Knowledge & Innovation in Molecular and Industrial Applications (KIMIA)
Vol. 01, No. 1, June 2026, Page 1-16
E-ISSN XXX-XXXX

Eco-Friendly Soap Innovation from Waste Cooking Oil of the Biscuit Industry: A Microbiological Quality Study as an Alternative to Commercial Soap

Mitha Fitriani Caniago[1]*, Allysha Diva Canceria[2], & Larisa Mandalini[3]
[1]*Universitas Bina Nusantara, Indonesia, [2]Politeknik ATI Padang, Indonesia, [3]Politeknik ATI Padang, Indonesia
*Co e-mail: mitha.caniago@binus.ac.id[1]

Article Information
Received: September 04, 2026
Revised: October 04, 2026
Online: October 20, 2026

Keywords
Waste Cooking Oil, Saponification, Liquid Soap, Microbiological Quality, Surface Sanitation

ABSTRACT
This study aimed to develop an eco-friendly liquid soap, EcoGrease, from waste cooking oil (WCO) generated at PT X (identity withheld), and to evaluate its microbiological quality and sanitation application compared with the commercial Soap AB. WCO was characterized, converted into soap by alkaline saponification with KOH, and analyzed for total plate count (TPC), Enterobacter, yeast and mold, Staphylococcus aureus, and Coliform, including swab sampling of WCO collection boxes. The WCO had a saponification value of 186.4 mg KOH/g and a free fatty acid (FFA) content of 4.86%, giving a KOH requirement of 19.72 g per 100 g WCO. yeast and mold counts increased from 213 to 324 CFU/g as air exposure increased from 7 to 18 h (R = 0.9901). The finished EcoGrease met the PT X internal hygiene standard for all parameters. After sanitation, the box cleaned with EcoGrease showed lower Enterobacter (30.5 vs. 257 CFU/cm²) and S. aureus (39 vs. 414 CFU/cm²) counts but a higher TPC (912.5 vs. 727.5 CFU/cm²) than the box cleaned with Soap AB. These descriptive results come from two different boxes without statistical testing. EcoGrease shows potential as an internal WCO valorization option for sanitation, pending validation with independent replicates.

INTRODUCTION

Large-scale food-processing industries generate substantial amounts of waste cooking oil (WCO) from repeated frying and heating processes. Repeated thermal use promotes oil degradation, including an increase in free fatty acid (FFA) content and changes in physicochemical and microbiological characteristics (Sanaguano-Salguero et al., 2018; Antonić et al., 2020, 2021; Al-Nima, 2025). If discharged without appropriate treatment, WCO can cause environmental pollution and drainage problems and may support the accumulation of contaminants around production facilities (Chirani et al., 2021; S. Rahayu et al., 2021). Therefore, practical approaches are needed to manage WCO within food-processing operations while adding value to the waste stream.

One potential approach is to convert WCO into soap through saponification. Previous studies have shown that WCO can be used as a raw material for bar and liquid soap, which supports

DOI: http://doi.org/10.69855/kimia
Available online at website: https://gpijournal.com/index.php/kimia

This work is licensed under a Creative Commons Attribution 4.0 International license

waste valorization and circular economy practices through the reuse of an industrial by-product (Adane, 2020; Maotsela et al., 2019; Antonić et al., 2020; S. Hartini et al., 2021; Mwamba et al., 2025). Other studies have added natural ingredients to improve product characteristics or antimicrobial properties (Wulandari et al., 2023; Zahra et al., 2024; Nababan et al., 2024; Glevitzky et al., 2026). However, most of these studies focused on laboratory-scale formulations, physicochemical properties, or antimicrobial activity against selected laboratory microorganisms, rather than on the performance of the soap under actual food-production sanitation conditions.

In an industrial context, a WCO-based soap should be evaluated not only for its raw material and saponification process but also for its intended application, especially when it is proposed as an alternative to a commercial cleaning product already used in a facility. The quality assessment therefore needs to consider parameters that are also relevant to the commercial benchmark. In food-processing environments, hygienic production equipment is important for controlling microbial contamination, and microorganisms such as *Staphylococcus aureus*, *Enterobacter*, Coliforms, and yeasts and molds are commonly included in product or sanitation specifications. In this study, microbiological parameters were therefore used for the quality assessment and practical evaluation of the developed soap. The microbiological quality of the finished soap is distinguished from its sanitation performance on equipment surfaces. The former describes the microbiological condition of the soap product, whereas the latter reflects the change in microbial contamination after the soap is applied during equipment cleaning. Studies that connect WCO utilization, soap development, finished-product assessment, and practical application on equipment surfaces within an actual food-processing facility, using the commercial product under the same production conditions as a comparison, remain limited.

To address this gap, the present study focused on the utilization of WCO as a raw material for an eco-friendly liquid soap, termed EcoGrease, through saponification. The resulting soap was evaluated using microbiological parameters as part of its quality assessment and compared with Soap AB, the commercial soap routinely used for equipment sanitation at the study site. Microbiological testing was thus used as an evaluation approach and not as the primary focus of the study. The study was conducted as a case study at a single food-processing facility, and WCO characterization and air-exposure observation were included as supporting information on the condition of the waste material. Accordingly, this study aimed to (1) characterize the initial condition of WCO and observe changes in its yeast and mold counts during different periods of air exposure; (2) develop EcoGrease liquid soap from WCO through saponification; and (3) evaluate the microbiological quality of the soap and its observed sanitation performance on a WCO collection box in comparison with Soap AB. The study examines whether the developed soap can meet the applicable quality requirements and give a sanitation outcome comparable to that of the commercial soap under the conditions examined.

METHODS

1. Research Design

This study used an experimental approach to investigate the utilization of WCO from a biscuit manufacturing process as a raw material for EcoGrease liquid soap through saponification. The study consisted of WCO characterization, EcoGrease formulation and production, evaluation of

This work is licensed under a Creative Commons Attribution 4.0 International license

the microbiological quality of the finished soap, and application of EcoGrease for sanitation of a WCO collection box (Figure 1). It was conducted at a national-scale biscuit and wafer manufacturing facility in Indonesia, referred to as PT X for confidentiality. The sanitation evaluation followed the routine sanitation practices of the Hygiene Sanitation (HS) division. Microbiological analysis was used to assess product quality and the sanitation condition of the tested surface, not as an investigation of antimicrobial activity.

