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# Commercial Probiotic Drink and Sumbawa Horse Milk as Lactic Acid Bacteria Source for Virgin Coconut Oil Extraction

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**Abstract** – Virgin coconut oil (VCO) can be produced without heat, without any alteration, requires minimal investments and energy during production, and has been reported to have many health benefits. We produced VCO with the addition of lactic acid bacteria (LAB) sources with combined method of chilling-thawing and fermentation. The LAB sources used were Sumbawa horse milk (SKL) and commercial probiotic milk (SPK). This study used a completely randomized design (CRD) using both LAB sources which were added to coconut milk, respectively 10 ml, 12.5 ml and 15 ml and compared with control or no treatment. LAB were also isolated from *blondo* and VCO using LAB specific medium, MRSA. The extracted VCO has the characteristics of clear color with a distinctive coconut smell and long shelf-life. Three LAB isolates were obtained, VCO1-3. VCO1 was found in all samples, with colony morphology of circular and entire margin, convex surface, small in size, and white in color. SKL10, SKL15 and SPK10 showed significant difference to the control, with VCO yield of 31.49-33.05 %, significantly higher than that of without LAB treatment. Both LAB sources of single (SPK) and mixed inoculums (SKL) showed higher yields, indicated their potential as starter for VCO extraction.

**Keywords** - Virgin Coconut Oil, VCO, Chilling-Thawing Extraction, Fermentation Extraction, Cold Pressed Oil, Lactic Acid Bacteria.

## INTRODUCTION

Indonesian coconut oil has the highest competitiveness in the global market based on stable export volume and supply [1]. [2], [3] predicted that in 2045, coconut-based product global demand will grow, this included coconut trees and the fruit products. [2] revealed that in Indonesia, coconut fruits are mainly produced as copra and Crude Coconut Oil (CNO) including virgin coconut oil (VCO). With Indonesia currently holding the title of the world's largest coconut producer [1], [3], it should naturally reflect the highest coconut oil production. However, this was not the case, because that large production was mostly consumed nationally for various purposes [1], [2]. This highlights Indonesia's capacity to emerge as a significant player in the global market for coconut oil and its derivatives, provided that processing technology continues to advance.

Sumbawa Regency is one of the largest coconut producing areas in West Nusa Tenggara Province, where 5.33% of coconut production was increasing since 2016 to 48,45 thousand tons in 2021 [4]. Just like many areas in Indonesia, coconut meat in Sumbawa Regency is mainly for food ingredients. This regency also used coconut in the production of medicinal coconut oil, that is commonly called, *Minyak Sumbawa* or Sumbawa Oil [5]. This traditional oil is produced by cooking coconut milk with spices and herbs until oil is formed. Many of coconut oil are produced by heating (hot extraction), to evaporate the water and break the protein that bind water and the oil [6]. High temperature extraction may impact the quality of coconut oil, causes changes physicochemical properties of coconut oil [7]. Moreover, this method causes the oil to be more brownish yellow in color and smell rancid quickly, and has higher energy requirements [6]. Some researches considered hot extracted coconut oil

without chemical interference is still VCO [8], [9]. But, low heat extraction methods can also be used, with the temperature of 60 °C according to SNI 7381:2008 and [10], 50 °C [10], [11], 30-40 °C [9], or without heat or at room temperature [9]. Whereas, in this study, we refer VCO only to the cold pressed extraction without pre- or post-treatment of heat and chemicals.

Several cold pressed methods are fermentation, enzymatic extraction, chilling-thawing [12]–[14] and physical extraction (centrifugation) [10], [14] and salting [15]. In terms of production, these methods are superior to others, primarily because they involve cost-effective processes with fewer preparation steps and minimal energy requirements [6]. In terms of quality, VCO is found to have the lowest unsaturated fatty acids but higher in saturated fatty acids compare other vegetable oils [7], [16]. Therefore, VCO has been reported to have many benefits, such as antiviral [17], antibacterial [17]–[22] and antiprotozoal properties [6], as adjuvant treatment for COVID-19 [23], antidiabetic [24] and other beauty and health benefits [25]–[27]. VCO supplementation in diet can also treat and prevent obesity, because of its medium chain fatty acids (MCFA) and lauric acid content [12], [28], [29].

