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OPTIMIZING VIRGIN COCONUT OIL YIELD AND QUALITY USING LACTIC ACID BACTERIA FROM *BLONDO* WITH CHILLING-THAWING METHOD

Peningkatan Rendemen dan Kualitas Minyak Kelapa Murni Menggunakan Bakteri Asam Laktat dari Blondo dengan Metode Chilling-Thawing

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ABSTRACT

Increasing demand for Virgin Coconut Oil (VCO) presents a promising opportunity to improve the economy in Indonesia. Various methods for VCO production have been explored to optimize yield and ensure desirable organoleptic and physicochemical qualities. Modifying the oil extraction process often necessitates costly equipment. In this study, VCO was extracted by combining the chilling-thawing method with the addition of lactic acid bacteria (LAB) isolated from blondo. The process began with the isolation and characterization of LAB isolates, followed by the application of the chilling-thawing method and addition of 2% LAB starter, then assessed for their consumer preference through a questionnaire. This innovative process yielded VCO at a rate of 35.78-46.65% using three LAB isolates (BAL 1-3) from blondo samples, representing an improvement over previous studies employing similar methodologies. Moreover, hedonic evaluation revealed a higher consumer preference for LAB-treated VCO compared to the control, further highlighting the effectiveness of LAB treatment in combination with the chilling-thawing method.

Keywords: *BAL, Chilling-thawing, Lactic Acid Bacteria, Oil extraction, VCO*

ABSTRAK

Meningkatnya permintaan minyak kelapa murni (VCO) menghadirkan peluang yang menjanjikan untuk meningkatkan perekonomian di Indonesia. Berbagai metode produksi VCO telah dieksplorasi untuk mengoptimalkan hasil dan memastikan kualitas organoleptik dan fisikokimia yang diinginkan. Ini melibatkan modifikasi proses ekstraksi minyak, yang seringkali memerlukan peralatan yang mahal. Dalam penelitian ini, VCO diekstraksi dengan menggabungkan metode *chilling-thawing* dengan penambahan bakteri asam laktat (BAL) yang diisolasi dari blondo. Prosesnya dimulai dengan isolasi dan karakterisasi isolat BAL, diikuti oleh penerapan metode *chilling-thawing* dan penambahan starter BAL 2%, kemudian dinilai untuk preferensi konsumen melalui kuesioner. Proses inovatif ini menghasilkan VCO sebanyak 35.78-46.65% menggunakan tiga isolat BAL (BAL 1-3) dari sampel blondo, yang menunjukkan peningkatan dibandingkan dengan penelitian sebelumnya yang menggunakan metodologi serupa. Selain itu, evaluasi hedonik mengungkap preferensi konsumen yang lebih tinggi terhadap VCO dengan perlakuan BAL dibandingkan dengan kontrol. Hasil ini menitikberatkan pada efektivitas pelakuan LAB yang dikombinasikan dengan metode *chilling-thawing*.

Kata Kunci: *BAL, Chilling-thawing, Bakteri Asam Laktat, Ekstraksi Minyak, VCO*

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INTRODUCTION

Global demand for Virgin Coconut Oil (VCO) has been steadily rising, a trend that has accelerated notably since the onset of the COVID-19 pandemic (Dacasin et al. 2021; Finnegan et al. 2023). VCO is highly sought after for its myriad health and beauty benefits (Satheeshan et al. 2020; Wallace 2018). Particularly in the health and wellness sector, VCO's market value is projected to soar from $2.54 billion in 2023 to an estimated $5.17 billion by 2032 (Fortune Business Insights 2025). Whereas, Indonesia's exports of coconut oil, including VCO and copra coconut oil, increased compared to the previous year, reaching $916.9 million (BPS 2025). VCO, in particular, held a favorable position in key importing countries such as China, Russia, Sri Lanka, and the United States (Oktania and Hardjanto 2023).