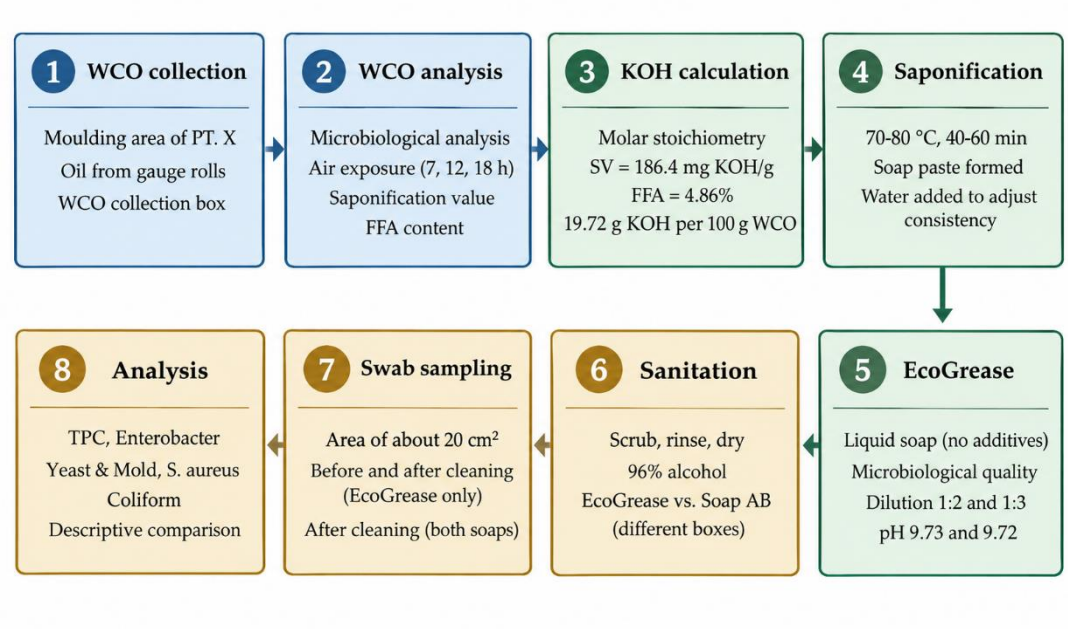


Figure 1. Research Workflow of EcoGrease Production and Its Evaluation at PT X

2. WCO Collection and Preparation

WCO was collected from the moulding area of PT X. During production, oil from several gauge rolls accumulated in trays and was collected in a WCO collection box, so the collected WCO represented a mixture of oil from several production streams. WCO was collected once for each analytical parameter and was used both as the raw material for EcoGrease production and for WCO characterization. Each sample was analyzed in duplicate from the same collected sample portion. Therefore, the duplicates were treated as analytical duplicates and not as independent sampling replicates.

3. WCO Characterization

The initial quality of the collected WCO was evaluated based on its saponification value (reflux-titration method) and FFA content (acidimetric titration), as well as TPC, *Enterobacter*, yeasts and mold, *S. aureus*, and Coliform, to characterize its condition prior to conversion into soap. In addition, the relationship between exposure time and yeast and mold counts was observed. The same collected WCO sample was exposed to ambient air and analyzed after 7, 12, and 18 h, and the resulting counts were evaluated descriptively.

This work is licensed under a Creative Commons Attribution 4.0 International license
Knowledge & Innovation in Molecular and Industrial Applications (KIMIA)
Vol. 01, No. 1, June 2026

4. EcoGrease Formulation and Saponification

EcoGrease was prepared by converting WCO into soap through alkaline saponification using potassium hydroxide (KOH) (Figure 2). The formulation consisted of 100 g WCO, 19.72 g pure KOH, 30-40 g water to dissolve the KOH in the first stage, and an additional 70-80 g water in the second stage to dissolve the soap paste and adjust its consistency. The amount of KOH was determined from the saponification value and FFA content of the WCO using a molar stoichiometric approach that accounts for KOH consumption in both triglyceride saponification and FFA neutralization. No additional additives were incorporated. The KOH was first dissolved in water, combined with the WCO, and processed at 70-80 °C for 40-60 min until a soap paste formed. Additional water was then added to obtain the desired consistency, giving a total water content of approximately 100-120 g per 100 g WCO. The resulting EcoGrease had a thick, paste-like consistency but remained sufficiently fluid to be categorized as a liquid soap. For sanitation, EcoGrease was diluted with water at ratios of 1:2 and 1:3 (soap:water), and the pH values of the dilutions were 9.73 and 9.72, respectively.

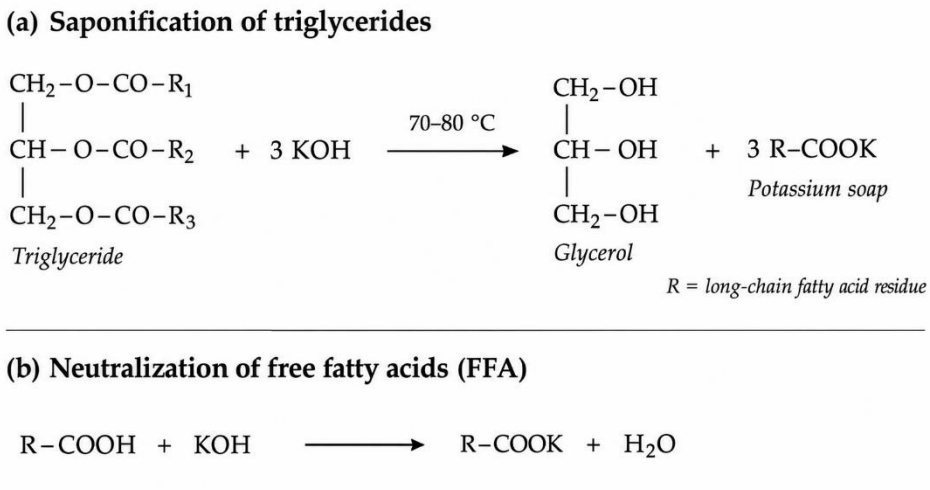


Figure 2. Chemical Reactions Involved in the Production of EcoGrease: (a) Saponification of Triglycerides with KOH; (b) Neutralization of Free Fatty Acids

5. Microbiological Analysis

Microbiological analyses were conducted for the characterization of WCO, the quality evaluation of the finished EcoGrease, and the sanitation evaluation of the WCO collection-box surface. Each sample was analyzed in duplicate from the same sample portion. The microbiological parameters, culture media, dilution factors, and incubation conditions are presented in Table 1.

