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Effectiveness of bromelain for VCO extraction by adding of metal effector and testing of its antimicrobial activity

Purkan Purkan[1,2], Nabila Rahma Octavia[1], Lailatul Hidayah[1], Agus Supriyanto[3], R Merlyn Sujatha[4], Sri Sumarsih[1,2], Bonifasius Adhika Dani Setyawan[1], Sofijan Hadi[1,2]

[1]Department of Chemistry, Faculty of Science and Technology, Airlangga University. Jl. Mulyorejo, Surabaya, Indonesia
[2]Research Group of Biochemical Engineering, Enzyme Biotechnology and Gene Cloning, Airlangga University. Jl. Mulyorejo, Surabaya, Indonesia
[3]Department of Biology, Faculty of Science and Technology, Airlangga University. Jl. Mulyorejo, Surabaya, Indonesia
[4]Department of Biochemistry, St. Peter's Institute of Higher Education and Research, Avadi, Chennai, Tamil Nadu, India

ABSTRACT: Antimicrobial resistance (AMR) is a major global health concern. Virgin coconut oil (VCO) shows potential as a natural antimicrobial due to its medium-chain fatty acids, including lauric, caprylic, and capric acids. This study optimized bromelain-based VCO production by adding Ca^{2+} and Mg^{2+} ions (as $CaCl_2$ and $MgCl_2$) and evaluated its antimicrobial activity. The optimal bromelain concentration (8% b/v) produced VCO with a 32.05% yield and good-quality parameters, including moisture content, free fatty acids (FFA), peroxide value, iodine value, and lauric acid content. The addition of 2.0 mM Ca^{2+} increased the VCO yield to 39.89%, while 1.5 mM Mg^{2+} yielded 36.58%; each effector also improved VCO quality during bromelain-assisted extraction. These results indicate that the addition of $CaCl_2$ and $MgCl_2$ effectors effectively enhanced bromelain activity, resulting in high-quality VCO. The enzymatic VCO exposed inhibition zones of 6.98, 4.58, and 3.37 mm to *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans,* respectively, while VCO from heating extraction appeared to have inhibition zones of 5.19, 3.61, and 3.01 mm. Meanwhile for antibiotic control using chloramphenicol for *S. aureus* and *E. coli* showed inhibition zones of 14.01 and 17.98 mm, respectively, then fluconazole antibiotic for Candida albicans gave an inhibition zone of 5.18 mm. Although less potent than antibiotics, enzymatic VCO demonstrated enhanced antimicrobial potential and quality.

1. INTRODUCTION

Antimicrobial resistance (AMR) is a real threat to global health. This condition makes pathogenic microbial infections difficult to treat, which also increases the spread of disease, making it more severe and fatal. AMR is a condition where microorganisms such as bacteria, viruses, fungi, and parasites become resistant to antimicrobial drugs (Hadi et al, 2023; Setditjen Farmalkes, 2024; WHO 2025). Bacterial infections are reported to be the second leading cause of death

* Corresponding author.
E-mail address: sofijan-h@fst.unair.ac.id (Sofijan Hadi)

worldwide after ischemic heart disease. If left untreated, this figure is estimated to reach 10 million per year by 2050 (Setditjen Farmalkes, 2024; WHO, 2025). The risk of a silent pandemic due to AMR results in higher mortality from sepsis than COVID-19 (Setditjen Farmalkes, 2024; WHO 2025).

The high number of cases of microbial resistance to antibiotics necessitates the provision of safe, natural, and effective alternative antimicrobials. Drug synthesis approaches and the exploration of natural-based drugs can be developed. Exploring natural materials is attractive because, in addition to contributing to the control of pathogenic microbes, this approach supports the trend of consumer preference for natural-based products.

Virgin coconut oil (VCO) is a metabolite compound found in coconuts that offers various health benefits due to its source of medium-chain fatty acids such as lauric, caprylic, and capric acids (Abbas et al., 2017; Colletti et al., 2021; Kusuma & Putri, 2020; Mohan et al., 2016). VCO is also needed in many industries, such as the food, cosmetics, and pharmaceutical industries, for various purposes (Nivya et al., 2023). Another advantage of VCO is its antimicrobial properties, which stem from the ability of its lauric acid component to be converted into monolaurin. Monolaurin has been shown to be effective against various pathogenic microorganisms, including gram-positive bacteria, lipid-lowering viruses, and fungi (Colletti et al., 2021; Nabilla & Advinda, 2022; Nasir et al., 2018). VCO has demonstrated antiviral effectiveness against Coronavirus-2 (SARS-CoV-2), better known as Covid-19 (Colletti et al., 2021; Imelda et al., 2024; Trisnawati, 2021). To date, VCO's potential remains untapped, making research and commercialization crucial.

VCO in coconut milk exists in an emulsion form with other components such as water, protein, and fat, with lipoprotein as the emulsifier. Lipoprotein emulsifiers bind coconut oil droplets in a thin layer, allowing the oil and water droplets to combine (Mela & Bintang, 2021). VCO can be removed/separated if the emulsion bonds are disrupted through various methods.

Hydrolysis of lipoprotein emulsifiers by heating reduces the quality and yield of VCO. Exposure to high temperatures in heating methods can reduce product quality due to the degradation of nutrients, particularly the antioxidants, vitamin E, and phenolic compounds in VCO (Nivya et al., 2023). The use of solvent dispersion methods to extract VCO is also prone to leaving chemical residues that can cause toxicity, quality instability, and complicate purification.

Innovative VCO production requires the development of precise and efficient methods to achieve high quality and yield. Enzymatic extraction is highly feasible due to the unique and specific nature of enzymes, which prevent the formation of byproducts. Enzymes also work under mild conditions, requiring no extreme temperatures or pH (Rahmalia & Kusumayanti, 2021), thus minimizing the damage to VCO's active components.

Bromelain is a proteolytic enzyme capable of hydrolyzing proteins into amino acids or small peptides (Varilla et al., 2021). This enzyme, derived from pineapple (Rahmalia & Kusumayanti, 2021; Varilla et al., 2021), can hydrolyze the emulsifier lipoprotein molecules in coconut milk and therefore can be used for VCO production. Several pioneering studies utilizing pineapple biomass for various applications have been developed in previous years, one of which is as a source of the bromelain enzyme (Colletti et al., 2021). Although the use of bromelain is promising, its performance in VCO production requires optimization based on various factors, such as reaction conditions and the involvement of effector compounds to maximize VCO production with high antimicrobial activity.

The use of bromelain for VCO production remains problematic, as optimal process conditions and the type of effector that supports it remain unsolved. The quality and antimicrobial activity of the resulting VCO are also unknown. This paper reports on the effectiveness of bromelain for VCO production and the product's antimicrobial properties.

2. MATERIALS AND METHODS

2.1. Materials

The chemicals used in this study included isolates of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* bacteria, best brand bromelain, distilled water, grated coconut, water, 95% ethanol, phenolphthalein (PP) indicator solution, cyclohexane, acetic acid, glacial acetic acid, Wijs solution, potassium iodide (KI), sodium thiosulfate ($Na_2S_2O_3$), chloroform, NaOH, helium gas, 15% BF_3 solution in methanol, starch indicator solution, NA powder, NB powder, MHA powder, and n-hexane.

2.2. Research procedures

2.2.1. Production of coconut milk

Coconut milk is made by removing the coconut flesh and grating it using a grater. The grated coconut is mixed with water in a 1:1 ratio, then squeezed through a cloth sieve to obtain the coconut milk. The coconut milk is then placed in a beaker, covered with aluminum foil, and left for 2 hours until two layers form: a creamy phase on top and a skim phase on the bottom. The cream and skim phases are separated using a pipette and centrifuged (Rahmalia & Kusumayanti, 2021).

2.2.2. Enzymatic extraction of VCO with bromelain

Hundred milliliters of coconut milk was added with 0.4 g of bromelain enzyme. The mixture is stirred for 5 minutes using a stirrer. The mixture is left in an incubator for 24 hours at 37°C until three layers form: an oily phase in the middle, a watery phase on top, and a watery phase at the bottom. The oil and water can be separated by filtration with filter paper or centrifugation at 5000 rpm for 5 minutes. The resulting coconut oil is then stored in a plastic bottle.

