

Physicochemical Characteristics and Bioactivity of *Lactobacillus casei* Fermented Coconut Oil

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Abstract. Virgin coconut oil from fermented lactic acid bacteria (VCO-LAB) was said to be oil with good characteristic of physicochemical and bioactivity among other coconut oils, but it has not been scientifically proven. The aimed of this study was to compare the characteristic of physicochemical and bioactivity of heavy metal chelating capacity and antibacterial activity on VCO-LAB with centrifugation (commercial VCO) and heating (traditional oil). Based on the physicochemical test, VCO-LAB has scent like a rancid coconut and there was no significant difference between VCO-LAB with commercial VCO and traditional oil. VCO-LAB has the weakest of heavy metal chelating capacity with value 49.87%. VCO-LAB has moderate antibacterial activity against *Escherichia coli* and *Stapylococcus aureus* with antibacterial activity 28.03% and 40.76% compared *o*-chloramphenicol as positive control.

Keywords: virgin coconut oil, *Lactobacillus casei*, fermentation, antibacterial activity

1. Introduction

Coconut (*Cocos nucifera* L.) is an economically important plant, it provides nutrition for human and become raw materials in several industries. Coconut can be found widely in tropical regions of the world, include Indonesia, that becomes world largest coconut producers with 19.1 million tons coconuts in 2014 [1]. Indonesia has 3.712 billion acre or 34% of world coconut plantation area [2]. Coconut can be made into various products, such as copra, *nata de coco*, and coconut oil.

It is common for the people in Indonesia to prepared coconut oil by heating process traditionally. Traditional coconut oil was made by dry rendering extraction process, the resulting oil will have low quality of physicochemical characteristics due to the 100 °C high temperature heating process [3]. The quality of coconut oil might be increased by converting it into virgin coconut oil (VCO), VCO is a processed coconut oil with better quality that can be used as high grade cooking oil or as antimicrobials agents [4].

Virgin coconut oil can be prepared through heating, fermentation, and enzymatic processes. The applied methods will correlate to the physicochemical characteristics and bioactivity of the oil. Centrifugation method will give a VCO with good physicochemical characteristics and long storability [5]. Fermentation method works by interrupting water-oil emulsion in coconut milk, the advantages of fermentation method are simple, low fuel usage, less by product residue, low rancidity with long storability, and fragrant aroma [6]. The process is possible by the use of microbes with proteolytic, amyolytic, and lipolytic activities [7]



Lactic acid bacteria (LAB) are able to induce the separation process of oil and water in an emulsion by interrupting the oil-water microstructure [8]. The advantages of VCO from lactic acid bacteria fermentation (VCO-LAB) are low saponification value, low peroxide value, low free fatty acid, and high antibacterial activity [9]. Lactic acid bacteria that have been used are *Lactobacillus plantarum* and *Lactobacillus casei*.

Rahmadi *et al.* [10] has produce VCO LAB from several lactic acid bacteria and showed the antibacterial activities of the oil, however they did not analyzed the physicochemical characteristics and did not compare the value with the coconut oil before treatment. The research objectives were to produce and analyze VCO-LAB by *Lactobacillus casei* fermentation as well as compare it with the base coconut oil and commercial VCO that was produce by centrifugation method.

2. Materials and methods

2.1. Materials

The samples on this research were coconut oil that was produced traditionally by heating process (UKM Healthy Project, Bekasi, Indonesia), VCO-LAB from the coconut oil that was fermented using *Lactobacillus casei* from Yakult, and commercial VCO from centrifugation process. Bacterial media for the research include deMann Rogosa Sharpe Agar (MRSA), deMann Rogosa Sharpe Broth (MRSB), Nutrient Agar (NA) and Nutrient Broth (NB). Treatment bacteria were *Escherichia coli* and *Staphylococcus aureus*. Other chemicals were CaCO₃, skim milk, ethanol, iodine, safranine, NaOH, HCl, KOH, phenolphthalein indicator, starch, Hanus reagents, KI, chloroform, Na₂S₂O₃, fluoroglucinol, acetic acid, Pb(NO₃)₂, HNO₃, H₂SO₄, chloramphenicol, zeolit, pepton, yeast extract, and bromine.

Instrumentations used in the research included light microscope, autoclave, laminar airflow cabinet, incubator, UVVis spectrophotometer, atomic absorption spectrophotometer (AAS) and other glassware.

