

Formulation and Antibacterial Activity Test of Liquid Hand Soap from Pineapple (*Ananas comosus* (L.) Merr.) Kombucha Against *Escherichia coli* Bacteria

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Abstract

Pineapple fruit (*Ananas comosus* (L.) Merr.) exhibits antibacterial activity due to flavonoids, phenols, saponins, bromelain, and tannins. Fermentation into kombucha generates organic acids that may further enhance this activity and can be formulated into liquid hand soap to help prevent diarrhea. This study evaluated the effects of different pineapple kombucha concentrations on the physical characteristics, physical stability, and antibacterial effectiveness of liquid soap against *Escherichia coli* using the well diffusion method. Soaps were prepared with pineapple kombucha at F0 (0%), F1 (20%), F2 (30%), and F3 (40%). All formulas were yellow to orange, had a thick liquid consistency, and produced a distinctive pineapple aroma with a slightly acidic note. All formulations met Indonesian National Standard (SNI) requirements, with pH 7.50–7.56, foam height 87.33–109.33 mm, viscosity 1398.33–3144 cPs, specific gravity 1.053–1.091 g/mL, and water content 25.32%–32.73%. Foam height and water content remained stable during testing. Antibacterial activity increased with higher kombucha concentration, reflected by larger inhibition zones. F3 showed the greatest inhibition zone (16.75 ± 0.204 mm), classified as strong. Overall, pineapple kombucha-containing liquid hand soap demonstrated potential to inhibit *E. coli* growth. These findings support the use of pineapple kombucha as an antibacterial additive in hand hygiene formulations.

Keywords: hand sanitizer, *Aloe vera*, 70% alcohol, microorganism colony count, glove juice method

1. INTRODUCTION

Infectious diseases continue to represent a significant global public health challenge, particularly in developing countries where sanitation and hygiene practices remain suboptimal. Infection occurs when pathogenic microorganisms invade and proliferate within the host, potentially leading to systemic or localized disorders. Among infectious conditions, diarrhea remains one of the most prevalent and life-threatening diseases in children. The World Health Organization (WHO) reports that diarrhea is among the leading causes of mortality in children under 59 months of age, with approximately 1.7 billion cases occurring annually worldwide. These cases contribute to an estimated 443,832 deaths among children under five years and 50,851 deaths among those aged 5–9 years (WHO, 2024). In Indonesia, the 2023 Indonesian

Health Survey (Survei Kesehatan Indonesia/SKI) documented 877,531 cases of diarrheal disease nationwide, including 29,331 cases in Lampung Province (Kementerian Kesehatan, 2023). These data emphasize the continued burden of diarrheal diseases and the importance of effective preventive interventions.

Escherichia coli is one of the principal bacterial pathogens associated with diarrhea. Although certain strains constitute normal intestinal microbiota, pathogenic variants can cause gastrointestinal infection when transmitted through contaminated food, water, or indirect contact with contaminated surfaces (Adhikari et al., 2020). When ingested in sufficient quantities, pathogenic *E. coli* can disrupt intestinal homeostasis and induce diarrhea (Hunowu et al., 2023). Inadequate hand hygiene significantly increases transmission risk, as hands function as a primary vector linking environmental contamination to oral exposure. Consequently, hand hygiene practices, particularly handwashing with soap, are critical components of diarrheal disease prevention strategies.

Liquid hand soap is widely utilized due to its practical and hygienic advantages compared with bar soap. Its closed packaging system reduces cross-contamination, while its liquid consistency facilitates even distribution on the skin and efficient rinsing (Abdilah, et al., 2022). Despite these advantages, the incorporation of natural antibacterial agents into commercial liquid soap formulations remains limited. Exploration of plant-based bioactive compounds may provide alternative or complementary antimicrobial strategies with potential public health benefits.

