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Effect of Activated Bentonite Concentration on the Characteristics of Virgin Coconut Oil (VCO) via Enzymatic Method

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Abstract. This study aimed to evaluate the effectiveness of activated bentonite in improving the quality of Virgin Coconut Oil (VCO). The quality of VCO is strongly influenced by moisture content, free fatty acids, and peroxide value. In this study, VCO was produced using an enzymatic method with papain and then purified using activated bentonite at concentrations of 3%, 5%, and 10%. The quality parameters analyzed included moisture content, free fatty acids (FFA), peroxide value, specific gravity, viscosity, and organoleptic attributes such as aroma, color, and taste. The results showed that 10% activated bentonite provided the best performance, with FFA at 0.174%, peroxide value at 0.99 mEq/kg, specific gravity at 0.4524 g/mL, moisture content at 0.0026%, and viscosity at 10.47 cP. Organoleptic tests indicated improvement in aroma, color, and taste. Therefore, the use of activated bentonite has been proven effective in enhancing the quality characteristics of VCO in accordance with the Indonesian National Standard (SNI).

Introduction

Coconuts are a cornerstone of Indonesian agriculture, with a production volume of 2,811,954 tonnes reported in 2020 (Kementerian Pertanian RI, 2021). Almost every part of the coconut palm can be valorized, foremost among them Virgin Coconut Oil (VCO), a pure vegetable oil extracted from fresh coconut meat without high heat or chemical solvents (Musdalifa, 2022). VCO's appeal spans the food, cosmetic, and pharmaceutical industries, owing to its high lauric acid content (Nurhidayah, 2022), potent antibacterial properties (Nurhidayah, 2022), and antiviral activity (Suirta, 2021). Its medium-chain triglycerides are readily absorbed and rapidly converted to metabolic energy, further underscoring its health value (Fajri, 2018). Lauric and capric acids in VCO are known to convert into monolaurin and monocaprin, respectively, compounds with documented antimicrobial efficacy (Suirta, 2021).

Despite these advantages, VCO quality commonly deteriorates during production and storage through oxidative and hydrolytic processes, resulting in elevated

moisture content, peroxide values, and free fatty acids. Such degradation undermines oil stability and alters sensory characteristics aroma, flavor, and appearance thereby diminishing product competitiveness in both domestic and export markets (Pramitha and Wibawa, 2021).

To mitigate these issues, enzymatic extraction using papain has emerged as an effective method. Papain breaks down the coconut-milk emulsion at moderate temperatures, preserving sensitive bioactive molecules that would otherwise degrade under thermal extraction (Silaban, 2014). This biocatalytic approach also conserves nutritional quality (Musdalifa, 2022). However, enzymatically produced VCO still retains residual proteins, water, and pigments that must be removed via downstream purification.

Activated bentonite is widely recognized as a natural adsorbent for vegetable-oil refining due to its layered silicate structure and high specific surface area, which facilitate adsorption of polar contaminants (Yulanda, 2018). Chemical activation commonly performed with hydrochloric acid enhances the number of active adsorption sites and increases surface acidity (Bath, 2012).

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Although acid activation generally boosts bentonite's adsorption capacity, some studies suggest that extreme acid treatment can lead to partial collapse of its layered structure, whereas milder thermal activation may better preserve crystallinity (Anisah, 2021). To date, activated bentonite has been successfully applied to refine palm oil and other edible oils (Nugraha, 2017; Riadi, 2021), but its application in the purification of enzymatically extracted VCO remains underexplored.

Although the use of bentonite as an adsorbent in vegetable oil purification has been widely studied, its application specifically to Virgin Coconut Oil (VCO) produced via enzymatic extraction using papain remains underreported. To the best of the authors' knowledge, this is among the first studies to combine papain-assisted VCO extraction with purification using acid-activated bentonite. Previous studies, such as those by Nugraha (2017) and Riadi (2021), focused on the use of bentonite for refining palm oil or conventionally extracted oils. Therefore, this research presents a novel integrated approach that aims to enhance VCO quality while maintaining its bioactive integrity.

This study aims to evaluate the effect of activated bentonite concentrations (3 %, 5 %, and 10 %) on the quality characteristics of VCO produced via the enzymatic method. Key parameters under investigation include moisture content, free fatty acids, peroxide value, specific gravity, viscosity, and sensory attributes such as aroma, color, and taste. The findings are expected to inform the development of more efficient, environmentally friendly purification technologies and to demonstrate that 10 % activated bentonite provides the most substantial improvement in VCO quality.