2.2.3. VCO extraction by the heating method

VCO is extracted by heating coconut milk at 100–120°C for 60 minutes until the water evaporates completely, leaving only oil and pulp. Heating denatures the proteins in the coconut milk, disrupting the stability of the milk emulsion. The pulp includes coagulated proteins. This is then separated by filtering through cheesecloth, and the remaining residue is further heated to extract more oil (Nivya et al., 2023).

2.2.4. VCO yield analysis

Yield analysis is performed by measuring and comparing the weight of the resulting oil with the initial weight of the coconut cream. The coconut oil yield can be calculated using the equation below (Razali et al., 2022):

$$\text{Yield VCO} = \frac{\text{Weight of coconut oil}}{\text{Initial weight of coconut cream}} \times 100\%$$

2.2.5. VCO characterization

VCO quality was determined physicochemically, including free fatty acid (FFA) content, iodine value (IV), peroxide value, and water content, in accordance with Indonesian National Standards (SNI). Meanwhile, the fatty acid content of VCO was determined using GC-MS analysis (Nivya et al., 2023).

2.2.6. Bacterial and fungal cultivation

Samples of gram-negative bacteria (*E. coli*), gram-positive bacteria (*S. aureus*), and fungi (*C. albicans*) were rejuvenated on nutrient agar (NA) media, followed by inoculation into nutrient broth (NB) media. Rejuvenation was performed aseptically in laminar air flow to prevent contamination of the cultures during the process. During cultivation, the cultures were incubated at 37°C for 24 hours (Nabilla & Advinda, 2022).

2.2.7. Antimicrobial testing

Mueller Hinton Agar (MHA) disc media were prepared to test microbial sensitivity to VCO. The antibacterial activity test was conducted using the paper disc method. The culture results of the test bacteria *E. coli*, *S. aureus*, and *C. albicans* were poured into separate Petri dishes containing MHA media using a 100-microliter micropipette, then swabbed evenly over the entire surface of the MHA. Paper discs were soaked in VCO extraction solutions of various concentrations for 15 minutes. Then, the paper discs were gently placed on the surface of the MHA media containing the test bacteria. The MHA media were then incubated for 3 × 24 hours at 37°C. Next, a paper disc dipped in the appropriate antibiotic solution was placed on each media, on a side opposite the previous disc, as a positive control solution. Other treatments were also performed to observe variations in contact time (Nabilla & Advinda, 2022; Nasir et al., 2018).

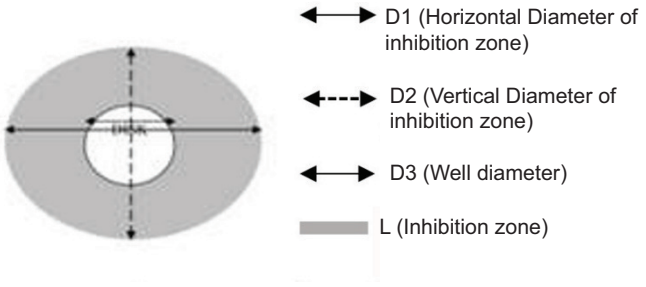
2.3. Calculation of inhibition zones and data analysis

The VCO inhibition zone around the paper discs was observed by measuring the diameter of each microbial sample, then compared with the positive control (antibiotic solution) (Nasir et al., 2018). The area of the inhibition zone formed in each treatment was measured using the formula in Figure 1.

3. RESULTS AND DISCUSSION

3.1. Enzymatic extraction of VCO using bromelain enzyme

The VCO was extracted from the cream of coconut milk. The cream was obtained after the coconut milk was left to stand at room temperature for 2 hours, forming two layers. The cream resides in the top layer, while the skim layer settled at the bottom (Figure 2). This separation phenomenon is related to the fundamental property of coconut milk as an oil-in-water emulsion system, which is physically unstable



$$L = \frac{(D1 - D3) + (D2 - D3)}{2}$$

$$L = \frac{(D1-6)+(D2-6)}{2}$$

Figure 1. Formula for calculating the inhibition zone.

and thus prone to destabilization during storage. Emulsion instability occurs due to the interaction between oil and water molecules, leading to physical changes in coconut milk such as creaming, flocculation, and coalescence (Hamad, 2011). The formation of two layers is influenced by several factors, one of which is the density difference between fat and water components. Coconut consists mainly of triglycerides, which have a lower density than water, causing fat globules to gradually rise to the surface and form the creamy layer, a process known as creaming (Yulinda et al., 2021).

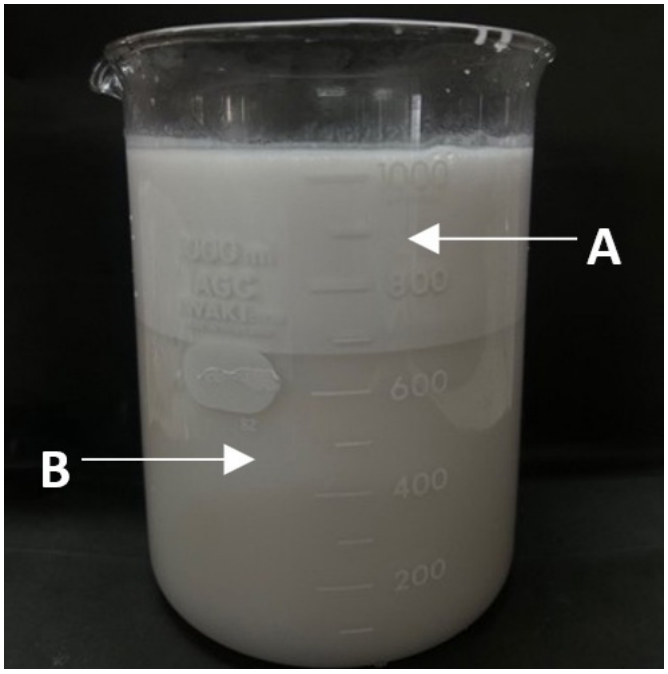


Figure 2. Coconut milk after a 2-hour settling period. A = coconut cream, B = coconut skim.

To extract the VCO, the coconut cream was treated by bromelain at various concentrations from 2.0–8.0 (% w/v), then incubated for 18 hours. The VCO appeared and separated from blondo and the aqueous phase after this incubation. The separation of VCO takes place by the proteolytic action of the enzyme. Bromelain is a sulfhydryl-type protease that is highly effective in cleaving peptide bonds of emulsifying proteins in coconut milk (Harimurti et al., 2024). Emulsifying proteins, which normally stabilize oil droplets in water, are hydrolyzed by bromelain into smaller peptide fragments, resulting in protein coagulation and flocculation. Consequently, emulsion stability is reduced, and oil droplets coalesce and rise to form an oil layer in the middle. Meanwhile, the precipitated solids between the phases form the blondo layer (Rahmalia & Kusumayati, 2021).

The productivity of enzymatic VCO extraction was reflected by an increase in yield with the rising concentration of bromelain. The VCO yield increased significantly along with the increase in bromelain enzyme concentration applied (Figure 3).

In the treatment without enzyme (0% w/v), a VCO yield of 24.27% was obtained, whereas in the treatments with bromelain enzyme at concentrations of 2.0, 4.0, 6.0, and 8.0 (% w/v), the yields obtained were 25.71, 29.43, 31.06, and 32.05 (%), respectively. Based on these yield values, the percentage increase of VCO yield reached 32.05%. This indicates that the addition of bromelain enzyme optimized the separation of oil from the coconut milk emulsion matrix. During the extraction process, the structure of coconut milk underwent modification, primarily through the cleavage of lipoprotein bonds responsible for binding most of the oil fraction. These bonds were actively hydrolyzed by bromelain, enabling the release of oil from the aqueous phase and facilitating its migration to the surface. Increasing the concentration and

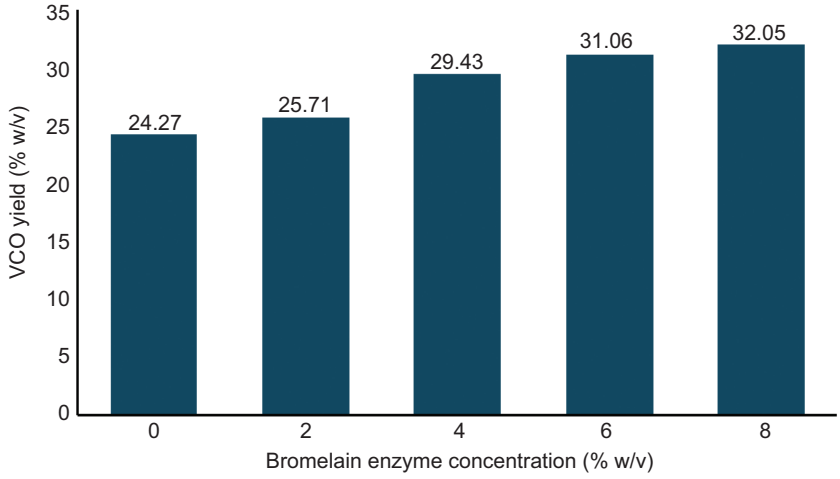


Figure 3. Productivity of VCO based on yield from enzymatic extraction with bromelain.

activity of bromelain accelerated the disintegration of the protein emulsion, thereby enhancing the amount of oil that could be extracted. The higher the concentration of enzyme added, the greater the number of peptide bonds in coconut milk proteins that were hydrolyzed, resulting in a stronger ability of the enzyme to release oil from the protein matrix. Thus, the addition of bromelain facilitated the breakdown of the coconut milk emulsion system through peptide bond hydrolysis, producing amino acids as degradation products (Supriyanto et al., 2023).