Table 1. Microbiological Analysis Conditions

Parameter	Medium	Incubation Temperature	Dilution	Incubation Time
TPC	Plate Count Agar (PCA)	35 ± 1°C	10^1	2 × 24h
Enterobacter	Violet Red Bile Glucose Agar (VRBGA)	35 ± 1°C	10^1	2 × 24h
Yeast and Mold	Potato Dextrose Agar (PDA) supplemented with 10%	25 ± 1°C	10^1	2 × 24h

This work is licensed under a Creative Commons Attribution 4.0 International license

Parameter	Medium	Incubation Temperature	Dilution	Incubation Time
	tartaric acid			
Staphylococcus aureus	Baird-Parker Agar (BPA) with egg-yolk tellurite	35 ± 1°C	Undiluted	2 × 24h
Coliform	Lactose Broth (LB)	35 ± 1°C	Up to 10^{-9}	2 × 24h

The results for WCO and EcoGrease were expressed as CFU/g or MPN/g, and the results for surface samples were expressed as CFU/cm² or MPN/cm² based on the sampled area. For the finished EcoGrease, a result of 0 CFU/g was reported according to the laboratory reporting practice and represents a result below the applicable detection limit. Samples reported as too numerous to count (TNTC) were retained as reported by the laboratory because counts sufficient for conversion were not available. The results for WCO and EcoGrease were compared with the PT X internal hygiene standard, which is set out in the company's work instruction and has been adjusted to ISO and SNI requirements.

Quality control of the microbiological analysis followed the standard practice of the laboratory. Sterile swabs, sterile diluent, and sterilized glassware were used, and all handling was carried out using aseptic technique. A sterile blank (an unused swab and diluent processed in the same way as the samples) was included as a negative control to verify that the swabs, diluent, and handling procedure were free from contamination, and the productivity of the culture media was verified by the laboratory using reference strains. Surface sampling followed the principles of SNI ISO 18593:2018 on horizontal methods for surface sampling (Badan Standardisasi Nasional, 2018). To prevent cross-contamination during swabbing, the sampler wore a clean laboratory coat, mask, and sterile gloves, which were changed between sampling locations. The sterile swab was opened immediately before sampling and was not allowed to touch non-target surfaces, and each used swab was returned to its tube, closed, labeled, and transported in a cool box (below 10 °C) to the laboratory for analysis.

6. Surface Sanitation Application of EcoGrease

The sanitation performance of EcoGrease was evaluated on a WCO collection box in the moulding area, which was selected because it receives oil from several gauge rolls during production. Surface sampling was conducted using a sterile swab over an area of approximately 20 cm². For the EcoGrease treatment, the same surface was sampled before and after cleaning. EcoGrease and the commercial soap (Soap AB) were applied to different WCO collection boxes located within the same general production area. Both soaps were used at a soap-to-water ratio of 1:2 or 1:3, which corresponds to the routine application range of Soap AB and was therefore adopted for comparison under practical sanitation conditions. The tested surface was scrubbed with the respective soap until it was considered clean according to the routine sanitation procedure, rinsed with water, and dried using oil-free compressed air and a blower. The compressed air was supplied by an oil-free compressor equipped with a filter to minimize contamination from oil and dust. After drying, the surface was sanitized with 96% alcohol. The cleaning procedure was controlled by the HS division and followed the sanitation standard operating procedure (SOP) of PT X. The duration

This work is licensed under a Creative Commons Attribution 4.0 International license

of scrubbing and the contact time were not separately quantified because the routine industrial SOP was followed.

7. Before-and-After Evaluation of EcoGrease Sanitation

To evaluate the change following EcoGrease application, swab samples were collected from the same WCO collection-box surface before and after cleaning and analyzed for TPC, *Enterobacter*, yeasts and molds, *S. aureus*, and Coliform. The before-and-after evaluation was conducted only for EcoGrease. A pre-cleaning baseline was not obtained for the Soap AB surface. Therefore, the EcoGrease and Soap AB results were treated as separate descriptive observations and not as a controlled paired comparison.

8. Data Analysis

The microbiological measurements from duplicate analyses were summarized using the reported values and arithmetic means where applicable. The results for WCO and EcoGrease were compared descriptively with the PT X internal hygiene standard. A numerical reference limit for the surface swab results was not available to the authors because surface hygiene criteria are managed by the HS division; therefore, the surface results were interpreted descriptively without a compliance remark. The relationship between WCO air-exposure time and yeast and mold counts was evaluated using simple linear correlation based on the mean counts at 7, 12, and 18 h, which gave a correlation coefficient of $R = 0.9901$. No inferential statistical test was performed to determine whether the differences between EcoGrease and Soap AB were statistically significant. Therefore, the differences between the two soaps are presented as descriptive observations and are not interpreted as statistically significant effects.

RESULTS

1. Microbiological Characteristics of WCO

The initial microbiological characteristics of the collected WCO are presented in Table 2. The mean TPC was 58.5 CFU/g, *Enterobacter* was not detected, and the mean Coliform count was below 3 MPN/g. In contrast, yeasts and molds were reported as TNTC, and *S. aureus* showed a mean count of 477.5 CFU/g. Based on the PT X internal hygiene standard, the initial WCO complied with the criteria for TPC, *Enterobacter*, and Coliform, whereas yeasts and molds and *S. aureus* did not comply.

Table 2. Initial Microbiological Analysis of PT X's Waste Cooking Oil

Parameter	Test 1	Test 2	Mean	Internal Hygiene Standard	Remark
TPC	57 CFU/g	60 CFU/g	58.5 CFU/g	1,000 CFU/g	Compliant
Enterobacter	0 CFU/g	0 CFU/g	0 CFU/g	10 CFU/g	Compliant
Yeast & Mold	TNTC	TNTC	TNTC	200 CFU/g	Non-compliant
S. aureus	485 CFU/g	470 CFU/g	477.5 CFU/g	100 CFU/g	Non-compliant
Coliform	<3 MPN/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	Compliant

This work is licensed under a Creative Commons Attribution 4.0 International license

The yeasts and molds response of WCO to ambient air exposure is shown in Table 3 and Figure 3. The mean count increased from 213 CFU/g at 7 h to 277 CFU/g at 12 h and 324 CFU/g at 18 h. A simple linear correlation between exposure time and Yeasts and molds count gave R = 0.9901, indicating a strong positive linear association within the tested exposure period. This observation describes the change in microbial count during exposure and is not interpreted as evidence of a causal relationship.