Pineapple (*Ananas comosus*) contains various bioactive compounds, and its antibacterial potential can be enhanced through fermentation into kombucha. Kombucha is produced through the symbiotic activity of acetic acid bacteria and yeast, resulting in the formation of organic acids, alcohols, and other metabolites. These fermentation products may exert antibacterial effects by disrupting lipid membranes, altering cell permeability, and destabilizing bacterial protein function through pH modification (Najah & Bintari, 2021; Rezaldi et al., 2022; Wang et al., 2022). Previous findings indicate that pineapple kombucha prepared with 35% sugar concentration demonstrated strong antibacterial activity against *E. coli*, with an inhibition zone diameter of 17.17 mm (Rezaldi et al., 2022).

However, prior research reported a reduction in antibacterial activity following formulation into liquid soap. Pineapple kombucha with 35% sugar concentration incorporated into liquid soap produced an inhibition zone of only 10.12 mm against *E. coli* (Fadillah et al., 2023). This decline suggests that formulation factors may influence the stability and bioavailability of active compounds, thereby affecting antibacterial efficacy.

Based on this background, the present study aims to evaluate the effect of varying concentrations of pineapple kombucha on the physical characteristics, stability, and antibacterial activity of liquid hand soap formulations. The findings are expected to provide empirical evidence supporting formulation optimization and to contribute to the development of natural antibacterial agents for hand hygiene applications.

2. METHOD

The equipment used included an Analytical Balance (METTLER TOLEDO ME204) jars, thermometers, Incubator (MEMMERT IN110), autoclave (GEA), Laminar Air Flow, pH meter (Milwaukee), Viscometer (IKA Germany ROTAVISC Hi-Vi I). The materials used comprised SLES (PT.Panchemie Berkah Cemerlang), NaCl (PT.Panchemie Berkah Cemerlang), Amphitol® (PT Panchemie Berkah Cemerlang), glycerin (PT Panchemie Berkah Cemerlang), EDTA (Raja Kimia), nipasol (Raja Kimia), pineapple fragrance (Pekon Hydro), coloring agents (Raja Kimia), distilled water (Raja Kimia), sugar (PT Gunung Madu Plantations), Sabun Nuvo® (Wings Group), SCOBY kombucha starter (Natsilver), pineapple, 0.9% NaCl (Raja Kimia), Nutrient Agar (NA) (Agavi Lab), 70% alcohol (Raja Kimia), aluminum foil (Natsilver), McFarland 0.5 solution (Agavi Lab), and *Escherichia coli* bacteria (Agavi Lab).

Phytochemical Screening

Flavonoid Test

A total of 1 mL of kombucha solution was taken with a pipette, followed by the addition of 1 mL of amyl alcohol, a small amount of magnesium powder, and 10 drops of concentrated hydrochloric acid. The mixture was gently shaken. The presence of flavonoids was indicated by a color change to red, orange, or purple, while the presence of flavones was marked by the appearance of a yellow color (Damayanti & Tahirah Hasan, 2024).

Saponin Test

A total of 2 mL of kombucha solution was taken and mixed with 10 mL of hot water, then cooled to a lower temperature. The solution was shaken for 10 seconds and left to stand for 10 minutes. The formation of stable foam or froth that persisted for 10 minutes indicated the presence of saponins (Damayanti & Tahirah Hasan, 2024).

Tannin Test

Kombucha solution was heated with water, filtered, and divided into two parts for testing. In the first part, the filtrate was treated with FeCl_3 solution, producing a blue-black color that indicated the presence of tannins and natural polyphenols. The second part was tested by adding 1% gelatin solution, where the formation of a white precipitate indicated the presence of tannins (Mulyani et al., 2021).

Bromelain Enzyme Identification Test

Kombucha was tested using Millon's reagent by adding the reagent to form a white precipitate, then heating the mixture to produce a reddish-brown color. The ninhydrin test was conducted by adding ninhydrin reagent, followed by heating, which produced a purple-blue color. The biuret test was performed by adding biuret reagent to the extract, resulting in a blue-purple coloration (Saptarini, Rahayu, & Herawati, 2019).

