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1 Article

A Comparative Study of Virgin Coconut Oil, Coconut 2 Oil and Palm Oil in Terms of their Active Ingredients 3

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17 Abstract: Global petroleum-based fuel reserves are declining, resulting in the necessity to find 18 alternatives, one of which is biofuel derived from sustainable sources. One of such fuels, i.e. 19 biodiesel, can be produced from palm oil and coconut oil, however, another alternative source of 20 fuel is Virgin Coconut Oil (VCO). This research aims to study the unique factors of VCO 21 compared to coconut oil and palm oil to determine whether VCO can be used as biodiesel. 22 Psychochemistry (Iodium umber, peroxide, % FFA) is analyzed using a general method. Lauric 23 acid content was analyzed by the Chromatographic Gas method. Isolation of LAB was conducted 24 by the dilution method using MRSA + 0.5% CaCO3 media. In addition, macromolecular 25 identification was conducted by 16S rRNA. VCO distinguished by more higher content of lauric 26 acid (C12:0) 41% - 54.5% as compared with coconut and palm oils: 0%: 0.11% rerspectively . The 27 VCO also contains LAB, namely Lac.plantarum and Lactobacillus paracasei, can inhibit the growth of 28 pathogenic bacteria, such as P.aeruginase, Kleibsiella, S.aureus, S.epidermidis, Proteus, E.coli, 29 Lis.monocytogenes, B. cereus, and S. typhosa. Compare with VCO is based on having a high content 30 of Lauric Acid, 54%, and LAB content. Nevertheless, based on the content of their water level, free 31 fatty acid, and Iodium number, these three kinds of oil are applicable to be used as biodiesel.

- 32 Keywords: Bacteriocin; Lactic Acid Bacteria (LAB), Lauric Acid; Virgin Coconut Oil (VCO)
- 33

34 1. Introduction

35 Global petroleum reserves have declined sharply in recent years, therefore, a solution being 36 attempted is searching for alternative fuels that are renewable and eco-friendly, such as biodiesels. 37 Commonly, biodiesels are usually produced from plant oils such as kapok (Ceiba pentandra), palm oil 38 (Elaeis), and coconut (Cocos nucifera). The tropical biodiversity has been contributed to many 39 industrial products including pharmaceutical drugs [1-3]. , biofuel and as well as energy storage 40 materials. . Raw materials of biodiesels should fulfill several criteria, such as having a low moisture (water) 41 and free fatty acid contents. High contents of both contribute to the occurence of saponification, meaning that 42 the oil is not suitable as a biodiesel[4].

43 Oils from plants such as Sterculia foetida, Jatropha curcas, Calophyllum inophyllum and Reutealis 44 trisperma have been converted to biodiesel to power internal combustion engines [2-5]. Using

- 2 of 12
- 45 plant-derived energy sources could reduce negative environmental impacts on the environment, 46 especially reducing carbon dioxide emissions to the atmosphere [6, 7]. Several materials from the 47 tropical biodiversity have also been tried for energy storage materials, which can replace batteries in 48 the future [8-10]. These can help store significant amounts of solar energy from tropical countries,
- which usually has plenty of sunlight [11]. Another exceptional plant from the tropical biodiversity
 that has been used for many purposes is the coconut, about which in this research the authors
 attempt to study coconut oil and its active ingredients.

52 Several possible alternatives for biodiesel include the oil of the coconut, normally used for cooking, which 53 originated from heated coconut milk and known as *coconut oil*, and the cooking oil made from the oil of the 54 palm kernel, known as *palm oil* [2-5]. In addition, recently the oil of the coconut processed without heating has 55 been identified as a possible biodiesel source, known as *Virgin Coconut Oil* (VCO) [5–12].

Virgin Coconut Oil (VCO)) can be made through several methods such as by fermenting coconut milk [12] and by adding microbes (*Lactobacillus fermentum* and *Lactobacillus plantarum*) as starter cultures [13-15]. VCO can also be produced through centrifugation and microwave processes [5], and by fermentation without the addition of microbes as a starter [17]. This oil is called virgin oil because it is made without any heating [18]

61 VCO has been used widely because it is believed to have benefits compared to coconut oil made 62 through a heating process and palm oil. VCO is useful against microbes, bacteria and virii [14], and 63 is useful for for helping lose weight in terms of metabolism. VCO contains medium chain 64 triglycerides, which is initially digested or processed in the body from carbohydrates that can cut 65 back hunger. Thus, it causes people to consume less carbohydrates, which eventually reduce body 66 weight [18]. VCO also affects the healing of ovariectomy [19], and can be used as an antioxidant [20]. 67 VCO can also reduce blood pressure [10]. In addition, VCO can also be used for skincare [22], as an 68 external drug such as wound medicine, and can function as a probiotic [23].

69 VCO and palm oil have different characteristics with different function. VCO gravitates more 70 towards medicine, probiotic, and cosmetics, whereas palm oil characteristics quite suitable to be 71 converted to diesel fuel [24]. Not only palm oil, some other vegetable oils have also been proven to 72 be economical and can be converted to biodiesel production in large scale [25-27]. The chemical 73 composition of VCO has been studied in [28] including the iodine number, the saponification 74 number, the amount of free fatty acids (% FFA), viscosity and colour. This chemical composition 75 needs to be examined prior consumption in order to follow Asian and Pacific Coconut Community 76 (APCC) standardisation because VCO is originally made from fresh coconut milk

VCO has been known for its high lauric acid content, which is between 46.36 - 48.42% [28].].
Processed from coconut milk, which is high in carbohydrates and proteins, the fermentation process
of VCO results in lactic acid bacteria (LAB) [29]. LAB from VCO have been isolated and their
antimicrobial ability has also been studied [17]. This atypical microbial ability exists because LAB
contains bacteriocin [30], [31] which can kill pathogenic bacteria. Further, bacteriocin from *Lactobacillus plantarum* [18] has also been isolated as well.

This research compares the psycho-chemistry (such as: acid, saponification, and Iod number) of coconut oil, palm oil, and VCO [3], [24 - 25] as the consideration in determining whether these oils are appropriate to be used as biodiesel. It also analyses the content of fatty acids such as lauric acid, palmitic acid, and stearic acid [20–22], as the indicator of the characteristics of each oil, besides determining the antimicrobial ability of VCO [5], [9][15]. These analysis are performed in order to examine whether VCO is more suitable as a biodiesel or as an oil with multiple function in health sector.

90 The newest fact referring to VCO which has yet acknowledged is the presence of lactic acid
91 bacteria (LAB) on the oil and blondo layers (VCO dregs). This LAB will be present when VCO is
92 made through traditional fermentation process or fermentation using the existing bacteria in the air.
93 Nevertheless, there have been many studies concerning palm oil as an alternative for biodiesel

94 [2-4]. In the meantime, the palm oil biomass and its fatty acid have been studied, besides the95 possibility of the palm oil to be used for dietary purposes [23].

96 2. Materials and Methods

97 **2.1.** *Materials:*

98 The samples were three types of virgin coconut oils, with different methods of 99 extraction/fermentation), coconut oil (derived from heating coconut milk), and consumer-grade 100 palm oil.

101

102 2.1.1. Chemical Material:

103 For Lauric Acid Analysis, -hexane (p.a), CHCl₃, Aquadest, reagent for sample preparation, namely 104 saturated NaCl, Na₂SO₄ anhydrate, BF₃. Methanol, and N₂gas to stop the oxidation occurred were

saturated NaCl, Na₂SO₄ anhydrate, BF₃, Methanol, and N₂gas to stop the oxidation occurred were
 used. The internal of fatty acid standard. Whereas for Acid Number analysis (%FFA), 95%Alcohol

106 (pa), blue bromothymol indicator, phenolphthalein indicator, HCl 0.5 N Standard Solution, and

107 KOH Standard Solution were used. KOH ethanolic, HCl 0.5 N standard solution was taken for

108 Saponification Number Analysis, and Potassium Iodide was used for Peroxide Number Analysis.

109 Meanwhile MRSA (deMann Rogosa Sharpe Agar) p.a + 0.5% CaCO₃ media was used for Isolation of

110 Lactaid Acid Bacteria and in order to increase their amount using MRS media.

111 **2.1.2.** Instruments:

112 The instrument used where ordinary laboratory glassware such as petri dish, Erlenmeyer, test 113 tube, and beaker glass, where all of them made from Pyrex. In addition, Gas Chromatography GC 114 GC-MS Shimadzu QP 2010S (Shimadzu Corp, Kyoto, Japan) was set for lauric acid analysis and

115 Autocklav Yamata SN 21 for sterilization and Laminar Flow as the working media for isolating the

116 bacteria and for performing antimicrobial analysis.

- 117 2.2. The standardization of VCO
- 118 2.2.1. The determination of pH

119 The pH of the sample is determined using the pH meter in accordance with the applicable 120 general procedure.

121 2.2.2. The analysis of fatty acid sample using GC-MS

122Prior to injecting the sample into GC-MS instrument, the oil sample was prepared by setting 50123gram of VCO sample, and adding 400 μ L of NaOH Metanolic . This mixture was vortexed and124heated at 50° C for 10 minutes. After undergoing the cooling process, 1mL CH₃COOH, 1mL distilled125water, and 1mL n-hexane were added respectively. Then this mixture was vortexed and cooled for126several minutes where two layers were formed as the result.

127 About 1 μ L at the top layer was taken as the sample to be injected and analyzed in GC-MS: 128 Shimadzu QP2010 which was equipped with capillary colum of (30m) x 0,25 mm ID; 0,25 im 129 (interspersed by DB5MS. Japan), by injecting the sample into the capillary colum. The carrier gas 130 was helium, where the injector and detector temperature was set at 280° C. The injection was 131 performed using the split mode (1:30). The column temperature was programmed to change from 132 50° C to 280 ° C at a rate of 5° C per minute. Fatty acid ethyl esters were separated at the constant 133 pressure (100kPa), and the peak was identified through the comparison of mass spectrum with mass 134 spectral as the database. The compound identification was as regards to the comparison of its mass 135 spectrum with NIST Mass Spectral Library 2008.

136 2.2.3. The determination of water content

A porcelain dish along with a loose-fitting cover were cleaned, and dried in the dryer oven at 138 105°C for 1 hour. Then they were taken using a pair of clamps and inserted into desiccators where 139 the lid was detached. Soon after the dish has cooled, the lid was tightened and weighed. A 2 gram

140 sample was weighed into the dish and undergone another eight hours's dry-heating in a hot-air

- 141 oven at 105°C, until reaching a constant weight. Another cooling process in desiccators was142 conducted for 30 minutes before determining its water level.
- 143 2.2.4. The determination of acid number

144 5 grams of the sample was weighed into a 300mL Erlenmeyer flask, then added 25 mL neutral 145 alcohol, after that the flask was connected to an upright condenser, and boiled for 30 minutes. After 146 cooling down, the sample was titrated with NaOH 0.1 M using pp indicator. The volume of NaOH 147 titrer was recorded.

148 2.2.5. The determination of Iod number

0.5 grams of oil was weighted in a covered Erlenmeyer flask, then 10 mL chloroform was added
and shaken. Then 15 mL Wij's solution was added and shaken slowly for another 30 minutes where
the Erlenmeyer continued to be covered. The lid and inner wall of Erlenmeyer have been washed
with 50 mL distilled water (initially heated and cooled). Next step was titration with 0,1 N Tio
(Na₂S₂O₃) until the color changed into light brown, and 2mL of 1% starch was used as indicator. The
titration was continuously conducted until the dark blue color disappeared.

