

PROCESSING OF SQUID (*Loligo sp.*) CARTILAGE WASTE AS ANTIBACTERIAL HANDWASH

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Abstract

Squid cartilage (*Loligo sp.*) is known to contain chitosan compounds that have antibacterial activity. This study aims to formulate chitosan from squid cartilage (*Loligo sp.*) in the form of *handwash preparations* with concentrations of 0.1%, 0.2%, and 0.3% which are physically and chemically stable, and see the optimum concentration in inhibiting bacterial growth. The method used is laboratory experiments through three stages of extraction, namely deproteination, demineralization, and deacetylation as well as physical quality tests and ability tests before and after *cycling tests* which include organoleptic tests, homogeneity tests, pH tests, viscosity tests, foam height tests and antibacterial activity tests against *Staphylococcus aureus* and *Escherichia coli* bacteria by the method of sumuran. The results obtained based on the ANOVA test on *Staphylococcus aureus* bacteria are 0.000 and on *Escherichia coli* bacteria are 0.000. Chitosan from squid cartilage (*Loligo sp.*) can be formulated in the form of a *handwash preparation* and the most effective concentration in inhibiting *Staphylococcus aureus* and *Escherichia coli* is a concentration of 0.3%.

Keywords: Squid cartilage (*Loligo sp.*), Chitosan, Handwash, Antibacterial.

1. Introduction

The world was shocked by the emergence of a new disease originating from Wuhan in early 2020, which rapidly spread to 190 countries. This outbreak was named coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus). Up until now, the number of deaths due to the corona virus infection has reached 3,889,723, while 459,015 COVID-19 patients have been declared recovered (WHO, 2021).

Hands are highly susceptible to the spread of infections and diseases in humans, as human hands frequently come into direct contact with the environment. Maintaining hand hygiene is one of the crucial steps in preventing the emergence and spread of diseases caused by microbial infections. Sanitation measures, such as washing hands with soap and clean water, aim to keep them clean and free from germs. Soap can be used as an alternative antibacterial agent to inhibit or even kill bacteria.

Squid (*Loligo sp.*) is one of Indonesia's fishery export commodities, along with stotong, fish, and octopus. Many people are unaware that this animal has squid cartilage, which is usually discarded when processing squid meat, but can actually be used in both food and non-food applications. The inner skin of squid cartilage contains chitin and chitosan compounds (Yulianis et al., 2020).

Staphylococcus aureus, *Bacillus cereus*, and *B. subtilis* are gram-positive bacteria, while *Escherichia coli*, *Salmonella choleraesuis*, *S. Typhimurium*, *Pseudomonas aeruginosa*, *Bacteroides fragilis*, and *Vibrio cholerae* are gram-negative bacteria. Chitosan has antibacterial properties that can inhibit the growth of pathogenic bacteria and microorganisms. Chitosan has positively charged polycations that can prevent the development of pathogenic bacteria. The antibacterial property of chitosan is just one of its many uses. Chitosan is a natural polysaccharide that is non-toxic, biodegradable, and biocompatible, produced from the deacetylation process of chitin and has free amino (NH₂) groups that make this polymer polycationic (Nadia et al., 2021).

In the testing of the antibacterial activity of squid cartilage chitosan against *Staphylococcus aureus* and *Escherichia coli* conducted by Nadia et al. (2021), with concentrations of 0.4%, 0.5%, 0.6%, 0.7%, and 0.8%, as well as 70% alcohol (positive control) and 0.1% acetic acid (negative control), it was found that chitosan at concentrations of 0.4%, 0.5%, 0.6%, and 0.7% exhibited moderate inhibitory activity against *Staphylococcus aureus*

with inhibition zones of 6.0 ± 0.00 , 7.3 ± 0.12 , 8.2 ± 0.10 , and 9.0 ± 0.00 , respectively, while at a concentration of 0.8% and 70% alcohol (positive control), it exhibited strong inhibitory activity with inhibition zones of 11.1 ± 0.12 and 10.2 ± 0.06 . As for *Escherichia coli*, it exhibited moderate inhibitory activity at concentrations of 0.4%, 0.5 %, and 0.6% with inhibition zones of 7.4 ± 0.12 , 9.1 ± 0.06 , and 9.6 ± 0.10 , respectively, while at concentrations of 0.7%, 0.8%, and 70% alcohol (positive control), it exhibited strong inhibitory activity with inhibition zones of 11.4 ± 0.12 , 12.8 ± 0.06 , and 12.2 ± 0.15 (Nadia et al., 2021).