Preparation of Pineapple Kombucha

A total of 250 g of honey pineapple was cut into small pieces and blended with a small amount of water. The blended pineapple was then squeezed and filtered to obtain 200 mL of juice. Next, 350 g of sugar was weighed and placed into a pan, followed by the addition of pineapple juice and water until the total volume reached 1000 mL. The mixture was boiled to a temperature of 60°C. After boiling, the solution was cooled to approximately 29–30°C and transferred into a sterilized container. A SCOBY and 100 mL of kombucha starter were added. The container was then covered with a clean cloth and placed in a dry location away from direct sunlight. Fermentation was carried out for 14 days (Rezaldi, et al., 2022).

Liquid Hand Soap Formula

Table 1. Formula of pineapple kombucha liquid hand soap

Ingredients	Concentration (%) b/v			
	F0	F1	F2	F3
Kombucha Solution	-	20	30	40
<i>Sodium Laury Ether Sulphate</i>	15	15	15	15
NaCl	5	5	5	5
Amphitol®	5	5	5	5
Gliserin	10	10	10	10
EDTA	0,05	0,05	0,05	0,05
Nipazole	0,05	0,05	0,05	0,05
Pineapple fragrance	0,1	0,1	0,1	0,1
Soap coloring	0,1	0,1	0,1	0,1
Water	ad 100	ad 100	ad 100	ad 100

Preparation of Liquid Hand Soap

All ingredients to be used, including kombucha, SLES, NaCl, Amphitol®, glycerin,

EDTA, nipazole, and water, were weighed. The soap preparation was carried out in two mixtures. Mixture 1: Water and NaCl were gradually added into a beaker containing SLES, and stirred until homogeneous. A portion of the water was reserved for Mixture 2. Mixture 2: EDTA, nipazole, glycerin, and Amphitol® were placed into another beaker and stirred until homogeneous. The remaining water and pineapple fragrance were added and stirred until evenly mixed. Mixture 2 was then poured into Mixture 1 and stirred until homogeneous. Pineapple kombucha and coloring were added, followed by further mixing until fully homogenized. The finished soap was transferred into soap bottles and labeled.

Evaluation of Liquid Hand Soap

Organoleptic Test

The organoleptic characteristics of the liquid soap were evaluated through direct observation of the product's color, aroma, and texture. According to the SNI (1996) standard, liquid soap must meet the criteria of a liquid form, appropriate color, and distinctive fragrance (SNI, 1996).

pH Test

A total of 1 g of soap sample was placed into a 1000 mL volumetric flask and diluted with CO₂-free distilled water until homogeneous. The solution was then transferred into a beaker and left at room temperature. The pH of the solution was measured using a digital pH meter until the value appeared on the screen. The expected pH range should fall within the standard of 4 to 10 (SNI, 2017).

Foam Height Test

A total of 1 g of liquid soap sample was added into a graduated cylinder containing 10 mL of distilled water. The cylinder was tightly sealed and shaken gently for 20 seconds. The foam height formed was then measured. According to SNI standards, the acceptable foam height for liquid soap ranges between 13 and 220 mm (SNI, 1996).

Specific Gravity Test

A clean, dry pycnometer was first weighed. It was then filled with water and left to stand for 10 minutes at 25°C. Afterward, the pycnometer was weighed again. The same procedure was repeated using the liquid soap sample in place of water. According to SNI standards, the acceptable specific gravity range for liquid soap is 1.01 to 1.1 g/mL (SNI, 1996).

Viscosity Test

The viscosity was measured using a Brookfield viscometer. The soap sample was placed

into a container, ensuring the rotor could rotate smoothly. Rotor number 3 was used in the measurement. Viscosity values within the range of 400 to 4000 cPs are considered acceptable according to SNI standards (SNI, 1996).

Moisture Content Test

A dish and its lid were preheated for one hour at 100°C, then cooled in a desiccator for 20–30 minutes before weighing. Subsequently, 1 g of soap sample was placed into the dish and heated in an oven at 105°C for two hours until a constant weight was achieved. Based on SNI standards, the moisture content of liquid soap must not exceed 60% (SNI, 1996).