155 2.2.6. The isolation of lactic acid bacteria

156 The oil sample was diluted utilizing saline solution with dilution up to 10^{-7} . Then this sample 157 was planted on the dish containing MRSA + 0,5 % CaCO₃ selective media, and incubated overnight 158 at 37°C. The growth was observed.

159 2.2.7. The molecular identification

Initially, the identification is began with isolating genomic DNA of lactic acid bacteria, then,
continued by amplying 16S rRNA gen. In order to determine the size of its DNA, this gen is
analyzed using Electrophoresis gel, and ended with sequencing.

163 2.2.8. Antimicrobial analysis

164 The antimicrobial analysis was carried out using agar-disk method, which has been modified 165 using a disk made from filter paper. It was assumed that 1 unit of antimicrobial activity was defined 166 as 1 mm² inhibited zone area or "halo" zone[23]. It began with testing bacteria grown on NA media 167 (Merck) of 1.5% agar concentration at 37°C. Incubated overnight, then 1 dose of testing bacteria was 168 moved into test tubes containing 10 mL sterile distilled water. After that, the cells numbers was 169 conformed to Mc Farland 0.5, which was estimated to be $10^6 - 10^7$ CFU mL⁻¹ number of cells. 170 Toothpicks wrapped with cotton and sterilized were dipped into testing bacteria (more or less 100 171 μ L). Left to dry, then took the filter paper which has been sterilized and perforated like a disk with 172 80 mm diameter, dipped it into LAB isolate, and stuck onto the solid media surface in petri dish 173 which has been smeared by testing bacteria. Incubated for the period of 3 x 24 hours, and observed 174 until the clear zone or "halo" zone was formed indicating the occurence of the growth inhibition of

- 175 pathogenic bacteria by lactic acid bacteria. Finally the diameter of clear zone is measured.
- 176 3. Results and Discussion
- 177 3.1. Composistion and properties VCO, coconut milk, and palm oil.

According to the experimental study presented in previous sections, it is shown that the peculiarity of Virgin Coconut Oil compared to coconut oil produced by heating coconut milk and palm oil sold in the market is in its high composition of lauric acid (54.06%), and lactic acid bacteria (Lactobacillus plantarum and Lactobacillus paracasei). In the presence of lactic acid bacteria containing bacteriocin, VCO is characterized as a antimicrobial, in contrast to coconut and palm oils. As shown in Table 1 below, the lauric acid content of VCO is the highest with 54.06%. In

184 contrast, coconut oil contains only 2.81%, and palm oil none (0%). Table 1 shows that there are two

210

185 types of fatty acid contents in VCO; 54.06% lauric acid and 12.06% stearic acid, and none (0%) 186 palmitic acid. The absence of palmitic acid is because VCO is not made of palm oil. Palmitic acid is 187 found in palm oil. The lauric acid content of VCO is considered high compared to what has been 188 obtained through this research [12]. This is because VCO is processed without heating, or through 189 fermentation. Thus the fatty acid carbon bonds are not broken, in other words, the fatty acid is in the 190 form of medium chain triglycerides, particularly lauric acid. On the other hand, coconut oil made from 191 cooked coconut milk has low lauric acid content, i.e. 2.81%, containing 2.65% saturated acids, due to the 192 production process involving heating. Conversely, palm oil has absolutely no lauric acid content, whereas its

193 palmitic acid is high; 2.28% compared to VCO and coconut oil.

From Table 1, it can be said that VCO has an acid number of 1.0165 which is higher than coconut milk (acid number of 0.39695), and palm oil (acid number of 0.39645) [13]. VCO water content is similar to palm oil, which is 0.11% whereas coconut oil processed through heating has a lower water content of 0.10%. However, the water content of the three samples still meet the limit standard of cooking oil 2177 SNI 3741 2013. In other words, the smaller number of the three samples' water content simplifies the heating process so the three samples can be used as biodiesels, in accordance with [18][25-27].

The analysis result of % FFA contains of 0.264 VC), 0.281 coconut oil, and 0.51 palm oil. It shows
 that VCO heating process is much better than other oil samples according to the study of [20]

The VCO saponification number is 348.003 whereas coconut milk has a saponification number of 269.6266 and palm oil 204.0045. The high saponification number reflects the number of the fatty acid molecules. The bigger the saponification number, the smaller the molecules, or consisting of smaller fatty acid molecules or shorter chain, and vice versa. Hence, the higher saponification number of the VCO is because it consists of medium chain triglyceride fatty acids [14], [15]. The higher saponification number compared to palm and coconut oils mean that the saponification occuring in VCO is greater, even though still within tolerable limits.

Type of the Oil	Type of Fatty acid	%	Acid number	Saponification number	%FFA	Iodin number	pН	Water conten
								%
VCO(A)	Lauric Acid	54.06	1.0165	348.003	0.264	5.3287	6.5	0,11
	Palmitat Acid	-	-					
	Stearat Acid	12.03	_					
VCO(B)	Lauric Acid	53.9	1.0322	345.705	0,258	5.2431	6.4	0,12
	Palmitat Acid	-	-					
	Stearat Acid	12.01	_					
VCO(C)	Lauric Acid	53.7	1.0274	346,645	0.262	5.2565	6.5	0,11
	Palmitat Acid	-						
	Stearat Acid	11.9						
Coconut	Lauric Acid	2.81	0.39695	269.6266	0.281	7.023	6.9	0.11
oil	Palmitat Acid	2.31	_					
	Stearat Acid	2.65						
Palm Oil	Lauric Acid	0.45	0.39645	204.0045	0.515	51.0042	6.6	0.09

Processes **2019**, *7*, x FOR PEER REVIEW

1	Palmitat Acid	2.88	_					
	Stearat Acid	-	_					
Palm Oil	Lauric Acid	-	0.39645	203.02595	0.733	49.71675	6.5	0.09
2	Palmitat Acid	24.42						
	Stearat Acid	-	-					

211

212 3.2. Result of LactidAcid Bacteria Isolation

Figure 1, below presents the isolated result of lactic acid bacteria onto VCO, coconut oil, and palm oil. It is shown that VCO contains lactic acid bacteria, as when it was being isolated using MRSA + CaCO₃ selective media, the lactic acid bacteria colony which can grow in "Halo" area is found. This area is a clear zone where it can produce lactic acid neutralising CaCO3. It is in line with [5], and as an additional, the results in Figure 2 and 3 points out the identification of lactic acid bacteria using 16S rDNA which turn out to be *Lacobacillus plantarum* and *Lactobacillus paracasei* as mentioned in [6], [7], and [8].



- 220
- 221

Figure 1. Lactid acid bacteria isolate grown in MRS + 0,5% CaCO3 media

It can be seen, in Figure 1, there is a clear area, in the middle there is a white dot which is a colony of lactic acid bacteria present in VCO oil. This is proof that VCO contains lactic acid bacteria [29] [32]

224 bacteria.[29], [32]



Figure 2. Result of lactid acid bacteria isolation from coconut oil and from palm oil with no evidence
 of growth.

Meanwhile, in Figure 2, is a petri dish filled with coconut oil and palm oil grown on the same media. No visible growth of lactic acid bacteria. This proves that lactic acid bacteria do not exist in coconut oil and palm oil. This is in accordance with[9], [14] [29]. But not so [35], which only uses MRSA media, without the addition of CaCO3. But growing colonies are not in the "Hello" area.

- 232 3.3. Molecular Identification of Lactid Acid Bacteria
- The molecular identification of lactic acid bacteria produces Lactobacillus plantarum as shown in the data below Figure 3
 - >CONTIQ_M16.4_1440bp_Lactobacillus plantarum_100% GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGGATA ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTAT CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTC AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGAC STGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCI STAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGG CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTAC SCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA ISGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATC AGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA AGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAAT CGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACCCCCCGTCACACCATGAGAGTTTGTAAC ACCCAAAGTC

235

236

Figure 3. Lactid acid bacteria gene sequence from isolation of Lactobacillus plantarum

1Į -	Aligoments Cowmload - GenBank Graphics Distance tree of results						
	Description	Max score	Total score	Query cover	E value	Ident	Accession
3	Bacterium strain A2 16S ribosomal RNA gene, partial sequence	276	276	100%	3e-71	100%	KX268350.1
	Lactobacillus paracasei strain HD1.7 16S ribosomal RNA gene, partial seguence	276	276	100%	3e-71	100%	EU249147.1
3	Ladobacillus rhamnosus strain L156.4.16S ribosomal RNA gene, partial sequence,	274	274	99%	1e-70	100%	KX644947.1
3	Lactobacillus casei strain LC5, complete genome	274	1372	99%	1e-70	100%	CP017065.1
3	Lactobacillus rhamnosus strain 4B15, complete genome	274	1372	99%	1e-70	100%	CP021426.1
	Lactobacillus paracasei strain IIA, complete genome	274	1372	99%	1e-70	100%	CP014985.1
3	Lactobacillus mamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
3	Lactobacillus rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1
3	Lactobacillus rhamnosus strain BFE5264, complete genome	274	1372	99%	1e-70	100%	CP014201.1
1	Lactobacillus casei strain K1C 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S	274	274	99%	1e-70	100%	KU954559.1
3	Ladobacilius mamnosus strain LRB, complete genome	274	1372	99%	1e-70	100%	CP016823.1
	Ladobacillus mamnosus gene for 16S ribosomal RNA, partial sequence, strain: NGRI 0110	274	274	99%	1e-70	100%	LC177236.1
5	Lactobacillus paracasei strain FS3 16S ribosomal RNA gene, partial seguence	274	274	99%	1e-70	100%	KU315064.1
	Ladobacillus rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

237

238 Figure 4. The result of molecular identification of lactid acid bacteria showing *Lactobacillus paracasei*.

239 3.4. Result of Anti Microbial Analysis.

The antimicrobial ability of VCO is also analyzed below. Antimicrobial analysis was performed
 with Lactobacillus paracasei and Lactobacillus plantarum lactid acid bacteria onto testing bacteria;
 Pseudomonas aeruginase, Klebsiella, Staphilococcus aureus, Staphilococcus epidermidis, Proteus,

243 from [31][35], Eschericia coli, Listeria monocytogenes, Bacillus cereus, and Salmonella typhosa.

As seen in Figure 4 below, clear zones show that the growth of pathogenic bacteria was inhibited by Lactobacillus plantarum and Lactobacilus paracasei.



Result of antimicrobial analysis of *Lactobacillus*`*plantarum* Lactid Acid Bacteria onto *E, coli* testing bacteria

Result of antimicrobial analysis of *Lactobacillus*`*plantarum* Lactid Acid Bacteria onto *S.aureus* testing bacteria

Figure 4. Result Antimicrobial analysis of *Lactobacillus*`*plantarum* Lactid Acid Bacteria onto *E, coli and S. aureustesting* bacteria

As illustrated in Figure 4, VCO has the antimicrobial ability derived from two types of lactic acid bacteria against nine testing bacteria. It is seen that these lactic acid bacteria have a good ability to kill pathogenic bacteria; *Listeria monocytogenes*, and *E. coli*, as stated in [20], where the antimicrobial ability of *Lacobacillus plantarum* is found to be most effective against *Listeria monocytogenes*, *E.coli* and *Bacillus subtilis* testing bacteria.

252

246

Table 2. Antimicrobial activity analysis of LAB in the form of clear zone diameter (mm).

