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Original Research Paper

# Comparison of Antibacterial Activity and Physicochemical Characteristics of Vco (Virgin Coconut Oil) With Different Manufacturing Methods

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**Abstract**

Cocos nucifera is an important plantation commodity in Indonesia with high economic value and considerable potential for the development of value-added products, including Virgin Coconut Oil. This study aimed to compare the physicochemical characteristics and antibacterial activity of VCO produced using heating, fermentation, and enzymatic methods. The research was conducted experimentally from June to August 2024 at the Advanced Biology Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram. VCO samples were evaluated through organoleptic tests and physicochemical analyses, including yield, moisture content, peroxide value, and free fatty acid content. Antibacterial activity was tested against *Staphylococcus aureus* and *Escherichia coli* using the disk diffusion method. The results showed that all VCO samples generally met Indonesian National Standard quality requirements, except for free fatty acid content in some treatments. The fermentation method produced the highest yield (31.88%), while the heating method resulted in the best overall quality, characterized by 0% moisture content, 0 mg Eq/kg peroxide value, and the highest organoleptic acceptance. All VCO samples exhibited antibacterial activity, although the inhibition zones were categorized as weak. The findings indicate that the heating method is the most suitable for producing high-quality VCO with antibacterial potential.

**Keywords:** Antibacterial activity, Physicochemical properties, Virgin Coconut Oil

## INTRODUCTION

Indonesia is a country endowed with abundant natural resources, particularly in the agricultural and plantation sectors (Omar Al et al., 2026). One of the strategic commodities in the plantation subsector is coconut (Cocos nucifera L.), which has high economic value (Agus et al., 2025) and is widely distributed across various regions. Based on data from the Directorate General of Estates in 2022, coconut productivity in Indonesia reached 11.29 quintals/ha, with a total plantation area of 3,342,113 ha, making it the third-largest commodity after oil palm and rubber (Najib & Assistant, 2022). The abundant availability of raw materials provides significant opportunities to develop value-added coconut-based products (Divate et al., 2026). Therefore, optimizing coconut utilization is essential to support local resource-based economic development and improve community welfare.

Coconut is widely known as the “tree of life” because almost all parts of the plant can be utilized for various human needs (Salum et al., 2020). One of the main uses of the coconut fruit is its processing into Virgin Coconut Oil (VCO) (Srivastava et al., 2018). VCO is produced without high heat or chemical processes, resulting in better physicochemical characteristics compared to conventional coconut oil (Mohammed et al., 2021). It is typically clear, has a distinctive coconut aroma, contains low moisture (Nasution et al., 2019) and free fatty acid levels, and has a longer shelf life (GUPTA et al., 2010). In addition, VCO contains bioactive compounds such as medium-chain fatty acids (MCFA), particularly lauric acid, which are known to possess biological activities, including antibacterial properties (Nasir et al., 2018). These characteristics make VCO not only economically valuable but also highly promising for health-related applications.

However, the quality and antibacterial activity of VCO are strongly influenced by the processing methods used (Widianingrum et al., 2019). Various methods, such as fermentation, heating, enzymatic treatment, and centrifugation, can produce different physicochemical characteristics (Yao et al., 2024). Previous studies have generally focused on a single processing method or have examined physicochemical properties without comprehensively linking them to antibacterial activity (Duman et al., 2024). Moreover, studies that simultaneously compare multiple processing methods across two main aspects physicochemical properties and antibacterial activity against both Gram-positive and Gram-negative bacteria remain limited (Neagu et al., 2024). The novelty of this study lies in its comparative approach, which integrates the analysis of the physicochemical characteristics and antibacterial activity of VCO produced using different processing methods within a single comprehensive framework. This approach is

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expected to provide a more holistic understanding of how processing methods influence both the quality and biological potential of VCO.

Given the above background, this study is important for obtaining more comprehensive scientific information on VCO quality. This study aims to compare the physicochemical characteristics of VCO produced using different processing methods. In addition, it also aims to analyze differences in the antibacterial activity of VCO against selected bacterial strains. The findings of this study are expected to provide a scientific basis for determining the optimal VCO processing method in terms of both quality and antibacterial effectiveness, thereby contributing to the development of high-value-added coconut-based products.