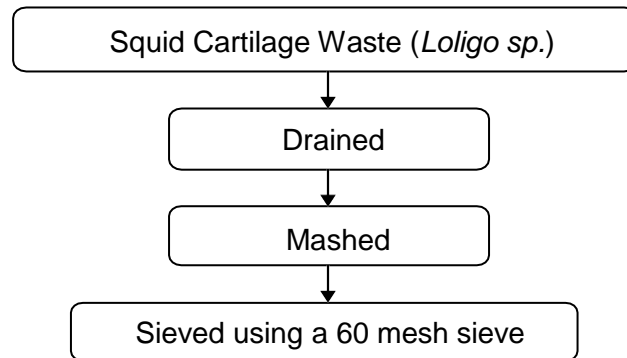
Based on the aforementioned background, the researcher chose to conduct a study on the formulation and evaluation of handwash preparations using squid cartilage extract (*Loligo sp.*) against *Staphylococcus aureus* and *Escherichia coli* bacteria.

2. Method

The following materials were used: squid cartilage extract (*Loligo sp.*), *Staphylococcus aureus* and *Escherichia coli* bacteria, distilled water, glycerin, sodium carboxymethylcellulose (Na CMC), NaOH, HCl, sodium benzoate, stearic acid, sodium lauryl sulfate (SLS), triethanolamine, rose oil.

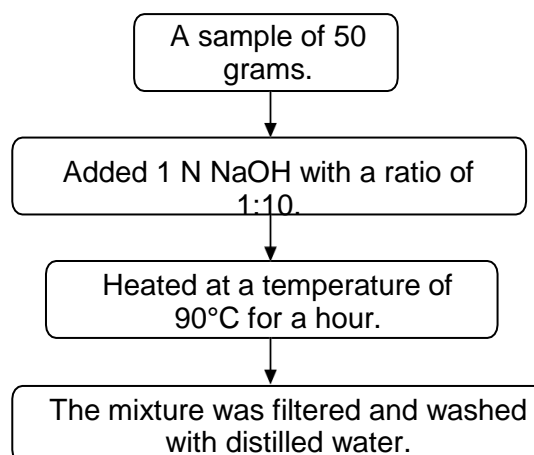
1. Research Procedure

a. Sample Preparation

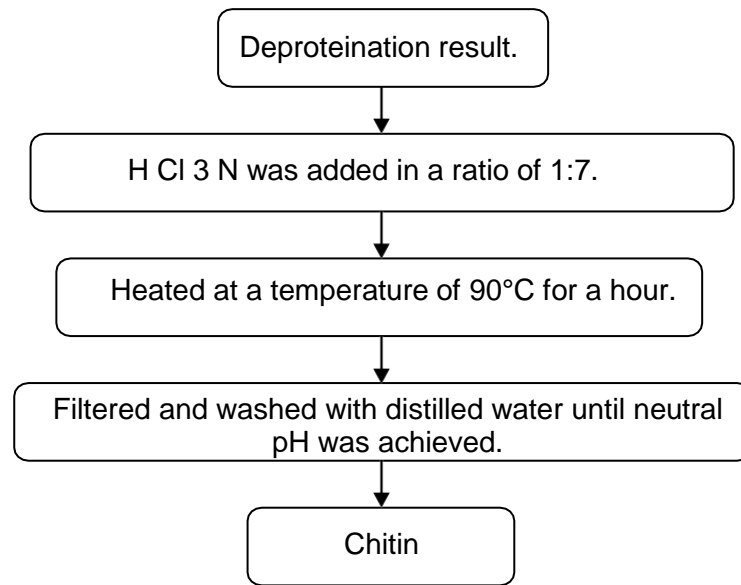


b. Extraction of Squid Cartilage (*Loligo sp.*)