No	Testing Bacteria	Lactobacillus	Lactobacillus
INO.		plantarum	paracasei
1.	Escherichia coli	16	16
2.	ListeriaMonocitogenes	17	18
3.	BacillusSubstiliss	15	11
4.	Salmonella Typhyphosa	12	11
5.	StaphillococcusAureus	11	11
6.	Pseudomonas aeruginase	17	14
7.	Klebsiella	13	12
8.	Staphilococcus epidermidis	13	12
9.	Proteus,	14	13

253

In Table 2 it can be seen that the lactic acid bacteria present in the VCO are able to inhibit the
 growth of pathogenic bacteria as *StaphillococcusAureus*, *Pseudomonas aeruginase*, *Klebsiella*,
 Staphilococcus epidermidis, and *Proteus*, in accordance with[31]

257 4. Conclusions

258 Compared to coconut oil and palm oil, VCO has a higher content of Lauric Acid and lactic acid 259 bacteria. Nevertheless, based on the content of water, free fatty acid, and Iodine number, all three 260 kinds of oil are applicable to be used as biodiesels. VCO has other features in the field of health

- 261 because it has lactic acid bacteria that can kill pathogens, characterizing it as a antimicrobial, in
- 262 contrast to coconut and palm oil. The lauric acid content of the VCO is the highest (54.06%)
- 263 compared to the lauric acid content of coconut oil (0.45%), and palm oil (0%). Having a high acid
- 264 number; 1.10165, and high saponification number and Iodine number demonstrate the characteristic
- 265 of VCO, as it contains lauric acid with small molecules which are medium chain triglycerides (MCT). 266
- In the future, a more specialized assessment needs to be done, in the economic field to examine 267 whether VCO is more appropriate as a fuel alternative or as an antimicrobial.

268 **Author Contributions**

269 Conceptualization: Suryani Suryani and Sariani Sariani; Resources: Marganof Marganof, and Suryani Suryani, 270 Methodology: Suryani Suryani and Marganof Marganof; Software: Suryani Suryani; Validation: Suryani 271 Suryani, Marganof Marganof; Formal analysis: Suryani Suryani and Marganof Marganof; Writing - original 272 draft preparation: Sariani Sariani, Teuku Meurah Indra Mahlia and Suryani Suryani; Writing - review and 273 editing: Suryani Suryani, Rahmawati, Femi Earnestly, and Sariani Sariani; Project administration: Sevindrajuta, 274 and Rahmawati.

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396

Comments and Suggestions for Authors

Abstract:

Line 22: "LABiawas" - should be separated

Lines 24-25: I suggest to reformulate sentence: "The results obtained show that the content of Lauric Acid is considered high; 41% - 54.5% for VCO, where 0% of coconut oil, and 0. 11% of palm oil."

To:

VCO distinguished by more higher content of lauric acid (C12:0) 41% - 54.5% as compared with coconut and palm oils: 0%; 0.11% respectively

Introduction:

Line 76: The Authors are encouraged to phrase a hypothesis and to state specific major novel contributions reported in their manuscript.

Materials and Methods

Line 85: Please explain what does it mean ? "The internal and external of fatty acid standard."

Authors should add information in the manuscript about used standard, internal or external standard ?

Authors prepared fatty acid methyl esters ?

More information should be added: about preparation sample for chromatographic analysis.

Lines 92-95: There is a lack of information about conditions of chromatographic separation of fatty acids. Figure 1 is not needed, Please delete it.

Results and Discussion

Line 105: the title of subchapter is too long. Please change it to more general:

e.g: Composition and properties of ...

General comments:

There is a lack of statistical analysis, number of samples. In Table 1 statistical analysis is missing. Because of that it is impossible to draw conclusion. Also, please check the grammar or format in the whole manuscript I found some mistakes:

Conclusions: not "...compared to.." but "compared with.."

Major revisions are necessary especially in the presentation. of the manuscript.

For example the word "psycho-chemistry" is used in both the abstract and other parts of the manuscript

The figures' quality is quite poor.

Scientific hypothesis must be more clear, the same applies to the results.

Review Report Form						
English language and style	 (x) Extensive editing o () Moderate English o () English language a () I don't feel qualified 	f Englis changes nd styl to judg	h language required e are fine/r e about th	e and style ninor spell e English l	e required check required anguage and style	3
		Yes	Can be improved	Must be improved	Not applicable	
Does the introd background and include	luction provide sufficient all relevant references?	()	()	(x)	()	
Is the research design appropriate?		()	(x)	()	()	
Are the methods adequately described?		()	(x)	()	()	
Are the results clearly presented?		()	()	(x)	()	
Are the conclusions su	pported by the results?	()	(x)	()	()	
Comments and Suggestions for Authors	Major revisions are neces For example the word "p manuscript The figures' quality is qui Scientific hypothesis mu	ssary e sycho⊣ ite poor st be n	specially i chemistry" : nore clear,	n the prese is used in the same	entation. of the ma both the abstract applies to the resu	nuscript. and other parts of the Ilts.
Submission Date	21 November 2019					

Reviewer's Report

Title of the paper:

A Comparative Study of Virgin Coconut Oil, Coconut Oil and Palm Oil in Terms of their Active Ingredients

Author(s):

Suryani^{1*,} Sariani², Femi Earnestly³, Marganof⁴, Rahmawati⁴, Sevindrajuta⁴, Teuku Meurah Indra Mahlia⁵

Reviewer:

Reviewer

Date: March 4, 2020 Comments and Suggestions for Authors

Comments

General comment:

Also I strongly suggest to revise the aim of the work which I asked in previous revision. I propose the aim of the work:

"Thus, the aim of the research was comparison of the psychochemical parameters and contents of fatty acids (lauric, palmitic and stearic) of coconut oil, palm oil, and VCO as the indicators of the characteristics of each oil. Also the antimicrobial ability of VCO was analyzed."

Authors are asked for delete previous aim:

"This research compares the psycho-chemistry (such as: acid, saponification, and Iod number) of coconut oil, palm oil, and VCO [24] [29][30] as the consideration in determining whether these oils are appropriate to be used as biodiesel. It also analyses the content of fatty acids

such as lauric acid, palmitic acid, and stearic acid [30][31][39][40], as the indicator of the characteristics of each oil, besides determining the antimicrobial ability of VCO. ."

• **Response:**

We would like to thank the reviewer for providing suggestions and positive feedback in improving the paper. We really appreciate for your constructive comments. We have revised the manuscript in more coherent and more concise way such as in manuscript as follows:

Paragph 5 Introduction	
"This research compares the	
psycho-chemistry (such as: acid.	
saponification, and Iod number) of	
coconut oil palm oil and VCO	
[24] $[29]$ $[30]$ as the consideration	
in determining whether these oils	
are appropriate to be used as	
hindiesel. It also analyses the	
content of fatty acids	
such as louris acid, relutio	
such as lauric acid, paimitic	
acid, and stearic acid	
[30][31][39][40], as the	
indicator of the characteristics	
of each oil, besides	
determining the antimicrobial	
ability of VCO"	
Was delete	
	Paragraph 5 Intoduction
	Has been revised to below
	"Thus, the aim of the research was
	comparison of the psychochemical
	parameters and contents of fatty acids (lauric,
	palmitic and stearic) of coconut oil, palm oil,

and VCO as the indicators of the
characteristics of each oil. Also the
antimicrobial ability of VCO was analyzed."





1 Article

A Comparative Study of Virgin Coconut Oil, Coconut 2 Oil and Palm Oil in Terms of their Active Ingredients 3 4 Suryani Suryani^{1,*}, Sariani Sariani², Femi Earnestly³, Marganof Marganof⁴, Rahmawati⁵, 5 Sevindrajuta⁶, and TeukuMeurah Indra Mahlia⁷ 6 ^{1,3} Department of Chemistry, Faculty of Science and Technology, Muhammadiyah University of West 7 Sumatera;suryanimdiah@yahoo.com 8 ² Department of English, Politeknik Negeri Padang;sarianipasni@yahoo.com 9 ³ Department of Forestry, Faculty of Foresttry, Muhammadiyah University of West Sumatera; 10 marganofkarani@ymail.com 11 ⁴Department of Agro Technology, Faculty of Agriculture, Muhammadiyah University of West Sumatera; 12 rahmawati_3007@yahoo.co.id 13 ⁵ Department of Agro Technology, Faculty of Agriculture, Muhammadiyah University of West Sumatera; 14 juta indra@yahoo.co.id 15 ⁷School of Information, Systems and Modelling, Faculty of Engineering and Information Technology, 16 University of Technology Sydney, Sydney, NSW 2007, Australia; tmindra.mahlia@uts.edu.au 17 18 * Correspondence: tmindra.mahlia@uts.edu.au; Tel.:+61-XXX-XXX-XXXX 19 Received: date; Accepted: date; Published: date 20 21 ABSTRACT 22 Virgin Coconut Oil (VCO) is very nutritious to the human health because it contains of Lauric Acid 23 and Lactic Acid Bacteria (LAB). The aims of this research is to study the uniqueness factors of VCO 24 compared to coconut oil and palm oil. Lauric Acid content was analyzed by Chromatographic Gas 25 method. Isolation of LAB ia was conducted by dilution method using MRSA + 0.5% CaCO3 media. 26 In addition, the macromolecular identification was conducted by 16S rRNA. The results obtained 27 shows that the content of Lauric Acid is considered high; 41% 54.5% for VCO, where 0% for 28 coconut oil, and 0. 11% forpalm oil.VCO 29 . The VCO also contains LAB, namely Lac.plantarum and Lactobacillus paracasei, which has the 30 ability to inhibit the growth of pathogenic bacteria, such as P.aeruginase, Kleibsiella, S.aureus, 31 S.epidermidis, Proteus, E.coli, Lis.monocytogenes, B. cereus, and S. typhosa. Compare to VCO, coconut oil 32 and palm oil do not have above mentioned bacteria. It is concluded that the uniqueness of VCO is 33 that it has a high content of Lauric Acid, 54% and contains LAB. 34 35 Keywords: Bacteriocin, Lactic Acid Bacteria (BAL), Lauric Acid, Virgin Coconut Oil (VCO). 36 37 38 1.Introduction

39 The tropical biodiversity has been contributed to so many industrial products including 40 pharmaceutical, biofuel and as well as energy storage materials. Some of sources from tropical 41 biodiversity have been converted to pharmaceutical product such as drugs [1]. Where some other 42 such as Sterculiafoetida, Jatropha curcas, CalophylluminophyllumandReutealistrispermahave been 43 converted to biodiesel to power internal combustion engine [2-5]. By using bioenergy such as 44 biodiesel could reduce negative environmental impact on the environment especially by reducing 45 carbon dioxide to atmosphere [6, 7]. Several of materials from tropical biodiversity have also been 46 used for energy storage material than can replace batteries in the future [8-10]. This can help to store 47 a significant amount of solar energy from tropical country which usually has plenty of sunrays[11]. 48 Anotherexceptional plant from tropical biodiversity that has been used for many purposes is 49 coconut, however, in this research the author attempts to studycoconut oil and their active 50 ingredients.

