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The impact of substituting water with eco enzyme in natural bar soap production

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ABSTRACT

The integration of natural and sustainable additives in soap production addresses concerns over synthetic chemicals and environmental impact. This study investigates the effect of substituting water with eco enzyme, a fermented organic waste product, in natural bar soap production using the cold process method. The research evaluates its dual function as a biocatalyst for the saponification reaction and a natural antimicrobial agent. Soap batches were produced with varying eco enzyme concentrations (0 g, 13 g, 26 g, and 39 g) and analyzed against the SNI 3532:2021 standard. Results demonstrated that eco enzyme addition significantly accelerated the saponification process, reducing the time to reach emulsion, light trace, and trace stages. All soap variations containing eco enzyme (13-39 g) fully complied with SNI standards, including a Total Plate Count of ≤10 CFU/g, indicating potent antimicrobial properties absent in the control sample (≤4000 CFU/g). Furthermore, the addition of 26 g of eco enzyme yielded the optimal balance, producing the hardest soap (2.03 N) with the lowest water content (6.75%) and detectable lipase (6.43 mU/g) and protease (0.0037 mU/g) enzymes. The study concludes that eco enzyme is a highly effective, multi-functional natural additive that enhances reaction kinetics, improves physicochemical qualities, and ensures the microbiological safety of natural bar soap.

1. Introduction

The COVID-19 pandemic has reshaped public awareness of personal hygiene, fostering the emergence of consistent behavioural changes in maintaining cleanliness. Soap has become a primary hygiene product due to its ability to remove microorganisms, oil, and dirt from the body or clothing. Despite its widespread use, many soap formulations still incorporate synthetic chemicals such as diethanolamine, parabens, sodium lauryl sulfate, and triclosan. These compounds are widely used as surfactants and preservatives but have been reported to cause adverse effects including skin irritation, dryness, and redness [1,2,3]. The accumulation of synthetic residues in water ecosystems also raises environmental concerns, leading to a growing demand for more natural and biodegradable alternatives in soap production.

The development of soap from natural materials has gained significant attention as consumers become increasingly concerned about health safety and environmental sustainability [4]. Vegetable oils such as palm oil, coconut oil, and olive oil are among the most commonly used raw materials in soap production due to their emollient and moisturizing properties. The type of oil used strongly influences the physical characteristics, hardness, and cleansing power of the final product. To enhance soap functionality, natural active ingredients have been introduced into formulations to improve antimicrobial and chemical properties [5,6]. One promising innovation is the integration of eco enzyme, a biocatalyst obtained from fermenting organic waste such as fruit peels, vegetable residues, water, and palm sugar.The fermentation process for producing eco enzyme occurs anaerobically over three to six months, resulting in a brown liquid with a distinctive sour aroma.

This liquid contains enzymes such as amylase, lipase, and protease that are capable of decomposing carbohydrates, lipids, and proteins during degradation [7,8]. Enzymes are known as natural catalysts that accelerate specific biochemical reactions by up to 10^6 times with remarkable selectivity [9,10]. The presence of these bioactive components enables eco enzyme to perform multiple functions, including cleaning, catalysis, and microbial control. In addition, eco enzyme filtrate is rich in acetic and amino acids that can serve as a natural cleaner or fertilizer, while its propionic nature effectively inhibits microbial growth, making it suitable even for food preservation [11,12].

This study highlights the novelty of utilizing eco enzyme in soap formulation not only as a natural antibacterial agent but also as a biocatalyst that may influence the saponification reaction. Previous studies have focused primarily on eco enzyme applications in cleaning solutions or agriculture, whereas its catalytic potential in cosmetic chemistry remains underexplored. The research seeks to examine the dual function of eco enzyme in soap production by evaluating its ability to control the reaction rate of saponification under different temperature and time conditions [13]. Findings from this study are expected to contribute to the advancement of eco-friendly soap technology, offering an innovative model for sustainable personal hygiene products with improved antibacterial and catalytic performance.

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2. Materials and Methods

2.1. Materials

Eco enzyme has been sampled from the Reaction and Separation Engineering Laboratory (Department of Chemical Engineering, University of Lampung, Indonesia), palm oil, coconut oil, olive oil, NaOH, glycerine, and distilled water.

Table 1. Formulation and composition of ingredients for making soap

Material	A1	A2	A3	A4
Coconut Oil (g)	90	90	90	90
Palm Oil (g)	90	90	90	90
Olive Oil (g)	120	120	120	120
NaOH (g)	43	43	43	43
Glycerine (g)	15	15	15	15
Distilled water (g)	84	71	58	45
Eco enzyme (g)	0	13	26	39

2.2. Methods

The formula and composition of the soap-making ingredients are shown in Table 1, which were prepared with varying additions of eco enzyme for each sample variation. The soap formulations are symbolised as A1, A2, A3, and A4, which denote the formulation without any eco enzyme addition and with eco enzyme additions of 13 g, 26 g, and 39 g, respectively. NaOH was dissolved in a mixture of distilled water and glycerin, which was then cooled to room temperature (30 °C). An eco enzyme was added to a mixture of coconut oil, palm oil, and olive oil in one container.