This surge is attributed to the growing preference for natural health and self-care products (Wallace 2018). Moreover, VCO serves as a key ingredient in many beauty products, particularly for enhancing skin health (Zhang et al. 2019; Satheeshan et al. 2020), as well as in massage oils (Songkro et al. 2010; Darmareja et al., 2020). Numerous studies have delved into the health benefits of consuming VCO, including its potential in preventing obesity (Adeyemi et al. 2020; de Vasconcelos et al. 2022), cancer (Illam et al. 2017), osteoporosis (Hayatullina et al. 2012; Malik et al. 2019), maintaining oral health (Peedikayil 2019), acting as an antidiabetic agent (Rahmawati et al. 2020), having antibacterial and anti-inflammatory properties (Zakaria et al. 2011; Rahmawati et al. 2020), exhibiting antiviral effects, especially in combating COVID-19 (Angeles-Agdeppa et al. 2021; Dacasin et al. 2021), and functioning as an immunomodulator (Widianingrum et al. 2019). Furthermore, VCO improves lactic acid bacteria and superior fatty acid content compared to regular coconut oil and palm oil (Suryani et al. 2020).

Nonetheless, coconuts are primarily sold as young coconuts or grated coconuts in local markets in many regions in Indonesia, mainly for personal consumption or local trade, resulting in relatively low prices. Consequently, coconut utilization for the extraction of natural vegetable oils, particularly VCO, remains limited, despite its significant potential to uplift the community's economy (Mela and Bintang 2021).

VCO, produced by either low-temperature heating (< 60 °C) or cold-pressing, exhibits a clear, slightly yellowish color with a distinct coconut flavor and aroma, meeting the safety standards outlined in SNI 7381-2008. Enzymatic, mechanical, and fermentative processes are employed for VCO (Ravindra and Bosco 2017), while coconut oil is typically obtained through heating (Wallace 2018). These methods aim to separate water and oil and reduce water content. Raghavendra & Raghavarao (2010) demonstrated that the enzymatic method, followed by chilling and thawing, yields the highest-quality VCO with elevated levels of short and medium-chain fatty acids compared to commercial oil. This finding highlights the potential for combining methods to enhance both the yield and quality of VCO.

Lactic acid bacteria (LAB) naturally present in the coconut meat and water (Maini and Lopez 2022). These LAB facilitates the breakdown of water and fat bonds during fermentation, converting coconut milk into VCO (Rasyid et al. 2021; Maini and Lopez 2022). Consequently, LAB can also be obtained from coconut milk residue after fementation, known as *blondo* (Rasyid et al. 2021; Chaidir et al. 2023) and can accelerate the breakdown process and maximizing oil yield (Asiah et al. 2019; Maini and Lopez 2022). Notably, research by Chaidir et al. (2023); Kusumawardani & Chaidir (2023) indicated that combining chilling-thawing with LAB supplementation significantly increases VCO production compared to methods lacking LAB. This study extends the findings of Chaidir et al. (2023) by exploring LAB sources obtained from *blondo*.

Virgin Coconut Oil (VCO) production, traditional and laboratory methods have typically relied on single techniques. However, there's ongoing interest in refining production methods to maximize VCO quality and efficiency. Studies have compared various approaches, with fermentation and supercritical fluid extraction (SFE) emerging as contenders. Fermentation shows promise

for its superior qualities, while SFE offers efficiency, particularly for large-scale operations (Aytaç 2022). Microbial involvement including yeasts, fungi (Hidayatulloh et al. 2020), and bacteria especially lactic acid bacteria (LAB), is mainly involve in fermentative extraction (Mansor et al. 2012; Yang et al. 2021). While most studies use LAB, there's limited exploration of combination methods in VCO production. Some researchers have found success with combinations of enzymatic, chilling, and mechanical methods (Prasanna et al. 2024). However, these often require expensive equipment.

A recent study by Chaidir et al. (2023) introduced a new approach by combining chilling-thawing with LAB supplementation. Their findings demonstrated improved VCO yield without the need for specialized equipment. Additionally, Maini and Lopez (2022) highlighted the importance of LAB, particularly, *Weissella* and *Lactobacillus*, in VCO fermentation, emphasizing the need to leverage natural LAB populations for optimal extraction.

The novelty of this research lies in its focus on using LAB isolates from *blondo* as potential starters. By combining the isolates with a controlled extraction method, the aim is to consistently produce high-quality VCO. This approach offers a practical and cost-effective solution for VCO production, particularly on a smaller scale or in home settings. Leveraging the coconut resources can optimize VCO production, thereby fostering positive economic and communal outcomes.