51 Virgin Coconut Oil (VCO) is one type of oil that can be made in several methods such asby 52 fermenting coconut milk [1] and by adding microbes (Lactobacillus fermentum and Lactobacillus 53 plantarum) as starter [2-4].TheVCO can also be produce through centrifugation and microwave 54 process [5], and by fermentation without the addition of microbes as a starter [6]. This oil is also 55 called virgin oil because it is made without any heating processes [7]

56 The VCO has been used widely by many people because people believe that the VCO has some 57 benefits compared to original coconut oil made from cooked coconut milk and palm oil. The VCO is 58 useful for anti-microbial, or antibacterial and antiviral [3], and is useful for losing weight or helping 59 obese people in terms of metabolism. It is due to that VCO contains Medium Chain Trigliserida 60 which is initially digested or processed in the body out from carbohydrates that can cut back hunger. 61 Thus, it causes obese people consume less carbohydrates which eventually reduce their weight [7]. 62 By consuming the VCO, it also affects the healing of ovarianectomy [8], and can also used as an 63 antioxidant [9]. The VCO can also reduce blood pressure [10]. In addition, VCO can also be used for 64 skin care [11], as an external drug such as wound medicine, and can function as a probiotic [12]. Both 65 VCO and palm oil have their own characteristics with different function. VCO charasteristics are 66 more to medicine, probiotic, and cosmetics, whereas palm oil characteristics The composite 67 characteristic of palmitat acid is excessive when it is utilized for thermal energy storage [15]. How 68 ever, biodiesel is considered more economical when it is originated from vegetable oil which is 69 inedible such as Ceiba petandra [16]. are more onto biodiesel biodiesel [13-14]. The chemical 70 composition of VCO has been studied in [17] including the iodine number, the saponification 71 number, the amount of free fatty acids (% FFA), viscosity and color. This chemical composition need 72 to be examined prior consumption in order to follow Asian and Pacific Coconut Community (APCC) 73 standarisation, because VCO is originally made from fresh coconut milk

The specialty of VCO has been known for its high Lauric Acid content, which is between 46.36 -48.42% [17]. VCO is the basic ingredient of coconut milk which is high in carbohydrates and protein, so if fermented there will be Lactic Acid Bacteria(LAB) [18]. Lactic Acid Bacteria from VCO have been isolated and their antimicrobial ability has also been studied [6]. This atypical microbial ability exists because LAB contain bacteriocin [19], [20] which can kill pathogenic bacteriaFurther, this bacteriocin Lactobacillus plantarum [18] has also been isolated as well.

To study the features of VCO, it is necessary to analyze its fatty acid content [22] as has been studied the fatty acid content of fabaceae seed oil. The content of these fatty acids is usually analyzed by the Mass Spectra Chromatography Gas method [23], as in pomegranate seed oil and grapes.

83 2. Materials and Methods

84 Materials:

The sample was Virgin Coconut Oil (derived from the fermented coconut milk), coconut oil (derivedfrom heating the coconut milk), palm oild which are sold in the market.

87

88 Chemical Material:

89 For Lauric Acid Analysis, -hexan (p.a), CHCl₃, Aquadest, reagent for sample preparation namely

- 90 saturated NaCl, Na₂SO₄ anhidrat, BF₃, Methanol, and N₂gas to stop the oxidation occured were used.
- 91 The internal and exeternal of fatty acid standard. Whereas for Acid Number analysis (%FFA),
- 92 95%Alcohol (pa), blue bromtimol indicator, phenolphtalein indicator, HCl 0.5 N Standard Solution,

- 93 and KOH Standard Solution were used.KOH ethanolics, HCl 0.5 N standard solution was taken for
- Saponification Number Analysis, and Potassium Iodide was used for Peroxide Number Analysis.
 Meanwhile MRSA + 0.5% CaCO₃ media was used for Isolation of Lactid Acid Bacteria, and in order to
- 96 increase their amount using MRS media.
- 97

98 Instruments:

- 99 The instrument taken were ordinary laboratory glassware like petri dish, erlenmeyer, test tube, and
- 100 beaker glass, where all of them made from pyrex. In addition, Gas Chromatography GC GC-MS
- 101 Shimadzu QP 2010S (Shimadzu Corp, Kyoto, Japan) was set for lauric acid analysis and Autocklav
- 102 Yamata SN 21 for sterilization.
- 103



104 105

- 106 **Figure 1**. Gas Chromatography GC GC-MS Shimadzu QP 2010S (Shimadzu Corp, Kyoto, Japan)
- 107
- 108 Methods:
- 109



- 110
- Figure2. Method of a comparativestudy of virgin coconut oil, coconut oil and palm oil in terms of
 their active ingredients
- 113 3. Results and Discussion

3.1.The result of Lauric Acid, acid number, acid number, Saponification Number, Iodine Number, Free Fatty Acid % FFA and pH of Virgin Coconut Oil, coconut milk, and palm oil.

According to the experimental study presented in previous Sections, it is proof that the peculiarity of Virgin Coconut Oil compared to coconut oil produced by heating the coconut milk, and the palm oil sold in the market is in the ingredients of its high Lauric Acid (54.06%), and its

119 lactid acid bacteria (Lactobacillus plantarum dan Lactobacillus paracasei). In the presence of Lactid Acid

Bacteria containing bacteriocin, VCO characterizes as antimicrobial, in contrast to coconut and palmoil.

122 Shown inTable 1. below that lauric acid ingredients of VCO is the highest which is 54.06%. In 123 contrast, the lauric acid ingredients of coconut oil is 2.81%, and none for palm oil. Table 1. Presents 124 that there are two types of fatty acid content in VCO; 54.06% lauric acid and 12.06% stearat acid, and 125 none for palmitic acid. The absence of this palmitic acid is because VCO is not formed by palm oil. It 126 is based on the fact that the highest content of palmitic acid will exist in the oil originally from palm 127 oil. The 54.06% of lauric acid content in VCO is considered high, compared to what has been 128 obtained through this research [1]. It is because VCO is processed without heating or by 129 fermentation. Thus the fatty acid carbon bonds are not broken, in other words, the fatty acid is 130 included into Medium Chain Trigliserida, particularly the lauric acid. For coconut oil made from 131 cooked coconut milk, apparently the lauric acid content is low which is 2.81%, containing 2.65% 132 stearat acid, and possibly due to the production process which is cooked. Conversely to palm oil, 133 absolutely there is no lauric acid content, whereas its palmitic acid is high; 2.28% compared to VCO 134 and coconut oil.

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	$\sim \sim$	

Table 1. The Result of Standardization and Ingridient sample

Type of the oil	Type of the fatty acid	%	Acid number	Saponification number	%FF A	Iodin number	pН
	Lauric Acid	54.06					
VCO	Palmitat Acid	-	1.0165	348.003	0.264	5.3287	6.5
	Stearat Acid	12.03					
	Lauric Acid	2.81	_				
Coconut	Palmitat Acid	2.31	0.39695	269.6266	0.281	7.023	6.9
oil	Stearat Acid	2.65					
	Lauric Acid	0.45	_				
Palm	Palmitat Acid	2.88	0.39645	204.0045	0.51	51.0042	6,6
Oil 1			_		5		
	Stearat Acid	-					
	Lauric Acid	-	_				
Palm	Palmitat Acid	24.42	0.39645	203.02595	0.73	49.71675	6.5
Oil 2			_		3		
	Stearat Acid	-					

136

From Table 1. above it can be said that VCO acid number is 1.0165 which is higher than coconut milk acid number in 0.39695, and palm oil acid number in 0.39645 [2]. It is as a result of the acid number showing that KOH and NaOH amount can neutralize the free fatty acid. It means that VCO acid number is higher than coconut oil and palm oil acid numbers caused by higher free fatty acid to number of VCO.

VCO saponification number is 348.003 whereas coconut milk saponification number is 269.6266 which is higher than palm oil saponification number; 204.0045 due to saponification number that shows the number of fatty acid molecule. The bigger the saponification number, the smaller the molecule, or consisting of smaller fatty acid molecule or shorter chain, and the other way around. Hence, the higher the saponification number of VCO is because it consists of Medium Chain Trigliserida fatty acid [3], [4].

148 3.2. Result of LactidAcid Bacteria Isolation



Palm oil



lactid acid bacteria isolation obtained from palm oil with no evidence of growth

161 3.3. Molecular Identification of Lactid Acid Bacteria

- 162 Figure 3 below shows the result of molecular identification of LAB at the verification stage showing
- the size of DNA is 1500bp. Moreover, Figure 4 highlights the DNA arrangement of LAB.



164 165

Figure3.Electrophoresis result on 4 isolates

>CONTIQ_M16.4_1440bp_Lactobacillus plantarum_100% SCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGGATA ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTAT CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTT AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACA STGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCT GTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGG CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTAC GCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATC AGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA AGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAAT CGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAAC ACCCAAAGTC



GGTGAAGTCGTAACAAGGTAGCCGTAA

174

175

sequences producing significant alignments:						
Select: All None Selected:0						
1 Alignments gellowmioad - Gentlank Graphics Lastance tree of results				-	n -	
Description	score	Total	cover	value	Ident	Accession
Bacterium strain A2 16S ribosomal RNA gene, partial seguence	276	276	100%	3e-71	100%	KX268350.1
Lactobacillus paracasei strain HD1.7 16S ribosomal RNA gene, partial sequence	276	276	100%	3e-71	100%	EU249147.1
Lactobacillus mamnosus strain L156.4.16S ribosomal RNA gene, partial sequence,	274	274	99%	1e-70	100%	KX644947.1
Lactobacillus casel strain LC5, complete genome	274	1372	99%	1e-70	100%	CP017065.1
Lactobacillus rhamnosus strain 4B15, complete genome	274	1372	99%	1e-70	100%	CP021426.1
Lactobacillus paracasei strain IIA, complete genome	274	1372	99%	1e-70	100%	CP014985.1
Lactobacillus mamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
Lactobacilius rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1
Ladobacillus rhamnosus strain BFE5264, complete genome	274	1372	99%	1e-70	100%	CP014201.1
Lactobacillus casei strain K1C 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and	1235 274	274	99%	1e-70	100%	KU954559.1
Ladobacillus rhamnosus strain LRB, complete genome	274	1372	99%	1e-70	100%	CP016823.1
Lactobacillus mamnosus gene for 16S ribosomal RNA, partial seguence, strain: NGRI 0110	274	274	99%	1e-70	100%	LC177236.1
Lactobacillus paracasei strain FS3 16S ribosomal RNA gene, partial seguence	274	274	99%	1e-70	100%	KU315064.1
Lactobacilius rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

CGAAGCCGGTGGCGTAACCCTTTTAGGGAGCGAGCCGTCTAAGGTGGGACAAATGATTAG

176 Figure 5. The result of molecular identification on lactid acid bacteria showing *Lactobacillus paracasei*.

177 **3.4. Result of Anti Microbial Analysis**.

178 Anti microbial analysis was oerformed between *Lactobacillus paracasei* lactid acid bacteria, and 179 *Lactobacillus plantarum* onto testing bacteria; *Pseudomonas aeruginase, Klebsiella, Staphilococcus aureus*,

180 Staphilococcus epidermidis, Proteus, from [24], Eschericia coli, Listeria monocytogenes, Bacillus cereus, and

181 Salmonella typhosa. Lactid Acid Bacteria which can inhibit the growth of testing or pathogenic bacteria

182 is pointed ut in

183 It is seen in Figure 5. below a clear zone determining that the growth of pathogenic bacteria can be

184 inhabited by *lactobacillus plantarum*, and *lactobacilus paracasei*.





Result of anti microbial analysis of *Lactobacillus*`*plantarum* Lactid Acid Bacteria onto *E,coli* testing bacteria

Result of anti microbial analysis of *Lactobacillus`plantarum* Lactid Acid Bacteria onto *S.aureus* testing bacteria

Figure 5. Result Antimicrobial analysis from *Lactobacillus*`*plantarum* Lactid Acid Bacteria onto *E,coli and S.aureus*testing bacteria

186 Illustrated in Table 3 below, here VCO has the antimicrobial ability derived from two lactic acid 187 bacteria against nine testing bacteria. It is seen that these two lactic acid bacteria have good ability to 188 kill pathogenic bacteria; *Listeria monocytogenes*, and then *E.coli* bacteria, as stated in [9], where the 189 antimicrobial ability of *Lacobacillus plantarum* is the best rather than *Listeria monocytogenes*, *E.coli* and 190 *Bacillus sbtillis* testing bacteria.