3.2. Effect of enzymatic extraction of VCO bromelain by addition of metal compounds

The productivity of enzymatic VCO extraction with the addition of $CaCl_2$ and $MgCl_2$ showed a significant increase in yield up to a certain concentration. A gradual increase in VCO yield was observed with the addition of $CaCl_2$ from 0 mM (without metal) up to 2.0 mM, followed by a decrease at 2.5 mM. The yields obtained at concentrations of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 (mM) were 32.05, 36.49, 37.27, 37.98, 39.89, and 37.76 (%), respectively (Figure 4). Overall, an increase of 24.46% in yield was achieved from the condition without metal addition to the optimum concentration at 2.0 mM.

The addition of metal ions acts as enzyme effectors that modulate the activity and conformational stability of bromelain, as well as influence intermolecular interactions within the emulsion system. $CaCl_2$ and $MgCl_2$ have been reported to play an important role in maintaining the structure of protease enzymes while also contributing to the mechanisms of protein flocculation and coagulation in complex media such as coconut milk.

The activity and stability of this enzyme are strongly influenced by its three-dimensional structure, particularly in the active site region and substrate-binding site. Calcium ions (Ca^{2+}) in $CaCl_2$ can bind to negatively charged amino acid residues such as aspartate or glutamate located around the active site, thereby helping to maintain the secondary and tertiary structural stability of the enzyme. This allows the enzyme to retain its active conformation, enabling the recognition and binding of substrate proteins such as emulsifiers present in coconut milk (Liu et al., 2011). The activity of bromelain increases significantly when Ca^{2+} ions are added in specific amounts, as these ions act as cofactors that stabilize the enzyme structure and strengthen the interaction between the enzyme and the substrate (Razali et al., 2022). However, at excessively high concentrations of Ca^{2+} ions, enzyme activity can decrease. This may be caused by conformational changes that disrupt the active site or by the formation of Ca^{2+} complexes with other compounds such as phosphate or free amino acids, which can hinder substrate access to the enzyme (Kaur et al., 2015).

Meanwhile, the addition of $MgCl_2$ (Mg^{2+}) exhibited a nonlinear pattern. The yield gradually increased with the addition of Mg^{2+} ions from 0 mM (without metal ions) up to 1.5 mM, followed by a decrease at 2.0 mM, and then a slight increase at 2.5 mM. The yields obtained at 0, 0.5, 1.0, 1.5, 2.0, and 2.5 (mM) were 32.05, 35.63, 35.65, 36.58, 36.16 (%), and 36.28%, respectively (Figure 5). Overall, an increase of 14.15 % was achieved from the condition without metal ion addition to the optimum concentration at 1.5 mM.

At the concentration of 2.0 mM, a decrease in yield was observed, which was explained by the ionic oversaturation phenomenon that disrupted the local structure of the active site or interfered with enzyme–substrate interactions.

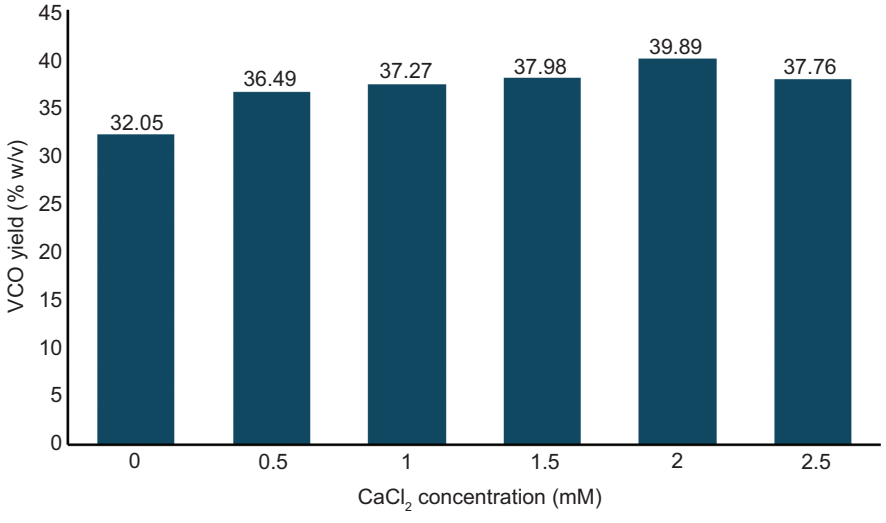


Figure 4. Productivity of VCO based on yield from extraction with bromelain enzyme and Ca^{2+} ($CaCl_2$) ion addition.

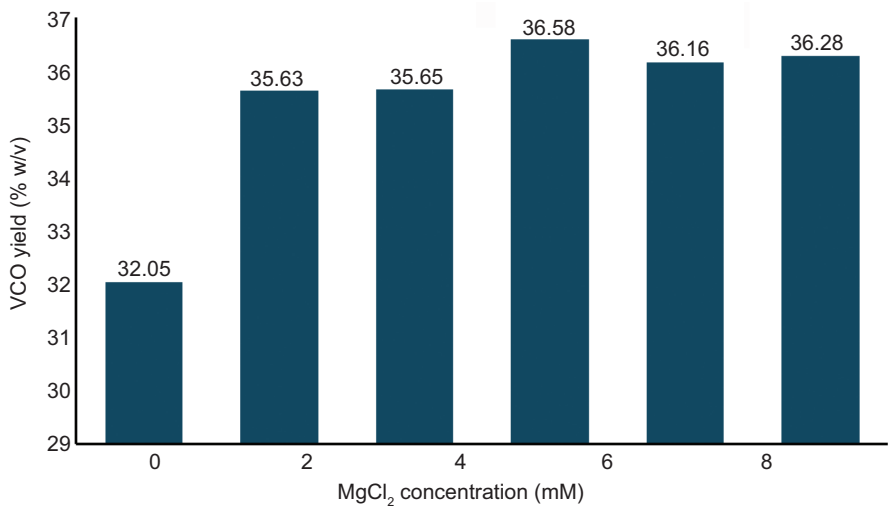


Figure 5. Productivity of VCO based on yield from extraction with bromelain enzyme and Mg^{2+} ($MgCl_2$) ion addition.

The kinetics of bromelain have been reported to be highly sensitive to metal ion modulators, in which ions such as Ca^{2+} and Cu^{2+} have been shown to induce K-type or V-type activation. However, at higher concentrations, competitive or uncompetitive inhibition may also be exhibited (Kaur et al., 2015). At the concentration of 2.5 mM, a slight increase in VCO yield was obtained, indicating that the enzymatic system eventually reached a more stable condition after being previously disturbed by the addition of metal ions. This stabilization was considered to occur through minor conformational adjustments of the enzyme, which enabled partial recovery of its activity. In addition, the flocculation of protein hydrolysis products may have facilitated the separation of oil from water. Such a pattern, in which activity initially increases, decreases at intermediate concentrations, and slightly increases again, has commonly been reported in enzymatic systems influenced by metal ions such as $CaCl_2$ (Kaur et al., 2015).