2.2. Production of virgin coconut oil by lactic acid bacteria fermentation (VCO-LAB)

2.2.1. *Preparation of Lactobacillus casei* [10]. *Lactobacillus casei* was growth on solid MRSA medium contain CaCO₃, incubation was done at 37 °C for 24 hours. The bacteria were then kept in 5 °C prior to usage.

2.2.2. *Optical density measurements* [11]. One percent of *L. casei* culture was inoculated in 30 mL MRSB and incubated at 37 °C for 30 hours. Optical density (OD) measurements were done every 3 hours from hour 0 to hour 30 using spectrophotometer at 441 nm.

2.2.3. *Regeneration of Lactobacillus casei* [10]. Small portion of *L. casei* was inoculated in 10 mL MRSB and incubated in 37 °C for 18 hours. One millilitre of the isolate was then moved into 200 mL skim milk medium contain 10% skim milk, 1% pepton, 2% glucose, and 2% yeast extract. The medium was then incubated in 37 °C for 48 hours. The resulting medium was used for VCO –LAB fermentation process.

2.2.4. *Production of virgin coconut oil by fermentation* [10]. Coconuts were grated and soaked in warm 50 °C water (1:2) for 30 minutes. Grated coconuts were filtered and the coconut milk was collected. Coconut milk was incubated in room temperature until oil-water emulsion was separated. Oily part of the emulsion is collected and 2% *L. casei* in skim milk was added. The solution was sealed and incubated at 37 °C. VCO-LAB was collected 24 hours later from the upper part of the solution.

2.3. Physicochemical Test

2.3.1. *Organoleptic test* [12]. Organoleptic tests were included color, taste, and odor.

2.3.2. Total acid number and free fatty acid percentage [13]. Five grams of oil were mixed with 100 mL ethanol 95% and 2 mL phenolphthalein indicator was added. Solution was shaken well and titration was done by the addition of 0.1 N NaOH.

$$TAN = \frac{V \times N \times 40}{W}$$

$$FFA = \frac{V \times N \times MW}{1000 \times W} \times 100\%$$

where TAN is total acid number, V is NaOH volume (mL), N is NaOH concentration (N), W is sample weight (g), FFA is percentage of free fatty acid (%) and MW is molecular weight of the free fatty acid.

2.3.3. Saponification value [14]. One milligram of oil sample was mixed with 12.5 mL 4% KOH in ethanol and incubated at 70 °C for 30 minutes. Solution was cooled and 100 µL phenolphthalein was added. Titration was done by the addition of 0.5 N HCl.

$$SV = \frac{56.1 \times N \times (V_{blank} - V_{sample})}{W}$$

where SV is saponification value, N is HCl concentration (N), V is HCl volume (mL), and W is sample weight (g).

2.3.4. Iodine value [13]. Oil sample of 125 mg was mixed with 5 mL chloroform and 5 mL Hanus reagents. Solution was incubated in dark cabinet. Five millilitres of 15% KI and 50 mL water was added after 30 minutes. Titration was done by the addition of 0.1 N Na₂S₂O₃.

$$IV = \frac{V_{blank} - V_{sample}}{W} \times N \times 12.69$$

where IV is the number of iodine (g) for addition of 100 g sample, V is Na₂S₂O₃ volume, W is sample weight (g), and N is Na₂S₂O₃ concentration (N).

2.3.5. Peroxide value [13]. Oil sample of 125 mg was mixed with 15 mL CH₃COOH-CHCl₃ (1:1) and 250 µL saturated KI solution and kept for 1 minute. Fifteen millilitres of water was added and titration was done using 0.1 N Na₂S₂O₃.

$$PV = \frac{V \times N \times 8}{W}$$

where PV is peroxide value, N is Na₂S₂O₃ concentration (N), and W is sample weight (g).

2.3.6. Surface tension test [13]. Oil sample was poured in a Perti disk and surface tension was measured using tensiometer.

2.3.7. Rancidity test [15]. Five millilitres oil was mixed with 5 mL of saturated HCl and 50 mg CaCO₃. Solution was put in a sealed Erlenmeyer flask and a filter paper that has been saturated with fluoroglucinol was laid in the upper part of the flask. Incubation was carried out for 20 minutes and rancidity was observed by the colour change of the paper into pink colour.

2.3.8. Potassium content measurements [16]. Five millilitres of oil was mixed with 2.5 mL saturated H₂SO₄ and 5 mL saturated HNO₃ and incubated for 1 hour. Solution was heated until the color faded

away and filtered. Fifty millilitres of water was added and absorbance was measured using AAS at 766.5 nm wavelength.