MATERIALS DAN METHODS

Time and Place of Study

This study were conducted in Laboratory of Faculty Life Science and Technology (FITH), Sumbawa University of Technology (UTS), West Nusa Tenggara, Indonesia (-8.571021457719608, 117.43148660949505), in February-September 2024.

Materials

The materials used in this study were lactic acid bacteria (LAB) media: De Man Rogosa and Sharpe (MRS) broth media, Agar powder, distilled water, 70% alcohol, 3% KOH solution, 3% hydrogen peroxide (H_2O_2) solution, grated coconut obtained from Seketeng Market (-8.502464658596569, 117.42933855442837), and water. *Blondo* samples for LAB isolation were obtained from VCO processed using the chilling-thawing method.

Methods

VCO production with Chilling-thawing method

The production of VCO involves a series of steps to extract oil from grated coconut. Initially, grated coconut was mixed with water at a ratio of 1:2 (Rahmadi et al. 2013). The chilling-thawing method were modification of Raghavendra and Raghavarao (2010) method. The extracted coconut milk was filtered and stored in plastic containers. The coconut milk is then left to incubate at room temperature for 2 hours, allowing for the separation of cream and skim. The coconut cream, which rises to the top, was carefully transferred to a separate plastic bag and refrigerated (~5 °C) for 24 hours until solidification occurs. Once solidified, the cream was filtered through a cheese cloth to isolate the oil, from water and *blondo*. The oil was collected using a pipette and transferred into bottles. Meanwhile, the *blondo* is stored in microtubes for further processing.

Isolation of LAB from *Blondo*

Isolation of LAB from *blondo* VCO was conducted using MRS Agar, with the addition of 1% $CaCO_3$ (Suryani et al. 2020). 1 gr of *blondo* samples, obtained from prior VCO production were diluted with distilled water to a 10^{-5} dilution. Subsequently, dilutions of 10^{-1}, 10^{-3}, and 10^{-5} were each plated in 100 µl on to the media. The inoculum was evenly spread using an L rod and incubated for 48 hours. Following incubation, macroscopic morphological observations were conducted to identify LAB colonies. Individual LAB colonies were isolated and streaked onto MRS agar + 1% $CaCO_3$ media, followed by incubation for 48 hours. This procedure was repeated until pure LAB isolates were obtained.

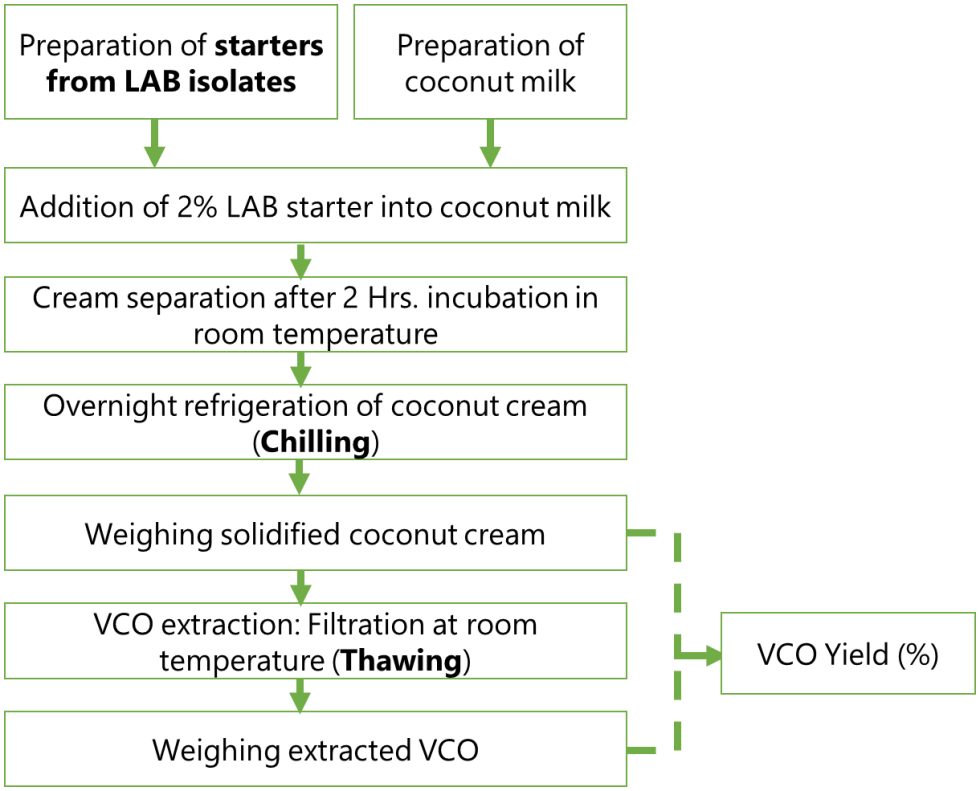

Figure 1. Overview of the chilling-thawing process and lactic acid bacteria (LAB) addition in the VCO extraction method