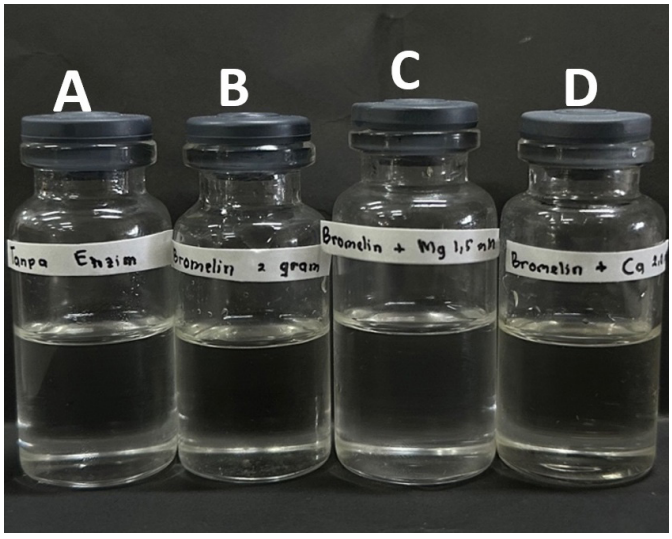


Figure 6. Results of virgin coconut oil (VCO) extraction. A = VCO without enzyme, B =VCO with enzyme, C = VCO with Mg^{2+}, and D = VCO with Ca^{2+}.

3.3. Characterization of VCO

The characterization of VCO was conducted to evaluate the quality of the oil obtained from the extraction process using bromelain and metal ion activators. Quality assessment is essential to ensure that the produced VCO meets established standards, such as the Indonesian National Standard (SNI) 7381:2008, particularly for parameters including moisture content, FFA value, IV, peroxide value, and fatty acid composition.

3.4. Organoleptic result

Organoleptic evaluation was conducted to assess the sensory quality of VCO, particularly in terms of aroma, color, and clarity. This evaluation provided information regarding the success of the extraction process as well as the stability of the product during storage. The results of the organoleptic evaluation of VCO under various treatments are presented in Figure 6. Based on the evaluation, the produced VCO was in accordance with the Indonesian National Standard (SNI), as no deviations in color, odor, or taste from the normal characteristics of VCO were observed.

Based on the extraction results of VCO, the organoleptic evaluation of each sample was carried out and is presented in Table 1. The evaluation included three main parameters, namely, aroma, color, and clarity, and was conducted to determine the extent to which the enzymatic and metal ion

Table 1
Result of organoleptic evaluation.

Without bromelain	Colorless	Fresh coconut aroma, not rancid
Bromelain 8% b/v	Slightly pale yellow	Fresh coconut aroma, not rancid
$CaCl_2$ 2.0 mM	Slightly pale yellow	Fresh coconut aroma, not rancid
$MgCl_2$ 1.5 mM	Colorless	Fresh coconut aroma, not rancid

treatments affected the sensory quality of VCO. The results showed that all VCO samples remained in accordance with the SNI and were classified as good quality.

3.4.1. Moisture content analysis

The analysis of moisture content in this study was conducted to evaluate the quality and storage stability of the produced VCO. A high moisture content in VCO may increase the risk of hydrolysis reactions, leading to the formation of glycerol and FFAs, which accelerate the deterioration of VCO quality. Conversely, a low moisture content tends to make VCO more stable and prolong its shelf life. In addition, the presence of residual proteins from the filtration step also has the potential to accelerate rancidity in VCO (Sari et al., 2024).

The moisture content analysis for VCO without enzyme exhibited the lowest moisture level of 0.1033%, whereas the treatment with bromelain showed a moisture content of 0.1500% (Figure 7). The addition of metal ions under the optimum enzyme condition further increased the moisture level, yielding 0.1866% for bromelain + $CaCl_2$ and 0.1933% for bromelain + $MgCl_2$. Although an increase was observed, the obtained moisture content still complied with the SNI standard, which specifies a maximum moisture level of <0.2%. The low moisture content in the control was attributed to the separation mechanism, which relied solely on gravity or mild heating; therefore, the emulsion structure (the bonds between proteins and lipids) remained relatively intact, causing water molecules to remain strongly bound within the emulsion matrix and not carried into the oil phase (Rahmalia & Kusumayanti, 2021).

In contrast, under enzymatic treatment, bromelain hydrolyzed peptide bonds in the protein layer surrounding the oil globules in the coconut milk emulsion system. The cleavage of these bonds destabilized the emulsion structure, facilitating the separation of oil from water, while also releasing water molecules that were previously entrapped within the protein matrix. The hydrolysis products in the form of peptides and amino acids are amphipathic, possessing both hydrophilic and hydrophobic groups, thereby increasing the solubility of water in oil. This phenomenon led to the retention of some water in the oil phase even after the separation process.

In treatments with $CaCl_2$ and $MgCl_2$, the higher moisture content was attributed to the role of these metal ions as enzyme activators, enhancing the efficiency of protein hydrolysis. Excessive enzymatic activity may also promote the formation of water-in-oil microemulsions, which are dispersions of microsized water droplets that cannot be easily separated by heating (Fadhilah et al., 2018).

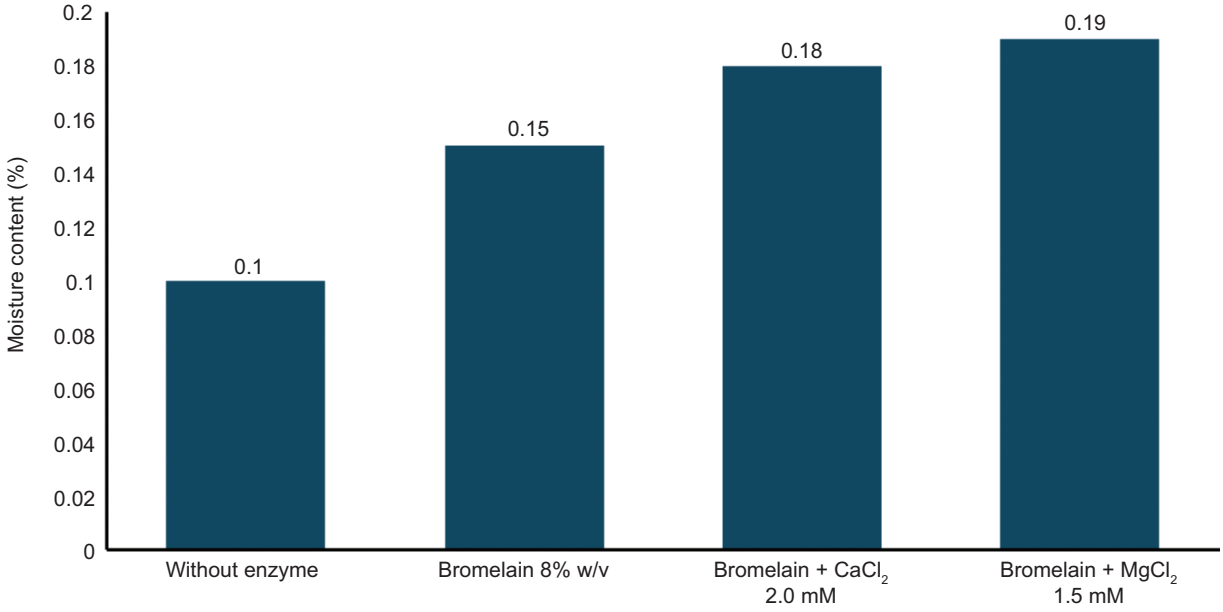


Figure 7. Moisture content of VCO obtained from extraction using bromelain and the addition of $CaCl_2$ and $MgCl_2$.

3.4.2. FFA Analysis

The determination of FFA content was carried out to evaluate the quality of the extracted VCO. A high FFA content is considered an indicator of triglyceride hydrolysis into FFAs and glycerol, which potentially reduces the oil quality. According to the SNI 01-7381-2008 standard, the FFA content in coconut oil should not exceed 0.2%. The measurement of FFA levels in VCO was performed using the acid–base titration method. This method was intended to determine the concentration of acidic compounds (in this case, FFAs) by reacting them with a standardized alkaline solution, namely, sodium hydroxide (NaOH).

FFA analysis in the control without enzyme was not measured due to the very small amount of oil obtained. The bromelain treatment produced the highest FFA content of 0.26%, followed by bromelain + $MgCl_2$ (0.24%) and bromelain + $CaCl_2$ (0.23%) (Figure 8). All values remained within the quality standard range for VCO as recommended by SNI for high-quality oil (≤0.2%), although the values obtained from enzymatic treatments slightly exceeded this limit. The increase in FFA content with the addition of bromelain was supported by the findings of Harimurti et al. (2024), who demonstrated that bromelain is capable of accelerating the degradation of triglycerides in coconut oil. Their study further emphasized that the use of enzymes in VCO production accelerates the hydrolysis reaction, thereby directly increasing the FFA level, provided that temperature and time parameters are well controlled. This confirms that the increase in FFA should not be interpreted as an absolute indicator of quality deterioration but rather as evidence of a controlled hydrolysis process.