Table 3. Yeast and Mold Growth in WCO by Air-Exposure Duration

Air-Exposure Duration (h)	Test 1 (CFU/g)	Test 2 (CFU/g)	Mean (CFU/g)
7	216	210	213
12	274	280	277
18	328	320	324

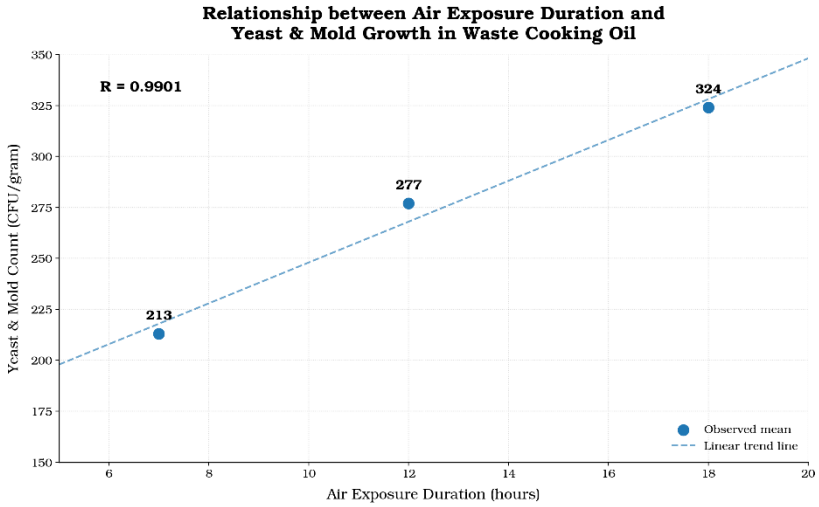


Figure 3. Relationship Between Air-Exposure Duration and Yeast & Mold Growth in Waste Cooking Oil

2. EcoGrease Formulation and Product Characteristics

EcoGrease was produced from the collected WCO through alkaline saponification using KOH. The WCO had a saponification value of 186.4 mg KOH/g and an FFA content of 4.86%, which gave a KOH requirement of 19.72 g per 100 g WCO. The formulation consisted of 100 g WCO and 19.72 g pure KOH, with water added in two stages (30-40 g to dissolve the KOH and 70-80 g to dissolve the soap paste and adjust its consistency). No additional additives were used. The resulting product had a thick, paste-like consistency but remained sufficiently fluid to be categorized as a liquid soap. For sanitation, EcoGrease was diluted at ratios of 1:2 and 1:3 (soap:water), and the measured pH values were 9.73 and 9.72, respectively.

3. Microbiological Quality of Finished EcoGrease

The microbiological quality of the finished EcoGrease is presented in Table 4. TPC, *Enterobacter*, Yeasts and molds, and *S. aureus* were reported as 0 CFU/g in both duplicate analyses, and Coliform was reported as <3 MPN/g in both analyses. Thus, all evaluated parameters were

This work is licensed under a Creative Commons Attribution 4.0 International license

within the PT X internal hygiene standard. The absence of reported colonies indicates that the samples were below the laboratory detection limit for the respective culture-based analyses. These results describe the microbiological quality of the finished soap and are not interpreted as evidence of intrinsic antimicrobial activity.

Table 4. Microbiological Analysis of the Finished EcoGrease Soap Product

Parameter	Test 1	Test 2	Mean	Internal Hygiene Standard	Remark
TPC	0 CFU/g	0 CFU/g	0 CFU/g	1,000 CFU/g	Compliant
Enterobacter	0 CFU/g	0 CFU/g	0 CFU/g	10 CFU/g	Compliant
Yeast & Mold	0 CFU/g	0 CFU/g	0 CFU/g	200 CFU/g	Compliant
S. aureus	0 CFU/g	0 CFU/g	0 CFU/g	100 CFU/g	Compliant
Coliform	<3 MPN/g	<3 MPN/g	<3 MPN/g	<3 MPN/g	Compliant

4. Surface-Sanitation Application of EcoGrease and Soap AB

The microbiological results after sanitation of the WCO collection boxes using EcoGrease and Soap AB are presented in Table 5 and Figure 4. For TPC, the mean count after EcoGrease treatment was 912.5 CFU/cm², compared with 727.5 CFU/cm² after Soap AB treatment. *Enterobacter* showed mean counts of 30.5 CFU/cm² after EcoGrease and 257 CFU/cm² after Soap AB, and *S. aureus* showed 39 and 414 CFU/cm², respectively. Yeasts and molds were reported as 0 CFU/cm² after both treatments, and Coliform was reported as <3 MPN/cm² for both. These results are post-cleaning observations from two different WCO collection boxes within the same general production area. Because no pre-cleaning baseline was obtained for the Soap AB surface, no numerical reference limit was available for the surface results, and no inferential statistical analysis was performed, the differences between EcoGrease and Soap AB are presented descriptively and not as statistically significant differences.

Table 5. Microbiological Results of WCO Collection Boxes After Sanitation Using EcoGrease and Soap AB

Parameter	EcoGrease Test 1	EcoGrease Test 2	EcoGrease Mean	Soap AB Test 1	Soap AB Test 2	Soap AB Mean
TPC	905 CFU/cm²	920 CFU/cm²	912.5 CFU/cm²	735 CFU/cm²	720 CFU/cm²	727.5 CFU/cm²
Enterobacter	31 CFU/cm²	30 CFU/cm²	30.5 CFU/cm²	254 CFU/cm²	260 CFU/cm²	257 CFU/cm²
Yeast & Mold	0 CFU/cm²	0 CFU/cm²	0 CFU/cm²	0 CFU/cm²	0 CFU/cm²	0 CFU/cm²
S. aureus	38 CFU/cm²	40 CFU/cm²	39 CFU/cm²	418 CFU/cm²	410 CFU/cm²	414 CFU/cm²
Coliform	<3 MPN/cm²	<3 MPN/cm²	<3 MPN/cm²	<3 MPN/cm²	<3 MPN/cm²	<3 MPN/cm²

This work is licensed under a Creative Commons Attribution 4.0 International license

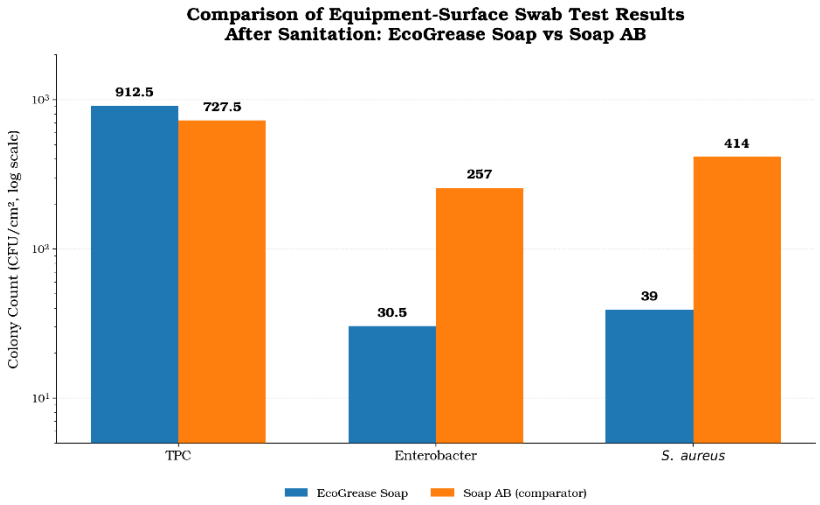


Figure 4. Comparison of Equipment-Surface Swab-Box Results After Sanitation Between EcoGrease and Soap AB (Logarithmic Scale)