191

185

192 193

Table 3. Anti microbial activity analysis of LAB on the form of clear zone diameter (mm)

No.	Testing Bacteria	Lactobacillus plantarum	Lactobacillus paracasei
1.	Escherichia coli	16	16
2.	ListeriaMonocitogenes	17	18
3.	BacillusSubstiliss	15	11
4.	SalmonellaTyphyphosa	12	11
5.	StaphillococcusAureus	11	11
6.	Pseudomonas aeruginase	17	14
7.	Klebsiella	13	12
8.	Staphilococcus epidermidis	13	12
9.	Proteus,	14	13

194 4. Conclusions

In the presence of Lactid Acid Bacteria containing bacteriocin, VCO characterizes as antimicrobial, in contrast to coconut and palm oil. The lauric acid content of VCO is the highest; 54.06% compared to lauric acid content; 0.45%, and palm oil which apparently has none. It means that VCO is exceptional, and can be the taken as medicine. Having high acid number; 1.10165, and high saponification number and Iodine number demonstrate the characteristic of VCO, as it contains lauric acid with small molecules which areMedium Chain Trigliserida (MCT), where initially will be digested in order to reduce weight.

202 Contribution Author.

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 Methodology: Suryani Suryani and Marganof Marganof; Software: Suryani Suryani;Validation: Suryani
 Suryani, Marganof Marganof; Formal analysis: Suryani Suryani and Marganof Marganof; Writing – original
 draft preparation: Sariani Sariani, Teuku Meurah Imdra Mahlia and Suryani Suryani;Writing – review and
 editing: Suryani Suryani, Rahmawati, Femi Earnestly and Sariani Sariani;Project administration:
 Sevindrajuta, and Rahmawati.

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299		




1 Article

12

20 21

A Comparative Study of Virgin Coconut Oil, Coconut 2 Oil and Palm Oil in Terms of their Active Ingredients 3 4 Suryani1*, Sariani², Femi Earnestly³, Marganof⁴, Rahmawati⁴, Sevindrajuta⁴, Teuku Meurah 5 Indra Mahlia⁵

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ABSTRACT

22 Virgin Coconut Oil (VCO) is very nutritious to human health because it contains Lauric Acid 23 and Lactic Acid Bacteria (LAB). The aims of this research are to study the unique factors of VCO 24 compared to coconut oil and palm oil. Lauric Acid content was analysed by the Chromatographic 25 Gas method. Isolation of LABiawas conducted by the dilution method using MRSA + 0.5% CaCO3 26 media. In addition, macromolecular identification was conducted by 16S rRNA. The results obtained 27 show that the content of Lauric Acid is considered high; 41% - 54.5% for VCO, where 0% of coconut 28 oil, and 0. 11% of palm oil. The VCO also contains LAB, namely Lac.plantarum and Lactobacillus 29 paracasei, which has the ability to inhibit the growth of pathogenic bacteria, such as P.aeruginase, 30 Kleibsiella, S.aureus, S.epidermidis, Proteus, E.coli, Lis.monocytogenes, B. cereus, and S. typhosa. Compare 31 to VCO, coconut oil and palm oil do not have above mentioned bacteria. It is concluded that the 32 uniqueness of the VCO is that it has a high content of Lauric Acid, 54% and contains LAB. 33

34 Keywords: Bacteriocin, Lactic Acid Bacteria (BAL), Lauric Acid, Virgin Coconut Oil (VCO).

35 36

37 1. Introduction

38 The tropical biodiversity has been contributed to so many industrial products including 39 pharmaceutical, biofuel and as well as energy storage materials. Some of the sources from tropical 40 biodiversity have been converted to pharmaceutical product such as drugs [1]. Where some other 41 such as Sterculiafoetida, Jatropha curcas, CalophylluminophyllumandReutealistrispermahave been 42 converted to biodiesel to power internal combustion engine [2-5]. By using bioenergy such as 43 biodiesel could reduce negative environmental impact on the environment, especially by reducing 44 carbon dioxide to atmosphere [6, 7]. Several of materials from tropical biodiversity have also been 45 used for energy storage material than can replace batteries in the future [8-10]. This can help to store 46 a significant amount of solar energy from tropical country which usually has plenty of sunrays [11]. 47 Another exceptional plant from tropical biodiversity that has been used for many purposes is 48 coconut, however, in this research the author attempts to study coconut oil and their active 49 ingredients.

50 Virgin Coconut Oil (VCO) is one type of oil that can be made in several methods such as by 51 fermenting coconut milk [12] and by adding microbes (Lactobacillus fermentum and Lactobacillus 52 plantarum) as a starter [13-15]. The VCO can also be produced through centrifugation and a 53 microwave process [5], and by fermentation without the addition of microbes as a starter [17]. This 54 oil is also called virgin oil because it is made without any heating processes [18]

55 The VCO has been used widely by many people because people believe that the VCO has some 56 benefits compared to original coconut oil made from cooked coconut milk and palm oil. The VCO is 57 useful for anti-microbial, or antibacterial and antiviral [14], and is useful for losing weight or helping 58 obese people in terms of metabolism. It is due to that VCO contains Medium Chain Trigliserida 59 which is initially digested or processed in the body out from carbohydrates that can cut back hunger. 60 Thus, it causes obese people to consume less carbohydrates, which eventually reduce their weight 61 [18]. By consuming the VCO, it also affects the healing of ovariectomy [19], and can also be used as 62 an antioxidant [20]. The VCO can also reduce blood pressure [10]. In addition, VCO can also be used 63 for skincare [22], as an external drug such as wound medicine, and can function as a probiotic [23]. 64 Both VCO and palm oil have their own characteristics with different function. VCO characteristics 65 are more to medicine, probiotic, and cosmetics, whereas palm oil characteristics quite suitable to be 66 converted to diesel fuel [24]. Not only palm oil, some other vegetable oils have also been proven to 67 be economical and can be converted to biodiesel production in large scale [25-27]. The chemical 68 composition of VCO has been studied in [28] including the iodine number, the saponification 69 number, the amount of free fatty acids (% FFA), viscosity and colour. This chemical composition 70 needs to be examined prior consumption in order to follow Asian and Pacific Coconut Community 71 (APCC) standardisation because VCO is originally made from fresh coconut milk

The specialty of VCO has been known for its high Lauric Acid content, which is between 46.36 -48.42% [28]. VCO is the basic ingredient of coconut milk, which is high in carbohydrates and protein, so if fermented there will be Lactic Acid Bacteria (LAB) [29]. Lactic Acid Bacteria from VCO have been isolated and their antimicrobial ability has also been studied [17]. This atypical microbial ability exists because LAB contains bacteriocin [30], [31] which can kill pathogenic bacteria further, this bacteriocin Lactobacillus plantarum [18] has also been isolated as well.

To study the features of VCO, it is necessary to analyse its fatty acid content [33] as has been studied the fatty acid content of Fabaceae seed oil. The content of these fatty acids is usually analysed by the Mass Spectra Chromatography Gas method [34], as in pomegranate seed oil and grapes.

82 2. Materials and Methods

83 Materials:

84 The sample was Virgin Coconut Oil (derived from the fermented coconut milk), coconut oil (derived85 from heating the coconut milk), palm oil which are sold in the market.

86

87 Chemical Material:

88 For Lauric Acid Analysis, -hexane (p.a), CHCl₃, Aquadest, reagent for sample preparation, namely

89 saturated NaCl, Na₂SO₄ anhydrate, BF₃, Methanol, and N₂gas to stop the oxidation occurred were

- 90 used. The internal and external of fatty acid standard. Whereas for Acid Number analysis (%FFA),
- 91 95%Alcohol (pa), blue bromothymol indicator, phenolphthalein indicator, HCl 0.5 N Standard
- 92 Solution, and KOH Standard Solution were used. KOH ethanolic, HCl 0.5 N standard solution was
- 93 taken for Saponification Number Analysis, and Potassium Iodide was used for Peroxide Number

- 94 Analysis. Meanwhile MRSA + 0.5% CaCO3 media was used for Isolation of Lactaid Acid Bacteria and
- 95 in order to increase their amount using MRS media.

96 Instruments:

- 97 The instrument has taken where ordinary laboratory glassware like a petri dish, Erlenmeyer, test tube,
- 98 $\,$ and beaker glass, where all of them made from Pyrex. In addition, Gas Chromatography GC GC-MS $\,$
- 99 Shimadzu QP 2010S (Shimadzu Corp, Kyoto, Japan) was set for lauric acid analysis and Autocklav
- 100 Yamata SN 21 for sterilization.



- Figure 1. Gas Chromatography GC GC-MS Shimadzu QP 2010S (Shimadzu Corp, Kyoto, Japan).
- 104 Methods:

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105



- 106
- Figure 2. Method of a comparative study of virgin coconut oil, coconut oil and palm oil in terms of
 their active ingredients.

109 3. Results and Discussion

110 3.1. The result of Lauric Acid, acid number, acid number, Saponification Number, Iodine

111 Number, Free Fatty Acid % FFA and pH of Virgin Coconut Oil, coconut milk, and palm oil.

According to the experimental study presented in previous Sections, it is proof that the peculiarity of Virgin Coconut Oil compared to coconut oil produced by heating the coconut milk, and the palm oil sold in the market is in the ingredients of its high Lauric Acid (54.06%), and its Lactaid acid bacteria (*Lactobacillus plantarum* dan *Lactobacillus paracasei*). In the presence of Lactaid Acid Bacteria containing bacteriocin, VCO characterizes as antimicrobial, in contrast to coconut and palm oil.

Shown in Table 1. Below that lauric acid ingredient of the VCO is the highest which is 54.06%.
In contrast, the lauric acid ingredients of coconut oil is 2.81%, and none of the palm oil. Table 1.

included into Medium Chain Triglycerides, particularly the lauric acid. Since coconut oil made from
cooked coconut milk, apparently the lauric acid content is low which is 2.81%, containing 2.65%
saturated acid, and possibly due to the production process which is cooked. Conversely to palm oil,
absolutely there is no lauric acid content, whereas its palmitic acid is high; 2.28% compared to VCO
and coconut oil.

				e e	-		
Type of the oil	Type of the fatty acid	%	Acid number	Saponification number	%FF A	Iodin number	pН
	Lauric Acid	54.06					
VCO	Palmitat Acid	-	1.0165	348.003	0.264	5.3287	6.5
	Stearat Acid	12.03					
	Lauric Acid	2.81					
Coconut	Palmitat Acid	2.31	0.39695	269.6266	0.281	7.023	6.9
oil	Stearat Acid	2.65	_				
	Lauric Acid	0.45					
Palm	Palmitat Acid	2.88	0.39645	204.0045	0.51	51.0042	6,6
Oil 1					5		
	Stearat Acid	-					
	Lauric Acid	-	_				
Palm Oil 2	Palmitat Acid	24.42	0.39645	203.02595	0.73 3	49.71675	6.5
	Stearat Acid	_	_		0		

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Table 1. The Result of Standardization and Ingredient sample

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From Table 1. above it can be said that VCO acid number is 1.0165 which is higher than the coconut milk acid number in 0.39695, and palm oil acid number in 0.39645 [13]. It is as a result of the acid number showing that KOH and NaOH amount can neutralize the free fatty acid. It means that the VCO acid number is higher than coconut oil and palm oil acid numbers caused by higher free fatty acid content of the VCO.

The VCO saponification number is 348.003 whereas coconut milk saponification number is 269.6266 which is higher than the palm oil saponification number; 204.0045 due to saponification number that shows the number of the fatty acid molecule. The bigger the saponification number, the smaller the molecule, or consisting of smaller fatty acid molecules or shorter chain, and the other way around. Hence, the higher the saponification number of the VCO is because it consists of Medium Chain Trigliserida fatty acid [14], [15].