The coconut milk conversion into VCO can be started naturally with its indigenous lactic acid bacteria (LAB) [30]. LAB in VCO extraction carry out a microstructural degradation process on proteins that bind oil, so that the oil will be separated from water [13]. LAB can produce lactic acid which can increase antimicrobial activity and lauric acid content in VCO [30], [31]. LAB also contributes to its distinct smell [32].

Through preliminary research, we found that adding commercial probiotic milk can increase VCO production. This result is in accordance with [33] that concluded *Lactobacillus casei* Shirota strain from the commercial milk, produced 11 % higher yield of VCO than non-LAB. Additionally, a potential LAB source for VCO extraction is Sumbawa horse milk. Sumbawa horse milk is mainly consumed for its health benefits, not only because it is known to have lower fat content but also because of its high LAB content. Sumbawa horse milk contained LAB species from Genus *Enterococcus*, *Lactococcus* [34] and also *Weissella* and *Lactobacillus* [35]. The quality and quantity of VCO extraction were found to be improved by the addition of LAB from the genus *Lactobacillus* [33], [36]. The same result is also reported for *L. casei* in

commercial probiotic drinks [13], [33], [37]. Therefore, in this study, we explored LAB sources of single inoculum of LAB in commercial probiotic milk and a mixed inoculum in Sumbawa horse milk to produce VCO as a diversification of coconut products in Sumbawa Regency.

## METHOD

This research was carried out at the Microbiology Laboratory, Faculty of Biotechnology, Sumbawa University of Technology, Sumbawa Regency, West Nusa Tenggara. Grated coconuts obtained from local market, Seketeng Market, Sumbawa. Other materials are Sumbawa horse milk (SKL), commercial probiotic milk/Yakult (SPK), distilled water, De Man, Rogosa and Sharpe Agar (MRSA), Nutrient Agar (NA).

### VCO Extraction Using LAB sources

Coconut milk is made by mixing water with grated coconut in a 7/10 w/v. The milk was manually extracted with its coconut water and topped up with water. For each treatment, 500 ml of coconut milk was prepared with varying concentrations. Rahmadi et al [33] found that the optimal concentration for VCO extraction in fermentation methods was 2 % of LAB inoculum. In this study, the variation of 2 % of 500 ml coconut milk, including 10 ml, 12.5 ml and 15 ml, as shown in Table 1. The LAB sources used are SPK and SKL. SKL used in this study was milked in the morning and stored in the refrigerator before application as well as SPK that contained single species inoculum of *L. casei* Shirota strain. Treatment concentrations can be seen in Table 1.

Table 1. Treatment of LAB addition

LAB Treatment	Treatment codes	
Sumbawa Wild Horse Milk (SKL)	10 mL	SKL10
	12.5 mL	SKL12.5
	15 mL	SKL15
Commercial Probiotics (SPK)	10 mL	SPK10
	12.5 mL	SPK12.5
	15 mL	SPK15
Control	No LAB	K

The method use in this study is a modified method of [14], that combining chilling-thawing followed by LAB fermentation. Commercial milk (SPK) and Sumbawa horse milk (SKL) were added to coconut milk according to their respective concentrations (Table 1). The mixtures were transferred into each treatment jars, stirred evenly and incubated for 2 hours. The coconut cream on the top called *kanil*, the

middle layer was mostly protein or *blondo*, and the bottom layer was water [15]. Treatment jars were then refrigerated (at ~4-5 °C) for 24 hours. The solids formed were mostly *kanil* and *blondo* were separated and weighted. Then, placed onto filter clothed container for 24 hours at room temperature to thaw and ferment. Each extracted oil from each treatment was then separated carefully from the remaining water using a plastic pipette and put into a bottle and weighted. Since every 500 mL coconut milk would produce different weights of solids, the extracted oils were compared to the solids in percentage.