The NaOH solution was poured into the mixture of oils and the eco enzyme, and then stirred until a homogeneous mixture was achieved, as indicated by the formation of an emulsion. Stirring was continued until the batter reached a light trace condition, at which point it started to thicken slightly. A modification to the production method was performed: after the batter reached the light trace condition (a thin trace), the container was covered with plastic wrap and left to stand for 5 minutes. This was intended to utilise the heat from the alkali and fatty acid mixture in the vegetable oils that had undergone the saponification reaction

The container cover was opened, and stirring was resumed until the batter reached a trace condition, characterised by a thick, soap-like batter that left a trail. Subsequently, the batter was poured into moulds and left to stand for 24 to 48 hours, until it had solidified. The resulting samples were then removed from the moulds and stored on an open rack at room temperature for two months before analysis. Data on the time and temperature of the batter were collected for every change in condition (emulsion, light trace, and trace).

The quality tests of the resulting soap include Water Content, Acidity (pH), Free Alkali, and Free Fatty Acid, which are analysed in accordance with the Indonesian National Standard SNI 3532:2021. Microbial contamination (Total Plate Count) is analysed using ISO 21149. After that, an enzymatic test is performed on one of the soap samples that has met the SNI 3532:2021 standard. The amylase and protease enzyme content in the soap is determined using the spectrophotometric method, while the lipase enzyme is determined using the acid-base titration method. Hardness analysis of the soap is also performed using a hardness measuring instrument (rheometer needle).

3. Result and Discussion

3.1. The effect of the eco enzyme addition on the saponification reaction

The saponification reaction in soap making involves the hydrolysis of triglycerides by an alkali, producing glycerol and fatty acid salts (soap). The reaction's efficiency depends on factors such as mixing time and the ratio of reactants, which influence the completeness of saponification and the soap's quality, particularly in terms of free alkali and fatty acid content. The addition of an eco enzyme serves as a catalyst to accelerate the reaction, helping the soap mixture reach emulsion, light trace, and trace stages more quickly through reduced mixing time and optimal temperature control.

3.1.1. The effect of eco enzyme addition on mixing time

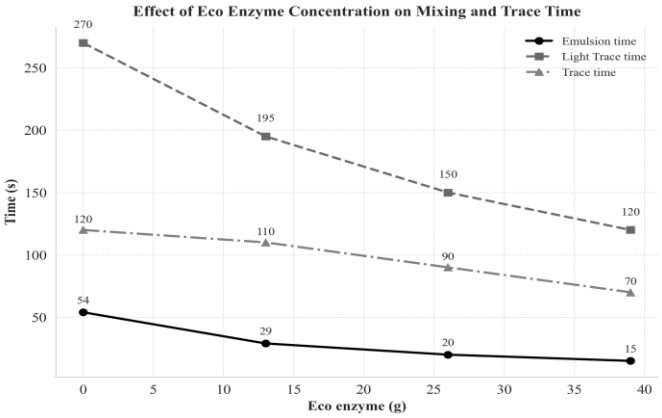

Fig. 1. Comparison of the eco enzyme variation against the emulsion time, light trace time, and trace time

The data presented in Figure 1 indicate a clear inverse relationship between eco enzyme concentration and the mixing times required to achieve emulsion, light trace, and trace conditions. At 0 g of eco enzyme, the emulsion, light trace, and trace times were 54, 270, and 120 seconds, respectively, while the addition of 40 g of eco enzyme reduced these times significantly to 15, 120, and 70 seconds. This trend demonstrates that the inclusion of eco enzyme accelerates the saponification process by enhancing the reaction kinetics between fatty acids and alkali. The catalytic nature of the eco enzyme facilitates faster hydrolysis of triglycerides, thereby promoting the earlier formation of soap micelles and reducing the energy and time required to reach stable emulsification.

From a chemical kinetics perspective, the acceleration observed can be attributed to the enzymatic action of natural compounds within the eco enzyme, which often contain protease, amylase, and lipase derived from the fermentation of fruit or vegetable waste [14,15]. These enzymes lower the activation energy of the saponification reaction, allowing the system to reach reaction equilibrium at a faster rate. The increase in reaction temperature observed alongside shorter mixing times aligns with the Arrhenius principle, where elevated temperatures enhance molecular collisions, thereby supporting the catalytic role of the eco enzyme [16,17,18]. The simultaneous decrease in light trace and trace times suggests that the enzyme improves miscibility and accelerates the conversion of triglycerides into fatty acid salts, resulting in a more rapid thickening of the soap mixture.