5. Before and After Microbiological Evaluation of EcoGrease Sanitation

The before-and-after results for the surface treated with EcoGrease are presented in Table 6 and Figure 5. Before cleaning, TPC, *Enterobacter*, and *S. aureus* were reported as TNTC. After cleaning with EcoGrease, the mean counts decreased to 344 CFU/cm² for TPC, 9.5 CFU/cm² for *Enterobacter*, and 20.5 CFU/cm² for *S. aureus*. The mean yeast count decreased from 91.5 to 59 CFU/cm², mold decreased from 29 to 0 CFU/cm², and Coliform decreased from 4 to <3 MPN/cm². These results show a descriptive reduction in the measured microbial indicators following EcoGrease application. However, because the pre-cleaning values for TPC, *Enterobacter*, and *S. aureus* were TNTC, the magnitude of reduction for these parameters could not be calculated. In addition, no inferential statistical test was performed, so the changes are not interpreted as statistically significant effects.

Table 6. Microbiological Results Before and After Cleaning with EcoGrease

Parameter	Before Cleaning Test 1	Before Cleaning Test 2	Before Cleaning Mean	After Cleaning Test 1	After Cleaning Test 2	After Cleaning Mean
TPC	TNTC	TNTC	TNTC	348 CFU/cm²	340 CFU/cm²	344 CFU/cm²
Enterobacter	TNTC	TNTC	TNTC	9 CFU/cm²	10 CFU/cm²	9.5 CFU/cm²
Yeast	93 CFU/cm²	90 CFU/cm²	91.5 CFU/cm²	58 CFU/cm²	60 CFU/cm²	59 CFU/cm²
Mold	28 CFU/cm²	30 CFU/cm²	29 CFU/cm²	0 CFU/cm²	0 CFU/cm²	0 CFU/cm²
S. aureus	TNTC	TNTC	TNTC	21 CFU/cm²	20 CFU/cm²	20.5 CFU/cm²
Coliform	4 MPN/cm²	4 MPN/cm²	4 MPN/cm²	<3 MPN/cm²	<3 MPN/cm²	<3 MPN/cm²

This work is licensed under a Creative Commons Attribution 4.0 International license
Knowledge & Innovation in Molecular and Industrial Applications (KIMIA)
Vol. 01, No. 1, June 2026

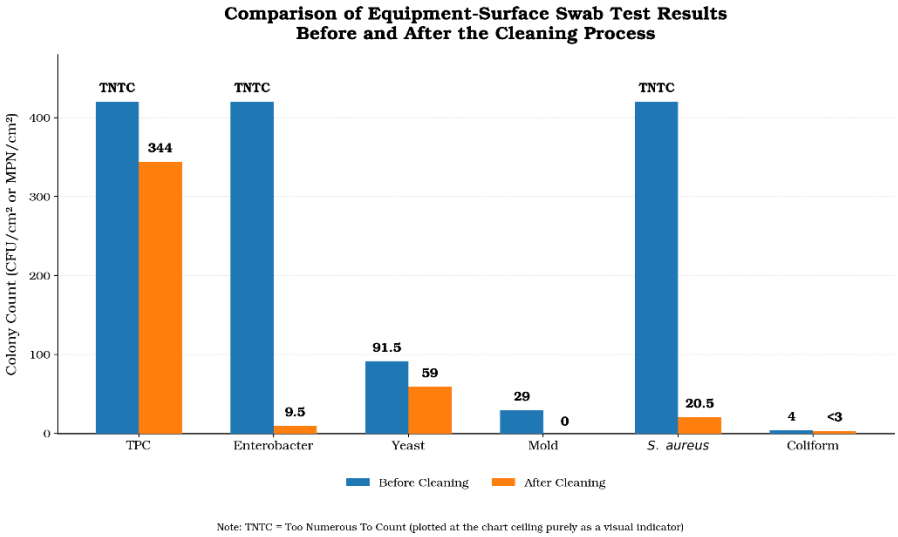


Figure 5. Comparison of Equipment-Surface Swab-Box Results Before and After the Cleaning Process Using EcoGrease

DISCUSSION

1. Microbiological Characteristics of WCO and the Effect of Air Exposure

The initial characterization showed that the WCO contained measurable microbial contamination, although the levels varied among the parameters. The mean TPC and Coliform were 58.5 CFU/g and <3 MPN/g, respectively, and *Enterobacter* was not detected. In contrast, Yeasts and molds were reported as TNTC, and the mean *Staphylococcus aureus* count of 477.5 CFU/g exceeded the internal hygiene standard. These findings indicate that WCO collected from the industrial process was not microbiologically uniform across parameters and contained microorganisms that may be relevant to its use as a raw material. The increase in yeast and mold counts during air exposure, from a mean of 213 CFU/g at 7 h to 277 CFU/g at 12 h and 324 CFU/g at 18 h, was accompanied by a strong positive linear correlation ($R = 0.9901$). This pattern indicates an association between longer exposure and higher yeast and mold counts under the conditions tested. However, because only one WCO sample with three time points was monitored and no inferential analysis was performed, the result should be treated as a descriptive observation and not as evidence of a causal relationship. It nevertheless supports the importance of controlling the condition of WCO before its conversion into a value-added product.

2. Chemical Basis of EcoGrease Formulation

The conversion of WCO into EcoGrease was based on alkaline saponification, in which triglycerides in the oil react with KOH to form soap and glycerol (Figure 2). KOH was selected to produce a liquid soap formulation. The formulation used 100 g WCO and 19.72 g KOH, and the alkali requirement was determined from the saponification value (186.4 mg KOH/g) and the FFA content (4.86%) of the WCO through a molar stoichiometric approach. Because part of the alkali is also consumed in neutralizing free fatty acids, incorporating both parameters gives a more specific basis for determining the KOH requirement than a fixed formulation. The detailed calculation is not

This work is licensed under a Creative Commons Attribution 4.0 International license

presented in full to maintain the focus of the manuscript. The saponification was conducted at 70-80 °C for 40-60 min until a soap paste formed. The relatively high temperature facilitates the reaction between the oil components and the alkali and promotes formation of the soap phase, and the subsequent addition of water adjusted the consistency of the paste into a thick but sufficiently fluid liquid soap. The pH values of the diluted EcoGrease were 9.73 (1:2) and 9.72 (1:3), which suggests that the two dilution ratios produced comparable alkaline conditions. Nevertheless, the alkaline nature of the formulation should be considered in practical use, particularly for repeated occupational contact, surface compatibility, and surfaces with specific pH limitations.