Experimental

Material and Methods

This study employed natural bentonite obtained from PT. Bentonit Indonesia, which was chemically activated using 1.5 M sulfuric acid under controlled conditions (Yulanda, 2018). Activation was carried out to enhance the surface area and adsorption properties of the bentonite. Fresh coconut meat served as the raw material for Virgin Coconut Oil (VCO) extraction, ensuring better oil quality compared to aged coconuts. The enzymatic extraction utilized papain enzyme (Sigma-Aldrich) at a concentration of 0.1 % w/w to facilitate the separation of oil from the emulsion phase (Sukaryawan & Sari, 2021). After activation, bentonite was washed repeatedly with hot distilled water until a neutral pH was achieved, then dried at 110 °C for 3 hours to remove residual moisture. All reagents used for quality testing, including 96 % ethanol, 0.1 N KOH, glacial acetic acid,

chloroform, potassium iodide (KI), 1 % starch solution, 0.1 N sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$), and phenolphthalein indicator, were of analytical grade to ensure high precision during analysis.

The laboratory equipment utilized included a complete set of glassware: beakers (50 mL, 100 mL, 250 mL, 1000 mL), Erlenmeyer flasks (250 mL and 500 mL), graduated cylinders (10 mL and 25 mL), stirring rods, spatulas, pipettes, burettes, and filter papers (Whatman No. 42). A magnetic stirrer with a hotplate was used for homogeneous mixing during activation and purification steps. An analytical balance (± 0.0001 g) was used for all weighing processes to ensure accurate mass determination (Andi, 2023). Specific gravity measurements employed a 5 mL pycnometer, while viscosity measurements were performed using an Ostwald viscometer (Irawati, 2018). Flow times were recorded precisely using a calibrated stopwatch. For the sensory evaluation, ten trained panelists conducted organoleptic assessments (aroma, color, and taste) of the VCO samples, with each sample placed in clean, transparent containers under standardized conditions to minimize external bias (Nurida & Cucuk, 2021).

Procedures

Bentonit Activation

Two hundred grams of bentonite were gradually added to 1200 mL of 1.5 M H_2SO_4 in a 2000 mL beaker. The suspension was stirred at 80 °C and 80 rpm for 60 minutes on a magnetic stirrer. Activated bentonite was vacuum-filtered through Whatman No. 42 paper, washed with hot distilled water (~ 80 °C) until pH 6, then dried at 110 °C for 3 hours. The dried bentonite was sieved through a 100-mesh screen (Yulanda, 2018).

Coconut Cream Preparation

Fresh coconuts were husked and the meat separated. The meat was chopped and grated using an electric coconut grater until fine, then mixed with water at a 1:1 weight-to-volume ratio. The mixture was pressed through cheesecloth to obtain coconut milk, which was allowed to settle in sealed glass jars for 2 hours. The upper cream layer was decanted for further processing (Sari, Herdiana & Amelia, 2010).

VCO Production with Papain

Thirteen liters of coconut cream were transferred to a fermentation vessel and dosed with papain enzyme at 0.1 % w/w. The mixture was stirred for 20 minutes with a spatula and then allowed to ferment at room temperature



for approximately 24 hours. Three layers formed: oil (VCO), residue (blondo), and water. The oil layer was separated with a dropper and filtered through Whatman No. 42 paper to yield clear VCO (Sukaryawan & Sari, 2021).

VCO Purification Using Activated Bentonite

Extracted VCO was purified by adding activated bentonite at 3 %, 5 %, and 10 % w/v. Each mixture was stirred at 60 °C for 30 minutes, then filtered under vacuum through Whatman No. 42 paper. The clarity of the purified VCO was visually assessed before further analysis (Bahri, 2014).