The observed increase in FFA content can also be attributed to enzymatic activation by divalent metal ions. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions act as natural cofactors that enhance the structural stability of proteolytic enzymes such as bromelain and strengthen the affinity between the enzyme and its substrate. These cations improve catalytic efficiency by stabilizing binding at the enzyme's active site, thereby accelerating the hydrolysis of triglyceride esters and generating FFAs (Maskey et al., 2025).

3.4.2. IV analysis

The determination of IV in VCO was conducted to measure the degree of unsaturation of the fatty acids contained in the oil. This parameter is essential because the IV reflects the number of double bonds present in the fatty acid molecules. A higher IV indicates a higher proportion of unsaturated fatty acids in the oil sample (Samanta et al., 2023). The measurement of the IV was carried out using the standard iodometric titration method, known as the Wijs method, which is based on the addition reaction of iodine (I_2) with C=C

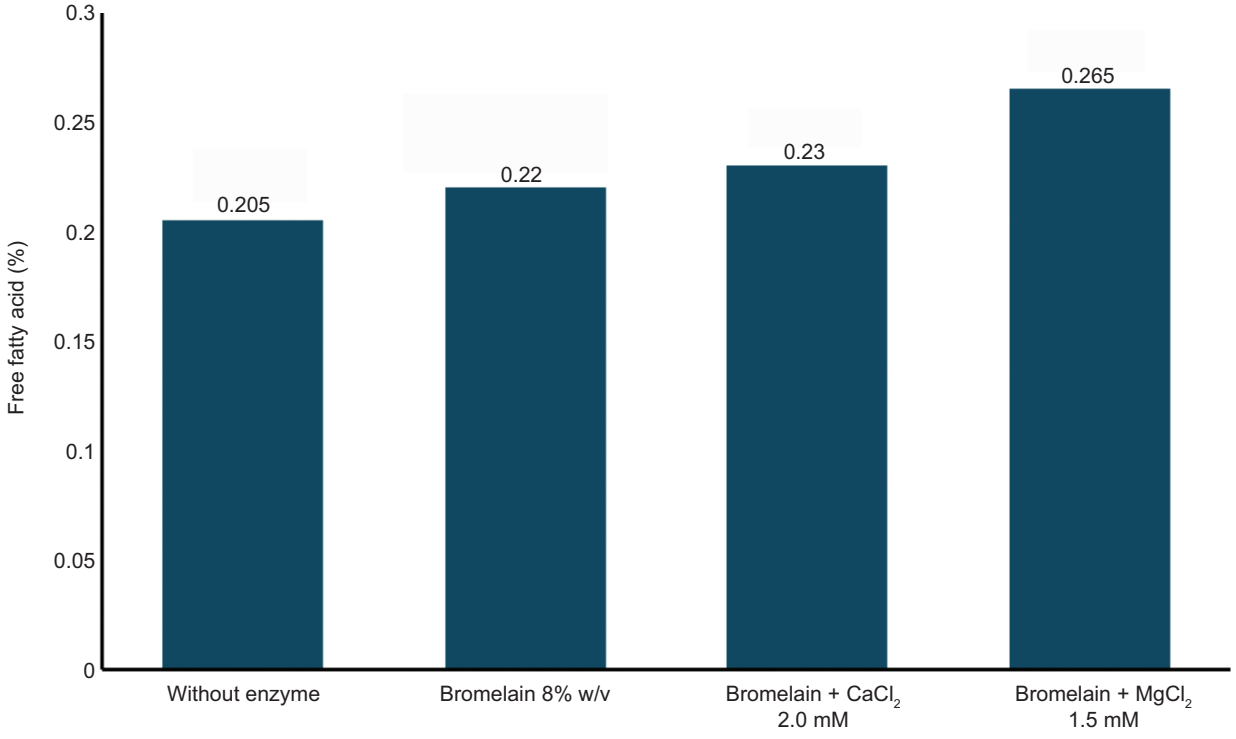


Figure 8. Free fatty acid (FFA) content of VCO obtained from extraction using bromelain with the addition of $CaCl_2$ and $MgCl_2$.

double bonds present in the unsaturated fatty acid chains (Peamaroon et al., 2021).

The addition of bromelain was observed to increase the IV from 8.66 (without enzyme) to 9.22 (g I_2/100 g) (Figure 9). This increase indicates that bromelain activity facilitated the release of unsaturated fatty acid fractions from the triglyceride structure in VCO. These findings are consistent with the study by Harimurti et al. (2024) entitled *Bromelain-Extracted Virgin Coconut Oil: Physical and Chemical Stability in Different Temperature During Storage,* which reported that the application of bromelain from *Ananas comosus* in VCO extraction enhanced the content of bioactive compounds and lipid unsaturation due to the breakdown of structural components during the enzymatic fermentation process.

Based on Figure 9, the addition of $CaCl_2$ (Ca^{2+}) at a concentration of 2 mM together with bromelain increased the IV to 10.50 g I_2/100 g, which represents the highest level among all treatments. This increase indicates a synergistic effect between Ca^{2+} ions and bromelain, while still remaining within the quality standards defined by SNI. Calcium ions in $CaCl_2$ are known to stabilize the three-dimensional structure of enzymes by strengthening electrostatic interactions at their active sites, thereby enhancing catalytic efficiency (Hardi et al., 2023). In contrast, the addition of $MgCl_2$ at a concentration of 1.5 mM also raised the IV to 9.17 g I_2/100 g, but the effect was less pronounced compared to $CaCl_2$. This difference can be attributed to the distinct stabilizing abilities of the two divalent ions on the enzyme structure. This finding is consistent with the study of Harimurti et al. (2024) in BIO Web of Conferences, which reported that although both Ca^{2+} and Mg^{2+} act as enzymatic cofactors, the binding affinity of Ca^{2+} to amino acid residues responsible for maintaining the structural conformation of bromelain is generally stronger than that of Mg^{2+}.

3.4.3. Peroxide value (PV) analysis

PV is a chemical parameter used to evaluate the early stage of oil deterioration, including VCO, by measuring the formation of peroxide compounds resulting from the oxidation of unsaturated fatty acids. This oxidation process can be triggered by exposure to air, light, or elevated temperatures. Peroxide formation occurs during the initial stage of oxidation (initiation), where external energy such as heat or light induces the release of a hydrogen atom from a methylene group ($–CH_2–$) located adjacent to a double bond. The loss of this hydrogen atom generates highly reactive free radicals, which subsequently react with nearby oxygen molecules to form peroxy radicals (active peroxides) (Nivya et al., 2023).

The addition of bromelain was observed to significantly increase the peroxide value compared to the sample without enzyme. The peroxide value rose from 1.2143 mg equiv/kg to 2.4278 mg equiv/kg following the addition of the