Moreover, the reduction in mixing time implies not only

higher process efficiency but also a potential improvement in product uniformity. The catalytic effect of the eco enzyme promotes consistent saponification, reducing the presence of unreacted alkali or fatty acid residues that may otherwise compromise soap quality. This observation is in agreement with findings by Rodriguez-Amaya et al. (2023), who reported that enzyme-assisted soap production yielded more homogeneous emulsions and improved stability in natural formulations [19]. Similarly, Pasha et al. (2021) demonstrated that enzymatic enhancement in saponification reactions reduces reaction time by up to 60%, consistent with the current data trend [20].

Overall, the addition of eco enzyme can be understood as both a chemical and process optimization factor in soap production. By accelerating molecular interactions through catalytic mediation and thermal facilitation, eco enzyme addition ensures a more efficient saponification pathway. This aligns with the broader principles of green chemistry, emphasizing reduced energy consumption and the utilization of biodegradable catalysts [21,22]. Thus, beyond its immediate effect on mixing time, the eco enzyme embodies an environmentally sustainable approach to improving reaction kinetics and process performance in soap manufacturing.

3.1.2. The effect of mixing time on temperature

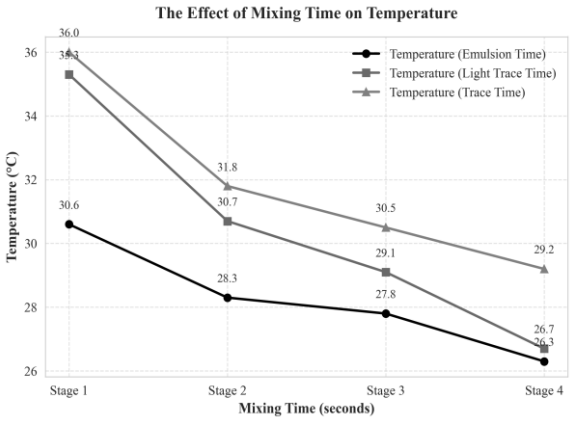

Fig. 2. Comparison of emulsion, light trace, and trace time against temperature

The visual data clearly establishes an inverse correlation between the Mixing Time stages (Stage 1 to Stage 4) and the resulting temperature drop across all critical saponification markers: Emulsion Time, Light Trace Time, and Trace Time. The overall trend shows a consistent and significant decrease in temperature as the mixing duration increases. Quantitatively, the most notable change occurs between Stage 1 and Stage 4. For instance, the Light Trace Time temperature drops substantially from 35.3 °C to 26.7 °C, indicating a difference of 8.6 °C. This data suggests that a longer, more extended mixing duration hypothesized to facilitate greater interaction with the *eco enzyme* contributes to enhanced thermal control and potentially a lower overall enthalpy of reaction (ΔH_{rxn}) realized within the system, a critical factor in mitigating the exothermic nature of the saponification process [23,24].

The observed temperature reduction in conjunction with increased mixing time strongly implies that the *eco enzyme* preparation acts as a biocatalyst, significantly altering the reaction kinetics. Saponification is a second-order, exothermic reaction where the base hydrolyzes triglycerides. The *eco enzyme* solution, being a fermentation product, is likely rich in lipase [15,25], which is known to catalyze the hydrolysis of triglycerides into fatty acids and glycerol. This enzymatic pre-hydrolysis effectively lowers the activation energy (E_a) required for the subsequent reaction with the alkali. By facilitating this rate-limiting step, the enzyme allows the saponification to proceed at a more controlled, lower-temperature trajectory. The longer mixing time (Stage 4 vs. Stage 1) provides increased contact time for the interfacial biocatalysis to occur, resulting in a more efficient and slower release of heat throughout the process, preventing the sharp thermal spikes often associated with rapid saponification. This stabilization of the thermal profile is crucial for high-quality soap production.

From a chemical kinetics perspective, the progressive reduction in temperature with prolonged mixing, especially the lower final Trace Time temperatures (e.g., 29.2 °C at Stage 4) demonstrates successful thermal management mediated by the catalyst. In the absence of a catalyst, higher initial temperatures would be required or a much faster, uncontrolled reaction would occur. Furthermore, the *eco enzyme* preparation is typically acidic (due to organic acid formation during fermentation). Introducing this acidic solution into the highly basic lye mixture likely results in a minor, initial neutralization event. While this reaction itself is exothermic, it consumes a small amount of the available alkali, potentially *extending the time* required for the remaining alkali to reach the necessary saponification concentration, thus prolonging the Mixing Time (time to trace). This physicochemical interaction, coupled with the enzymatic catalysis, spreads the total heat release over a longer duration, leading to the lower measured temperatures at the critical *trace* points. Therefore, the data strongly supports the hypothesis that the addition of *eco enzyme* provides a dual mechanism for reaction control: enzymatic acceleration of the rate-limiting step and physicochemical modification of the lye concentration, both contributing to a more sustainable and thermally stable saponification process [26].