3. Microbiological Quality of Finished EcoGrease

The finished EcoGrease showed no detectable TPC, *Enterobacter*, Yeasts and molds, or *S. aureus*, and the Coliform result was <3 MPN/g. All parameters were therefore within the PT X internal hygiene standard, which indicates that the saponification and subsequent processing produced a soap that met the microbiological quality criteria applied in this study. However, these results should not be interpreted as evidence that EcoGrease has intrinsic antimicrobial activity. No direct antimicrobial assay, such as agar diffusion or minimum inhibitory and bactericidal concentration testing, was conducted. The analysis was performed to evaluate the quality of the finished soap and to provide a basis for comparison with the commercial soap, so the absence of detectable microorganisms demonstrates microbiological quality under the tested conditions rather than antimicrobial efficacy.

4. Surface-Sanitation Outcomes of EcoGrease and Soap AB

The post-sanitation results showed different microbiological profiles between the collection boxes cleaned with EcoGrease and those cleaned with Soap AB. EcoGrease gave a higher mean TPC (912.5 vs. 727.5 CFU/cm²) but lower *Enterobacter* (30.5 vs. 257 CFU/cm²) and *S. aureus* (39 vs. 414 CFU/cm²) counts, while Yeasts and molds and Coliform were the same for both soaps. These values are surface swab results after cleaning and should not be confused with the microbiological quality of the finished soap, which was 0 CFU/g for TPC (Table 4). The higher TPC after EcoGrease sanitation should not be interpreted as evidence that EcoGrease was intrinsically less effective than Soap AB. The two soaps were applied to different collection boxes, and no pre-cleaning baseline was available for the Soap AB surface. Differences in the initial soil or microbial load, the intensity of manual scrubbing, the amount of soap solution applied, and the rinsing and drying conditions may have contributed to the variation. In addition, the physical characteristics of the two formulations may have influenced their application and distribution on the surface. Furthermore, no numerical reference limit was available for the surface results, so compliance with a surface hygiene criterion could not be judged. The results therefore provide a descriptive comparison under the specific conditions tested and not a statistically established comparison of cleaning performance.

5. Interpretation of the Before-and-After EcoGrease Sanitation Results

The before-and-after evaluation on the EcoGrease-treated collection box showed a substantial change in the observed counts. TPC, *Enterobacter*, and *S. aureus* were reported as TNTC before cleaning and decreased to mean values of 344, 9.5, and 20.5 CFU/cm², respectively. Yeast

This work is licensed under a Creative Commons Attribution 4.0 International license

decreased from 91.5 to 59 CFU/cm², mold from 29 to 0 CFU/cm², and Coliform from 4 to <3 MPN/cm². These observations indicate that the sanitation procedure using diluted EcoGrease was associated with lower microbial counts on the tested box. However, the TNTC results before cleaning prevented calculation of an exact percentage or logarithmic reduction for TPC, *Enterobacter*, and *S. aureus*. The changes also cannot be attributed solely to the chemical action of EcoGrease, because the procedure involved mechanical scrubbing, water rinsing, drying with oil-free compressed air and a blower, and sanitization with 96% alcohol. Therefore, the before-and-after results should be interpreted as an evaluation of the complete sanitation procedure involving EcoGrease and not as a direct measurement of the intrinsic antimicrobial action of the soap.

6. Practical Implications for Internal WCO Valorization

The results show a potential route for utilizing WCO generated within the studied biscuit manufacturing facility as a raw material for EcoGrease. Instead of being treated only as waste, the chemical characteristics of the WCO were used to determine the KOH requirement and convert the oil into a value-added soap that met the microbiological quality criteria and could be applied to the sanitation of a WCO collection box under the tested conditions. The findings should, however, be seen as a potential internal application at the studied facility and not as evidence that EcoGrease can generally replace Soap AB or other commercial sanitation products. Different facilities may generate WCO with different characteristics and may use different sanitation procedures, equipment, water quality, soap formulations, and hygiene requirements. Consequently, the suitability of WCO-derived soap should be evaluated for the specific conditions of each facility.

7. Limitations and Future Research

Several limitations should be considered when interpreting the findings. The study was conducted at a single industrial site using WCO collected during one sampling occasion. The surface evaluation was performed on WCO collection boxes rather than production equipment, and EcoGrease and Soap AB were applied to different boxes. Only the EcoGrease-treated surface had a before-and-after evaluation, and a pre-cleaning baseline was not available for the Soap AB surface. A numerical reference limit for the surface swab results could not be obtained because surface hygiene criteria are managed by the HS division, so these results were compared descriptively. The measurements were performed in duplicate from the same sample portion and should not be interpreted as independent replicates. The study also did not include inferential statistical testing or direct antimicrobial assays. Consequently, the results do not establish statistical superiority, equivalence, or intrinsic antimicrobial activity of EcoGrease. Future studies should include independent sampling replicates, standardized pre- and post-cleaning measurements for both EcoGrease and commercial soap, controlled cleaning conditions, and surface reference limits. Further evaluation of physicochemical stability, cleaning performance, material compatibility, occupational and skin safety, biodegradability, and economic feasibility is also needed before broader implementation. Multi-site validation using WCO from different production conditions would determine the consistency and practical applicability of EcoGrease.

This work is licensed under a Creative Commons Attribution 4.0 International license

CONCLUSIONS

EcoGrease was successfully developed from waste cooking oil (WCO) through alkaline saponification using a KOH amount determined from the saponification value and free fatty acid content of the WCO. The finished EcoGrease met the PT X internal hygiene standard, with no detectable TPC, *Enterobacter*, Yeasts and molds, or *Staphylococcus aureus* and a Coliform count of <3 MPN/g. Its application in the sanitation of a WCO collection box was associated with lower observed microbial counts after cleaning; however, the results were descriptive and did not establish statistical superiority, equivalence, or intrinsic antimicrobial activity compared with the commercial Soap AB. Therefore, the findings indicate the potential of EcoGrease as an internal WCO valorization option for sanitation under the specific conditions tested at PT X, and not as a general replacement for commercial sanitation products. Further studies with independent replicates and multiple industrial sites are needed to validate its consistency and applicability, together with evaluation of physicochemical stability, cleaning performance, toxicity, skin compatibility, biodegradability, material compatibility, and production cost.

ACKNOWLEDGMENT

The authors would like to express their gratitude to PT X for providing the waste cooking oil (WCO) samples and access to the facilities required for this research. The authors also sincerely thank the laboratory and production personnel who assisted with sample collection, experimental activities, and data collection. The authors appreciate all parties who provided constructive suggestions and technical assistance during the development of this study and the preparation of the manuscript.