144 **3.2. Result of LactidAcid Bacteria Isolation**

Table 2. below presents the isolated result of lactic acid bacteria onto VCO, coconut oil, and palm oil. It is shown that VCO contains lactic acid bacteria, as when it was being isolated using MRSA + CaCO₃ selective media, the lactic acid bacteria colony which can grow in "Halo" area is found. This area is a clear zone where it can produce lactic acid neutralising CaCO3. It is in line with [5], and as an additional, the results in Figure 2 and 3 points out the identification of lactic acid

- 150 bacteria using 16S rDNA which turn out to be Lacobacillus plantarum and Lactobacillus paracasei as
- 151 mentioned in [6], [7], and [8].



Table 2. Result of LactidAcid Bacteria Isolation

153 3.3. Molecular Identification of Lactid Acid Bacteria

154 Figure 3 below shows the result of molecular identification of LAB at the verification stage showing

155 the size of DNA is 1500bp. Moreover, Figure 4 highlights the DNA arrangement of LAB.



4. Bac4

Consensus:

CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACC CGAAGCCGGTGGCGTAACCCTTTTAGGGAGCGAGCCGTCTAAGGTGGGACAAATGATTAG GGTGAAGTCGTAACAAGGTAGCCGTAA

11	Alignments Download - GenBank Graphics Distance tree of results						4
	Description	Max score	Total score	Query cover	E value	Ident	Accession
1	Bacterium strain A2 16S ribosomal RNA gene, partial sequence	276	276	100%	3e-71	100%	KX268350.1
1	Lactobacillus paracasei strain HD1.7 16S ribosomal RNA gene, partial seguence	276	276	100%	3e-71	100%	EU249147.1
3	Lactobacilius rhamnosus strain L156.4 16S ribosomal RNA gene, partial sequence,	274	274	99%	1e-70	100%	KX644947.1
3	Laclobacillus casel strain LC5, complete genome	274	1372	99%	1e-70	100%	CP017065.1
3	Ladobacilius rhamnosus strain 4B15, complete genome	274	1372	99%	1e-70	100%	CP021426.1
1	Ladobacillus paracasei strain IIA, complete genome	274	1372	99%	1e-70	100%	CP014985.1
3	Laclobacillus mamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
1	Laclobacillus rhamnosus strain WO2 genome	274	274	99%	1e-70	100%	CP020016.1
3	Ladobacillus rhamnosus strain BFE5264, complete genome	274	1372	99%	1e-70	100%	CP014201.1
1	Lactobacillus casel strain K1C 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S	274	274	99%	1e-70	100%	KU954559.1
3	Ladobacillus rhamnosus strain LRB, complete genome	274	1372	99%	1e-70	100%	CP016823.1
	Lactobacillus rhamnosus gene for 16S ribosomal RNA, partial sequence, strain: NGRI 0110	274	274	99%	1e-70	100%	LC177236.1
1	Lactobacillus paracasei strain FS3 16S ribosomal RNA gene, partial seguence	274	274	99%	1e-70	100%	KU315064.1
n	Ladobacillus rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

169

170 Figure 5. The result of molecular identification of lactid acid bacteria showing Lactobacillus paracasei.

171 3.4. Result of Anti Microbial Analysis.

172 Antimicrobial analysis was performed between Lactobacillus paracasei lactid acid bacteria, and 173 Lactobacillus plantarum onto testing bacteria; Pseudomonas aeruginase, Klebsiella, Staphilococcus aureus, 174 Staphilococcus epidermidis, Proteus, from [35], Eschericia coli, Listeria monocytogenes, Bacillus cereus, and

175 Salmonella typhosa. Lactid Acid Bacteria which can inhibit the growth of testing or pathogenic

176 bacteria is pointed out in

177 It is seen in Figure 5. below a clear zone determining that the growth of pathogenic bacteria can be 178

inhabited by lactobacillus plantarum, and lactobacilus paracasei.



Result of antimicrobial analysis of Lactobacillus'plantarum Lactid Acid Bacteria onto E, coli testing bacteria



Result of antimicrobial analysis of Lactobacillus`plantarum Lactid Acid Bacteria onto *S.aureus* testing bacteria

Figure 5. Result Antimicrobial analysis of Lactobacillus' plantarum Lactid Acid Bacteria onto E, coli and S. aureustesting bacteria

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As illustrated in Table 3, VCO has the antimicrobial ability derived from two lactic acid bacteria against nine testing bacteria. It is seen that these two lactic acid bacteria have good ability to kill pathogenic bacteria; *Listeria monocytogenes*, and then *E.coli* bacteria, as stated in [20], where the antimicrobial ability of *Lacobacillus plantarum* is the best rather than *Listeria monocytogenes*, *E.coli* and *Bacillus sbtillis* testing bacteria.

184 185

Table 3. Antimicrobial activity analysis of LAB in the form of clear zone diameter (mm) Lactobacillus Lactobacillus Testing Bacteria No. plantarum paracasei 1. Escherichia coli 16 16 2. ListeriaMonocitogenes 17 18 3. BacillusSubstiliss 15 11 4. SalmonellaTyphyphosa 12 11 5. **StaphillococcusAureus** 11 11 6. Pseudomonas aeruginase 17 14 7. Klebsiella 13 12 8. 12 Staphilococcus epidermidis 13 9. Proteus, 14 13

186 4. Conclusions

187 In the presence of Lactid Acid Bacteria containing bacteriocin, VCO characterizes as antimicrobial, in 188 contrast to coconut and palm oil. The lauric acid content of the VCO is the highest; 54.06% compared

to lauric acid content; 0.45%, and palm oil which apparently has none. It means that VCO is exceptional, and can be then taken as medicine. Having a high acid number; 1.10165, and high

saponification number and Iodine number demonstrate the characteristic of VCO, as it contains

192 lauric acid with small molecules which are Medium Chain Triglycerides (MCT), where initially will

193 be digested in order to reduce weight.

194 Contribution Author.

195 Conceptualization: Suryani Suryani and Sariani Sariani; Resources: Marganof Marganof, and Suryani Suryani,
196 Methodology: Suryani Suryani and Marganof Marganof; Software: Suryani Suryani; Validation: Suryani
197 Suryani, Marganof Marganof; Formal analysis: Suryani Suryani and Marganof Marganof; Writing – original
198 draft preparation: Sariani Sariani, Teuku Meurah Indra Mahlia and Suryani Suryani; Writing – review and
199 editing: Suryani Suryani, Rahmawati, Femi Earnestly, Sariani Sariani and Wahyu Caesarendra; Project
200 administration: Sevindrajuta, and Rahmawati.

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Article

A Comparative Study of Virgin Coconut Oil, Coconut Oil and Palm Oil in Terms of Their Active Ingredients

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ABSTRACT: This research aims to study the unique factors of virgin coconut oil (VCO) compared with coconut oil (i.e., coconut oil processed through heating the coconut milk and palm oil sold on the market). Its novelty is that it (VCO) contains lactic acid bacteria and bacteriocin. Lauric acid content was analyzed by the Chromatographic Gas method. Isolation of lactic acid bacteria (LAB) was conducted by the dilution method using MRSA + 0.5% CaCO3 media. Iodium number, peroxide, and %-FFA were analyzed using a general method, and isolation bacteriocin by the deposition method using ammonium sulfate. In addition, macromolecular identification was conducted by 16S rRNA. VCO was distinguished by a higher content of lauric acid (C12:0) 41%-54.5% as compared with $\frac{0\%}{2}$ coconut and palm oils $\frac{60\%}{2}$ and $\frac{0.11\%}{2}$, respectively). The VCO also contains LAB, namely Lactobacillus plantarum and Lactobacillus paracasei, and can inhibit the growth of pathogenic bacteria, such as Pseudomonas aeruginosa, Klebsiella, Staphylococcus aureus, S. epidermidis, Proteus, Escherichia coli, Listeria monocytogenes, Bacillus cereus, Salmonella typhosa and bacteriocin. Comparison with VCO is based on having a high content of lauric acid, 54%, and LAB content. The difference between VCO and coconut oil and palm oil is fatty acids. In VCO there are lauric acid and stearic acid, namely lauric acid VCO (A) 54.06%, VCO (B) 53.9% and VCO (C) 53.7%. The content of stearic acid VCO (A) is 12.03%, VCO (B) 12.01% and VCO (C) 11.9%. Coconut oil contains a little lauric acid, which is 2.81%, stearic acid 2.65% and palmitic acid 2.31%. Palm oil can be said to have very little lauric acid, namely in palm oil 1, 0.45%, and even in palm oil 2, 0%; in turn, palmitic acid palm oil 1 has 2.88% and palm oil 2 palmitic acid 2 has 24.428% and 88%.

Keywords: bacteriocin; lactic acid bacteria (LAB); lauric acid; virgin coconut oil (VCO)

1. Introduction

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Virgin coconut oil (VCO) can be made through several methods, such as by fermenting coconut milk [1–3] and by adding microbes (*Lactobacillus fermentum* and *Lactobacillus plantarum*) as starter cultures [4,5]. VCO can also be produced through centrifugation [6] and microwave processes [7], and by fermentation without the addition of microbes as a starter [8]. This oil is called virgin oil because it is made without any heating [9].

VCO has been used widely because it is believed to have benefits compared to coconut oil, made through a heating process, and palm oil. VCO is useful against microbes, bacteria and viruses [10], and is useful for helping one lose weight in terms of metabolism. VCO contains medium chain triglycerides [11,12], which is initially digested or processed in the body from carbohydrates that can cut back hunger [13]. Thus, it causes people to consume less carbohydrates, which eventually reduces body weight [14,15]. VCO also affects the healing after an ovariectomy [16] and can be used as an antioxidant [17]. VCO can also reduce blood pressure. In addition, VCO can also be used for skincare [18–20], as an external drug, such as wound medicine, and can function as a probiotic [4,21–23].

VCO and palm oil have different characteristics with different functions [24–26]. VCO gravitates more towards medicine, probiotics and cosmetics, whereas palm oil characteristics are quite suitable to be converted to diesel fuel. Other vegetable oils than palm oil have also been proven to be economical and can be converted to biodiesel production at a large scale [27–29]. The chemical composition of VCO has been studied in including the iodine number, the saponification number, the amount of free fatty acids (%-FFA), viscosity and color. This chemical composition needs to be examined prior to consumption in order to follow Asian and Pacific Coconut Community (APCC) standardization, because VCO is originally made from fresh coconut milk [30]

VCO has been known for its high lauric acid content, which is between 46.36% and 48.42% [31] Processed from coconut milk, which is high in carbohydrates and proteins, the fermentation process of VCO results in lactic acid bacteria (LAB) [32–35]. LAB from VCO have been isolated and their antimicrobial ability also has been studied [36]. This atypical microbial ability exists because LAB contains bacteriocin, which can kill pathogenic bacteria. Further, bacteriocin from *Lactobacillus plantarum* [34,35,37,38] has been isolated as well. Thus, the aim of the research was comparison of the psychochemical parameters and contents of the fatty acids (lauric, palmitic and stearic) of coconut oil, palm oil and VCO as indicators of the characteristics of each oil. The antimicrobial ability of VCO was also analyzed.

The newest fact referring to VCO, which is yet to be acknowledged, is the presence of lactic acid bacteria (LAB) in the oil and blondo layers (VCO dregs). This LAB will be present when VCO is made through traditional fermentation processes or fermentation using the existing bacteria in the air [36].