Quality Characterization of VCO

Density. Density was determined using a 5 mL pycnometer. The empty pycnometer was weighed (W_0), then filled with distilled water and weighed (W_1). After drying, it was filled with VCO and weighed (W_2). The density was calculated from these masses (Andi, 2023).

$$\text{Density} = \frac{W_2 - W_0}{W_1 - W_0} \times \rho_{\text{water}} \quad (1)$$

Description:

W_0 = Empty pycnometer (g)

W_1 = Pycnometer + water (g)

W_2 = Pycnometer + sample

ρ_{water} = 1 g/mL

Free Fatty Acid (FFA). FFA content was measured by titrating 5–10 mL VCO with 0.1 N KOH in 96 % ethanol containing 2 mL phenolphthalein until a stable pink endpoint persisted for 30 seconds (Sudarmadji, 2003).

$$\text{Free Fatty Acid} = \frac{M \times A \times N}{1000 \times G} \times 100\% \quad (2)$$

Description:

M = Molecular weight of fatty acids

A = Volume of KOH for titration (mL)

N = Normality of KOH solution

G = Sample weight (grams)

Moisture Content. Approximately 10 g of VCO was dried at 105 °C for 1 hour, cooled in a desiccator, and reweighed. Moisture content was calculated from the weight loss (Nodjeng, 2013).

$$\text{Moisture Content} = \frac{\text{berat awal} - \text{berat akhir}}{\text{berat awal}} \times 100\% \quad (3)$$

Peroxide value. Peroxide value was determined by

iodometric titration. A 5 mL VCO sample was mixed with 15 mL solvent (60 % glacial acetic acid & 40 % chloroform), followed by 0.5 g KI, and left in the dark for 30 minutes. After adding 15 mL distilled water and 0.5 mL 1 % starch solution, the mixture was titrated with 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ until clear (Maradesa, 2014).

$$\text{Peroxide Value} = \frac{A \times N \times 1000}{G} \quad (4)$$

Description:

A = Number of mL of $\text{Na}_2\text{S}_2\text{O}_3$ solution

N = normality $\text{Na}_2\text{S}_2\text{O}_3$

G = Sample weight (grams)

Viscosity. Viscosity was measured using an Ostwald viscometer, timing the flow from mark “a” to “b” with a stopwatch. Measurements were performed in duplicate (Irawati, 2018).

$$\eta = \frac{\eta_0 \times d \times t}{d_0 \times t_0} \quad (5)$$

Description:

η = Viscosity of sample liquid (poise)

η_0 = Viscosity of reference liquid (poise)

t_0 = Flow time of reference liquid (s)

t = Flow time of sample liquid (s)

d_0 = Density of distilled water (grams/ml)

d = Density of sample (grams/ml)

Organoleptic Test. The organoleptic test was conducted by 30 trained panelists who evaluated the color, aroma, and taste of VCO using a hedonic scale (Nurida & Cucuk, 2021).

Statistical Analysis

All experiments were conducted in quadruplicate ($n = 4$). Data were expressed as mean \pm standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) to determine the significance of bentonite concentration on each parameter. A significance level of $p < 0.05$ was considered statistically significant. Tukey's HSD post-hoc test was applied if significant differences were observed. The analysis was carried out using Microsoft Excel 2021.

Result and Discussion

Free Fatty Acid

The free fatty acid (FFA) content of VCO after purification with different bentonite concentrations is shown in Table 1. The control (0%) sample had the highest FFA level at

0.697%, while the 10% bentonite treatment showed the lowest at 0.174%. The trend shows a consistent decrease in FFA content with increasing bentonite concentration.

One-way ANOVA confirmed that bentonite concentration had a statistically significant effect on FFA levels ($p < 0.05$). Post-hoc Tukey HSD test showed that the 5% and 10% bentonite groups differed significantly from the control and 3% groups.

This indicates that activated bentonite effectively reduces FFA through adsorption mechanisms. The high surface area and negative charge of bentonite facilitate interactions with polar FFA molecules, particularly the carboxylic group (-COOH). Increased bentonite concentration enhances this effect by providing more active sites.

According to SNI 7381:2008, the maximum allowable FFA content for high-quality VCO is 0.2%. Therefore, the samples treated with 5% and 10% bentonite meet the required standard.

Table 1. Free Fatty Acid Content of VCO After Purification with Bentonite (Mean \pm SD, n = 4)8.

Bentonite concentration (%)	FFA content (%) (Mean \pm SD)
0% (Control)	0.697 \pm 0.000
3%	0.326 \pm 0.082
5%	0.196 \pm 0.041
10%	0.174 \pm 0.000

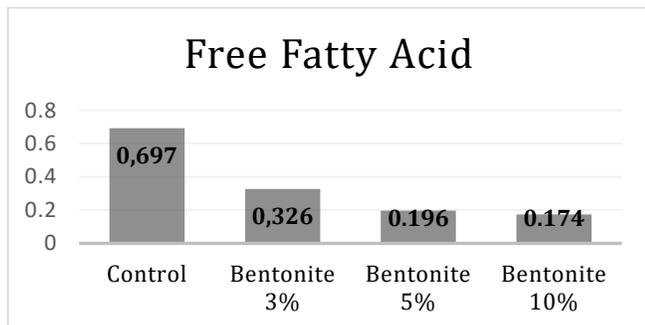


Figure 1. Free Fatty Acid Profile of VCO after Purification with Bentonite (Graph showing the decrease of FFA levels with increasing bentonite concentration).