LAB Characterization

LAB characterization involved conducting gram and catalase tests to determine the characteristics of the isolates. The gram test was performed using a 3% KOH solution by placing 1-2 drops on a glass slide. A bacterial colony was then picked using a tube and mixed with the KOH solution, observed whether the colony exhibited mucus. The presence of mucus indicated a gram-negative bacteria, whereas the absence of mucus indicated a gram-positive bacteria (Elvira et al. 2016). Gram staining were also conducted to confirm and to observe the cells.

Similarly, the catalase test was conducted using a 3% hydrogen peroxide (H_2O_2) solution. A bacterial colony was exposed to the H_2O_2 solution, and the presence of bubbles or foam indicated the production of catalase enzyme, characteristic of gram-negative bacteria. Conversely, the absence of foam indicated a lack of catalase enzyme production, typical of gram-positive bacteria (Detha et al. 2019).

LAB treatment in VCO extraction

LAB treatment were conducted following the method of Chaidir et al. (2023). LAB isolates were prepared by culturing into 10 ml MRS Broth + 1 % coconut milk and incubated for 48 hours, as starters. Then, coconut milk was prepared with a 1:2 ratio of coconut to water, then inoculated with a 2% LAB starters of 500 mL coconut milk. The selected LAB concentration was based on the findings of (Rahmadi et al. 2013; Andrianto et al. 2018), who reported that a 2% concentration resulted in the highest VCO yield. This approach is further supported by studies conducted by Kusumawardani and Chaidir (2023) and Chaidir et al. (2023), who applied LAB using similar methodologies.

After 2 hours, the cream layer was separated and chilled at ~5 °C in a refrigerator for 24 hours to solidify. The solidified coconut cream were then thawed without centrifugation at room temperature, until the oil were extracted. The extracted VCO was observed for their physical appearance, by color and aroma. The solidified coconut cream were weighed and compared with the extracted oil to calculate the yield. The yield was calculated using the formula:

$$\text{Yield } (\%) = \frac{Weight\ of\ VCO}{Weight\ of\ solidified\ cream}\ x\ 100\%$$

All LAB treatments were conducted in triplets and analyzed by One-way ANOVA and followed by Duncan posttest. Overall steps of the combination method are as followed:

Moisture content of VCO

The VCO was placed in a pre-weighed heat-resistant glass/container and heated in an oven at 105 ℃ for 1 hour. After heating, the VCO cooled and was reweighed to determine the weight loss. This heating process was repeated until a constant weight was achieved, as indicated by Soo et al. (2020). After obtaining the constant weight after heating, the moisture content of the VCO was calculated using the formula:

$$\text{Moisture content } (\%)\ = \frac{m1-m2}{m1}\ x\ 100\%,$$

where m1 represents the weight before heating and m2 represents the weight after heating by (Aziz et al. 2017).

Hedonic test

Hedonic test was conducted on 30 participants, comprising students and other untrained respondents from the general public (17 % males, 83 % females), aged between 20-23 years old. It is difficult to find regular consumers of VCO or similar products, however, the majority of respondents are familiar with coconut oil and have used it or coconut oil-based products at least once. The hedonic test was performed on VCO within 1 month of storage periods.

Each respondent evaluated VCO samples, both with and without the addition of LAB starter isolated from *blondo*. The assessed hedonic properties included color, aroma, taste, and texture, on a structured 7-point hedonic scale (1-dislike extremely, 2-dislike very much, 3-dislike, 4-neither like nor dislike, 5-like, 6-like very much and 7-like extremely). Data collection was facilitated through Google Form.