## RESEARCH METHODS

### Time and place

This research was conducted from June to August 2024 at the Advanced Biology Laboratory, Microbial Technology Room, Faculty of Mathematics and Natural Sciences, University of Mataram.

### Research design

This study employed an experimental research design. Experimental research is a method used to determine the effect of specific treatments on other variables under controlled conditions (Mutz & Pemantle, 2015). In this study, the treatments consisted of different methods of Virgin Coconut Oil (VCO) production, namely heating, fermentation, and enzymatic methods (Ramesh et al., 2020). All variables were measured using standardized procedures and instruments to ensure the validity and reliability of the data obtained.

### Population and research sample

The population in this study comprised all Virgin Coconut Oil (VCO) products derived from coconut milk processing. The samples consisted of VCO produced by three processing methods: heating, fermentation, and enzymatic, each using 800 mL of coconut cream as the initial material. A purposive sampling technique was applied, in which samples were selected to meet the research objective of comparing VCO quality across different processing methods. The independent variable in this study was the method of VCO production. In contrast, the dependent variables included physicochemical characteristics (yield, moisture content, peroxide value, and free fatty acids), organoleptic properties (color, aroma, and taste), and antibacterial activity against Staphylococcus aureus and Escherichia coli (Ailli et al., 2024). Data were collected through laboratory experiments using equipment such as an oven, analytical balance, incubator, autoclave, Laminar Air Flow (LAF), micropipettes, and glassware. The materials used included coconut meat, distilled water, bacterial starters (Streptococcus thermophilus and Lactobacillus bulgaricus), papain enzyme, Mueller-Hinton Agar (MHA), chemical reagents ($Na_2S_2O_3$, KOH, KI, ethanol), and bacterial cultures.

### Research procedure

#### *VCO Production*

Fresh coconut meat was grated and weighed (4 kg), then mixed with 8 liters of water and squeezed to obtain coconut milk. The coconut milk was left undisturbed for approximately 2.5 hours to separate into two layers: cream and water. The cream (2400 mL) was separated and divided into three equal portions (800 mL each) for the following treatments:

#### *Heating Method*

The cream was heated at a temperature below 90°C until oil and residue (blondo) formed. The oil was then filtered to obtain VCO (Djalil et al., 2019)

#### *Fermentation Method*

The cream was added with 2.5% bacterial starter (Streptococcus thermophilus and Lactobacillus bulgaricus) and incubated at 37°C for 24 hours. After incubation, three layers were formed, and the oil was separated and filtered (Kozłowska et al., 2022).

#### *Enzymatic Method*

The cream was mixed with 0.15% papain enzyme and homogenized, then left for 24 hours until oil separation occurred. The oil was then filtered to obtain VCO (Yuniarti et al., 2021).

#### *Organoleptic Test*

Organoleptic evaluation was conducted using a hedonic test involving 20 untrained panelists to assess color, aroma, and taste. The selected panelists met specific criteria, including being aged 18–50 years, having no sensory impairments, no allergies to coconut, and not consuming medications affecting sensory perception (Pandiselvam et al., 2024). The evaluation results were recorded using a questionnaire.

### Physicochemical Parameters Analysis

The physicochemical parameters analyzed in this study included yield, moisture content, peroxide value, and free fatty acids (FFA), referring to quality standards established by BSN (2008).

Yield represents the percentage of virgin coconut oil (VCO) obtained from the initial cream weight. It was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Initial cream weight}}{\text{Weight of VCO}} \times 100\% \quad (1)$$

Moisture content indicates the amount of water present in the VCO sample. It was determined using the equation:

$$\text{Moisture Content (\%)} = \frac{(BS+BCK)-(BC+I)}{BS} \times 100\% \quad (2)$$

where:

BS = sample weight
BCK = weight of container + dried sample
BC = weight of empty container
I = weight loss during drying

Peroxide value reflects the extent of lipid oxidation in VCO. It was calculated using the formula:

$$\text{Peroxide Value} = \frac{A \times N \times 1000}{G} \quad (3)$$

where:

A = volume of titrant (mL)
N = normality of titrant
G = sample weight (g)

FFA indicates the level of hydrolytic degradation of oil. It was determined using the equation:

$$\text{FFA (\%)} = \frac{M \times A \times N}{1000 \times G} \times 100\% \quad (4)$$

where:

M = molecular weight of fatty acid (usually lauric acid)
A = volume of titrant (mL)
N = normality of titrant
G = sample weight (g)

### Antibacterial Activity Test

Antibacterial activity was evaluated using the disk diffusion method with two replications (Klančnik et al., 2010). Mueller Hinton Agar (MHA) medium was prepared and sterilized at 121°C for 15 minutes. Bacterial suspensions of *Staphylococcus aureus* and *Escherichia coli* were standardized to 0.5 McFarland.