REFERENCES

Abera, B. H., Diro, A., & Beyene, T. T. (2023). The synergistic effect of waste cooking oil and endod (Phytolacca dodecandra) on the production of high-grade laundry soap. Heliyon. https://doi.org/10.1016/j.heliyon.2023.e16889

Adam, S., Sufyan, M. B., & Ahmad, M. (2026). SUSTAINABLE PRODUCTION AND PHYSICOCHEMICAL EVALUATION OF SOAP FROM LOCALLY SOURCED MATERIALS: A COMPARATIVE STUDY WITH COMMERCIAL PRODUCTS. FUDMA JOURNAL OF SCIENCES. https://doi.org/10.33003/fjs-2026-1009-5320

Adane, L. (2020). Preparation of Laundry Soap from Used Cooking Oils: Getting value out of waste. Scientific Research and Essays. https://doi.org/10.5897/sre2019.6649

Adriyani, L. W., Rizki, N. A., & Khairunisa (2025). FORMULATION AND ORGANOLEPTIC EVALUATION OF SOLID SOAP BASED ON VEGETABLE OIL, WASTE COOKING OIL, AND ECO-ENZYME. Nusantara Hasana Journal. https://doi.org/10.59003/nhj.v5i2.1697

Agustina, N., & Piska, F. (2023). Physical Characteristics of Organic Soap Formulated from Waste Cooking Oil and Areca Nut Extract (Areca catechu L.) Using Soapnut (Sapindus rarak DC.) Fruit Biosurfactant. https://doi.org/10.34012/agroprimatech.v7i1.4416

Ahadito, B., & Afriani, S. R. (2024). Soap Production from Waste Cooking Oil: A Review. Indonesian Journal of Fundamental and Applied Chemistry. https://doi.org/10.24845/ijfac.v9.i2.96

This work is licensed under a Creative Commons Attribution 4.0 International license

Al-Nima, A. (2025). Physicochemical Comparison of Soaps Produced from Fresh, Cooked, and Naturally Treated Oils. Iraqi Journal of Pharmacy. https://doi.org/10.33899/iraqij.p.2025.157726.1131

Alfiannissa, A. N., Wahyuni, T., & Asih, F. (2026). IMPLEMENTATION OF PROJECT-BASED LEARNING WITH AN EDU-ECOPRENEURSHIP FOCUS: STUDENTS' PRACTICAL SKILLS AND THE QUALITY OF BAR SOAP MADE FROM USED COOKING OIL WITH PAPAYA EXTRACT. UNESA Journal of Chemical Education. https://doi.org/10.26740/ujced.v15n2.p157-164

Antonić, B., Dordević, D., Jančíková, S., Tremlová, B., & Kushkevych, I. (2020). Physicochemical Characterization of Home-Made Soap from Waste-Used Frying Oils. Processes. https://doi.org/10.3390/pr8101219

Antonić, B., Dordević, D., Jančíková, S., Tremlová, B., Nejezchlebová, M., Goldová, K., & Treml, J. (2021). Reused Plant Fried Oil: A Case Study with Home-Made Soaps. Processes. https://doi.org/10.3390/pr9030529

Asahy, N. R., Nuha, F. K., Dhiyaulhaq, M., Putri, R. S., & Hamzah, A. J. M. (2023). Soap CharOl (Soap Charcoal Oil) Innovation of Oil and Used Cooking Oil Soap. Jurnal Integrasi Sains dan Qur'an (JISQu). https://doi.org/10.64477/21103-108

Azme, S. N. K., Yusoff, N., Chin, L. Y., Mohd, Y., Hamid, R., Jalil, M. N., ... Zain, Z. M. (2023). Recycling Waste Cooking Oil into Soap: Knowledge Transfer through Community Service Learning. Cleaner Waste Systems. https://doi.org/10.1016/j.clwas.2023.100084

Badan Standardisasi Nasional. (2018). SNI ISO 18593:2018: Mikrobiologi rantai pangan - Metode horizontal untuk teknik pengambilan contoh dari permukaan. BSN.

Chirani, M. R., Kowsari, E., Teymourian, T., & Ramakrishna, S. (2021). Environmental impact of increased soap consumption during COVID-19 pandemic: Biodegradable soap production and sustainable packaging. The Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2021.149013

Ferdian, M., Perdana, R. G., & RAHARDJO, P. P. (2023). Refinery technology of used cooking oil by utilizing coffee dregs and sugar cane bagasse as raw materials for making antiseptic transparent soap of guava leaf extract. International Journal of Agriculture, Environment and Food Sciences. https://doi.org/10.31015/jaefs.1109072

Glevitzky, M., Corcheş, M., Şerban, S., Strugariu, M., Kiss, I., & Vică, M. (2025). Sustainable Recycling of Used Cooking Oils Through the Production of Biodegradable Antimicrobial Soaps. Applied Sciences. https://doi.org/10.3390/app152111472

Glevitzky, M., Dumitrel, G., Pană, A.-M., Rusu, G., Corcheş, M., & Vică, M. (2026). Valorization of Waste Cooking Oils into Antimicrobial Soaps with Honey, Propolis, and Essential Oils. ChemEngineering. https://doi.org/10.3390/chemengineering10020031

Harefa, M. S., Try, D., Siagian, N., Simarmata, G. S., & Adha, N. (2025). Utilization of Waste Cooking Oil in the Development of Eco-Friendly Liquid Soap through the Substitution of Synthetic Surfactants. Jurnal Pendidikan Ilmu Pengetahuan Alam (JP-IPA). https://doi.org/10.56842/jp-ipa.v6i2.778

This work is licensed under a Creative Commons Attribution 4.0 International license

Hartini, S., Widharto, Y., Indarto, S., & Murdikaningrum, G. (2021). Eco-efficiency analysis of waste cooking oil recycling into liquid dish soap using life cycle assessment. IOP Conference Series: Earth and Environmental Science. https://doi.org/10.1088/1755-1315/896/1/012066

Hartini, E., Kurniatie, M., & Izzati, D. (2025). Innovation in the utilization of used cooking oil waste into soap in Pendrikan Kidul Village, Semarang. Community Empowerment. https://doi.org/10.31603/ce.12265

Maotsela, T., Danha, G., & Muzenda, E. (2019). Utilization of Waste Cooking Oil and Tallow for Production of Toilet "Bath" Soap.. Procedia Manufacturing. https://doi.org/10.1016/j.promfg.2019.07.008