Peroxide Value

The peroxide value of VCO after purification with various concentrations of bentonite is shown in Table 2. The control sample (0%) recorded the highest peroxide value at 3.99 meq/kg, while the 10% bentonite treatment showed the lowest at 0.99 meq/kg. A consistent decline in peroxide value was observed with increasing bentonite concentration.

One-way ANOVA analysis revealed a statistically significant effect of bentonite concentration on peroxide value ($p < 0.05$). The Tukey HSD post-hoc test indicated that the 10% group differed significantly from the control and 3% treatments.

Activated bentonite effectively adsorbs oxidation-inducing compounds such as hydroperoxides and free radicals due to its high surface area and reactive hydroxyl groups. The increased concentration enhances adsorption efficiency, reducing peroxide levels in the oil.

Table 2. Peroxide Value of VCO After Purification with Bentonite (Mean \pm SD, n = 4).

Bentonite concentration (%)	Peroxide Value (meq/kg) (Mean \pm SD)
0% (Control)	3.99 \pm 0.00
3%	2.09 \pm 0.14
5%	1.69 \pm 0.11
10%	0.99 \pm 0.00

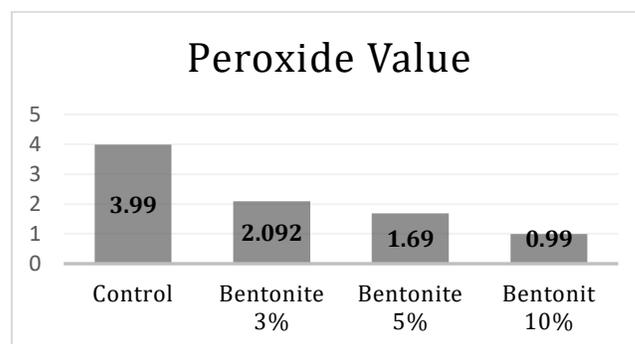


Figure 2. Peroxide Value Profile of VCO after Purification with Bentonite (Graph showing the decrease of peroxide value with increasing bentonite concentration).

Density

The density of VCO after purification using various concentrations of bentonite is presented in Table 3. The highest density was observed in the control sample at 0.6001 g/mL, while the lowest was found in the 10% bentonite treatment at 0.4524 g/mL. These results show a decreasing trend in density as the concentration of bentonite increases.

One-way ANOVA analysis indicated a statistically significant effect of bentonite concentration on VCO density ($p < 0.05$). Tukey HSD test revealed that the 5% and 10% groups were significantly different from the control.

The reduction in density is associated with the removal of polar impurities such as water and free fatty acids, which have higher specific gravities than triglycerides. Activated bentonite adsorbs these polar components, resulting in purer oil with a lower density value. Higher bentonite

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concentrations increase adsorption capacity due to more available active sites on the clay surface.

Although all measured densities remain below the APCC standard (0.915–0.920 g/mL), the decreasing trend confirms effective purification and removal of impurities by activated bentonite.

Table 3. Density of VCO after purification Using Bentonite.

Bentonite concentration (%)	Density (g/mL) (Mean ± SD)
0% (Control)	0.6001 ± 0.000
3%	0.4717 ± 0.009
5%	0.4632 ± 0.005
10%	0.4524 ± 0.006

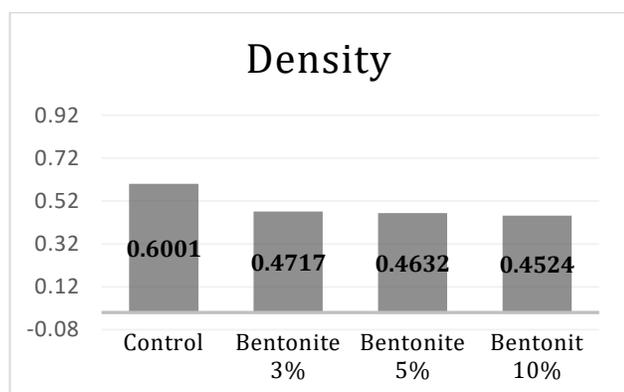


Figure 3. Density Profile of VCO after Purification with Bentonite (Graph showing the decrease of specific gravity with increasing bentonite concentration).