Paper disks soaked in VCO samples (heating, fermentation, enzymatic), chloramphenicol (positive control), and distilled water (negative control) were placed on inoculated media. The plates were incubated at 37°C for 24 hours. The inhibition zones were measured in millimeters (mm) using the following formula (Tjiptoningsih, 2020):

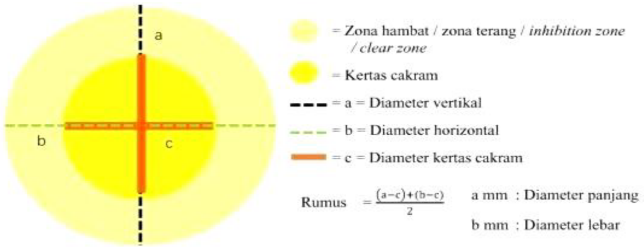


**Figure 1.** Antibacterial Inhibition Zone Measurement.

According to Kumowal et al. (2019), antibacterial inhibitory abilities can be grouped into various categories as follows (Table 1)

**Table 1.** Classification of Antibacterial Activity Based on Inhibition Zone Diameter

| Inhibition Zone Diameter (mm) | Inhibition Category |
|---|---|
| < 10 mm | Weak |
| 10 – 15 mm | Moderate |
| 16 – 20 mm | Strong |
| > 20 mm | Very Strong |

### Data Analysis

The data obtained were analyzed using descriptive quantitative methods. Physicochemical data were presented in tabular form and compared with the Indonesian National Standard (BSN, 2008). Organoleptic test results were analyzed based on the average preference scores of panelists. Antibacterial activity data were analyzed based on inhibition zone diameters and categorized accordingly. All results were presented in tables and figures to facilitate interpretation and discussion.

## RESULTS

### VCO Production

VCO is produced using three methods: heating, fermentation, and enzymatic processing. Before this, the coconut cream obtained from the grated coconut flesh is separated. Approximately 4 kg of coconut flesh is used, combined with 8 liters of distilled water, to produce 2400 mL of coconut cream. After obtaining the coconut cream, the three VCO production methods are then combined with the components used, resulting in VCO products with distinct characteristics (Figure 2), as described in Table 2.

**Figure 2.** Results of the third method of VCO after filtration. A) Heating VCO, B) Fermentation of VCO, C) Enzymatic VCO.

According to the National Standardization Agency (SNI 7381:2008), a good VCO has a clear to pale yellow color, a fresh, non-rancid coconut aroma, and a distinctive coconut oil flavor. Table 4.1 shows that the characteristics of each VCO product meet the SNI standards and therefore qualify as good VCOs. The yellowish color of VCO is produced by the addition of enzymes and by heating. The distinctive aroma and flavor of fresh coconut indicate that the VCO product is still in good condition and undamaged.

**Table 2.** Characteristics of VCO Products with Different Manufacturing Methods.

| Characteristics | Heating Method | Fermentation Method | Enzymatic Method |
|---|---|---|---|
| Color | Clear, slightly yellowish | Pale yellow | Yellow |
| Aroma | Typical fresh coconut aroma | Typical fresh coconut aroma | Typical fresh coconut aroma |
| Taste | Normal (typical coconut oil) | Normal (typical coconut oil) | Abnormal |

### Organoleptic Testing

This organoleptic test was conducted by 20 untrained panelists to assess the quality and determine the acceptability of the resulting VCO product based on their individual preference (hedonic) level. The selected panelists met several criteria, including no olfactory or taste disorders; being aged 18-50 years; having no allergies to coconut or coconut-derived products; and not currently taking medications that affect the sense of smell or taste. Based on the tests conducted, the panelists obtained varying assessments, shown in Table 3.