3.2. Quality and feasibility of bar soap based on SNI 3532:2021

The widespread distribution of commercial soap products occurs because they have achieved good quality according to the parameters set by the National Standardization Agency. Quality requirements are established to ensure that the marketed product meets high standards of quality and does not harm consumers who use it.

Table 2. Quality requirements for bar soap

Parameter test	SNI 3532:2021 requiment
Water content	Max 23 %
Acidity (pH)	6.0 – 11.0
Free Alkali	Max 0.1 %
Free fatty acid	Max 2.5 %
Total plate count	Max 1 x 10^3 CFU/g

3.2.1. Water content

The Water Content is a crucial quality parameter for solid soap, directly influencing its physical properties, structural integrity, and long-term stability. The primary objective of this test is to quantify the amount of residual moisture within the saponified product. A low water content is desirable as it correlates with increased hardness, reduced dissolution rate during use, and a prolonged shelf life. Conversely, an elevated water content results in a softer bar, making it prone to rapid wear and creating a favorable humid microenvironment susceptible to microbial contamination, specifically the proliferation of fungi or mold. For quality assurance, the Indonesian National Standard

(SNI 3532:2021) mandates a maximum allowable water content of 23% for bar soap. The following analysis examines how varying concentrations of eco enzyme influence this critical parameter.

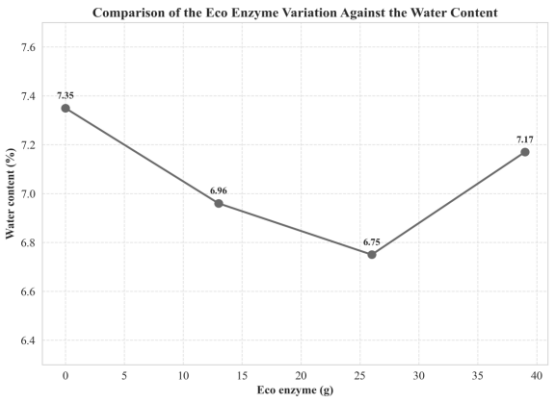

Fig. 3. Comparison of the eco enzyme variation against the water content

The experimental results, as depicted in the figure (showing the transition from a bar to a line chart), reveal a non-linear relationship between the concentration of eco enzyme addition and the final water content of the soap. The control sample (0g *eco enzyme*) exhibited the highest water content at 7.35%. Upon the initial increase in *eco enzyme* concentration to 13g and 26g, the water content progressively decreased to 6.96% and the minimum value of 6.75%, respectively. This initial decline suggests that the *eco enzyme* facilitates a more efficient and complete reaction, or influences the matrix structure to reduce moisture retention. However, a further increase to 39g *eco enzyme* resulted in an unexpected rebound, with the water content increasing significantly to 7.17%. Notably, all measured values are substantially below the maximum limit set by SNI 3532:2021 (23%).

The observed trend (a decrease followed by an increase in water content) can be mechanistically explained by the dual physicochemical and kinetic effects of the *eco enzyme* on the saponification matrix.

1. Enhanced Reaction Efficiency (Low Concentrations, 0g to 26g):
   At low to moderate concentrations, the lipase and esterase activity present in the eco enzyme promotes the hydrolysis and subsequent saponification reaction, as discussed in the previous section. By acting as a biocatalyst, the enzyme ensures a more complete conversion of triglycerides into soap (salt of fatty acids) and glycerol. Since water is a byproduct of the initial hydrolysis step (though consumed later) and is the solvent for the lye, a more complete conversion leads to the formation of a more compact crystalline soap structure. This denser, more ordered matrix entraps less free water, reducing the overall water content [27,28]. The increased hydrophobic character of the well-formed soap crystals essentially excludes residual moisture more effectively, explaining the decrease from 7.35% to 6.75%.
2. Dilution and Matrix Disruption (High Concentration, 39g):
   The increase in water content at the highest concentration (39g) is likely due to the physicochemical effects of the bulk additive. Eco enzyme is a complex aqueous solution containing organic acids, sugars, and water. When a large volume of this solution is introduced, it significantly increases the total initial water load in the reaction mixture, thereby lowering the effective concentration of the lye. Furthermore, high concentrations of non-soap compounds (such as unreacted organic acids or residual sugars from the eco enzyme) can disrupt the formation of the ordered soap crystal structure. This matrix disruption creates larger intercrystalline spaces or amorphous regions within the soap structure, which are less efficient at shedding or holding water tightly, leading to a higher retention of residual moisture (i.e., the water content rebounds to 7.17%) [29]. This non-linear behavior highlights a critical optimization point where the catalytic benefits (26g) are outweighed by the structural and dilution drawbacks (39g) of the additive itself.