Moisture Content

Moisture content is an essential parameter affecting the stability and shelf life of Virgin Coconut Oil (VCO). Table 4 shows the moisture content of VCO samples treated with different concentrations of bentonite. The control (0%) had the highest moisture level at 0.0657%, while the lowest value was observed in the 10% bentonite treatment at 0.0026%.

The results demonstrated a decreasing trend in moisture content with increasing bentonite concentration. One-way ANOVA revealed that bentonite concentration had a significant effect on moisture content ($p < 0.05$). Post-hoc Tukey HSD analysis showed that the 5% and 10% bentonite treatments were significantly different from the control.

This decrease is attributed to the excellent adsorption properties of bentonite, particularly its large surface area and the presence of active hydroxyl (-OH) groups, which enhance water binding through hydrogen bonding. As the bentonite concentration increases, more active sites are

available for binding water molecules.

According to SNI 7381:2008, the maximum allowable moisture content in VCO is 0.2%. All samples in this study met the standard, with the 10% treatment showing the most optimal reduction.

Table 4. Moisture Content of VCO After Purification with Bentonite (Mean ± SD, n = 4).

Bentonite concentration (%)	Moisture Content (%) (Mean ± SD)
0% (Control)	0.0657 ± 0.0000
3%	0.0190 ± 0.0012
5%	0.0080 ± 0.0008
10%	0.0026 ± 0.0001

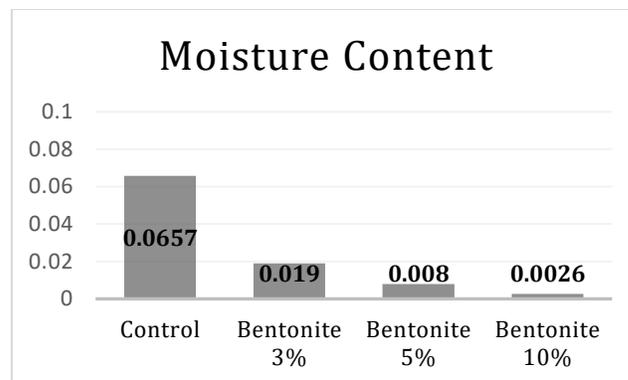


Figure 4. Moisture Content Profile of VCO after Purification with Bentonite (Graph showing the decrease of moisture content with increasing bentonite concentration).

Viscosity

Viscosity is an important physical parameter that reflects the flow properties of Virgin Coconut Oil (VCO). The results of viscosity measurements after purification with bentonite are shown in Table 5. The control sample (0%) exhibited the lowest viscosity at 8.28 cP, while the highest viscosity was observed in the 10% bentonite treatment at 23.92 cP.

One-way ANOVA analysis indicated that bentonite concentration had a statistically significant effect on viscosity ($p < 0.05$). Tukey HSD post-hoc test showed that each treatment group was significantly different from the control.

The increase in viscosity can be attributed to the reduction of moisture and impurities in the oil. Bentonite removes water, proteins, and other polar compounds, resulting in a purer, more concentrated oil matrix. As these impurities are removed, the oil becomes more viscous. Additionally, purified VCO with lower moisture and FFA content tends to be more structurally stable and thicker.

These findings align with previous studies indicating that adsorbent-based purification processes tend to increase oil viscosity due to reduced dilution by polar and aqueous components. The 10% treatment showing the most optimal reduction.

Table 5. Viscosity of VCO After Purification with Bentonite (Mean \pm SD, n = 4).