RESULT AND DISCUSSION

Isolation and Characterization of LAB

Figure 2 presents three LAB isolated from *blondo*. Individual isolates can only be observed on 10^{-5} dilution, since the rest are too dense. Consequently, LAB could be identified macroscopically, resulting in the isolation of three distinct LAB isolates exhibiting varying morphological characteristics. Furthermore, the morphology of three LAB isolates from *blondo* is detailed in Table 1.

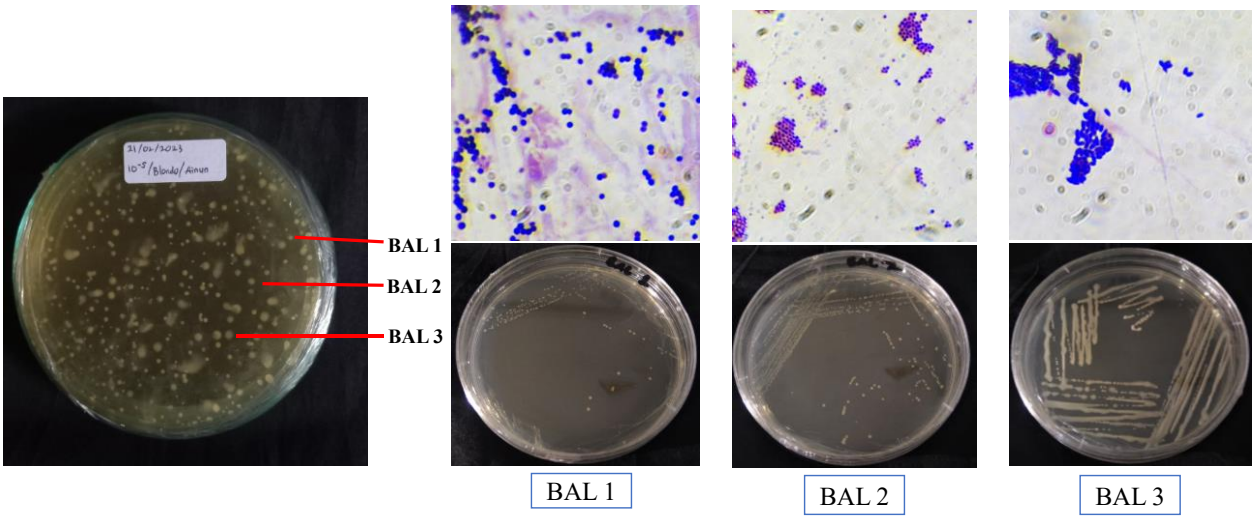

Figure 2. Lactic acid bacteria (LAB) colonies isolated from blondo. BAL 1-3 exhibit similar colony morphology and are identified as Gram-positive bacilli

Table 1. Colony morphology of Lactic Acid Bacteria (BAL 1-3) isolated from *blondo* sample

Isolate	Size	Color	Form	Elevation	Margin	Gram	Katalase
BAL 1	Punctiform (1 mm)	Whitish	Circular	Convex	Entire	+	+
BAL 2	Small (> 1 mm)	Cream	Circular	Convex	Entire	+	+
BAL 3	Small (> 1 mm)	Whitish cream	Circular	Convex	Entire	+	+

Based on the findings presented in Figure 2 and Table 1, the isolated LAB exhibited common characteristics, including small to medium size, white or cream coloration, and a convex, round surface. These observations align with the research conducted by Rasyid et al. (2021), where LAB grown on MRS Agar demonstrated similar traits, such as cream and white coloring, flat edges, round shape, and convex elevation.

The isolate's lack of reaction to 3% KOH confirms its gram-positive nature (Table 1), a definitive characteristic of LAB. Typically, LAB are gram-positive and do not produce catalase, resulting in a negative reaction to H_2O_2 and the absence of air bubble formation (Detha et al. 2019). This inability stems from LAB's incapacity to break down H_2O_2 into water and oxygen (Hamidah et al., 2019). Whereas all LAB isolates in this study tested positive for catalase (Table 1). Figure 3 (a-c) demonstrates the catalase test reaction of LAB isolated from *blondo*, revealing a weak positive reaction characterized by slight air bubble formation. This outcome aligns with research by Bulu et al. (2019) and Nanasombat et al. (2017), which identified several catalase-positive LAB isolates, including *Lactobacillus*, *Streptococcus*, *Pediococcus*, and *Leuconostoc*.