Panelists' assessments of the color, aroma, and taste of VCO products were based on each characteristic listed in Table 2, with individual preferences varying. Based on the assessment of 20 untrained panelists, the results showed that the color of heated, fermented, and enzymatic VCO was generally rated as "liked." Panelists rated the aroma of heated VCO as "very liked," the aroma of fermented VCO as "liked," and the aroma of enzymatic VCO as "somewhat liked." Meanwhile, the taste of heated VCO was rated as "very liked" and "disliked" for fermented and enzymatic VCO. Based on these assessments, it can be seen that the quality of VCO in terms of color, aroma, and taste that was most preferred by the average panelist was heated VCO.

**Table 3.** Organoleptic Test Results of VCO Color, Aroma and Taste Based on Level of Preference (Hedonic)

| Test Parameter | Heating Method | Fermentation Method | Enzymatic Method |
|---|---|---|---|
| Color | Like | Like | Like |
| Aroma | Very like | Like | Slightly like |
| Taste | Very like | Dislike | Dislike |

### Physicochemical Testing

This physicochemical testing is based on several parameters that need to be tested to determine the quality of the resulting VCO product based on its physical and chemical properties. The results of the physicochemical testing can be seen in Table 4.

**Table 4.** Results of Physicochemical Tests of VCO in Various Manufacturing Methods

| Test Parameter | Heating Method | Fermentation Method | Enzymatic Method |
|---|---|---|---|
| Yield (%) | 0,802 | 31.88 | 26.88 |
| Moisture Content (%) | 00.00 | 00.02 | 00.04 |
| Peroxide Value (mg Eq/kg) | 00.00 | 00.00 | 00.00 |
| Free Fatty Acids (%) | 00.32 | 0,043 | 00.42 |

Table 4 presents the results of physicochemical tests on VCO conducted by heating, fermentation, and enzymatic methods, across four parameters: yield, water content, peroxide value, and free fatty acids. The yield value meets the reference standard, which is >10%. The highest yield in fermented VCO is due to the addition of yogurt. The low water content is due to the VCO having undergone a filtering process and is 0 due to heating, which meets the reference standard, which is a maximum of 0.2%. The peroxide value is 0 because the VCO produced is fresh and has not been damaged, which meets the reference standard, which is a maximum of 2.0 mgEq/kg. The relatively high free fatty acid value, exceeding the reference standard of 0.2%, is due to the influence of temperature and hydrolysis reactions during the manufacturing process.

### Antibacterial Testing

The results of testing the antibacterial activity of VCO using three different manufacturing methods showed that all VCO products produced inhibition zones with varying diameters, as shown in Table 5.

**Table 5.** Antibacterial Activity Test Results of VCO on the Growth of Escherichia coli and Staphylococcus aureus Bacteria

| Test Parameter | Heating Method | Fermentation Method | Enzymatic Method |
|---|---|---|---|
| Yield (%) | 0,80208333 | 31.88 | 26.88 |
| Moisture Content (%) | 00.00 | 00.02 | 00.04 |
| Peroxide Value (mg Eq/kg) | 00.00 | 00.00 | 00.00 |
| Free Fatty Acids (%) | 00.32 | 0,043 | 00.42 |

Table 5 shows that the antibacterial activity of VCO was demonstrated for both test bacteria, E. coli and S. aureus, as indicated by the measurable diameter of the inhibition zone. The average inhibition zone value for both bacteria fell into the weak category. The resulting clear zone is shown in Figures 3 and 4. The clear zone seen in the VCO test samples was very small, almost invisible, due to VCO's weak inhibitory effect on E. coli and S. aureus. This can be compared with a positive control for the same test bacteria, 0.1% chloramphenicol, which has very strong inhibitory activity.