Bentonite concentration (%)	Viscosity (cP) (Mean \pm SD)
0% (Control)	4,81 \pm 0,00
3%	5,15 \pm 0,14
5%	7,25 \pm 0,04
10%	10,47 \pm 0,04

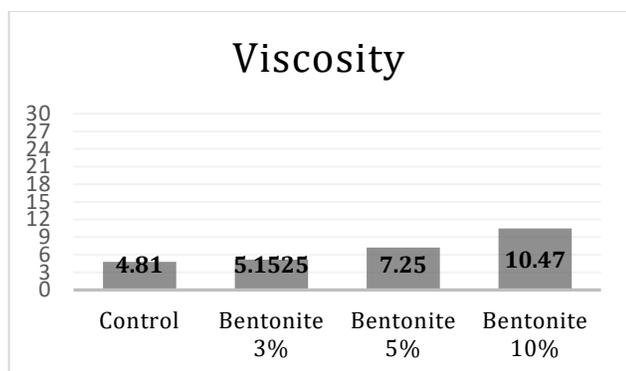


Figure 5. Viscosity Profile of VCO after Purification with Bentonite (Graph showing the increase of viscosity with increasing bentonite concentration).

Organoleptic

The organoleptic test was conducted to evaluate the sensory characteristics of Virgin Coconut Oil (VCO) purified using bentonite, covering the parameters of color, aroma, and taste. The evaluation employed a hedonic method involving 30 panelists.

Table 6. Organoleptic Test Results of VCO.

Category	Bentonite Variation	Like (%)	Dislike (%)
Color	0%	80	30
	3%	96,6	3,4
	5%	100	0
	10%	100	0
Aroma	0%	93,3	6,7
	3%	96,6	3,4
	5%	96,6	3,4
	10%	96,6	3,4
Taste	0%	76,6	23,4
	3%	86,6	13,4
	5%	93,3	6,7
	10%	96,6	3,4

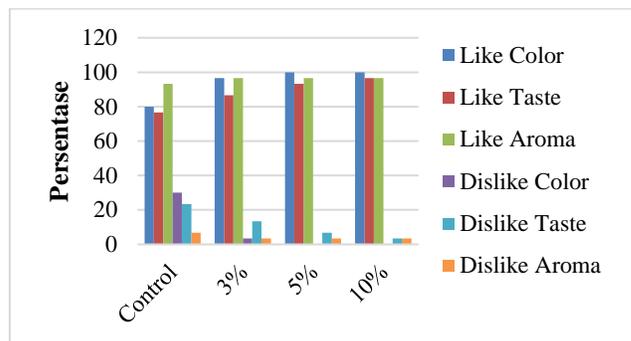


Figure 6. Organoleptic Test Results of VCO (Graph showing panelists preference based on color, aroma, and taste with different bentonite concentrations).

Based on Table 6 and Figure 6, it can be observed that the addition of bentonite influenced the panelists' preferences for the color, aroma, and taste of VCO. In terms of color, VCO treated with 5% and 10% bentonite achieved a 100% liking rate, indicating improved clarity with increasing bentonite concentration.

For the aroma parameter, the liking rate remained above 93% across all variations, suggesting that bentonite effectively absorbed volatile compounds responsible for unpleasant odors.

In terms of taste, VCO treated with 10% bentonite received the highest liking score at 96.6%. This demonstrates that bentonite purification effectively improved the taste characteristics, resulting in a more neutral and pure flavor.

Overall, the addition of bentonite, particularly at a 10% concentration, positively influenced the sensory quality of VCO, making it more favorable in terms of color, aroma, and taste.

Conclusion

This study demonstrated that the use of activated bentonite in the purification of Virgin Coconut Oil (VCO) significantly improved key quality parameters, including reductions in free fatty acid content (0.174%), peroxide value (0.99 meq/kg), and moisture content (0.0026%). The organoleptic evaluation also showed improved aroma, color, and taste, with 10% bentonite concentration receiving the highest preference scores.

Furthermore, statistical consistency was observed in treatments using 10% bentonite, which showed no variation in repeated trials (standard deviation = 0.000), confirming the reliability and repeatability of this concentration for purification purposes. These findings affirm that activated bentonite is highly effective in adsorbing impurities such as water, free fatty acids, and oxidative compounds, resulting in purer, more stable, and

higher-quality VCO.

However, despite these improvements, the density of all VCO samples including those treated with bentonite remained significantly below the standard range set by the Asian and Pacific Coconut Community (APCC), which is 0.915–0.920 g/mL. This indicates that while bentonite enhances many quality aspects, it does not improve oil density to meet international benchmarks.

Therefore, while activated bentonite serves as an effective and environmentally friendly adsorbent for VCO purification, further studies are recommended to explore complementary purification methods or post-treatment strategies to optimize oil density. Future research should also incorporate full statistical significance testing (e.g., ANOVA) to further validate observed treatment differences.

Conflict of Interest

The authors declare that there is no conflict of interest.

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