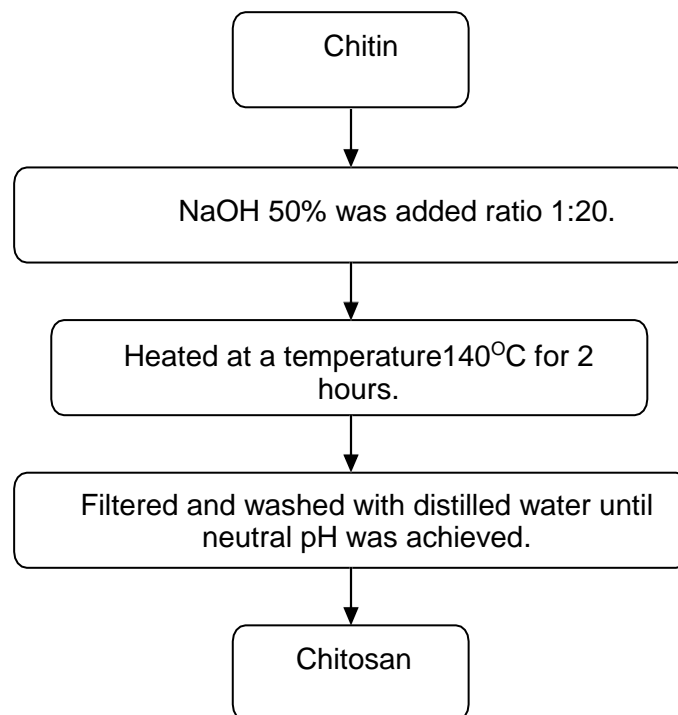
1) Deproteinization



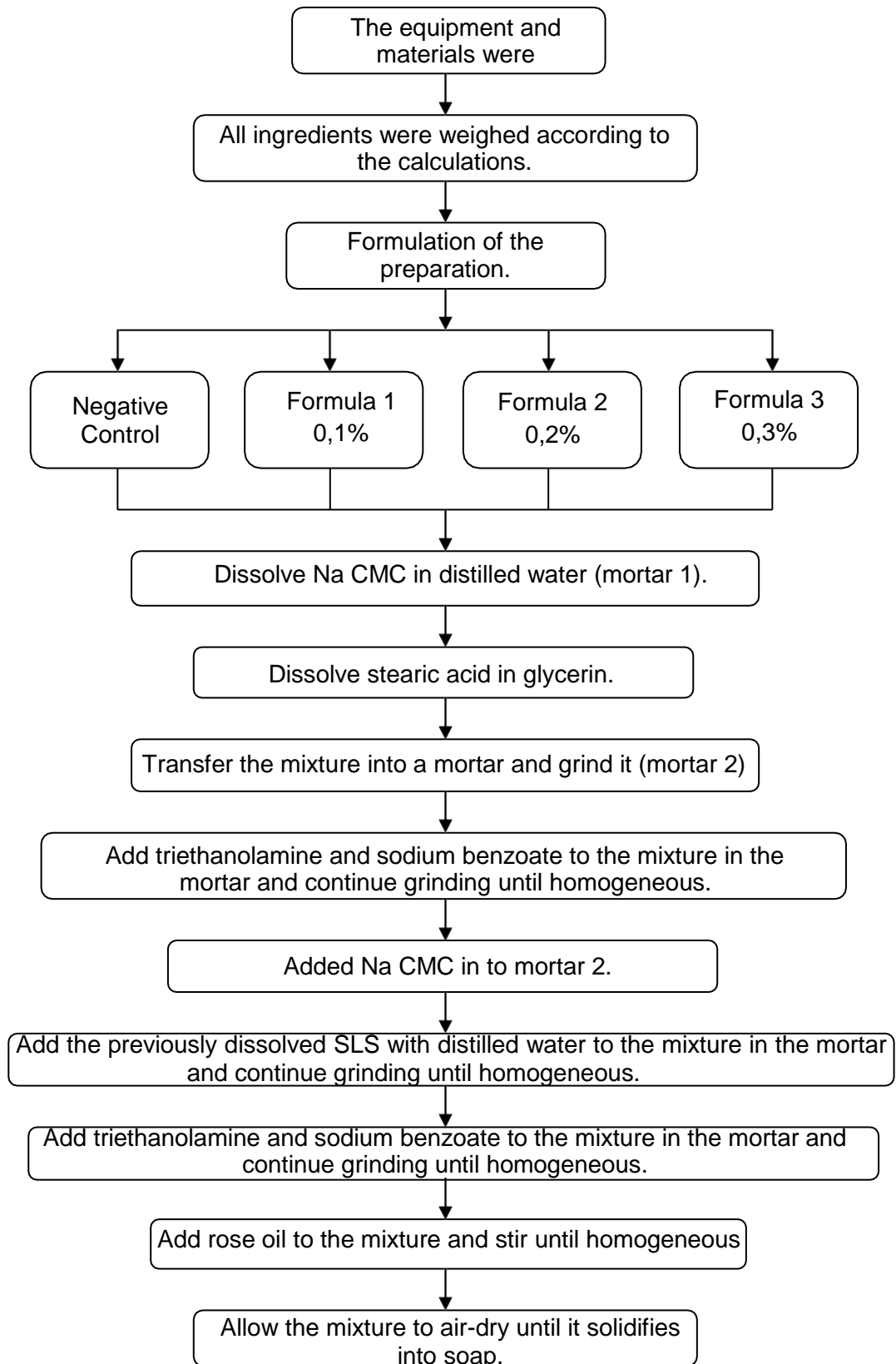
2) Demineralization



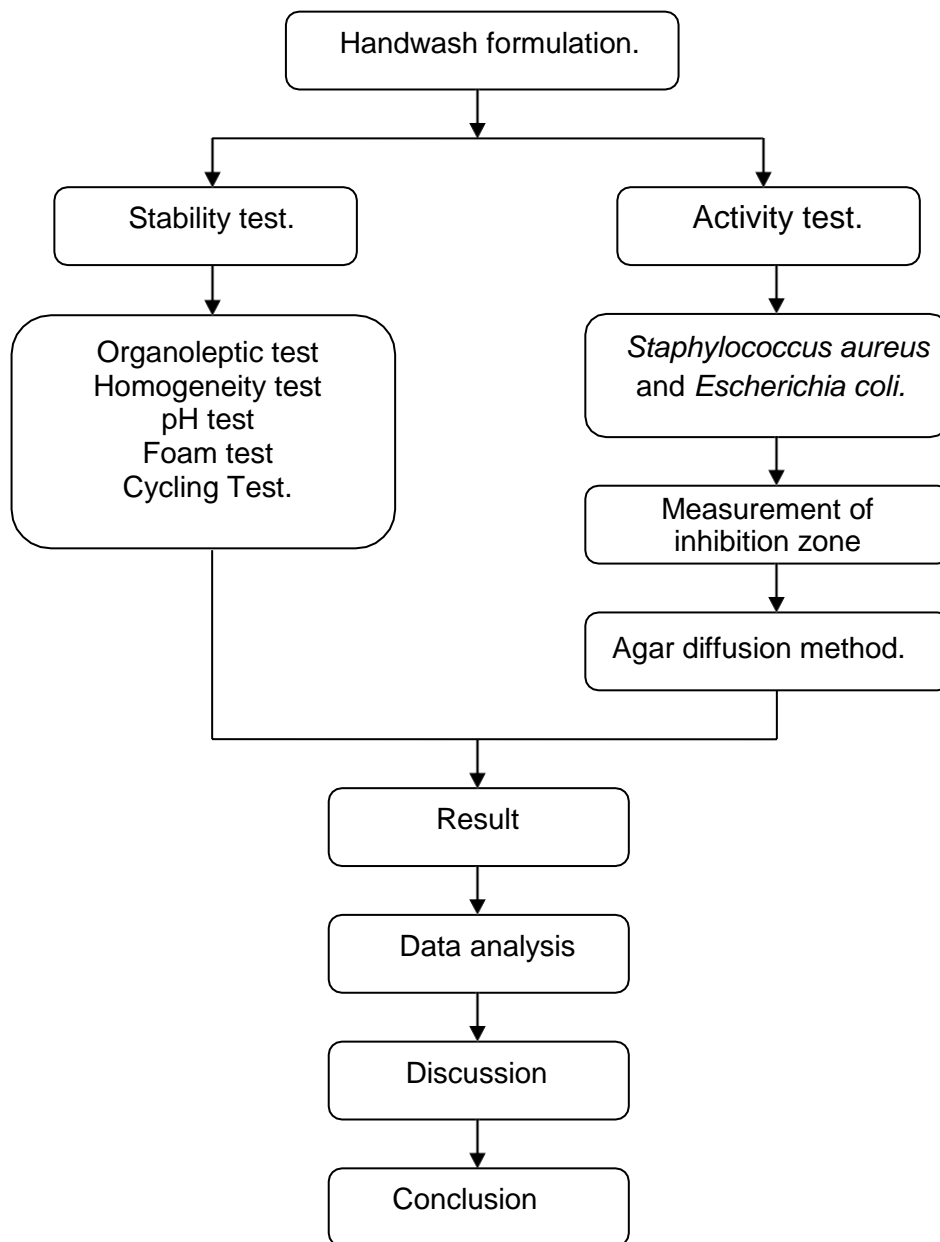
3) Deacetylation



c. Preparation of Handwash Formulation



d. Testing the Handwash Preparation



2. Data Analysis Technique

The data analysis technique in this study involves sample testing. The researcher conducted an experiment by processing two kilograms of squid cartilage waste (*Loligo sp.*) into an antibacterial handwash formulation.

3. Result and Discussion

The formulation and effectiveness test based on the results of the conducted research, it can be concluded that the handwash formulation made from squid cartilage (*Loligo sp.*) at concentrations of 0.1%, 0.2%, and 0.3% is physically and chemically stable. The handwash formulation from squid cartilage (*Loligo sp.*) at concentrations of 0.1%, 0.2%, and 0.3% showed the best inhibition of the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria, with the concentration of 0.3% having an average inhibition zone diameter of 13.43 mm and 13.4 mm, indicating strong antibacterial activity. An antibacterial handwash made from dried squid cartilage (*Loligo sp.*) is the focus of this study. The drying process aims to