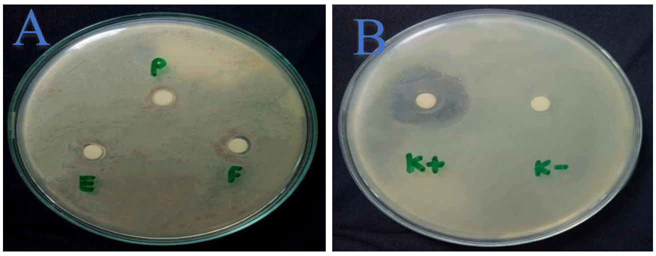


**Figure 3.** Visualization of antibacterial activity testing against Escherichia colion MHA media, incubated for 24 hours at 37°C. A) Test sample or VCO (P=heating; E=enzymatic; F=fermentation, B = Control.

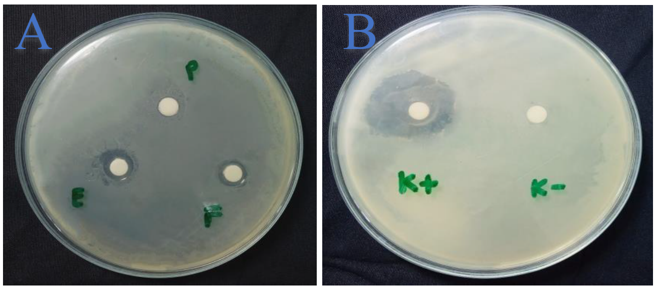


**Figure 4.** Visualization of antibacterial activity test against Staphylococcus aureus on MHA media, incubated for 24 hours at 37°C. A) Test sample or VCO (P=heating; E=enzymatic; F=fermentation), B) Control.

## DISCUSSION

VCO is produced using three methods: heating, fermentation, and enzymatic. The heating method is used at temperatures below 90°C for 30 minutes until a white blondo forms (Varghese et al., 2024). The purpose of this heating is to remove the water content from the coconut cream used and to separate the oil from the blondo. In this heating method, the coconut cream is heated and stirred continuously to prevent burning, resulting in a clear, yellowish oil and a white blondo (David & Rajabalaya, 2024). In the fermentation method of VCO production, a starter culture consisting of bacteria derived from yogurt is used to accelerate the fermentation process, thereby increasing the yield, resulting in a greater volume of oil produced in a shorter time. The type of bacteria contained in the yogurt starter used.

The bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are two types of lactic acid bacteria (LAB). Fermentation of coconut milk using LAB can produce extracellular protease enzymes that degrade the protein structure acting as an emulsifier surrounding the oil, thereby allowing the oil to separate and coalesce (S. Zhang et al., 2025). The third method used was the enzymatic method. In this method, coconut cream was supplemented with papain enzyme powder, which is a type of protease enzyme. Similar to the protease enzymes produced by LAB, papain functions to increase yield by catalyzing protein breakdown through hydrolysis of peptide bonds into simpler compounds.

Based on the three methods applied, the average characteristics of the resulting VCO were relatively similar and met the Indonesian National Standard (SNI), particularly in terms of color, aroma, and taste. The VCO produced ranged from clear to yellow in color, had a characteristic fresh coconut aroma, and exhibited a typical coconut oil taste (Table 2). Organoleptic testing conducted on 20 panelists showed that the highest preference level was observed in VCO produced by the heating method, as it exhibited the best characteristics compared to the other methods, aligning with the standard criteria. These characteristics include a clear appearance, fresh coconut aroma, and typical coconut oil taste. Wahyuzan et al. (2019) reported similar findings, where VCO produced by heating was preferred by panelists, with aroma scores ranging from 3.63–4.13 (liked) and a color score averaging 4.19 (liked). This is attributed to the absence of additional components in the heating method, resulting in a more natural coconut characteristic. (Xing et al., 2025), also stated that heating treatment enhances the emergence of the characteristic coconut aroma and taste.

In contrast, the fermentation and enzymatic methods involved the addition of external components, which resulted in slightly different characteristics, particularly in taste, which was considered abnormal and not in accordance with SNI standards that require a typical fresh coconut oil flavor. The highest yield was obtained from the fermentation method (31.88%). This is due to the addition of bacterial starters from yogurt, which degrade proteins in coconut cream, facilitating oil separation and increasing yield. Kusumawardani and Chaidir (2023) reported similar results, with fermentation-based VCO yielding approximately ±40%. This indicates that fermentation enhances yield due to microbial activity (Jakfar et al., 2023).

