* cheese to decrease. The P- 1, P-2, and P-3 formulations showed no more equal pH ranges because the application of a mixture of kefir and *whey* cheese to each formulation results in a decreased pH. Meanwhile, the preparation of the K- soap base showed high pH results allegedly due to the use of aquades so that the saponification reaction was not perfect.

3. Free Alkali Value

Free alkali testing is used to determine the amount of free alkalicontained in solid soap kefir *whey* cheese. Free alkali is a strong base in soap that is not bound to the fatty acids that make up the soap. If the alkalicontent exceeds the standard 0.1% it will cause irritation to the skin and if the free alkali content is too high it will cause the skin to become dry. In addition, its free fatty acid content will cause a rancid odor.

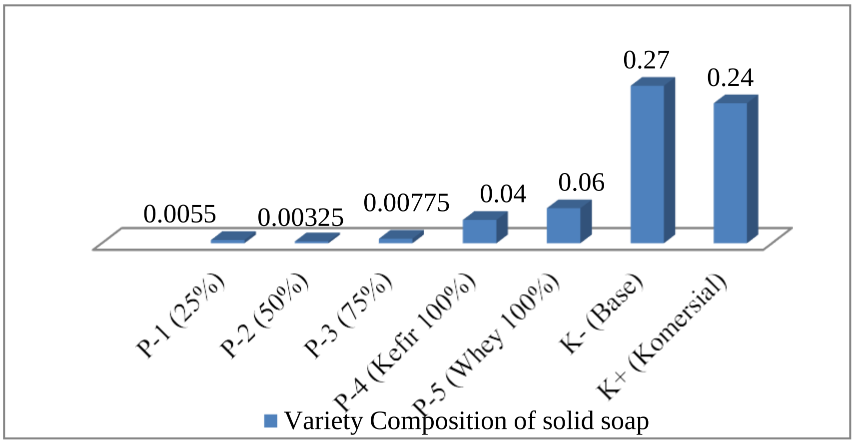

Figure 2. The test results of free alkali values for solid soap with various compositions

The results of solid soap preparations of kefir *whey* cheese P-1, P-2, P-3, P-4, and P-5 meet the SNI standard of less than 0.1%. This was because the saponification reaction has taken place optimally where all bases react perfectly and are exhausted to react with fatty acids at the time of soap formation. After mixing kefir *whey* cheese the results of free alkalicontent decreased further in P-1, P-2, and P-3 formulations compared to P-4 and P-5 which were only 100% kefir and 100% *whey* cheese. Meanwhile, the results of negative controls did not meet SNI standards. This was cause by alkalis condition did not react perfectly with fatty acids during the saponification process.

3. **Antimicrobial activity**

The antimicrobial activity test was performed according to the inhibition growth, as shown in Figure 3. Data analysis of ANOVA with a 95% confidence level showed that the variations in yoghurt significantly affected thediameter of inhabitation zone. Of the fiveformulations, the more *whey* cheese content given, it would increase the diameter of the inhibitory zone formed as in the P-1, P-2, and P-3 formulations (medium inhibitory power). Presumably in addition to lactic acidbacteria which are able to inhibit bacteria, there is a lactoferrin content in whey that has the potential to inhibit *Staphylococcus aureus* bacteria. Lactoferrin interacts with the target bacterial cells through absorption of iron, and the permeability of the bacterial cell wall. Lactoferrin will bind to lipopolysaccharides on specific receptors to inhibit bacterial cell growth. In addition, lysozyme and lactoferrin synergistically inactivate *Staphylococcus aureus* bacteri.

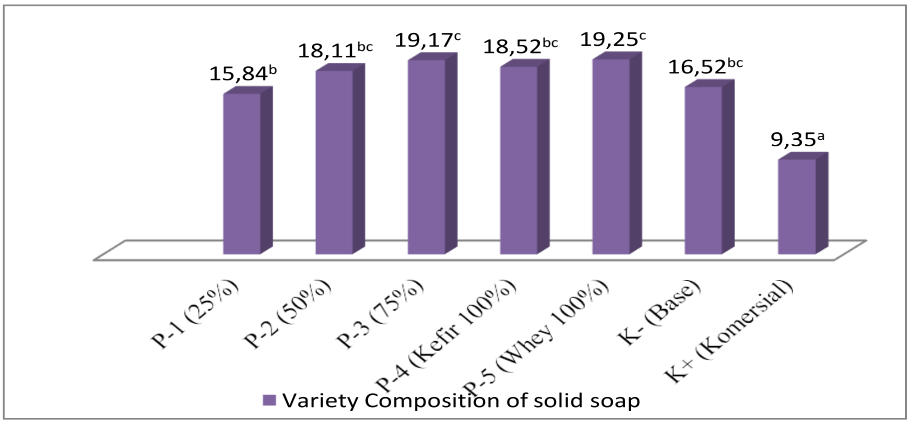

Figure 3. The test results of the antimicrobial activity for solid soap with various compositions

The bacterium used for testing kefir *whey* cheese solid soap is the bacterium *Staphylococcus aureus*. *Staphylococcus aureus* bacteria include gram-positive bacteria that have teichoic acid on the surface of the cell wall. Teichoic acid is a compound contained in the bacterial cell wall that could be passed by antibacterial compounds that play a role in the process of activating enzymes, enzymes will be used in the process of bacterial protein synthesis. OH^- ions present in antibacterial compounds will interact with OH^+ ions with teichoic acid. This interaction will cause inhibition of teichoic acid in activating enzymes so that the protein synthesis process is inhibited and causes lysis.

4. Conclusion

All formula of solid soap from kefir whey showed good physicochemical characteristics, pH 10.22 – 11.55 (ASTM requirement 9-11), water content 5.9– 11 % (SNI requirement less than 15%), free alkali 0.0055 – 0.024% (SNI requirement maximum 2.5%), so it can be concluded that all formulas meet the requirements established by SNI 3532: 2016 and ASTM-

D 2002. The Best formula for solid soap variant kefir is 100 % whey. These soaps can further be used as a cosmetic product in the treatment of bacterial skin infections.

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