3.2.2. Acidity (pH)

The determination of Acidity (pH) is a critical quality control measure that assesses the residual alkalinity or acidity of the final soap product. The pH value is paramount as it dictates the soap's interaction with the human integumentary system. An excessive alkaline pH can compromise the skin's natural acid mantle (typically ranging from pH 5.4 to 5.9), which functions as a vital physicochemical barrier against pathogenic microorganisms and environmental stressors. Damage or over-neutralization of this mantle increases the potential for skin irritation, dryness, and susceptibility to allergic reactions. The inherent nature of saponification, which involves the reaction of triglycerides with a strong base (alkali), results in soap products that are typically alkaline, with many commercial samples ranging from pH 9.3 to 10.7. For quality and safety compliance, the Indonesian National Standard (SNI 3532:2021) specifies a broad acceptable pH range of 6.0 to 11.0 for bar soap. The following analysis investigates how varying concentrations of the *eco enzyme* additive influence this crucial parameter.

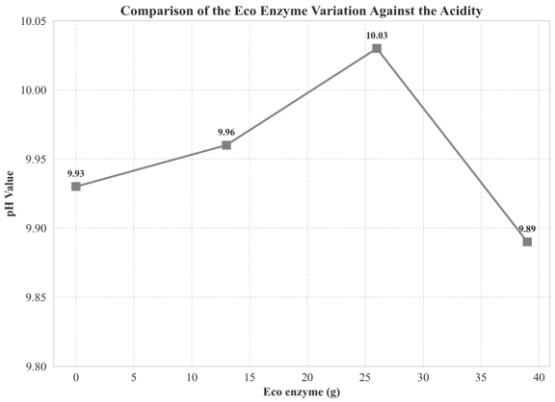

Fig. 4. Comparison of the Eco Enzyme Variation Against the Acidity

The pH results for the saponified product show a distinct non-linear, parabolic trend influenced by the concentration of *eco enzyme*. The pH value initially rises from the control sample (0g) pH of 9.93, peaking sharply at 10.03 when 26g of *eco enzyme* is incorporated. This maximum value is the highest alkaline reading observed. Subsequently, increasing the *eco enzyme* concentration to 39g causes a significant drop in alkalinity, resulting in the lowest pH value of 9.89. All samples remain within the established SNI quality range (6.0-11.0), confirming they are technically safe; however, the shift in alkalinity demonstrates a direct influence of the additive on the final chemical equilibrium.

The fluctuation in the final soap pH can be attributed to a complex interplay between the kinetics of saponification catalyzed by the enzyme and the acid-base chemistry introduced

by the *eco enzyme* solution itself.

1. Increased pH at moderate concentration (26g):
   The increase in pH from the control (9.93) to the peak (10.03) at 26g is counter-intuitive if one considers the eco enzyme purely as an acidic additive, but it can be explained by its catalytic efficiency. As discussed previously, the lipase in the eco enzyme facilitates a more complete and efficient hydrolysis of triglycerides into free fatty acids (FFA) and glycerol. A more complete and rapid conversion of the oil (which consumes the alkali, e.g., NaOH) results in a more homogeneous mixing and crystallization structure, but also a cleaner reaction. In a typical cold process soap, some unreacted NaOH might be isolated or poorly incorporated. However, the enzyme's efficiency ensures the NaOH is optimally utilized, forming RCOONa (soap). If this optimal conversion ensures that only trace amounts of free alkali remain, the resulting pH (which is measured by the hydrolysis of the soap salt RCOONa + H_2O = RCOOH + NaOH) will be slightly higher because the sodium salt itself is alkaline, and the structure is compact. Furthermore, research indicates that the presence of high-quality glycerol (a co-product of efficient saponification) can stabilize the system's pH by acting as a solvent and buffering agent, potentially slightly elevating the measured alkaline value [30,31].
2. Decreased pH at high concentration (39g):
   The sharp drop in pH to 9.89 at the highest concentration (39g) is primarily due to the overwhelming effect of the acidic components within the bulk eco enzyme additive. Eco enzyme is produced via fermentation and contains organic acids (like acetic acid or lactic acid) which lower its intrinsic pH [32,33]. When a large quantity is added, these acids consume the residual free NaOH through a simple neutralization reaction, effectively "quenching" the residual alkalinity of the system:

   $$RCOOH_{organic\ acid} + NaOH_{free} \rightarrow RCOONa + H_2O$$

   This direct acid-base reaction diminishes the concentration of the strong base (NaOH) available to contribute to the final alkalinity, thus shifting the equilibrium towards a lower pH value. This demonstrates that while low concentrations enhance catalytic conversion, high concentrations fundamentally alter the final acid-base stoichiometry, providing a means of fine-tuning the soap's mildness for dermatological safety.