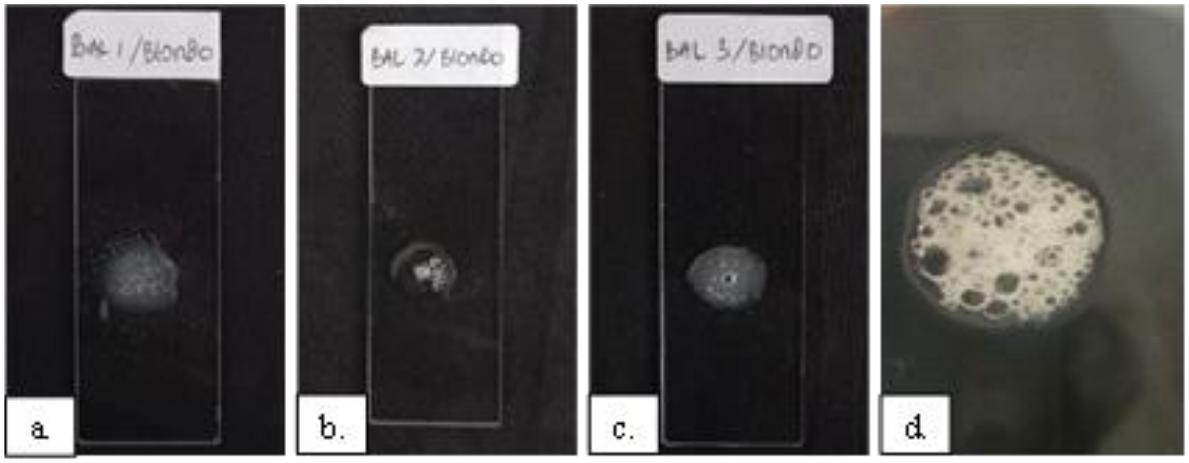

Figure 3. Weakly positive catalase reaction of LAB isolates (**a-c**) compared to **d**. a positive reaction by other bacteria isolated by Pulungan and Tumangger (2018)

Based on characteristics such as the halo zone formation on MRS Agar with 1% $CaCO_3$, LAB morphology, gram-positive and weak catalase reaction, we concluded that isolates BAL 1-3 are lactic acid bacteria (LAB).

Lactic Acid Bacteria (LAB) treatment on VCO Extraction

All treated VCO samples exhibited similar physical characteristics, such as color and aroma, as shown in the following figure:

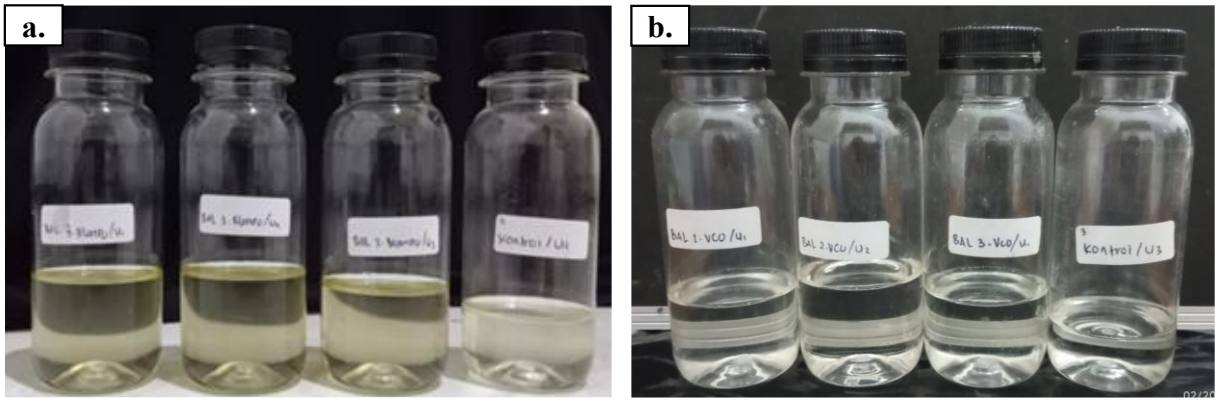

Figure 4.a. VCO extracted using BAL 3 compared to VCO without LAB addition (control); **b.** Color changes observed in LAB-treated VCO during storage

VCO treated with 2% BAL 1-3 met the strict Indonesian National Standard of SNI-7381-2008. All treated VCO exhibited similar physical apprearance as BAL 3 (Fig. 4.a), namely clear yellowish color, typical coconut aroma, with soft texture and tasteless. In contrast, the control VCO appears clear but has a slightly cloudy appearance, along with a slight rancid smell and a taste that deviated from typical coconut oil.