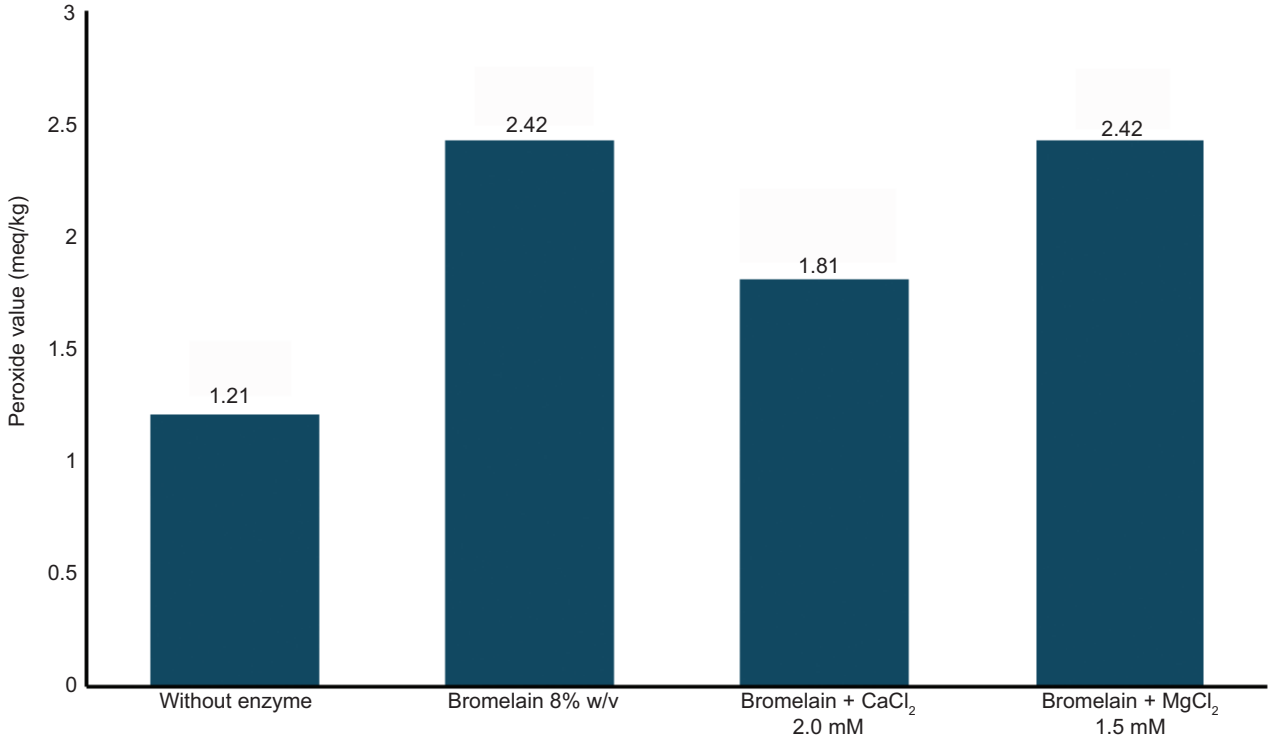


Figure 9. Iodine value (IV) of VCO obtained from enzymatic extraction using bromelain with the addition of $CaCl_2$ and $MgCl_2$.

optimum bromelain, indicating that the proteolytic activity of the enzyme accelerated the initial oxidation of fatty acids in VCO. Interestingly, significant changes were observed when metal ions such as $CaCl_2$ and $MgCl_2$ were added to the enzymatic system. The addition of $CaCl_2$ at a concentration of 2.0 mM resulted in a peroxide value of 2.4278 mg equiv/kg in the first titration, followed by 1.2114 mg equiv/kg in the second titration (Figure 10). This suggests that the presence of calcium ions may inhibit the oxidative reaction or alter the activity of bromelain, thereby reducing the formation of peroxide products. Bromelain possesses calcium-binding sites that contribute to enzymatic conformational stabilization and protection against denaturation under reactive conditions (Mala & Anal, 2021).

In contrast, the addition of $MgCl_2$ at a concentration of 1.5 mM did not exhibit a significant inhibitory effect on the peroxide value, which remained at 2.4278 mg equiv/kg, identical to the sample treated with bromelain alone. This indicates that $MgCl_2$ does not play a significant role in modulating bromelain activity during the initial oxidation of VCO. This observation is supported by literature reporting that Mg^{2+} is less effective than Ca^{2+} in stabilizing proteolytic enzymes due to differences in ionic radius and binding affinity to enzyme carboxylate groups (Liu et al., 2025).

3.4.4. Analysis of fatty acid composition

The analysis of fatty acid composition was conducted using a gas chromatography–mass spectrometry (GC–MS) instrument. GC–MS offers high sensitivity and selectivity for detecting volatile compounds such as fatty acid methyl esters (FAMEs), which are produced through the derivatization of fatty acids (Gangtian et al., 2019). FFAs in the oil are converted into their methyl ester forms to increase volatility and prevent degradation at the high temperatures of the chromatographic column. The fatty acid composition of VCO is influenced by carbon chain length, which is closely related to their boiling points. In general, fatty acids with shorter carbon chains have lower boiling points, making them more volatile or prone to loss during processing, whereas fatty acids with longer carbon chains have higher boiling points, rendering them more stable and retained within the oil.

As shown in Table 2, in sample A (VCO without bromelain), the lauric acid content was recorded at 37.29%, which was the lowest among all treatments. This low content is attributed to the fact that oil separation relied solely on gravity or mild heating, without protein hydrolysis by the enzyme, leaving triglycerides containing lauric acid largely trapped within the protein–lipid matrix. The use of bromelain in sample B increased the lauric acid content to 45.24%.

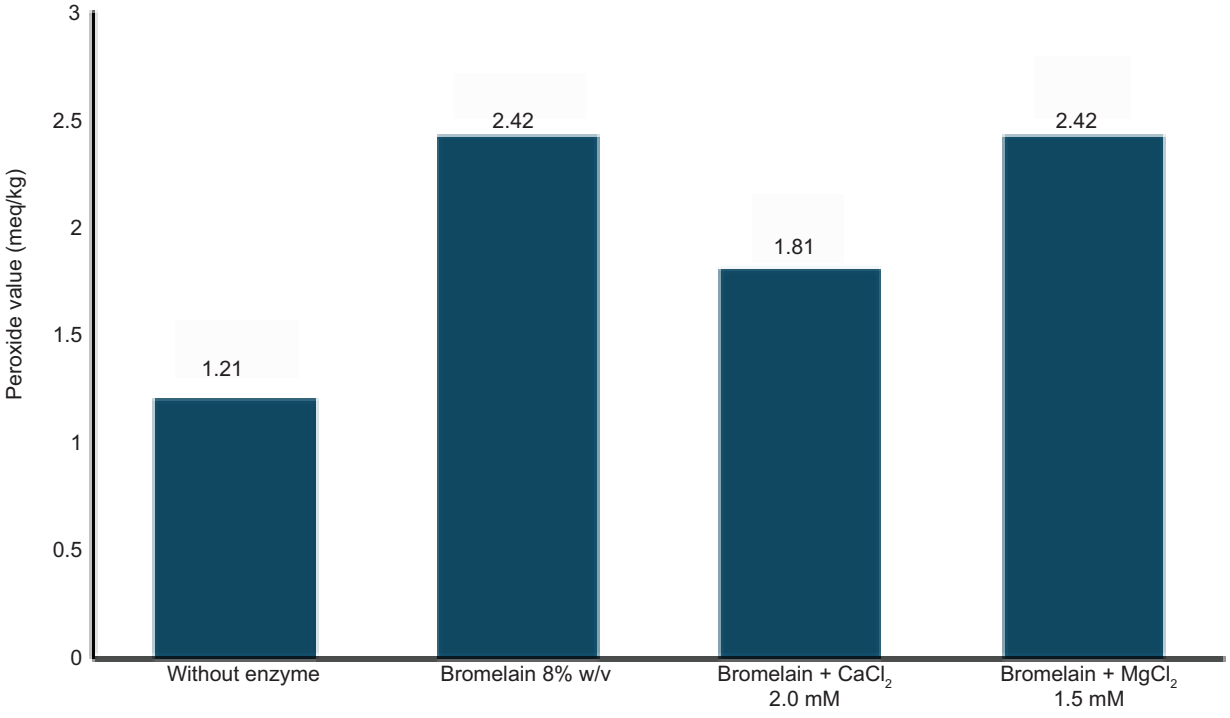


Figure 10. Peroxide value (PV) of VCO obtained from enzymatic extraction using bromelain with the addition of $CaCl_2$ and $MgCl_2$.

Table 2
Fatty acid composition analysis of VCO .

Fatty acid	Relative area (%)				SNI (%)
	A	B	C	D	
Saturated fatty acids					
Caproic acid	0.69	0.53	–	0.44	0–0.7
Caprylic acid	8.62	5.89	6.61	7.15	4.6–10.0
Capric acid	8.15	6.58	6.02	6.09	5.0–8.0
Lauric acid	37.29	45.24	45.31	46.3	45.1– 53.2
Myristic acid	18.40	18.86	19.21	16.5	16.8–21
Palmitic acid	8.25	7.9	8.21	9.05	7.5–10.2
Stearic acid	4.30	3.74	3.01	3.96	2.0–4.0
Unsaturated fatty acids					
Linoleic acid	1.86	1.83	1.77	1.79	1.0–2.5
Oleic acid	8.38	8.30	9.58	8.05	5.0–10.0

Annotation: A = VCO without bromelain, B = VCO with bromelain, C = VCO with bromelain + $CaCl_2$, and D = CO with bromelain + $MgCl_2$.