3.2.3. Free alkali

Bar soap is a soap produced from the saponification reaction between Sodium Hydroxide (NaOH) and fatty acids, resulting in a solid, bar product. NaOH is classified as a strong base with a high alkali content. Free alkali is defined as the amount of base that is not bound to fatty acids. Free alkali has harsh properties; therefore, soap with a high free alkali content can cause skin irritation. This is because sodium hydroxide is hygroscopic, meaning it can absorb water from the environment, which causes skin moisture to dry out faster. Based on SNI 3532:2021 standard, the maximum quality of free alkali in bar soap is 0.1%. According to the test results, all variations of the bar soap, produced with eco enzyme additions of 0 g, 13 g, 26 g, and 39 g, did not contain any free alkali content. This indicates that the saponification reaction, involving NaOH as the alkali, the vegetable oils used (palm oil, coconut oil, and olive oil), and the fatty acids derived from these oils, as well as the variations in the addition of the eco enzyme, proceeded completely.Several factors are suspected to be the reason why the resulting soap does not contain free alkali, namely the formulation of the ingredients used and the mixing time performed [34,35].

The stoichiometric calculation for the ingredients used was correct. Using an incorrect reactant ratio can result in soap that fails to meet standard specifications, as indicated by the values of free alkali or free fatty acid. During the mixing process from the emulsion phase until the trace phase, mass transfer occurs between the fatty acid and the eco enzyme mixture and the NaOH solution, which can eliminate mass transfer limitations, allowing the reaction between the reactants to proceed without hindrance and all alkali molecules to react stoichiometrically with the fatty acids, forming soap and glycerin [36]. Therefore, the concentration of free alkali tends to decrease as the mixing time continues.

3.2.4. Free fatty acid

The Free Fatty Acid (FFA) content is a critical quality indicator, quantifying unreacted fatty acids present in the final soap product. High levels of FFA are typically undesirable as they indicate an incomplete saponification reaction or the presence of non-saponifiable matter. FFA, while forming soap via neutralization with residual alkali, can contribute to soap rancidity over time through oxidation, potentially degrading the soap's texture, odor, and shelf life (AOCS Official Method Ca 5a-40). Furthermore, excessive FFA may require a higher alkali concentration in the initial formulation, which could lead to safety issues if not fully reacted. An ideal, high-quality soap product should minimize the concentration of FFA. This analysis explores how the catalytic influence of the eco enzyme impacts the efficiency of triglyceride conversion, thereby affecting the final FFA concentration.

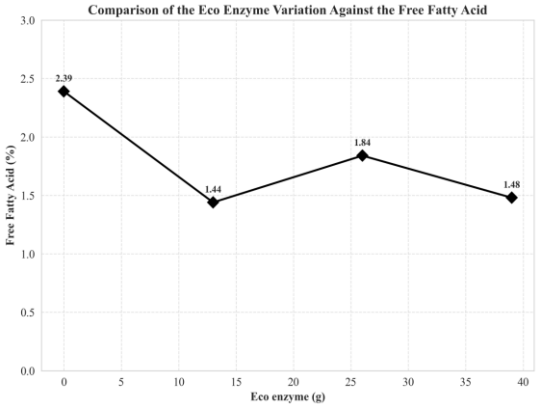

Fig. 5. Comparison of the eco enzyme variation against the free fatty acid

The data on Free Fatty Acid (FFA) content, when plotted against the varying eco enzyme concentrations, reveals a pattern of significant reduction compared to the control, followed by slight fluctuations. The control sample (0 g eco enzyme) exhibited the highest FFA content at 2.39%. The introduction of 13 g of eco enzyme immediately resulted in a sharp drop to 1.44%, representing a reduction of approximately 40% from the control. Increasing the concentration to 26 g caused a slight increase in FFA to 1.84%, indicating a less efficient conversion at this point compared to the 13 g dosage. Finally, at the highest concentration of 39 g, the FFA content dropped again to 1.48%. The primary finding is that any addition of eco enzyme leads to a substantial decrease in FFA content, underscoring its role as a process promoter.

The sharp initial drop in FFA from 2.39% (control) to 1.44% (13 g) provides the strongest evidence for the biocatalytic function of the eco enzyme. The high FFA content in the control is likely due to the inherent presence of some free fatty acids in the initial oil/fat feedstock or minimal pre-hydrolysis during the

initial mixing phase. The lipase and esterase activity within the eco enzyme solution effectively promotes the hydrolysis of triglycerides, which is the first step in converting the oil into its constituent FFA and glycerol. Importantly, this promotion appears to be so efficient that the produced FFA is quickly neutralized by the lye to form soap. This is an example of an accelerated reaction pathway where the enzyme optimizes the interfacial area between the oil and the aqueous lye phase, leading to a much more complete utilization of the oil and thus a lower residual FFA [37,38]. This reduction confirms the enzyme's ability to ensure a more complete saponification, which directly contributes to the long-term stability and reduced susceptibility to oxidative degradation (rancidity) of the soap.