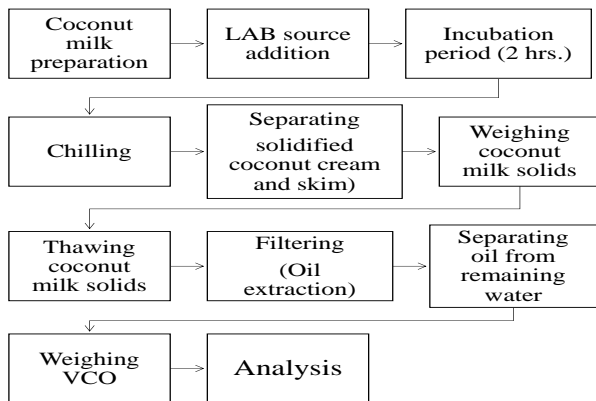


Figure 1. Overall process of coconut extraction with the addition of Lactic Acid Bacteria sources

Each treatment was replicated three times. Observational data were analyzed using One Way ANOVA with 5% Alpha and followed by Tukey’s honestly significant differences (HSD) method.

### Isolation of LAB from Residual Coconut Milk Solids

The remaining coconut solids after filtration that are mostly *blondo*, were sampled from treatments with the highest oil yield. 1 gram of *blondo* were diluted in series of 10<sup>-6</sup> and dilution of 10<sup>-4</sup>-10<sup>-6</sup> were plated on to De Man Rogosa Sharpe Agar (MRSA). The oil from the highest yield was also inoculated onto MRSA. Bacterial colonies were counted and observed for their colony morphology on MRSA after 48 hours incubation at 35° C.

## RESULTS AND DISCUSSION

### VCO Extraction Using LAB Sumbawa Horse Milk and Commercial Probiotic Milk

The VCO yields by commercial probiotic milk (SPK) and Sumbawa horse milk (SKL) treatments, have the following characteristics:

Table 2. VCO Average Yields and Characteristics

Treatment	Color	Scent	Storage time (Since extracted)	*Average Yields (%VCO/coconut milk solids)
SKL10	Clear	Coconut scent	> 6 months	31.50 ± 0.0205 <sup>c</sup>
SKL12.5	Clear	Coconut scent	> 6 months	29.46 ± 0.0165 <sup>b</sup>
SKL15	Clear	Coconut scent	> 6 months	33.05 ± 0.0278 <sup>c</sup>
SPK10	Clear	Coconut scent	> 6 months	31.49 ± 0.0242 <sup>c</sup>
SPK12.5	Clear	Coconut scent	> 6 months	30.71 ± 0.0370 <sup>b</sup>
SPK15	Clear	Coconut scent	> 6 months	28.65 ± 0.0143 <sup>b</sup>
K	Clear	Coconut scent	> 6 months	24.38 ± 0.0121 <sup>a</sup>

\*Note: Different letters in each column indicate statistically significant difference by the Tukey’s test (p<0.05).

Table 2 showed that all treatments had higher percentage VCO yields after chilling-thawing and fermentation methods than that of without the addition of LAB source. ANOVA test shows that the treatments have significant correlation to VCO yields with p value of 0.008611645 (p < 0.05). Whereas, Table 2 also showed that addition of 10 and 15 mL of SKL and 10 mL of SPK were significantly higher than control (SD) and that there is no significant difference to other group of treatments.

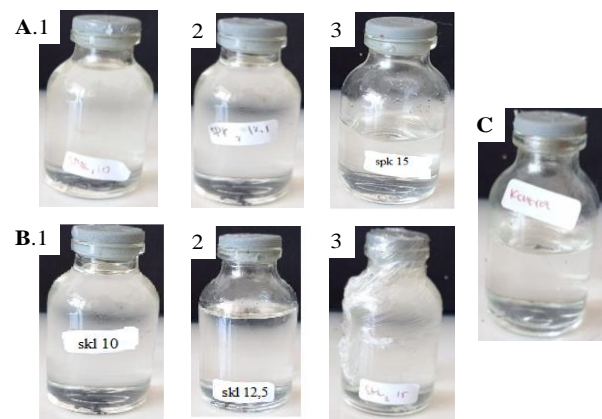


Figure 2. Virgin Coconut Oil (VCO) physical yield for treatment: A. Commercial Probiotic Milk (SPK) at concentration of A.1 10 mL, A.2 12.5 ml and A.3 15 mL, B. Sumbawa Wild Horse Milk (SKL) at concentration of B.1 10 mL, B.2 12.5 ml and B.3 15 mL and C. Control (K) or without treatment.