Physical Stability Test

An accelerated storage method was used to evaluate stability by alternately storing the product at very high and very low temperatures. Stability was assessed based on organoleptic properties, pH, specific gravity, viscosity, moisture content, and foam height. Tests were conducted before and after 6 storage cycles, where each cycle consisted of storage at 4°C (refrigerator) and 40°C (oven) for 24 hours (Suryani et al., 2017).

Antibacterial Activity Test

Preparation of NA Media

A total of 6 g of NA powder was dissolved in 100 mL of distilled water until fully homogeneous. The solution was then sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the medium was cooled to approximately 45°C before being poured into Petri dishes with a volume of 15–20 mL per plate (Lestari et al., 2020).

Rejuvenation of *E. coli*

A loopful of *Escherichia coli* culture was taken and inoculated into a tube containing NA medium. After opening the tube, the bacteria were evenly spread on the NA surface, then the tube was resealed and placed in a slanted position. The culture was incubated at 37°C for 24 hours in an incubator (Lestari et al., 2020).

Preparation of *E. coli* Suspension

Rejuvenated bacteria were taken from the medium using an inoculating loop and inoculated into a test tube containing 10 mL of 0.9% NaCl solution. The suspension was homogenized until its turbidity matched the McFarland 0.5 standard (Hutauruk et al., 2020).

Inhibition Test

The prepared *Escherichia coli* suspension (100 µL) was inoculated in a zigzag manner

across the surface of the medium. The medium was divided into four groups: negative control (F0) containing distilled water, F1 containing hand soap with 20% kombucha solution, F2 with soap containing 30% kombucha solution, and F3 with soap containing 40% kombucha solution. Wells with a diameter of 4 mm were made in each section, and 20 μ L of the respective treatment solution was placed into each well. The Petri dishes were then incubated at 37°C for 24 hours. Clear zones formed around the wells were measured using a caliper, in millimeters (mm), to evaluate antibacterial activity (Retnaningsih et al., 2019).

Data Analysis

One Way ANOVA was used to compare the effectiveness of various concentrations of pineapple kombucha liquid soap on the growth of *Escherichia coli*. If the data obtained were not normally distributed, the non-parametric Kruskal-Wallis test was used as an alternative. A Paired T-Test was employed to evaluate differences between data before and after the stability test. In addition, the relationship between quantitative variables was analyzed using Pearson's correlation test, adjusted according to the data distribution pattern.

3. RESULTS AND DISCUSSION

Phytochemical Screening of Pineapple Kombucha

The results of secondary metabolite content testing of pineapple kombucha are presented in Table 2.

Table 2. Phytochemical screening result of pineapple kombucha

Metabolite Coumpound	Reagent	Result	Documentation	
			Control	Result
Flavonoid	Mg powder+ HCl + amil alkohol	+		
Saponin	Shaking	+		
Tanin	FeCl ₃	+		
	Gelatin	-		

Metabolite Coumpound	Reagent	Result	Documentation	
			Control	Result
Bromelain	Millon's reagent	+		
	Ninhydrin reagent	-		
	Biuret reagent	-		

The phytochemical screening results showed that bromelain gave a positive reaction with Millon's reagent, indicating the presence of tyrosine in its structure (Saptarini, et al., 2019). However, biuret and ninhydrin reagents showed negative results, which may be due to bromelain denaturation under acidic conditions during kombucha fermentation (pH 2–4), whereas bromelain exhibits optimal stability at pH 6–7 (Syakbandini et al., 2018; Varilla et al., 2021). Under acidic conditions, the amine group becomes protonated into NH_3^+ , disrupting protein structure (Sartika, 2016).

Evaluation of Liquid Hand Soap

Organoleptic Test

The results of the organoleptic test on liquid hand soap are shown in Figure 1. Based on the observations, the color of F0 differed from F1, F2, and F3, where F0 appeared yellow while F1, F2, and F3 were yellowish-orange. In terms of aroma, each formulation exhibited a characteristic pineapple scent. F1, F2, and F3 presented a slightly acidic aroma due to the addition of pineapple kombucha in these formulations. In terms of texture, all formulations showed the same thick consistency.