This increase indicates that bromelain effectively hydrolyzed the peptide bonds in the emulsifying proteins of coconut milk, thereby disrupting the binding layer between water and oil and facilitating the release of lauric acid–rich triglycerides into the oil phase. Proteolytic enzymes can accelerate the liberation of medium-chain fatty acids from emulsion systems (Peamaroon et al. 2021).

In sample C, the addition of $CaCl_2$ together with bromelain resulted in the highest lauric acid content at 45.31%. This is attributed to the role of Ca^{2+} ions as activators, which stabilize the enzyme conformation and reduce the activation energy of the proteolytic reaction, thereby allowing protein hydrolysis to proceed more rapidly and thoroughly (Fadhilah et al., 2018).

Meanwhile, in sample D with the addition of $MgCl_2$, the lauric acid content was 46.3%. Although Mg^{2+} also acts as an activator to enhance lauric acid release, its ability to stabilize the enzyme conformation is not as strong as that of Ca^{2+} (Nasyanka et al., 2024). This difference explains why the increase observed in the $MgCl_2$ treatment is slightly lower compared to $CaCl_2$. Bromelain functions by cleaving the ester bonds of triglycerides into FFAs and their methyl esters, while Mg^{2+} from $MgCl_2$ can interact with the enzyme's active site or the substrate, thereby influencing hydrolysis efficiency. In addition, Mg^{2+} ions may stabilize the enzyme structure and enhance the solubility of certain lipid components, which in turn affects the fatty acid profile detected.

This distribution pattern indicates that thermal stability plays a crucial role in determining the final fatty acid composition of VCO. Fatty acids with high boiling points, particularly lauric acid, remain predominant even after enzymatic extraction, whereas fatty acids with low boiling points tend to decrease due to their volatility during processing. The efficiency of medium- and long-chain fatty acid release is enhanced in the presence of bromelain and metal ion activators, which disrupt protein–lipid bonds and facilitate the release of triglycerides into the oil phase more effectively (Fadhilah et al., 2018).

3.5. Antimicrobial activity of VCO

Antimicrobial activity tests against three types of microorganisms were conducted to evaluate the effectiveness of VCO extracted using the heating and enzymatic bromelain methods. The tests were conducted on *S. aureus* and *E. coli*, gram-positive and gram-negative bacteria, respectively, and *C. albicans*, a representative of pathogenic fungi.

The antimicrobial test results against *S. aureus* showed that VCO extracted using the bromelain and heating methods both exhibited inhibitory activity with varying strengths. VCO produced with the bromelain enzyme produced higher inhibition zones against *S. aureus, E. coli*, and *C. albicans* compared to VCO extracted with heating. Against these three microbes, the enzymatically extracted VCO produced inhibition zones of 6.98, 4.58, and 3.37 mm, while VCO extracted with heating produced inhibition zones of 5.19, 3.61, and 3.01 mm (Figure 11). The test was also controlled by using chloramphenicol antibiotics for *S. aureus* and *E. coli*; then fluconazole antibiotics for *C. albicans*. The test results with chloramphenicol for *S. aureus* and *E. coli* showed inhibition zones of 14.01 and 17.98 mm, respectively, while fluconazole antibiotics for *C. albicans* gave an inhibition zone of 5.18 mm (Figure 11). The antimicrobial activity of enzymatic VCO is still better than that of extraction by heating, but it was still below the antibiotic activity of chloramphenicol and fluconazole. These results indicate that enzymatic VCO possesses higher antimicrobial potential, particularly against *S. aureus*, *E. coli*, and *C. albicans*, although both were still less potent than synthetic antibiotics.

4. CONCLUSION

This study demonstrates that enzymatic extraction of VCO using bromelain reached an optimum concentration of 8% (w/v), yielding 32.05% (w/v). The addition of metal ion effectors enhanced the yield, with $CaCl_2$ at an optimum of 2.0 mM producing 39.89% (w/v) and $MgCl_2$ at 1.5 mM producing 36.59% (w/v). These results indicate that the addition of $CaCl_2$ and $MgCl_2$ effectors effectively enhanced bromelain activity,

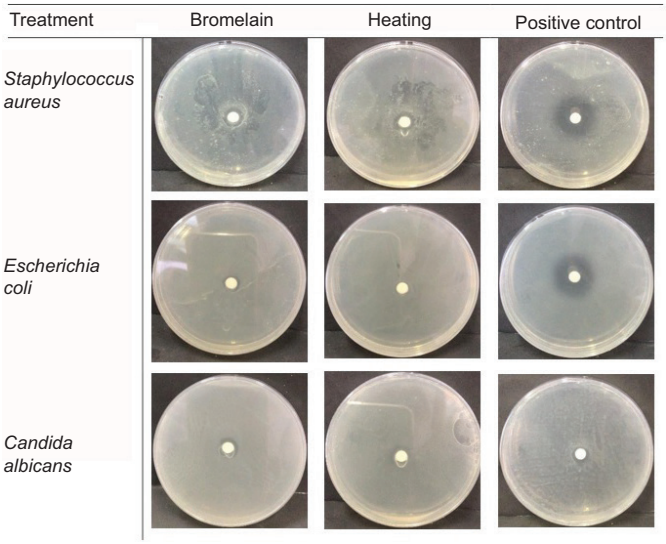


Figure 11. Inhibition zone results of the antimicrobial assay.

resulting in high-quality VCO. Quality parameters of the resulting VCO, including moisture content, FFAs, peroxide value, and IV, varied among treatments but remained within SNI standards. GC–MS analysis of fatty acid composition revealed lauric acid as the predominant component in each VCO product, which are at 45–46%. Enzymatic VCO exhibited greater inhibitory activity against *S. aureus*, *E. coli*, and *C. albicans*, indicating its higher potential as a natural antimicrobial agent.

AUTHOR CONTRIBUTIONS

Purkan Purkan did research concept and design, collection and/or assembly of data, data analysis and interpretation, and writing the article; Nabila Rahma Octavia was responsible for collection and/or assembly of data, data analysis and interpretation; Lailatul Hidayah looked into collection and/or assembly of data; Agus Supriyanto did data analysis and interpretation, critical revision of the article; R Merlyn Sujatha was responsible for writing the article, critical revision of the article; Sri Sumarsih was concerned with writing the article and final approval of the article; Bonifasius Adhika Dani Setyawan was responsible for collection and/or assembly of data; Sofijan Hadi was concerned with research concept and design, data analysis and interpretation, writing the article, and final approval of the article.

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CONFLICTS OF INTEREST

The authors confirm that they have no competing interests.

DATA AVAILABILITY

Data supporting the findings of this study are available within the article.

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ORCID

Purkan Purkan 0000-0002-3784-7008
Nabila Rahma Octavia 0009-0004-4020-1322
Lailatul Hidayah 0009-0005-2384-813X
Agus Supriyanto 0009-0003-2450-078X
R Merlyn Sujatha 0009-0007-0944-3077
Sri Sumarsih 0000-0001-9254-6008
Bonifasius Adhika Dani Setyawan 0009-0006-6125-3409
Sofijan Hadi 0000-0002-0653-8667

REFERENCES

Abbas, A.A., Assikong, E.B., Akeh, M., Upla, P., & Tuluma, T. 2017. Antimicrobial activity of coconut oil and its derivative (lauric acid) on some selected clinical isolates. International Journal of Medical Science and Clinical Invention. 4(8), 3173–3177. https://doi.org/10.18535/ijmsci/v4i8.12

Colletti, A., Li, S., Marengo, M., Adinolfi, S., & Cravotto, G. 2021. Recent advances and insights into bromelain processing, pharmacokinetics and therapeutic uses. Applied Sciences. 11(18), 8428. https://doi.org/10.3390/app11188428

Fadhilah, Y., Shoobihah, A., Setiasih, S., Handayani, S., & Hudiyono S. 2018. The effect of Ca^{2+}, Mg^{2+} ions, cysteine, and benzoic acid on the activity of purified bromelain from pineapple core extract (Ananas comosus [L]. Merr). AIP Conference Proceedings. 2049(1), 020029. https://doi.org/10.1063/1.5082434

Gangtian, Z., Fei, L., Peiyang, L., Sheng, H., Shukui, Z., Qiang, G., Yuqi, F. 2019. profiling of fatty acids in vegetable oils using gas

chromatography–mass spectrometry. Food Chemistry. 1(297), 124998. https://doi.org/10.1016/j.foodchem.2019.124998