The slight increase in FFA at 26 g (1.84%) followed by a return to near-minimum at 39 g (1.48%) highlights a concentration-dependent complexity often seen with biological additives. The rebound at 26 g might be linked to a phenomenon where the high concentration of non-soap organic matter (sugars, salts, minor acids) from the eco enzyme interferes with the lye-FFA interaction. Specifically, these extraneous compounds may form micelles or complexes that sterically hinder the alkali from neutralizing the free fatty acids, temporarily leaving more FFA unreacted. Conversely, the decrease back at 39 g could be due to the much higher volume of eco enzyme added, providing a sufficient mass of organic acid components that, while reducing alkalinity (as seen in the pH data), also drive the overall equilibrium to consume any remaining free sodium hydroxide or even complex with some of the FFA [14,39]. This non-linear behavior suggests that 13 g represents an optimal concentration where the catalytic benefits maximize the conversion efficiency while minimizing the inhibitory or interference effects of the bulk additive mass.

3.2.5. Total plate count

The Total Plate Count (TPC), often expressed in Colony-Forming Units per gram (CFU/g), is a standard microbiological assay used to quantify the overall viable population of aerobic bacteria and fungi in the final soap product. TPC serves as a crucial indicator of hygienic quality and microbiological safety. Elevated TPC values suggest poor manufacturing conditions, inadequate preservation, or susceptibility to microbial proliferation, which can lead to product spoilage, reduced shelf life, and pose a dermatological risk to consumers, especially to those with compromised skin barriers. While SNI 3532:2021 sets the limit for bar soap, a TPC limit of 1000 CFU/g is often applied to high-quality cosmetic preparations to ensure consumer safety and product integrity [40,41]. The following analysis evaluates the impact of eco enzyme addition on the microbial load of the soap, assessing its potential as a natural preservative.

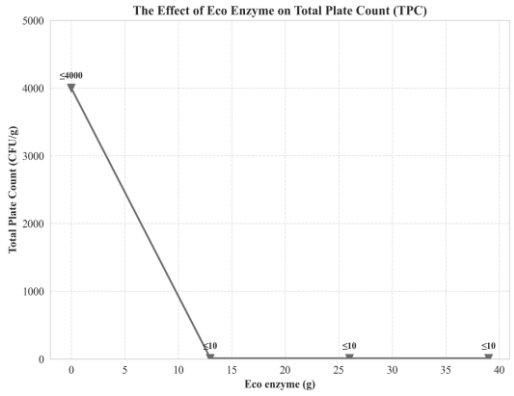

Fig. 6. Comparison of the eco enzyme variation against the total plate count

The data comparing the eco enzyme variation against the Total Plate Count (TPC) demonstrates an exceptionally strong and definitive antimicrobial effect resulting from the incorporation of the additive. The control sample (0 g eco enzyme) registered a TPC value of less than or equal to 4000 CFU/g, indicating a significant presence of viable microorganisms, potentially due to environmental contamination or non-sterilized ingredients. In stark contrast, the addition of even the lowest concentration of eco enzyme (13 g) immediately reduced the TPC count to the limit of detection, recorded as less than or equal to 10 CFU/g. This dramatic reduction is maintained across all higher concentrations (26 g and 39 g), confirming that eco enzyme effectively suppresses the microbial load to levels considered microbiologically safe and excellent for cosmetic products, far exceeding the general safety standard of 1000 CFU/g.This robust antimicrobial performance is primarily attributed to the complex physicochemical composition of the eco enzyme. The product of fruit/vegetable waste fermentation is rich in organic acids (such as acetic acid, lactic acid, and propionic acid), as established in previous studies on its antimicrobial properties [42,43]. The mechanism of antimicrobial action by these organic acids involves disruption of the microbial cell membrane. Specifically, the organic acids in their undissociated form (RCOOH) can readily penetrate the cell wall and membrane of microorganisms. Once inside the neutral pH of the microbial cytoplasm, the acid dissociates, releasing H^+ ions. This rapid influx of H^+ ions drastically lowers the intracellular pH, inhibiting essential metabolic enzymes, disrupting proton motive force, and ultimately leading to the microbial cell's death. The fact that the minimal concentration of 13 g is sufficient to achieve maximum microbial suppression suggests that the threshold concentration of active organic acids needed for bactericidal/fungicidal activity has been reached at this low dosage. This is a critical finding, positioning the eco enzyme not only as a processing aid but also as a highly effective natural preservative system, offering a cleaner label alternative to synthetic preservatives while simultaneously ensuring superior hygienic quality of the final soap product.

Table 3. Enzyme content in soap with 26 g of eco enzyme

Amylase Enzyme (U/g)	Lipase Enzyme (mU/g)	Protease Enzyme (mU/g)
Undetected	6.43	0.0037

Based on the data presented in Table 3, the addition of an eco-enzyme is proven to provide enzyme content in the resulting soap, including lipase and protease enzymes. The lipase enzyme content in the soap is 6.43 mU/g. This figure indicates that 1 gram of soap with added eco enzyme contains 6.43 mU of lipase enzyme. Meanwhile, the protease enzyme content in the soap is 0.0037 mU/g. This figure indicates that 1 gram of soap with added eco enzyme contains 0.0037 mU of protease enzyme. As for the amylase enzyme, its presence and quantity were undetected. This could be due to the minute amount of amylase enzyme in the soap, which is below the detection limit of 0.112 U/g, making the spectrophotometer unable to detect its presence.