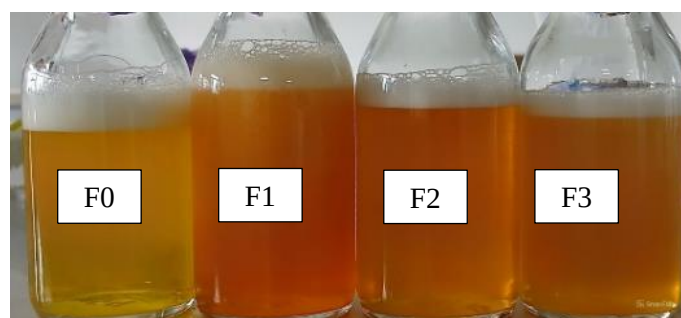


Figure 1. Organoleptic test result

pH Test

The pH test results of liquid hand soap are shown in Figure 2.

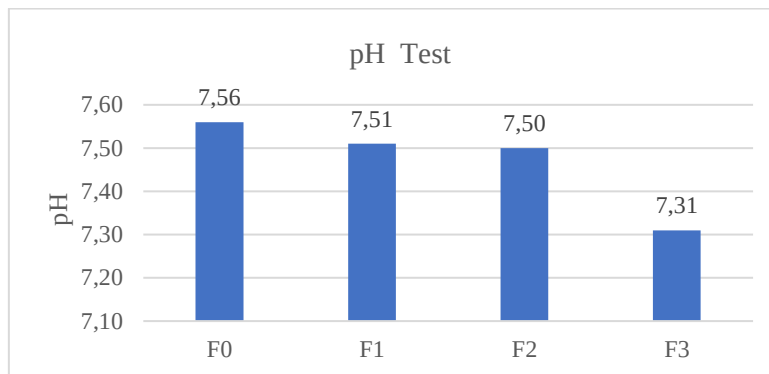


Figure 2. pH test result

All formulations met the pH standard according to SNI, which is 4–10. Statistical analysis showed a significance value of 0.000, indicating that there were significant differences in pH values among all formulations. The pH values of each formulation decreased with increasing concentrations of pineapple kombucha. This is due to the acidic pH of pineapple kombucha, which ranges from 2–3 (Syakbandini et al., 2018). The significant decrease in pH (towards acidity) in Formula 3 may result from the accumulation of organic acids in pineapple kombucha during fermentation, such as acetic acid, amino acids, glucuronic acid, and lactic acid (Puspitasari et al., 2017).

Foam Height Test

The foam height test results of pineapple kombucha liquid hand soap are presented in Figure 3. The test results showed that all formulations had foam heights that met the SNI requirements of 13–220 mm. Statistical analysis produced a significance value of 0.000, indicating that there were significant differences in foam height among all formulations. Foam height testing showed that all formulations of pineapple kombucha liquid hand soap were capable of cleaning dirt from hands. Although foam height does not always reflect cleaning effectiveness, it plays an important role in keeping dirt particles separated until rinsing. Foam height and stability are influenced by the presence of surfactants, namely SLES and Amphitol®. SLES, as an anionic surfactant, has high cleansing ability but may cause skin irritation due to electrostatic interactions with proteins in the stratum corneum (Darusman et al., 2023; Lips et al., 2006). To reduce irritation potential, SLES was combined with Amphitol®, an amphoteric surfactant that is milder, biodegradable, and has low toxicity (Blagojević et al., 2016; Suhail et al., 2019).

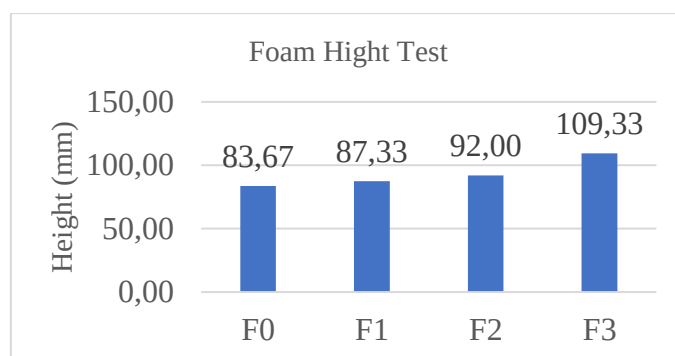


Figure 3. Foam Height test results

Specific Gravity Test

The specific gravity test results of liquid hand soap are shown in Figure 4.