VCO extracted through enzymatic (Soo et al. 2020; Ng et al. 2021) and fermentation (Raudya et al. 2023) are also showing clear to slightly yellow in color, this might be due to coconut husk contamination (Raudya et al. 2023), the use of pineapple as protease source (Prayitno 2019), or the use of MRS Broth as the starter medium (Affan et al. 2019). Whereas, Andrianto et al. (2018) and Chaidir et al (2023) produced clear color VCO with *Lactobacillus casei* starter. In our study, MRS Broth with coconut milk served as the growth medium for the LAB starter isolated from *blondo*, resulting in the characteristic yellowish VCO (Figure 4.a). In contrast, the control VCO, made with the same coconut milk without LAB treatment, remained clear in color.

From our observation, after several months of storage (> 6 months) at room temperature, all LAB-extracted VCO were changed into clear color, as shown in Figure 4.b. Several studies have examined the stability of virgin coconut oil (VCO), with a focus on oxidative potency and fatty acid composition during storage (Karouw et al. 2021; Mudiyanselage and Wickramasinghe 2023), as well as under high temperature conditions (Lu and Tan 2009; Srivastava and Semwal 2013). VCO has consistently demonstrated a lower total oxidation value (TOTOX) relative to extra virgin olive oil (EVOO) and grape seed oil (GSO), reflecting its superior oxidative stability even after prolonged frying test and storage exceeding 12 months (Nyam and Chew 2014; Karouw et al. 2021). However, to our knowledge, no study to date has specifically investigated the mechanisms underlying color changes in VCO. Although oxidation is suspected to play a role, this remains an underexplored and demands further investigation.

Table 2. VCO yield and moisture content

Treatment	Average VCO yield (%)	Moisture content (%)
BAL 1	42.13±6.38^{ab}	0.05±0.003
BAL 2	46.65±5.70^{b}	0.16±0.009
BAL 3	35.78±8.17^{ab}	0.02±0.001
Control (No LAB)	29.82±4.48^{a}	0.3±0.044

Table 2 compares the yield of VCO from each treatment and the control. Notably, VCO treated with BAL 2 exhibited the highest average yield of 46.65%, significantly surpassing the control VCO, which had a yield of 29.82%. Chaidir et al. (2023) reported that fermenting coconut milk with commercial probiotic and mixed

bacterial inoculum of wild horse milk as starter yielded VCO at 28-33%. Arisanti and Angelia (2020) achieved a 13.37% yield through fermentation using 1% commercial LAB dry culture. In contrast, Affan et al. (2019), who fermented coconut milk with 10% pure culture of *Lactobacillus sp*. isolated from VCO and mixed with pineapple, attained a yield of 43.17%. Mohammed et al. (2021) obtained a yield of 72% using *Saccharomyces cerevisiae*, although the chilling-thawing method was reported to produce VCO with preferred physicochemical properties. Whereas, Jasman et al. (2021) documented a 25.71% yield using the same yeast. Raudya et al. (2023) compared various yeasts and found tempeh yeast yielded 33.35%, tape yeast 24.41%, baker's yeast 28.88%, and no yeast at 28.6%.

When compared to other combined extraction methods, chilling-thawing followed by centrifugation has been reported to yield between 20–92%, while enzymatic treatment in combination with chilling-thawing and centrifugation produced yields ranging from 60.09% to 94.5%. Additionally, centrifugation followed by freezing–thawing and subsequent centrifugation achieved yields of up to 86.28% (Prasanna et al. 2024). Oseni et al. (2017) reported a 77.67% VCO yield using a combination of *L. plantarum* starter and centrifugation, whereas chilling-thawing followed by centrifugation resulted in a yield of 69.31%. The method applied in this study demonstrated competitive yields relative to other single-method extractions; however, physical separation techniques such as centrifugation tend to produced higher yields. Notably, residual oil was detected in the *blondo* following thawing, likely due to manual oil separation steps, indicating that further recovery processes may enhance overall extraction efficiency.