3.3. Hardness of bar soap

The Hardness of bar soap, measured in Newtons (N) or a similar force unit, is a paramount physical attribute that directly correlates with the soap's durability, structural integrity, and dissolution rate during use. A high hardness value is desirable as

it indicates a product that is resistant to breakage and less prone to dissolving quickly when exposed to water, thus extending its overall economic shelf life and usage longevity. Conversely, a soft soap is often associated with high water content, rapid wear, and poor handling properties. Hardness is primarily determined by two factors: the fatty acid profile of the raw materials (the ratio of saturated to unsaturated fatty acids) and the efficiency of the saponification process, which influences the formation of a dense, crystalline soap matrix. The subsequent analysis investigates the dose-dependent effect of the eco enzyme on soap hardness, linking the change in physical structure to its influence on reaction kinetics and final moisture content.

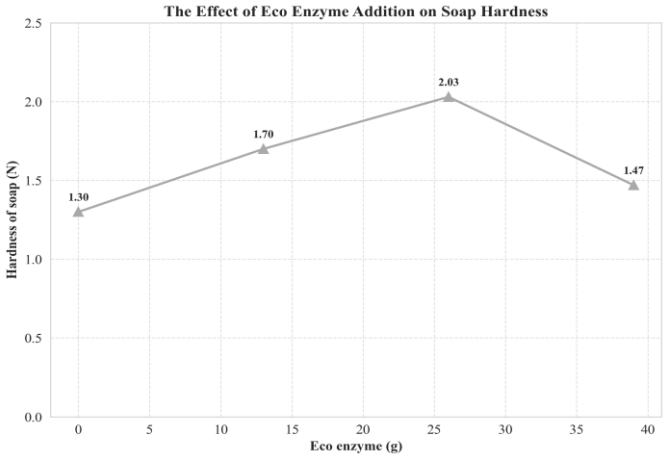

Fig. 11. Comparison of the eco enzyme variation against the hardness of soap

The data on Hardness of bar soap reveals a distinct non-linear, parabolic relationship with the concentration of eco enzyme added, closely mirroring the trend observed in the water content and pH analysis. The control sample (0 g eco enzyme) registered a hardness of 1.30 N. As the eco enzyme concentration increased to 13 g and 26 g, the hardness progressively increased, reaching a peak value of 2.03 N at the 26 g concentration. This maximum hardness represents a significant improvement in structural integrity, approximately a 56% increase compared to the control. However, further increasing the concentration to 39 g resulted in a sharp decrease in hardness, falling to 1.47 N, a value only marginally higher than the control.This parabolic trend is primarily explained by the interplay between saponification efficiency and matrix integrity, which is directly linked to the water content results. The initial increase in hardness (from 1.30 N to 2.03 N) is a direct consequence of the eco enzyme's role as an effective biocatalyst.

As established in the FFA and temperature analyses, the enzyme promotes a more complete and efficient saponification reaction. This efficiency leads to the formation of a higher yield of the salt of the fatty acids (soap), which can then organize into a denser, more uniform crystalline matrix [29,44,45]. Critically, this efficient crystallization simultaneously minimizes the entrapment of residual water, as confirmed by the minimal water content (6.75%) observed at the same 26 g concentration. The lower the water content and the denser the crystal packing, the higher the mechanical hardness of the bar.The sharp decline in hardness at the 39 g concentration (to 1.47 N) is explained by the detrimental physicochemical interference of the high volume of eco enzyme additive. As seen in the water content data (which rebounded at 39 g), the high concentration of non-soap organic matter (sugars, minor acids) disrupts the optimal packing of the soap crystals. This structural disruption leads to the formation of a less ordered, more amorphous soap structure with increased intercrystalline spaces. These spaces readily retain more moisture (the water content rebounded to 7.17%), leading to a weaker physical structure that is easily deformed, thus reducing the measured hardness. Therefore, the 26 g concentration represents the optimum balance where the catalytic benefits maximize structural efficiency, leading to the hardest and most durable soap bar.

4. Conclusion

In conclusion, this study unequivocally demonstrates that the substitution of water with eco enzyme in natural bar soap production confers significant multifunctional advantages, serving simultaneously as an efficient biocatalyst that accelerates the saponification reaction by reducing mixing times and moderating the process temperature, and as a potent natural preservative that suppresses microbial contamination to levels far exceeding regulatory standards. The optimal incorporation of 26 g of eco enzyme was identified as the critical point for maximizing soap quality, resulting in a product with superior structural hardness, minimal water content, and a balanced pH, all of which are attributable to the enzyme-facilitated, more complete and efficient saponification reaction that yields a denser soap matrix. Consequently, eco enzyme emerges as a viable and innovative green additive for the cosmetic industry, enabling the production of high-quality, dermatologically safer, and environmentally sustainable cleansing products with enhanced functional properties.

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