2.3.9. Heavy metal chelating activity [17]. One mL sample was dissolved in 50 mL of 50 ppm Pb solution and incubated for 30 minutes. Solution was filtered and the soluble Pb was measured using AAS at 283.3 nm wavelengths. Zeolit was used as a control.

2.4. Antibacterial Activity [18]

The assay used *E. coli* and *S. aureus*. Five hundred microliters of bacterial suspension (10^6 cells/mL) was poured in Petri disk contain NA medium. Disk filter paper was dipping in samples or chloramphenicol 10 mg/mL as a control until saturated. Incubation was carried out in 37 °C for 24 hours and antibacterial activity was measured as the clear area surrounding the disk filter paper.

2.5. Data Analysis

The data in this research were done in triplicate. Data were analyzed using one way analysis of variance with Duncan multiple range test ($p < 0.05$).

3. Results

We have produced VCO by fermentation using *Lactobacillus casei* (VCO-LAB) in this research. Prior to VCO production, we observed the growth rate of *L. casei* from its optical density (figure 1). The data showed that *L. casei* has a short adaptation phase and the optimum growth rate was observed until hour 18th at the end of log phase.

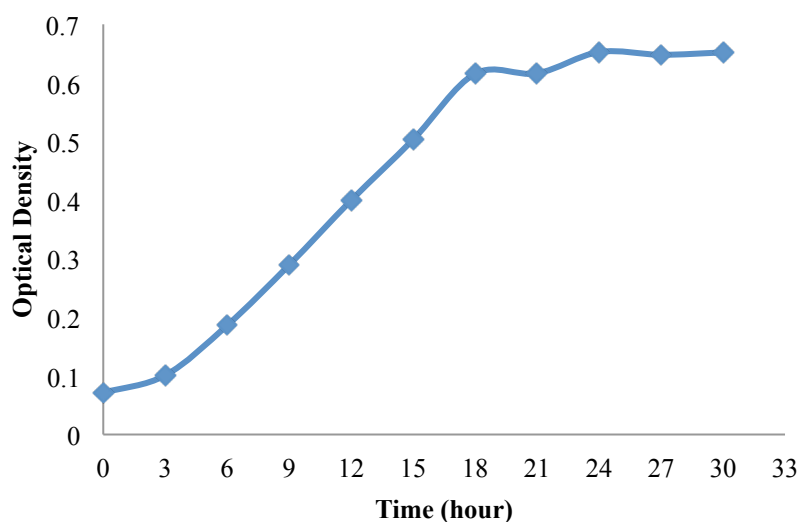


Figure 1. Growth rate *Lactobacillus casei*, lag phase is hour 0-3, log phase is hour 3-18, and stationary phase starts from hour 18th

Our VCO production yield is $17.27 \pm 4.51\%$ from the coconut milk raw material. We use coconut oil that was prepared traditionally as negative control and commercial VCO as positive control. Based on organoleptic observation, our VCO-LAB has similar color and taste to the commercial VCO, but the smell is rancid (table 1). However, based on rancidity test, there are no sample that is rancid include the VCO-LAB sample (table 2). VCO-LAB showed the highest antibacterial activity against *S. aureus* compared to other oil preparation (table 3).

Table 1. Organoleptic observation of traditional coconut oil, commercial virgin coconut oil (VCO), and virgin coconut oil by *L. casei* fermentation (VCO-LAB).

Samples	Color	Taste	Aroma
Traditional coconut oil	Clear yellow	Tasteless	Coconut
Commercial VCO	Clear	Tasteless	Coconut
VCO-LAB	Clear	Tasteless	Rancid coconut

Table 2. Physicochemical properties of traditional coconut oil, commercial virgin coconut oil (VCO), and virgin coconut oil by *L. casei* fermentation (VCO-LAB).

Characteristics	Samples		
	Traditional coconut oil	Commercial VCO	VCO-LAB
Rancidity	Not detected	Not detected	Not detected
Density (g/mL)	0.9178	0.9182	0.9179
Total acid number	1.253±0.19 ^a	1.600±0.00 ^c	1.387±0.09 ^b
Free fatty acid (%)	0.802±0.12 ^a	1.024±0.00 ^c	0.887±0.06 ^b
Saponification value	722.755±20.00 ^a	979.745±20.65 ^b	704.990±7.36 ^a
Iodine value	10.491±5.52 ^b	7.445±0.96 ^a	7.107±2.87 ^a
Peroxide value (mEq)	1.280±0.00 ^a	2.130±0.30 ^b	1.280±0.00 ^a
Surface tension (mN/m)	27±0.55 ^b	30.9±0.48 ^c	20.4±0.48 ^a
Heavy metal chelating activity (%)	80.45	79.30	49.87
Potassium content (µg/mL)	15.50	5.23	5.91

Table 3. Antibacterial activities of traditional coconut oil, commercial virgin coconut oil (VCO), and virgin coconut oil by *L. casei* fermentation (VCO-LAB) against *Escherichia coli* and *Staphylococcus aureus*.