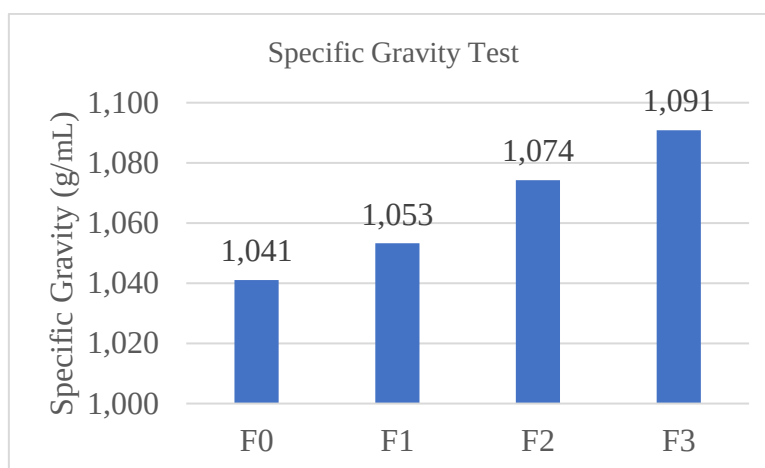


Figure 4. Specific gravity test results

Based on the results, all formulations met the SNI requirements for liquid soap specific gravity, which is 1.01–1.1 g/mL. The Kruskal-Wallis test showed a significance value of 0.016, indicating that there were significant differences in specific gravity values among all formulations. The results indicated an increase in specific gravity with higher concentrations of pineapple kombucha. This increase is attributed to the content of compounds in pineapple kombucha, such as organic acids (including acetic acid, amino acids, glucuronic acid, and lactic acid), residual sugars from fermentation, and secondary metabolites from pineapple such as flavonoids, saponins, tannins, and phenols (Puspitasari et al., 2017; Rezaldi, Desmak Pertiwi, et al., 2022). Increasing concentrations raised the number of particles in the solution, making them more compact and dense, thereby contributing to higher specific gravity values (Sani et al., 2014).

Viscosity Test

The viscosity test results of liquid hand soap are shown in Figure 5.

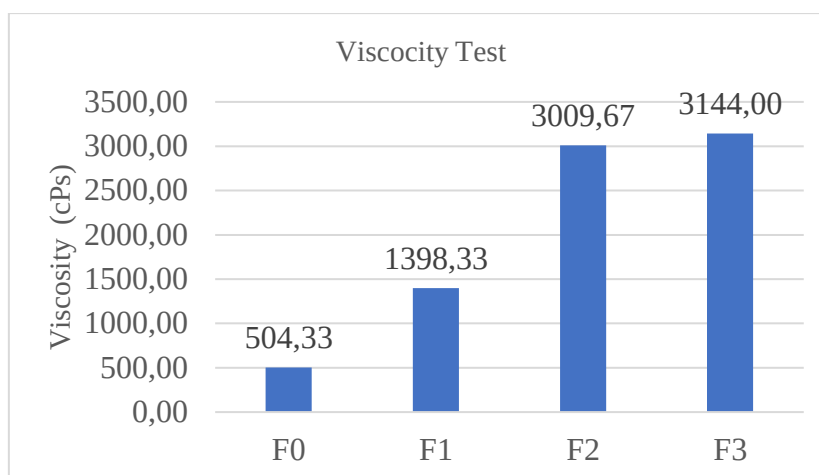


Figure 5. Viscosity test results

All formulations met the SNI requirements for liquid soap viscosity, which is 400–4000 cPs. Statistical analysis produced a significance value of 0.000, indicating significant differences in viscosity values among all formulations. The viscosity results showed an increase in viscosity along with higher concentrations of pineapple kombucha in the formulation, as higher solute concentrations increase the number of dissolved particles, leading to greater intermolecular friction and slower flow (Sinila, 2016). Viscosity is also influenced by formulation components, such as the anionic surfactant SLES and NaCl. SLES forms negatively charged micelles that change into cylindrical structures when NaCl is added, increasing thickness due to micelle entanglement (Sudarman et al., 2021). In addition, mixing technique, stirring speed, and water volume also affect the final viscosity of liquid soap (Silsia et al., 2017).