Our result showed characteristics of soft tasteless oil with a distinct coconut smell (Table 2) and clear color as shown in Figure 2. These organoleptic characteristics are in accordance with Indonesian National Standard (SNI 7381:2008) and many VCO researches ([6], [8], [10], [14], [21], [24], [30], [32],

[33], [36]–[42]. All treatments also showed no physical changes even after 6 months from their extraction (Table 2). According to Azevedo et al [21], VCO is not easily rancid because of its high saturated fatty acid content, so that the oxidation process is slower, and has a shelf life of more than 12 months. Villarino et al [32] indicated that LAB activity is responsible for its initial nutty, natural coconut smell of VCO that was extracted through fermentation methods. Yet, this activity can cause acidic or pungent smell of VCO, due to acetic acid accumulation with time and temperature during storage. Unfortunately, in their study, other physical changes were not studied. Whereas, Rahmadi et al [33] and Andrianto et al [39] also reported LAB extracted VCO found to have acidic to rancid coconut smells and yellowish color. We also observed slightly acidic smell in 15 mL LAB treatment in both SPK and SKL at the initial extraction, but return normal VCO smell during storage in room temperature storage. Villarino et al [32] suggested that storing VCO at lower temperature (<35 °C) might preserved its distinct aroma, whereas Mulyadi et al [43] suggested that 30 °C potentially preserves VCO quality.

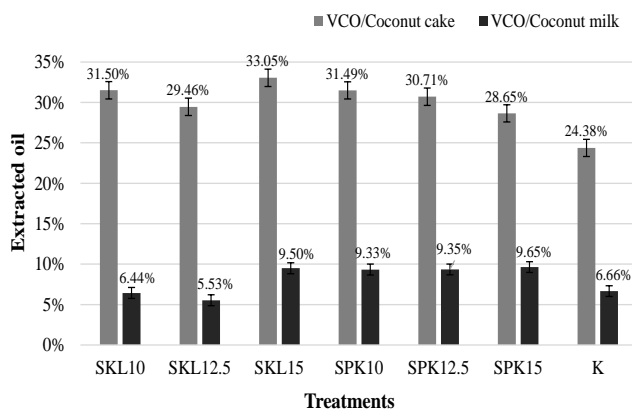


Figure 3. Average of extracted virgin coconut oil (%) of from coconut milk solids (in gray) and of 500 mL of coconut milk (in black).

Raghavendra et al [14] found that combined treatment of enzymatic and chilling-thawing method is the most effective destabilization of protein that bound water and oil that reflected in the highest oil yields compared to other single method of extraction. Our result showed lower results compare to that of [14], when compared with 500 mL coconut milk (Figure 3). This might be due to manual extraction and the distribution of coconut cream, as compared to mechanical extraction with centrifugation [14], [41]. Comparing the extracted coconut oil to the refrigerated solids may be a more suitable method for describing VCO yields.

The addition of 15 mL SKL were the highest yields of  $33.05 \pm 0.0278$  % (Table 2; Figure 3). Whereas control had the lowest yield of 24.38 % compared to the LAB treatment. Rahmadi et al [33] reported that 2% precultured *L. casei* from the same SPK produced 34.5% yield out of the total mixture used. Whereas, Arisanti et al [42] obtained 10.5-13.3 % yield out of the coconut cream with fermentation method by adding dry cultures of LAB. In this study, LAB treatment produced on average 6.43% higher yield compared to no treatment. Addition of 2 % or 10 mL LAB treatment with these combined methods can produce roughly 31.50% VCO of solids. Whereas, Raghavendra et al [14] recovered up to 94.1% or approximately 36.75 % VCO. This indicates that the addition of 2% LAB (SPK/SKL) using combination method is effective in increasing VCO yields, although it requires an improved oil recovery method.

Coconut oil in mature coconut contains about 20.86-44.01 %, meaning 1 L of coconut milk may produce roughly 200-440 mL oil [13]. Thus, several aspects need to be taken in to account in VCO production, such as coconut variety, coconut harvest age, method of milk extraction, the fat content, method of water-oil disruption (oil extraction) [7], [13], [25] and especially the final process of oil-water separation [44]. Based on our observation, repeating chilling-thawing post extraction as recovery method without centrifugation, can maximize VCO yields. This combined method, Chilling-thawing and fermentation methods, potentially produce higher yield and may increase VCO quality [14], [45]. However further study is required to assess the physicochemical quality of the extracted VCO.