remove or reduce the water content in the sample. The dried sample is then processed into chitosan following the procedures described by Nadia et al. (2021), which include three processes: deproteination, demineralization, and deacetylation. Deproteinization is performed to remove proteins from squid cartilage (*Loligo sp.*). Demineralization is carried out to eliminate minerals and obtain chitin. Deacetylation of chitin is done to obtain chitosan.

Four formulations of handwash from squid cartilage chitosan (*Loligo sp.*) were prepared: formulation I with a concentration of 0.1%, formulation II with a concentration of 0.2%, formulation III with a concentration of 0.3%, and a negative control (without chitosan). The selection of concentrations is based on previous research conducted by Nadia et al. (2021), which stated that squid cartilage chitosan (*Loligo sp.*) exhibited antibacterial activity against both gram-positive and gram-negative bacteria at a minimum concentration of 0.4%. Therefore, lower concentrations were chosen and tested against gram-positive and gram-negative bacteria.

In addition to the active ingredient, the soap formulation also includes additional ingredients such as Na CMC as a thickening agent, sodium benzoate as a preservative to prevent product degradation, glycerin as a moisturizing agent (humectant), stearic acid as a foam stabilizer, triethanolamine as a moisturizer to provide skin hydration, sodium lauryl sulfate as a foaming agent, and rose oil as a fragrance.

A cycling test method was conducted to assess the stability of the formulations by accelerating the degradation process and testing them under different conditions (Pratasik et al., 2019). The cycling test involved storing the samples in a refrigerator at 4°C for 24 hours, followed by placing them in an oven at 40°C for 24 hours, repeating this cycle six times. Physical changes were observed before and after the cycling test, including organoleptic examination, homogeneity, pH measurement, viscosity, and foam height.