On the other hand, moisture content is a crucial factor in determining VCO quality. According to SNI-7381-2008, exceeding 0.2%, can be a breeding ground for bacteria and trigger hydrolysis between oil and water (Andrianto et al. 2018; Mansor et al. 2012; Rohman et al. 2021), leading to a rancid odor and reduced shelf life. Table 2 indicated that the water content in treated VCO ranges from 0.02% to 0.16%, while the control VCO contains 0.3%. The higher moisture content in the control VCO may explain its slightly rancid aroma and unfamiliar taste as previously mentioned. All LAB-treated VCO fall below 0.2%, meeting the SNI-7381-2008 standard. This result underscores the efficacy of incorporating LAB starter in enhancing VCO quantity and quality.

Hedonic tests

The hedonic test yielded the average preference levels of respondents for the color, taste, aroma, and texture of VCO with and without the addition of LAB starter from *blondo*, as shown in the graphic below.

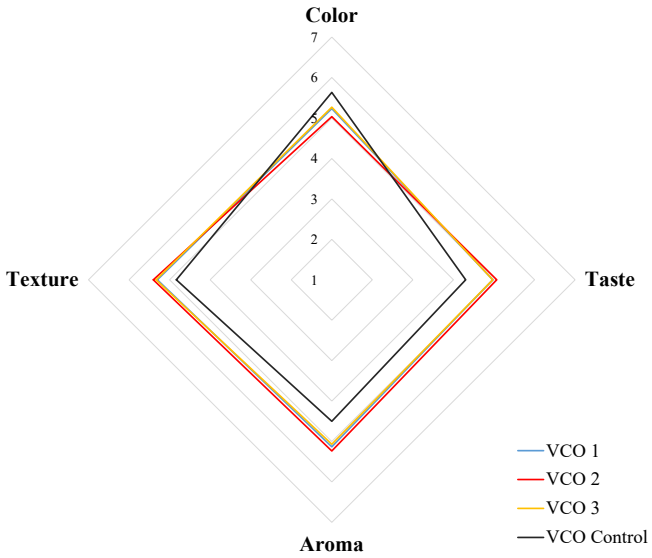

Figure 5. Hedonic evaluation of VCO treated with BAL 1-3 (VCO 1-3) compared to untreated VCO (control)

Figure 5 illustrates the preferences of 30 respondents for color, taste, aroma, and texture in both treated and control VCO. Treated VCO scored between 5.03-5.63 for color, with VCO control reaching the highest at 5.63 point in Likert positive with the scale of 1-7. Whereas, VCO treated with BAL 1-3 obtained the highest point on taste, aroma and texture compared to control, even though Control is preferred on the color parameter. All treated VCO obtained similar preference points by the respondents and commented about the clear yellowish color they produced, where microbial extraction often results in yellowish oil (Anwar et al. 2020).

In accordance with Table 2, VCO control has highest moisture content that was not aligned with Indonesian standard for VCO (SNI-7381-2008). High moisture content will increase enzymatic and microbial activity that potentially hydrolyzed free fatty acids and acetic acid production, resulting in rancid odor (Elizabeth et al. 2011; Villarino et al. 2020). Villarino et al. (2020) also suggested to keep VCO at 40-45°C to reduce rancidity during storage. In contrast, LAB-treated VCO samples remained within the acceptable limits for all organoleptic parameters according to the VCO standard (SNI-7381-2008), even after several months of storage.

CONCLUSION

This study isolated three strains of lactic acid bacteria (LAB) from *blondo* VCO, labelled as BAL 1, BAL 2, BAL 3. These LAB exhibited characteristic morphologies: small to medium-sized, white and cream in color, with round convex shapes and edges. The addition of these LAB starters significantly optimizing VCO yields to 35-46.65%, compared to the control VCO yield of 29.82%. LAB-treated VCO maintained moisture content within acceptable standards and was preferred by respondents in terms of taste, aroma, and texture. These findings confirm the potential of LAB, particularly BAL 2, to improve both the efficiency and sensory quality of VCO extraction. Further investigation is required to develop these LAB isolates into user-friendly starters.

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