2. Materials and Methods

2.1. Materials

The samples were three types of virgin coconut oils, with different methods of extraction/fermentation, coconut oil derived from heating coconut milk and consumer-grade palm oil.

2.1.1. Chemical Material

For lauric acid analysis, -hexane (p.a), CHCl₃ and Aquadest reagent for sample preparation, namely saturated NaCl, Na₂SO₄ anhydrate, BF₃, methanol and N₂gas to stop the oxidation occurring, were used. The internal fatty acid standard was used. Whereas, for acid number analysis (%FFA), 95% alcohol (pa), blue bromothymol indicator, phenolphthalein indicator, HCl 0.5 N standard solution and KOH standard solution were used. KOH ethanolic, HCl 0.5 N standard solution was taken for saponification number analysis, and potassium iodide was used for peroxide number analysis. Meanwhile MRSA (deMann Rogosa Sharpe Agar) p.a + 0.5% CaCO₃ media was used for isolation of lactic acid bacteria and in order to increase their amount using MRS media. The materials

2 of 11

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used for isolation bacteriocin were MRSB media (Merck), *Lactobacillus plantarum* M0, ammonium sulfate (NH4) 2SO4, phosphate buffer, DEAE cellulose, butanol, SDS stacking gel and markers.

2.1.2. Instruments

The instrument used where ordinary laboratory glassware, such as petri dishes, Erlenmeyer flasks, test tubes and beaker glass, all of them made by Pyrex. In addition, gas chromatography (GC) GC–MS Shimadzu QP 2010S (Shimadzu Corp, Kyoto, Japan) was set for lauric acid analysis and Autocklav Yamata SN 21 for sterilization and laminar flow as the working media for isolating the bacteria and for performing antimicrobial analysis.

2.2. The Standardization of the VCO

2.2.1. The Determination of pH

The pH of the sample is determined using the pH meter in accordance with the applicable general procedure.

2.2.2. The Analysis of Fatty Acid Sample Using GC-MS

Prior to injecting the sample into a GC–MS instrument, the oil sample was prepared by setting 50 g of VCO sample and adding 400 μ L of NaOH Metanolic. This mixture was vortexed and heated at 50 °C for 10 min. After undergoing the cooling process, 1 mL CH₃COOH, 1 mL distilled water and 1 mL n-hexane were added, respectively. Then this mixture was vortexed and cooled for several minutes where two layers were formed as the result.

About 1 μ L at the top layer was taken as the sample to be injected and analyzed in a GC–MS, Shimadzu QP2010 which was equipped with capillary column of (30 m) × 0.25 mm ID; 0.25 im (interspersed by DB5MS. Japan), by injecting the sample into the capillary column. The carrier gas was helium, where the injector and detector temperatures were set at 280 °C. The injection was performed using the split mode (1:30). The column temperature was programmed to change from 50 °C to 280 °C at a rate of 5 °C per minute. Fatty acid ethyl esters were separated at the constant pressure (100 kPa), and the peak was identified through the comparison of mass spectra with mass spectral as the database (internal standard). The compound identification was with regard to the comparison of its mass spectrum with the NIST Mass Spectral Library 2008.

2.2.3. The Determination of Water Content

Porcelain dishes along with loose-fitting covers were cleaned and dried in the dryer oven at 105 °C for 1 h. Then they were taken using a pair of clamps and inserted into desiccators where the lid was detached. Soon after the dish has cooled, the lid was tightened and weighed. A 2 g sample was weighed into the dish and undergone another eight hours dry-heating in a hot-air oven at 105 °C, until reaching a constant weight. Another cooling process in the desiccators was conducted for 30 min before determining its water level.

2.2.4. The Determination of Acid Number

A total of 5 g of the sample was weighed into a 300 mL Erlenmeyer flask, and then 25 mL neutral alcohol was added; after that the flask was connected to an upright condenser, and boiled for 30 min. After cooling down, the sample was titrated with NaOH 0.1 M using a pp indicator. The volume of the NaOH titer was recorded.

2.2.5. The Determination of Iodine Number

A total of 0.5 g of oil was weighted in a covered Erlenmeyer flask, then 10 mL chloroform was added and shaken. Then 15 mL Wij's solution was added and shaken slowly for another 30 min where the Erlenmeyer continued to be covered. The lid and inner wall of the Erlenmeyer were washed with 50 mL distilled water (initially heated and cooled). The next step was titration with 0.1

3 of 11

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N Tio (Na₂S₂O₃) until the color changed into light brown, and 2 mL of 1% starch was used as indicator. The titration was continuously conducted until the dark blue color disappeared.

2.2.6. The Isolation of Lactic Acid Bacteria

The oil sample was diluted utilizing saline solution with dilution up to 10^{-7} . Then this sample was planted on the dish containing MRSA + 0.5% CaCO₃ selective media, and incubated overnight at 37 °C. The growth was observed.

2.2.7. The Molecular Identification

Initially, the identification began with isolating genomic DNA of lactic acid bacteria, then, continued by amplifying 16S rRNA gen. In order to determine the size of its DNA, this gen was analyzed using electrophoresis gel and ended with sequencing.

2.2.8. Antimicrobial Analysis

The antimicrobial analysis was carried out using the agar-disk method, which has been modified using a disk made from filter paper. It was assumed that 1 unit of antimicrobial activity was defined as the 1 mm² inhibited zone area or "halo" zone. It began with testing bacteria grown on NA media (Merck) of 1.5% agar concentration at 37 °C. Incubated overnight, 1 dose of testing bacteria was moved into test tubes containing 10 mL sterile distilled water. After that, the cell numbers were conformed to McFarland 0.5, which was estimated to be 10^{6} – 10^{7} CFU mL⁻¹. Toothpicks wrapped with cotton and sterilized were dipped into testing bacteria (more or less 100 µL). After being left to dry, we took the filter paper that was sterilized and perforated it like a disk with a 80 mm diameter, dipped it into LAB isolate, and stuck it onto the solid media surface in petri dishes, which were smeared by testing bacteria. Samples were incubated for the period of 3 × 24 h and observed until the clear zone or "halo" zone was formed, indicating the occurrence of the growth inhibition of pathogenic bacteria by lactic acid bacteria. Finally, the diameter of the clear zone was measured.

3. Results and Discussion

3.1. Composistion and Properties of VCO, Coconut Oil and Palm Oil

From the research conducted on virgin coconut oil (VCO), coconut oil and palm oil, differences among the three were found. Virgin coconut oil contains lauric acid (53.70%–54.06%), stearic acid (2.65%–12.10%) and lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus paracasei*). Coconut oil contains very little lauric acid (and stearic and palmitic acids), while palm oil contains only palmitic acid. Because of the presence of lactic acid bacteria containing bacteriocin, VCO is characterized as an antimicrobial, in contrast to coconut and palm oils.

As shown in Table 1 below, the lauric acid content of VCO is the highest with 54.06%. In contrast, coconut oil contains only 2.81% and palm oil none (0%). Table 1 shows that there are two types of fatty acid contents in VCO; 54.06% lauric acid and 12.06% stearic acid, and no (0%) palmitic acid. The absence of palmitic acid is because VCO is not made of palm oil. Palmitic acid is found in palm oil. The lauric acid content of VCO is considered high compared to what has been obtained through this research [12]. This is because VCO is processed without heating, or through fermentation. Thus, the fatty acid carbon bonds are not broken, in other words, the fatty acid is in the form of medium chain triglycerides, particularly lauric acid. On the other hand, coconut oil made from cooked coconut milk has a low lauric acid content, 2.81%, containing 2.65% saturated acids due to the production process involving heating. Conversely, palm oil has absolutely no lauric acid content, whereas its palmitic acid is high (2.28%) compared to VCO and coconut oil.

Type of	T // A • 1	0/	Acid	Saponification	0/ 77	Iodine		Water
the Oil	Fatty Acids	%	Number	Number	%FFA	Number	pН	Content %
	Lauric Acid (C12:0)	54.06						
VCO (A)	Palmitic Acid	-	1.01	348.00	0.26	5.32	6.50	0.11
	Stearic Acid(C18:0)	12.03						
	Lauric Acid (C12:0)	53.90						
VCO (B)	Palmitic Acid (C16:0)	-	1.03	345.70	0,25	5.24	6.40	0.12
	Stearic Acid (C18:0)	12.01						
	Lauric Acid (C12:0)	53.70						
VCO (C)	Palmitic Acid (C16:0)	-	1.02	346.64	0.26	5.25	6.50	0.11
	Stearic Acid (C18:0)	11.9						
	Lauric Acid (C12:0)	2.81						
Coconut oil	Palmitic Acid (C16:0)	2.31	0.39	269.62	0.28	7.02	6.90	0.11
	Stearic Acid (C18:0)	2.65						
	Lauric Acid (C12:0)	0.45						
Palm Oil 1	Palmitic Acid (C16:0)	2.88	0.39	204.00	0.51	51.00	6.60	0.09
	Stearic Acid (C18:0)	-						
	Lauric Acid (C12:0)	-						
Palm Oil 2	Palmitic Acid (C16:0)	24.42	0.39	203.02	0.73	49.71	6.50	0.09
	Stearic Acid (C18:0)	-						

Table 1. The results: Composition and properties of virgin coconut oil, coconut oil and palm oil.

From Table 1 above it can be said that VCO has an acid number of 1.0165, which is higher than coconut milk (acid number of 0.39695) and palm oil (acid number of 0.39645). VCO water content is similar to palm oil, which is 0.11%, whereas coconut oil processed through heating has a lower water content of 0.10%. However, the water content of the three samples still meet the limit standard of cooking oil 2177 SNI 3741 2013. In other words, the smaller number of the three samples' water content simplifies the heating process so the three samples can be used as biodiesels, in accordance with current guidelines.

The analysis result of %-FFA contains 0.264 VCO, 0.281 coconut oil and 0.51 palm oil. It shows that VCO heating process is much better than other oil samples according to the study of the VCO saponification number being 348.003, whereas coconut milk has a saponification number of 269.6266

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and palm oil 204.0045. The high saponification number reflects the number of the fatty acid molecules. The bigger the saponification number, the smaller the molecules, or consisting of smaller fatty acid molecules or shorter chains, and vice versa. Hence, the higher saponification number of the VCO is because it consists of medium chain triglyceride fatty acids [14,42]. The higher saponification number compared to palm and coconut oils mean that the saponification occurring in VCO is greater, even though still within tolerable limits.

3.2. Result of Lactid Acid Bacteria Isolation

Figure 1 below presents the isolated result of lactic acid bacteria onto VCO, coconut oil and palm oil. It is shown that VCO contains lactic acid bacteria, as when it was being isolated using MRSA + CaCO₃ selective media, the lactic acid bacteria colony that can grow in the "Halo" area is found. This area is a clear zone where it can produce lactic acid, neutralizing CaCO₃. It is in line with, and as an addition to, the results in Figures 2 and 3, pointing out the identification of the lactic acid bacteria using 16S rDNA, which turned out to be *Lactobacillus plantarum* and *Lactobacillus paracasei* as mentioned.



Figure 1. Lactid acid bacteria isolate grown in MRS + 0.5% CaCO3 media.

It can be seen, in Figure 1, there is a clear area; in the middle there is a white dot that is a colony of lactic acid bacteria present in the VCO oil. This is proof that VCO contains lactic acid bacteria [29,32].

Meanwhile, in Figure 2, is a petri dish filled with coconut oil and palm oil grown on the same media. No visible growth of lactic acid bacteria was observed. This proves that lactic acid bacteria do not exist in coconut oil and palm oil. This is in accordance with [8,35,43] but not so in [35], who only uses MRSA media without the addition of CaCO3. But growing colonies are not in the "Halo" area.