### Lactic Acid Bacterial Isolates

Table 3. Isolation of Lactic Acid Bacteria from *blondo* samples.

Sample code	No. of BAL (CFU/mL)	Colony Morphology					Isolate code
		Shape	Margin	Elevation	Size	Color	
SPK12.5	$6 \times 10^4$	Circular (Cir.)	Entire (Ent.)	Convex (Con.)	Small (S)	White	VCO1
SKL15	$4.4 \times 10^4$	Cir.	Ent.	Con.	S	Cream	VCO2
		Cir.	Ent.	Con.	S	White	VCO1
K	<25	Cir.	Ent.	Con.	S	White	VCO1
VCO of SKL15	<25	Cir.	Ent.	Con.	Medium	White	VCO3
		Cir.	Ent.	Con.	S	White	VCO1

The highest VCO yield were used as samples for LAB isolation. *Blondo* from SPK12.5 and SKL15 and the oil from control (K) and SKL15 were use for LAB isolation. Overall, the colony morphology of

LAB was similar, as shown in Table 3. VCO1 were found in all samples with colony morphology of circular shape, entire margin, convex elevation and white in color, as shown in Figure 4.

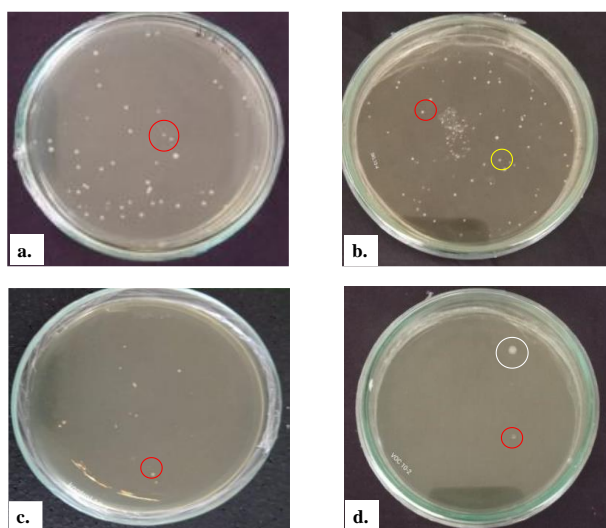


Figure 4. Isolated lactic acid bacteria from *blondo* of **a.** SPK12.5, **b.** SKL15, and oil from **c.** Control (K) and **d.** VCO of SKL15. Circle indicates isolate morphology: VCO1 (red), VCO2 (yellow) and VCO3 (white).

Isolates were differentiated by their colony morphology as shown in Figure 4. As observed *blondo* samples from LAB treatment, SPK12.5 and SKL15, has the highest growth, compared to control and the VCO of SKL15. Higher LAB content in press cake (*blondo*) samples of SPK and SKL might indicate higher rate of breaking down of oil-water emulsion. Whereas in control (K), isolated colonies, VCO1, might be the indigenous LAB present in the coconut milk and coconut water. In natural fermentation, VCO is extracted by its natural microbes that started with *Leuconostoc* genus and continued by other LAB from genus *Weissella*, *Enterococcus*, *Lactococcus* *Streptococcus*, and *Lactobacillus* [46]. Suryani et al [30] reported that *L. plantarum* and *L. paracasei* are responsible for the natural fermentation and were found in fermentation extracted VCO. In relation with the yield, the mixed LAB inoculum (SKL) has slightly higher overall yield compared to single LAB inoculum (SPK/*L. casei* Shirota strain). Asiah et al [44] found that mixed culture in the fermentation method met the criteria of available standards for VCO (SNI and APCC), unfortunately yield was not revealed in their study. We observed that improvement of VCO quality and quantity is possible by adjusting the right combination of LAB during oil extraction. This indicated that there is potential in developing VCO starter from LAB especially from Sumbawa horse

milk (SKL) for coconut product diversification in Sumbawa.

## CONCLUSION

LAB treatment of SPK and SKL showed significant higher yield compared to control (no treatment) with combined extraction method, Chilling-thawing and fermentation. Results showed that both LAB sources were potentially be used as VCO starter. Three isolates were also obtained, namely VCO1, VCO2 and VCO3. These isolates need be further studied to develop VCO starter to produce higher quality VCO.

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