Treatments	Antibacterial Activity (%)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Chloramphenicol	100	100
Traditional coconut oil	25.39	29.88
Commercial VCO	30.09	33.94
VCO-LAB	28.03	40.76

4. Discussion

The density of our VCO-LAB is 0.9179 g/mL (table 2) and it is acceptable in the range of 0.915-0.920 g/mL based on Asia Pacific Coconut Community [19]. Density is one of the parameter to check the purity of oil [12]. This result was better compared to the fermented VCO by Rahmadi *et al.* [10] with the density of 0.84 g/mL for *L. casei* fermented VCO and 0.87-0.89 g/mL for *L. plantarum* fermented VCO. The higher density was caused by the different time in regeneration time of *L. casei*, we use 18 hours or the end of lag phase while Rahmadi *et al.* [10] use 24 hours. At 24 hours, *L. casei* has already enter stationary phase and produce more secondary metabolites so the quality of the resulting VCO will be decreased. Rancid smell might be caused by lactic acid as the sugar fermentation product by *L. casei* [20].

The best value for total acid number and free fatty acid was shown by traditional coconut oil. Rahmadi *et al.* [10] had much lower free fatty acid content of 0.11% for *L. plantarum* fermented VCO and 0.12% for *L. plantarum* fermented VCO, however Che-Man *et al.* [21] had much higher free fatty

acid content of 2.45% for *L. plantarum* fermented VCO. High total acid number and total free fatty acid showed that there were free fatty acids from hydrolyze process of triglyceride in oil [22]. The hydrolysis process was possible because of water contamination in the oil.

Saponification value and iodine value of VCO-LAB was lower compared to the other samples for 704.99 and 7.11. However all samples had saponification value higher than APCC requirements [20]. Saponification number showed the number of ester bond from triglyceride and correlate with the average molecular weight in oil [23]. High saponification value means there are many short chain fatty acid in the triglyceride molecules in our samples.

Iodine value correlates with the number of unsaturated fatty acid in oil samples. Double bond in unsaturated fatty acid has the ability to bind iodine and increase the iodine value [22]. Our VCO-LAB iodine value was lower than traditional coconut oil and commercial VCO, but it was still higher compared to *L. plantarum* fermented VCO that was reported previously by Che Man *et al.* of 4.9 [21].

VCO-LAB and traditional coconut oil showed similarly low peroxide value of 1.28 mEq. This results was much lower than *L. plantarum* fermented VCO that was reported previously by Che Man *et al.* of 58 mEq [21] but higher than Marina *et al.* [24]. The value is a very important characteristic in describing oil quality, high peroxide content indicate the unsaturated fatty acid ion oil has been oxidized and the oil has become rancid [22].

VCO-LAB showed the best surface tension of 20.4 mN/m compared to other oils so it will be able to dissolve many organic compounds. However it has the lowest heavy metal chelating activity compared to other samples.

Potassium needs to be tested because it is often added in traditional coconut oil preparation to reduce free fatty acid content in oil. Table 2 shows that traditional oil had high potassium content and it was reduce about 70% in VCO commercial and our VCO-LAB.

All samples in this research have antibacterial activities with the highest antibacterial activity against *E. coli* was shown by commercial VCO, while highest antibacterial activity against *S. aureus* was shown by our VCO-LAB (table 3). VCO has higher phenolic content up to 3 times of the coconut oil. Many phenolic have been reported to show antibacterial activities against *E. coli* and *S. aureus*. Furthermore, Suryono [25] showed that VCO from fermentation by lactic acid bacteria contains bacteriocin that was able to inhibit the growth of *E. coli*, *S. aureus*, and *Listeria monocytogenes*. Bacteriocin works by making pores in cell membrane and disrupt the exchange of compounds across cell membrane [26]

5. Conclusion

Virgin coconut oil has been able to produce by fermentation from *Lactobacillus casei* (VCO-LAB) with the better quality than previously reported. The VCO-LAB also showed potential antibacterial activity against *Staphylococcus aureus* with inhibition activity 40.76%. However we suggest the detection of other secondary metabolites from *L. casei* in VCO that causing the rancid aroma in our VCO-LAB.

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