Hadi, S., Purkan, P., Sumarsih, S., Silalahi, R.R.E., Ifada, C., & Panjaitan, T.M. 2023. Discovering of pyrazinamide resistance in local strain of mycobacterium tuberculosis clinical isolates by molecular detection of pncA gene encoding PZase. Rasayan Journal of Chemistry. 16(1), 355–360. http://doi.org/10.31788/RJC.2023.1618198

Hamad, M. 2011. Analytical solution of natural convection flow of a nanofluid over a linearly stretching sheet in the presence of magnetic field. International Communications in Heat and Mass Transfer. 38, 487–492. https://doi.org/10.1016/j.icheatmasstransfer.2010.12.042

Hardi, J., Buheli, R.A.P., & Ridhay, A. 2023. Repeated use of bromelain immobilized on alginate membrane for virgin coconut oil production. AIP Conference Proceedings. 2719(1), 030028). https://doi.org/10.1063/5.0133259

Harimurti, S., Prayogo, G.K., Widada, H., Orbayinah, S., Sukamdi, D.P., Erviana, R., & Tho, D.C.M.V. 2024. Degradation study of bromelain-extracted virgin coconut oil based on peroxide and fatty acid formation under the elevated temperatures. In BIO Web of Conferences (Vol. 144, p. 05003). EDP Sciences. https://doi.org/10.1051/bioconf/202414405003

Imelda, A.A., Jacus, S., Nacis., Fabian, M., Dayrit., Keith, V.T. 2024. Virgin coconut oil (VCO) supplementation relieves symptoms and inflammation among COVID-19 positive adults: A single-blind randomized trial. Journal of Nutritional Science. 13(eA5), 1–9. https://doi.org/10.1017/jns.2023.118

Kaur, T., Kaur, A., & Grewal, R.K. 2015. Kinetics studies with fruit bromelain (Ananas comosus) in the presence of cysteine and divalent ions. Journal of Food Science and Technology. 52(9), 5954–5960. https://doi.org/10.1007/s13197-014-1639-5

Kusuma, M.A., and Putri, N.A. 2020. Asam lemak virgin coconut oil (VCO) dan manfaatnya untuk kesehatan. *Jurnal Agrinika.* 4(1), 93–107. https://doi.org/10.30737/agrinika.v4i1.1128

Liu, S.Q., Tao, Y., Meng, Z.H., Fu, Y.X., & Zhang, K.Q. 2011. The effect of calciums on molecular motions of proteinase K. Journal of Molecular Modeling. 17(2), 289–300. https://doi.org/10.1007/s00894-010-0724-6

Liu, X., Mao, S., Yuan, Y., Wang, Z., Tian, Y., Tao, L., & Dai, J. 2025. Antin-diabetic cognitive dysfunction effects and underpinning mechanisms of phytogenic bioactive peptides: A review. Frontiers in Nutrition. 11, 1517087. https://doi.org/10.3389/fnut.2024.1517087

Mala, T., & Anal, A.K. 2021. Protection and controlled gastrointestinal release of bromelain by encapsulating in pectin-resistant starch based hydrogel beads. Frontiers in Bioengineering and Biotechnology. 9, 757176. https://doi.org/10.3389/fbioe.2021.757176

Maskey, B., Shrestha, S., Shrestha, A., Sangroula, P., Karki, D.B., & Shrestha, N.K. 2025. Single step three phase partitioning for the purification of bromelain enzyme from pineapple (*Ananas comosus*) fruit. *Journal of Food Measurement and Characterization*. 9, 1–11. https://doi.org/10.1007/s11694-025-03379-3

Mela, E., & Bintang, S.D. 2021. Virgin coconut oil (VCO): Pembuatan, keunggulan, pemasaran dan potensi pemanfaatan pada berbagai produk pangan. Jurnal Penelitian dan Pengembangan Pertanian. 40(2), 103–110. https://doi.org/10.21082/jp3.v40n2.2021.p103-110

Mohan, R., Sivakuma, V., Rangasamy, T., & Muralidharan, C. 2016. Optimisation of bromelain enzyme extraction from pineapple (Ananas comosus) and application in process industry. American Journal of Biochemistry and Biotechnology. 12(3), 188–195. https://doi.org/10.3844/ajbbsp.2016.188.195

Nabilla, A.N., & Advinda, L. 2022. Antimicrobic activities of solid soap against *Staphylococcus aureus* dan *Escherichia coli* human pathogen bacteria. *Jurnal Serambi Biologi*. 7(4), 306–310.

Nasyanka, A.L., Na'imah, J., Firmani, U., Octavia, P., & Azizah, V.A. 2024. Characteristics of collagen-based milkfish bone waste extracted with bromelain with cofactor Ca^{2+}. *Jurnal Kesehatan Indonesia*. 14(1), 23–31. https://doi.org/10.22435/jki.v14i1.6625

Nasir, N.A.M.M., Abllah, Z., Jalaludin, A.A., Shahdan, I.A., & Abd Manan, W.N.H.W. 2018. Virgin coconut oil and its antimicrobial properties against pathogenic microorganisms: A review. In International dental conference of sumatera utara 2017 (IDCSU 2017), pp. 192–199. Atlantis Press. https://doi.org/10.2991/idcsu-17.2018.51

Nivya, E.M., Panjikkaran, S.T., Aneena, E.R., Sharon, C.L., Gopal, K.S., Berin, Pathrose D.S.K., & Rajeesha, C.R. 2023. Quality evaluation of virgin coconut oil extracted from different processing methods. The Pharma Innovation. 12(1), 44–48. https://doi.org/10.22271/tpi.2023.v12.i1a.18106

Peamaroon, N., Jakmunee, J., & Moonrungsee, N. 2021. A simple colorimetric Procedure for the determination of iodine value of vegetable oils using a smartphone camera. Journal of Analysis and Testing. 5(4), 379–386. https://doi.org/10.1007/s41664-021-00168-x

Rahmalia I., & Kusumayanti, H. 2021. The optimization of addition of bromelain enzyme catalyst on the fermentation of coconut milk to VCO (virgin coconut oil) using tempeh yeast. Journal of Vocational Studies on Applied Research. 3(2), 31–37. https://doi.org/10.14710/jvsar.v3i2.11949

Razali R., Fikran A.F., Vijay K.S., Kazufumi T., & Cahyo B. 2022. Heterologous expression and catalytic properties of codon-optimized small-sized bromelain from MD2 pineapple. Molecules. 27(19), 6634. https://doi.org/10.3390/molecules27196634

Samanta, A. Kataria, N., Dobhal, K., Joshi, N.C., Singh, M.P., & Verma, S. (2023). Wijs, potassium iodate, and AOCS official method to determine the iodine value (IV) of fat and oil. *Biomedical and Pharmacology Journal.* 16(2), 1201–1210. https://doi.org/10.13005/bpj/2700

Sari, N.K.D.M.I., Wibawa, A.A.C., Pramitha, D.A.I., & Megawati, F. 2024. Perbandingan mutu fisikokimia virgin coconut oil (VCO) dengan metode enzimatis dan fermentasi alami. Usadha. 3(3), 15–20. https://doi.org/10.36733/usadha.v3i3.7498

Setditjen Farmalkes, R.I. 2024. Resistensi Antimikroba Jadi Ancaman Nyata Kesehatan Global. https://farmalkes.kemkes.go.id/2024/11/resistensi-antimikroba-jadi-ancaman-nyata-kesehatan-global/

Supriyanto, S. 2023. Effect of bromelin enzyme concentration on coconut oil quality (Cocos nucifera). Agroindustrial Technology Journal. 7(2). https://doi.org/10.21111/atj.v7i2.9609

Trisnawati, I. 2021. VCO as adjuvant therapy for Covid-19 in Indonesia. https://ugm.ac.id/en/news/

Varilla, C., Marcone, M., Paiva, L., & Baptista, J. 2021. Bromelain, a group of pineapple proteolytic complex enzymes (Ananas comosus) and their possible therapeutic and clinical effects. A summary. Foods. 10(10), 2249. https://doi.org/10.3390/foods10102249

WHO (World Health Organization). 2025. *WHO global research agenda on AMR: Supporting Indonesia's fight against drug resistance. WHO.* https://www.who.int/indonesia/news/detail/21-03-2025-who-global-research-agenda-on-amr--supporting-indonesia-s-fight-against-drug-resistance

Yulindha, Y., Legowo, A.M., & Nurwantoro, N. 2021. Karakteristik fisik santan kelapa dengan penambahan emulsifier biji ketapang. Jurnal Pangan dan Gizi. 11(1), 1–14.