The first test conducted was organoleptic evaluation, which assessed the appearance, color, and aroma of the squid cartilage (*Loligo sp.*) handwash formulations. The organoleptic evaluation was performed through visual observation using the human senses. The results showed that the handwash formulations containing 0.1%, 0.2%, and 0.3% concentrations of squid cartilage (*Loligo sp.*) chitosan, as well as the negative control, had a slightly liquid form, white color, and distinctive rose aroma.

Homogeneity testing was carried out to determine the uniform dispersion of ingredients in the handwash formulations. The results of homogeneity testing for the squid cartilage (*Loligo sp.*) handwash at each concentration (0.1%, 0.2%, 0.3%) and the negative control showed good homogeneity both before and after the cycling test. This indicates that the handwash formulations meet the requirement of not having visible particles in the formulation (Fitri, 2020).

pH testing was conducted using a pH meter to determine if the formulated handwash pH is suitable for skin. The pH values of the handwash formulations before the cycling test were 6.86 for the negative control, 7.49 for 0.1% concentration, 7.62 for 0.2% concentration, and 7.70 for 0.3% concentration. There was a decrease in pH values after the cycling test, which could be due to various factors such as the degradation of chitosan and the presence of other ingredients. However, the pH values of all formulations remained within the acceptable range for skin (Fitri, 2020), which is between 4 and 7.

Viscosity testing was conducted to determine the flow properties of the handwash formulations. The viscosity values of the squid cartilage (*Loligo sp.*) handwash formulations before the cycling test were 4863 cP for the negative control, 5713 cP for 0.1% concentration, 6527 cP for 0.2% concentration, and 7460 cP for 0.3% concentration. There was a decrease in viscosity values after the cycling test, indicating a decrease in thickness. However, all formulations still met the viscosity requirements for handwash products, which is around 3000-10,000 cP (Fitri, 2020).

Foam height testing was conducted to evaluate the foaming properties of the handwash formulations. The foam height values of the squid cartilage (*Loligo sp.*) handwash formulations before the cycling test were 5.3 cm for the negative control, 6.6 cm for 0.1% concentration, 7.8 cm for 0.2% concentration, and 8.9 cm for 0.3% concentration. The foam height decreased

after the cycling test, but all formulations still met the foam height requirements for handwash products, which is more than 4 cm (SNI 06-4084-1996).

The antibacterial activity of the handwash formulations was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the agar diffusion method. The results were compared to the negative control (without chitosan) and the positive control (Dettol original handwash). The handwash formulations containing squid cartilage (*Loligo sp.*) chitosan exhibited antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, with increasing inhibition zones observed as the chitosan concentration increased.

Overall, this study successfully formulated and tested the effectiveness of an antibacterial handwash made from squid cartilage (*Loligo sp.*) chitosan. The handwash formulations exhibited improved clarity and provided cleanliness and comfort to users. Squid cartilage (*Loligo sp.*) chitosan showed antibacterial activity against both gram-positive and gram-negative bacteria. The handwash formulations passed quality testing, including evaluation of appearance, color, aroma, homogeneity, pH, viscosity, and foam height.

4. Conclusion

Based on the results of the conducted research, it can be concluded that the handwash formulation made from squid cartilage (*Loligo sp.*) at concentrations of 0.1%, 0.2%, and 0.3% is physically and chemically stable. The handwash formulation from squid cartilage (*Loligo sp.*) at concentrations of 0.1%, 0.2%, and 0.3% showed the best inhibition of the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria, with the concentration of 0.3% having an average inhibition zone diameter of 13.43 mm and 13.4 mm, indicating strong antibacterial activity.

The authors would like to express their sincere gratitude to all individuals and institutions that played a pivotal role in the completion of this research. Special thanks are extended to the teachers and the principal for their valuable contributions for this research. Their insights and expertise greatly enriched the research process. We are also indebted to Megarezky University for their locality support, which enabled the successful execution of this study. Their commitment to advancing scientific research is truly commendable.

Furthermore, we extend our appreciation to my teachers and lecturers for their assistance during the data collection and analysis phases. Their guidance and input were instrumental in shaping the outcomes of this research.

The collaborative efforts of these individuals and institutions have undoubtedly contributed to the quality and rigor of this research.

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