The enzymatic method produced a lower yield compared to fermentation, possibly due to shorter incubation time despite the addition of papain enzyme. Saina et al. (2023) stated that longer incubation enhances emulsion breakdown and increases the rate of protein hydrolysis, leading to higher VCO yield. Meanwhile, the heating method resulted in the lowest yield, likely due to insufficient heating duration and the absence of an incubation phase, which limited oil extraction. This is supported by (Thaweewong & Anuntagool, 2023), who found that the lowest yield occurred at 24-hour incubation, while the highest yield was achieved at 60 hours.

Moisture content analysis is essential to determine oil quality and shelf life. Higher moisture content can reduce shelf life and increase susceptibility to spoilage (Habiba et al., 2025). Table 3 shows that all VCO samples had low moisture content, with the lowest value (0%) observed in the heating method. This reduction is attributed to evaporation during heating (Strizhak et al., 2018). In contrast, fermentation and enzymatic methods retained small amounts of moisture (0.02% and 0.04%, respectively), likely due to the absence of heating and incomplete filtration (He et al., 2019).

Peroxide value analysis was conducted to assess the oxidation level of the oil. A low peroxide value indicates good oil quality and minimal oxidation (N. Zhang et al., 2021). The results showed that all VCO samples had a peroxide value of 0 mg Eq/kg, indicating high freshness and stability. This low value is likely associated with low moisture content, as water can facilitate microbial growth and enzymatic oxidation (Naraharasetti et al., 2025).

Free fatty acid (FFA) content is another important quality parameter. According to SNI (2008), the maximum FFA level for VCO is 0.2%. However, the highest FFA value in this study was 0.62% in the fermentation method. This is due to the presence of bacterial cultures (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) that produce enzymes such as lactase, protease, amylase, and lipase. Lipase plays a key role in breaking down fats into free fatty acids and glycerol. Hydrolysis reactions are accelerated by heat, water, acidity, and enzymatic catalysts, leading to increased FFA levels over time (Pramitha & Juliadi, 2019). The enzymatic method produced an FFA value of 0.42%. (Sun et al., 2025), reported that papain does not significantly influence oxidation processes, suggesting that residual moisture contributes to hydrolysis and FFA formation. In the heating method, increased FFA levels are associated with temperatures above 70°C, which accelerate triglyceride breakdown into free fatty acids and glycerol (Bazina et al., 2025).

The antibacterial activity test aimed to evaluate the inhibitory effect of VCO produced by different methods. VCO contains bioactive compounds such as lauric acid, which is converted into monolaurin, known for its antibacterial, antiviral, antifungal, and antiprotozoal properties (Nitbani et al., 2022). The results showed that all VCO samples exhibited antibacterial activity against both test bacteria, although the inhibition zones were relatively small (Table 4). Based on classification criteria (<10 mm = weak; 10–15 mm = moderate; 16–20 mm = strong; >20 mm = very strong)(Makimoto et al., 2005), all VCO samples fell into the weak category.

For *Staphylococcus aureus*, the highest antibacterial activity was observed in the enzymatic method (4.75 mm), whereas for *Escherichia coli*, the highest activity was observed in the heating method (3.75 mm). These results are consistent with Ma'ruf (2016), who reported lower inhibition against *E. coli* (3.61 mm) and higher inhibition against *S. aureus* (6 mm). This indicates that VCO is more effective against Gram-positive bacteria than Gram-negative bacteria. The cell wall structure of *E. coli* is more complex, consisting of multiple layers, whereas *S. aureus* has a simpler, single-layered structure, making it more susceptible to antibacterial agents. Therefore, the differences in antibacterial activity are likely due to variations in bacterial cell wall structure.

## CONCLUSION

This study showed that different processing methods significantly influenced the physicochemical characteristics and antibacterial activity of Virgin Coconut Oil. The fermentation method produced the highest yield (31.88%), while the heating method showed the best physicochemical quality with 0% moisture content, 0 mg Eq/kg peroxide value, and the highest panelist preference in organoleptic testing. In antibacterial assays, the enzymatic method showed the highest inhibition against Staphylococcus aureus (4.75 mm), whereas the heating method showed the highest inhibition against Escherichia coli (3.75 mm). These findings provide scientific information for determining the most suitable VCO processing method for producing high-quality oil with antibacterial potential.

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