Mapparessa, S. B., Sabara, Z., Suryanto, A., Lisa, & Lestari, T. (2023). Evaluation of Antimicrobial Activity, Moisturizing Effect, Irritation Potential, and Foam Height of Hand Soap Formulated with Waste Cooking Oil and Olive Oil. KOVALEN: Jurnal Riset Kimia. https://doi.org/10.22487/kovalen.2023.v9.i2.16449

Musa, I., Uduma, U. A., Uduma, M. B., & Mamman, J. (2025). Production and analysis of eco-friendly soap from millet, maize, and guinea corn stalks using blended oils. Journal of Basics and Applied Sciences Research. https://doi.org/10.4314/jobasr.v3i6.2s

Mwamba, B. W., Brobbey, M. S., Maritz, R. F., Leodolff, B., Peters, S., Teke, G. M., & Mapholi, Z. (2025). Valorising Waste Cooking Oil and Citrus Peel Waste for Sustainable Soap Production: A Techno-Economic and Environmental Life Cycle Assessment Study. Waste and Biomass Valorization. https://doi.org/10.1007/s12649-025-03048-y

Nababan, B. P., Mulmeyda, R., & Insyani, N. P. (2024). Utilization of Used Cooking Oil in Making Liquid Soap with the Addition of Water Hyacinth (Eichhornia crassipes) Leaf Extract as an Antibacterial. Chemistry and Materials. https://doi.org/10.56425/cma.v3i2.76

Octarya, Z., Yenti, E., Utami, L., & Yusbarina (2025). Sustainable solid soap production using recycled cooking oil with ecoenzyme and lemongrass extract. Acta Biochimica Indonesiana. https://doi.org/10.32889/actabioina.191

Oloruntele, I., Adeoye, S., Jaji, B., Ibrahim, M. M., & Hamza, M. (2025). Assessment of the Physico-Chemical Characteristics, Phytochemicals, Biological Activities, and Soap-Making Process Using the Pulp and Seed Oils of Avocados (Persea americana). UMYU Scientifica. https://doi.org/10.56919/usci.2541.045

Pauhesti, P., Yulia, P. S., Widiyatni, H., Sutadiwiria, Y., Jane, G., & Yanti, W. (2024). Soap Bar Making from Waste Cooking Oil for Residents of 07 Area, Duri Pulo Sub-district, Jakarta. International Journal Of Community Service. https://doi.org/10.51601/ijcs.v4i2.265

Rachmadani, K. A., Fatonah, S., & Wijayanggo, A. (2023). Ecoenzyme Is An Anti-Bacterial Material For Making Multi-Purpose Soap From Used Cooking Oil Case Study : Implementation Of The CSR Program Pt Pertamina Patra Niaga Dppu Adi Sumarmo. International Journal of Environmental, Sustainability, and Social Science. https://doi.org/10.38142/ijesss.v4i4.585

Rahayu, S., Pambudi, K., Afifah, A., Fitriani, S., Tasyari, S., Zaki, M., & Djamahar, R. (2021). Environmentally safe technology with the conversion of used cooking oil into soap. Journal of Physics: Conference Series. https://doi.org/10.1088/1742-6596/1869/1/012044

Rahayu, S., Yusuf, A., Nabila, D. A., Kaulika, S. A., Delina, M., Kardiman, Y., & Khamis, N. (2024). Promoting a Green Community at Pusat Komuniti Desa, Pekan Nenas, Pontian, Johor Bahru,

This work is licensed under a Creative Commons Attribution 4.0 International license

Malaysia, through Training on the Utilization of Waste Cooking Oil for Bar Soap Production. PengabdianMu: Jurnal Ilmiah Pengabdian kepada Masyarakat. https://doi.org/10.33084/pengabdianmu.v9i3.6135

Riansih, C., Purwanti, R., Ratnaningsih, D., & Susilani, A. T. (2024). Innovative Technology for Making Solid Soap from Used Cooking Oil as a Solution to the Problem of Household Waste and Home Industry Barukan Village, Manisrenggo, Klaten, Central Java. Mattawang: Jurnal Pengabdian Masyarakat. https://doi.org/10.35877/454ri.mattawang2470

Sanaguano-Salguero, H., Tigre-León, A., & Bayas-Morejòn, I. F. (2018). Use of waste cooking oil in the manufacture of soaps. International Journal of Ecology & Development.

Septiani, R., & Sundari, S. (2025). Transforming Organic Waste into Value-Added Products: Development of Eco-Friendly Liquid Soap. INSOLOGI: Jurnal Sains dan Teknologi. https://doi.org/10.55123/insologi.v4i1.4860

Soni, H., Bhattu, M., Verma, M., Kaur, M., Al-Kahtani, A. A., Lone, I. H., ... Ubaidullah, M. (2024). From kitchen to cosmetics: Study on the physicochemical and antioxidant properties of waste cooking oil-derived soap. Journal of King Saud University - Science. https://doi.org/10.1016/j.jksus.2024.103483

Sulastri, Khairunnasirah, Fazlia, R., Rahmayani, I., Muzainah, & Murfid, A. (2026). Formulation and in vitro antibacterial evaluation of eco enzyme hand soap enriched with pogostemon cablin oil. IOP Conference Series: Earth and Environmental Science. https://doi.org/10.1088/1755-1315/1641/1/012019

Wulandari, R., Nugraheni, I., & Kiptiah, M. (2023). Betel leaf extract as an anti-bacterial agent in solid soap formulation and characterisation. Jurnal Pijar Mipa. https://doi.org/10.29303/jpm.v18i3.4940

Zahari, N., Sabran, S., & Ruslan, N. (2024). The efficacy of home-made soap from used cooking oil and pineapple waste for eco-friendly household product. Food Research. https://doi.org/10.26656/fr.2017.8(5).9

Zahra, S. N., Rafah, R. N., Lestari, V. J., Nurhalizah, Kenaya, F. G., Fazri, A. A., ... Muhab, S. (2024). Preparation of Liquid Soap Utilising Used Cooking Oil with Aloe Vera as an Antibacterial Agent. Chemistry and Materials. https://doi.org/10.56425/cma.v3i1.71

Zayed, L., Gablo, N., Kalcakova, L., Dordević, S., Kushkevych, I., Dordević, D., & Tremlová, B. (2024). Utilizing Used Cooking Oil and Organic Waste: A Sustainable Approach to Soap Production. Processes. https://doi.org/10.3390/pr12061279

Zhumagulova, G., Kocherov, E., Korganbayev, B., Ivakhnyuk, G., Ramatullayeva, L., Ikramov, I., ... Mutasheva, G. (2026). Recycling waste cooking oil into soap: physicochemical characterization and multivariate assessment of experimental formulations. EQA - International Journal of Environmental Quality. https://doi.org/10.60923/issn.2281-4485/24650