Moisture Content Test

The moisture content test results of liquid hand soap are shown in Figure 6. All formulations met the SNI requirement for liquid soap moisture content, which is less than 60%. The Kruskal-Wallis test showed a significance value of 0.016, indicating that there were significant differences in moisture content among all formulations. The water content of liquid soap is influenced by the concentration of ingredients used and the speed of mixing. The results showed that the higher the concentration of pineapple kombucha used, the lower the water content in the liquid soap. This occurs because pineapple kombucha contains more solutes than solvents, thereby reducing the water content. Besides influencing quality, stability, and shelf life, water content also affects viscosity, where reduced water content increases viscosity, making the soap thicker (Rosmainar, 2021). This may affect the ease of use of the liquid soap.

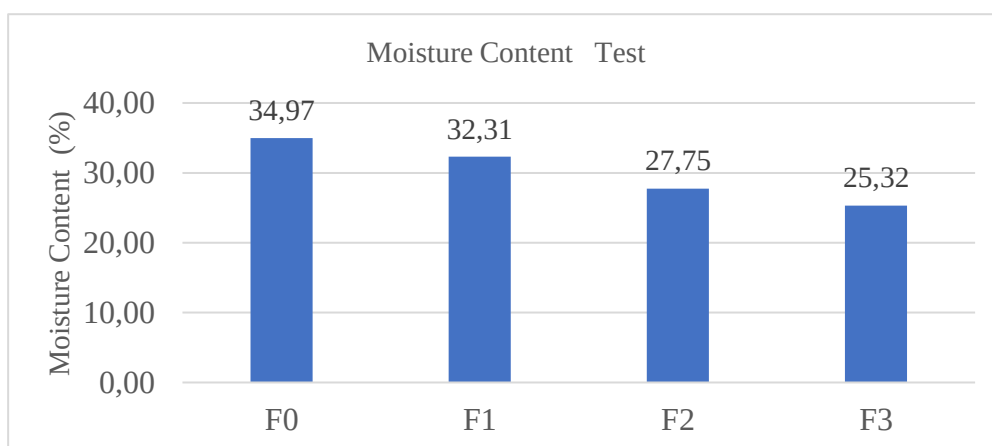


Figure 6. Moisture content test results

Stability Test

The stability test of liquid hand soap showed significant physical changes in organoleptic parameters in terms of texture (becoming more liquid), pH, specific gravity, and viscosity. Meanwhile, foam height and moisture content showed no significant physical changes. These findings indicate that the pineapple kombucha liquid hand soap has relatively low stability.

The decrease in pH in pineapple kombucha liquid soap formulations is caused by environmental factors such as temperature and CO₂ exposure during storage. CO₂ gas can dissolve in water and form carbonic acid, which dissociates and releases H⁺ ions, thus lowering the pH (Kurniati, et al., 2020). In addition, hydrolysis of polyphenols and glycosides in kombucha may produce free polyphenols with more acidic properties, further contributing to pH reduction (Chandra et al., 2024).

Extreme temperatures during the stability test (cycling test) may affect surfactant stability, such as that of SLES. Higher temperatures accelerate molecular movement and collisions among surfactant molecules, increasing the likelihood of foam formation (Wang et al., 2017).

The decrease in specific gravity was due to the degradation of glycosidic bonds at high temperatures during storage. Thermal hydrolysis accelerates the breakdown of bonds between sugar moieties and aglycones, producing lighter compounds and lowering solution density, thus reducing soap specific gravity (Permanadewi et al., 2021).

The reduction in viscosity was caused by elevated temperatures that weakened NaCl's thickening effect, resulting in a thinner consistency (Ikhsan et al., 2023). High temperatures increased NaCl molecular mobility, reducing intermolecular friction and ultimately lowering viscosity (Cahyaningsih et al., 2019).

The increase in water content was due to extreme temperatures promoting water droplet formation and the hygroscopic nature of excipients such as glycerin. With three hydroxyl (-OH) groups, glycerin attracts and binds water vapor from the air through hydrogen bonding, increasing water content in the formulation (Cahyaningsih et al., 2019).

Antibacterial Activity Test

The inhibition zone measurement results of pineapple kombucha liquid hand soap against *E. coli* are shown in Table 3.

Table 3. Antibacterial activity test results of pineapple kombucha liquid hand soap

Test Sample	Inhibition Zone Diameter (mm) $\pm$ SD	Conclusion on Inhibition Ability
F0	6,83 $\pm$ 0,656	Moderate
F1	16,25 $\pm$ 0,612	Strong
F2	16,33 $\pm$ 0,312	Strong
F3	16,75 $\pm$ 0,204	Strong
K+ (Nuvo®)	15,75 $\pm$ 0,408	Strong
K- (aquadest)	-	None

Based on the results, all pineapple kombucha liquid hand soap formulations exhibited antibacterial potential against *Escherichia coli*. The formulation with 40% kombucha concentration (Formula 3) produced the largest inhibition zone diameter of 16.75 $\pm$ 0.204 mm, even greater than the inhibition zone of the positive control (Nuvo®). This finding indicates that increasing kombucha concentration enhances antibacterial effectiveness, consistent with previous studies (Fadillah et al., 2023).

Active compounds in pineapple kombucha such as bromelain, flavonoids, saponins, phenols, acetic acid, lactic acid, and amino acids play important roles in antibacterial mechanisms. Bromelain degrades bacterial cell walls via protein hydrolysis, while flavonoids disrupt cell membranes and protein synthesis. Saponins cause membrane leakage due to their detergent properties, and phenols damage peptidoglycan structures and hydrophobic interactions in bacterial membranes.

Organic acids such as acetic and lactic acids are active under acidic conditions because they can penetrate bacterial membranes in their non-ionized form, disrupt enzymatic functions, and lower intracellular pH. Positively charged amino acids such as lysine and arginine may damage negatively charged bacterial membranes by forming pores that cause leakage of cellular

contents.

In addition to the antibacterial compounds of kombucha, other formulation components also contributed to antibacterial activity. Formula 0, which contained no kombucha, still showed an inhibition zone of 6.83 ± 0.656 mm due to the presence of surfactants SLES and Amphitol®, which can damage bacterial cell membranes by reducing surface tension, disrupting permeability, and causing bacterial cell dysfunction (Fagundes et al., 2020).

Although the antibacterial activity of pineapple kombucha liquid soap was slightly lower than that of pure kombucha (17.17 mm), the soap formulation still exhibited significant activity (16.75 mm). Unlike kombucha-based shower gel soap, which showed a drastic reduction in inhibition zone to 10.12 mm, the liquid soap maintained strong antibacterial effectiveness, making it suitable for use as a natural hand cleanser (Fadillah et al., 2023; Rezaldi, et al., 2022).

4. CONCLUSION

The variation in pineapple kombucha solution concentrations influenced the physical properties and physical stability of pineapple kombucha liquid soap. Increasing concentrations of pineapple kombucha solution led to higher foam height, specific gravity, and viscosity, while pH and moisture content decreased. The use of pineapple kombucha solution in hand soap formulations resulted in a reduction of organoleptic properties, pH, specific gravity, and viscosity after accelerated stability testing. In addition, the concentration of pineapple kombucha solution in the formulation affected the inhibition zones produced, where higher concentrations led to larger inhibition zones.

5. ACKNOWLEDGMENT

The author would like to express profound gratitude to Institut Teknologi Sumatera for the support provided, both in the form of facilities, services, and academic guidance throughout the research process. Without the contribution and active involvement of this institution, the completion of this research would not have been carried out optimally.

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