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Figure 2. Result of lactid acid bacteria isolation from coconut oil and from palm oil with no evidence of growth.

3.3. Molecular Identification of Lactid Acid Bacteria

The molecular identification of lactic acid bacteria produced *Lactobacillus plantarum*, as shown in the data below Figure 3.

_		_
	>CONTIQ_M16.4_1440bp_Lactobacillus plantarum_100%	-
	GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAA	Ó
	GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGAT/	ŧ
	ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTA	Ę
	CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGA	Ģ
	CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTT	ł
	CACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGA	ŧ
	AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA	ŧ
	SCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG	Ę
	AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGAO	Υ
	GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC	l
	GTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGA	ſ
	GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACG	1
	CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTA	1
	SCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA	٩
	GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTAT(2
	AGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGGATGACGTCAA	1
	TCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGG	ł
	AGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAA	ļ
	USUSGATLAGUATGUUSUSGTGAATAUGTTUUUSGGUUTTGTACACCCCCCCGTCACACCATGAGAGTTTGTAA	ł
	ACCCAAAGTC	l

Figure 3. Lactid acid bacteria gene sequence from isolation of Lactobacillus plantarum.

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ÂŶ	Alignments Download - GenBank Graphics Distance tree of results				_		<
	Description	Max score	Total score	Query cover	E value	Ident	Accession
5	Bacterium strain A2 16S ribosomal RNA gene, partial seguence	276	276	100%	3e-71	100%	KX268350.1
	Lactobacillus paracasei strain HD1.7.16S ribosomal RNA gene, partial seguence	276	276	100%	3e-71	100%	EU249147.1
6	Lactobacillus mamnosus strain L156.4.16S ribosomal RNA gene, partial sequence.	274	274	99%	1e-70	100%	KX644947.1
	Lactobacillus casei strain LC5, complete genome	274	1372	99%	1e-70	100%	CP017065.1
2	Lactobacillus mamnosus strain 4B15, complete genome	274	1372	99%	1e-70	100%	CP021426.1
۵	Lactobacillus paracasei strain IIA, complete genome	274	1372	99%	1e-70	100%	CP014985.1
6	Lactobacillus mamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
	Ladobadillus rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1
6	Lactobacillus rhamnosus strain BFE5264, complete genome	274	1372	99%	1e-70	100%	CP014201.1
2	Lactobacillus casei strain K1C 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S	274	274	99%	1e-70	100%	KU954559.1
6	Lactobacillus rhamnosus strain LRB, complete genome	274	1372	99%	1e-70	100%	CP016823.1
	Lactobacillus mamnosus gene for 16S ribosomal RNA, partial sequence, strain: NGRI 0110	274	274	99%	1e-70	100%	LC177236.1
6	Laciobacillus paracasei strain FS3 16S ribosomal RNA gene, partial seguence	274	274	99%	1e-70	100%	KU315064.1
1	Ladobacillus rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

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Figure 4. The result of molecular identification of lactid acid bacteria showing *Lactobacillus paracasei*.

3.4. Result of Antimicrobial Analysis

The antimicrobial ability of VCO is also analyzed below. Antimicrobial analysis was performed with *Lactobacillus paracasei* and *Lactobacillus plantarum* lactic acid bacteria onto the following testing bacteria: *Pseudomonas aeruginosa, Klebsiella, Staphylococcus aureus, Staphylococcus epidermidis, Proteus,* from [31,35], *Escherichia coli, Listeria monocytogenes, Bacillus cereus,* and *Salmonella typhosa*.

As seen in Figure <u>54</u> below, clear zones show that the growth of pathogenic bacteria was inhibited by *Lactobacillus plantarum* and *Lactobacillus paracasei*.



Result of antimicrobial analysis of *Lactobacillus plantarum* lactid acid bacteria onto *E. coli* testing bacteria.



Result of antimicrobial analysis of *Lactobacillus plantarum* lactid acid bacteria onto *S. aureus* testing bacteria

Figure 54. Results of the antimicrobial analysis of *Lactobacillus plantarum* lactid acid bacteria onto *E. coli* and *S. aureus* testing bacteria.

As illustrated in Figure 4, VCO has the antimicrobial ability derived from two types of lactic acid bacteria against nine testing bacteria. It is seen that these lactic acid bacteria have a good ability to kill pathogenic bacteria, e.g., *Listeria monocytogenes* and *E. coli*, as stated in [8], where the antimicrobial ability of *Lactobacillus plantarum* is found to be most effective against *Listeria monocytogenes*, *E.coli* and *Bacillus subtilis* testing bacteria.

In Table 2 it can be seen that the lactic acid bacteria present in the VCO are able to inhibit the growth of pathogenic bacteria as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella*, *Staphylococcus epidermidis*, and *Proteus*, in accordance with [36].

No.	Testing Bacteria	Lactobacillus plantarum	Lactobacillus paracasei
1.	Escherichia coli	16	16
2.	Listeria monocytogenes	17	<mark>18</mark>
3.	Bacillus substiliss	<mark>15</mark>	<mark>11</mark>
4.	Salmonella typhyphosa <mark>Salmonella typhy</mark>	12	11
5.	Staphylococcus aureus	11	11
6.	Pseudomonas aeruginosa	17	14
7.	Klebsiella	<mark>13</mark>	<mark>12</mark>
8.	Staphylococcus epidermidis	13	12
9.	Proteus	14	13

Table 2. Antimicrobial activity analysis o	f LAB in the form of clear zone diameter (r	mm).
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4. Conclusions

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Compared with coconut oil and palm oil, virgin coconut oil (VCO) has a higher content of lauric acid and lactic acid bacteria. Nevertheless, based on the content of water, free fatty acid and iodine number, VCO has other features in the field of health because it has lactic acid bacteria that can kill pathogens, characterizing it as an antimicrobial, in contrast to coconut and palm oil. The lauric acid content of the VCO is the highest (54.06%) compared to the lauric acid content of coconut oil (0.45%) and palm oil (0%). Having a high acid number, 1.10165, and high saponification number and iodine number demonstrate the characteristic of VCO, as it contains lauric acid with small molecules, which are medium-chain triglycerides (MCT). In the future, a more specialized assessment needs to be done, especially in the economic field to examine whether VCO is more appropriate as a fuel alternative or as an antimicrobial.

Author Contributions: Conceptualization: S.S. (Suryani Suryani) and S.S. (Sariani Sariani); resources: M.M., and S.S. (Suryani Suryani); methodology: S.S. (Suryani Suryani) and M.M.; software: S.S. (Suryani Suryani); validation: S.S. (Suryani Suryani), M.M.; formal analysis: S.S. (Suryani Suryani) and M.M.; writing—original draft preparation: S.S. (Sariani Sariani), T.M.I.M. and S.S. (Suryani Suryani); writing—review and editing: S.S. (Suryani Suryani), R.R., F.E., S.S. (Sariani Sariani) and A.F.; project administration: S.S. (Sevindrajuta Sevindrajuta) and R.R.

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Conflicts of Interest:

"The authors declare no conflict of interest."

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Article

A Comparative Study of Virgin Coconut Oil, Coconut Oil and Palm Oil in Terms of their Active Ingredients

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ABSTRACT

. This research aims to study the unique factors of Virgin Coconut Oil (VCO) compared with coconut oil (i.e., coconut oil processed through heating the coconut milk and palm oil sold on the market. Its novelty is that it (VCO) contains lactic acid bacteria and bacteriocin. Lauric acid content was analyzed by the Chromatographic Gas method. Isolation of LAB was conducted by the dilution method using MRSA + 0.5% CaCO3 media. Iodium number, peroxide, % FFA is analyzed using a general method Isolation bacteriocin by deposition method using ammonium sulfate. In addition, macromolecular identification was conducted by 16S rRNA. VCO distinguished by more higher content of lauric acid (C12:0) 41% - 54.5% as compared with coconut and palm oils: 0%: 0.11% rerspectively. The VCO also contains LAB, namely Lac.plantarum and Lactobacillus paracasei, can inhibit the growth of pathogenic bacteria, such as P.aeruginase, Kleibsiella, S.aureus, S.epidermidis, Proteus, E.coli, Lis.monocytogenes, B. cereus, and S. typhosa, and bacteriocin .. Compare with VCO is based on having a high content of Lauric Acid, 54%, and LAB content. The difference between VCO and coconut oil and palm oil is fatty acids. In VCO there are lauric acid and stearic acid, namely lauric acid VCO (A) 54.06%; VCO (B) 53.9% and VCO (C) 53.7%. The content of stearic acid VCO (A) is 12.03%, VCO (B) 12.01% and VCO (C) 11.9%. Coconut oil contains a little lauric acid which is 2.81%, stearic acid 2.65%, and palmitic acid 2.31%. Palm oil can be said to have very little lauric acid namely in palm oil 1 (0.45%) even in palm oil 2 (0%) and palmitic acid palm oil 1 (2.88%) and palm oil palmitic acid 2 (28, 88%).

Keywords: Bacteriocin, Lactic Acid Bacteria (LAB), Lauric Acid, Virgin Coconut Oil (VCO).

1. Introduction

Virgin Coconut Oil (VCO)) can be made through several methods such as by fermenting coconut milk [1] [2] [3] and by adding microbes (*Lactobacillus fermentum* and *Lactobacillus plantarum*) as starter cultures [4][5]. VCO can also be produced through centrifugation [6] and microwave processes[7], and by fermentation without the addition of microbes as a starter [8]. This oil is called virgin oil because it is made without any heating [9].

VCO has been used widely because it is believed to have benefits compared to coconut oil made through a heating process and palm oil . VCO is useful against microbes, bacteria and virii [10], and is useful for for helping lose weight in terms of metabolism. VCO contains medium chain triglycerides[11][12], which is initially digested or processed in the body from carbohydrates that can cut back hunger [13]. Thus, it causes people to consume less carbohydrates, which eventually reduce body weight[14][15]. VCO also affects the healing of ovariectomy [16], and can be used as an antioxidant [17]. VCO can also reduce blood pressure. In addition, VCO can also be used for skincare [18][19][20], as an external drug such as wound medicine, and can function as a probiotic[21][22][23][4].

VCO and palm oil have different characteristics with different function [24][25][26]. VCO gravitates more towards medicine, probiotic, and cosmetics, whereas palm oil characteristics quite suitable to be converted to diesel fuel . Not only palm oil, some other vegetable oils have also been proven to be economical and can be converted to biodiesel production in large scale [27][28][26][29]. The chemical composition of VCO has been studied in including the iodine number, the saponification number, the amount of free fatty acids (% FFA), viscosity and colour. This chemical composition needs to be examined prior consumption in order to follow Asian and Pacific Coconut Community (APCC) standardisation because VCO is originally made from fresh coconut milk [30]

VCO has been known for its high lauric acid content, which is between 46.36 - 48.42% [31] Processed from coconut milk, which is high in carbohydrates and proteins, the fermentation process of VCO results in lactic acid bacteria (LAB) [32][33][34][35]. LAB from VCO have been isolated and their antimicrobial ability has also been studied [36]. This atypical microbial ability exists because LAB contains bacteriocin , which can kill pathogenic bacteria. Further, bacteriocin from *Lactobacillus plantarum*[34] [35][37][38] has also been isolated as well.

"Thus, the aim of the research was comparison of the psychochemical parameters and contents of fatty acids (lauric, palmitic and stearic) of coconut oil, palm oil, and VCO as the indicators of the characteristics of each oil. Also the antimicrobial ability of VCO was analyzed."

The newest fact referring to VCO which has yet acknowledged is the presence of lactic acid bacteria (LAB) on the oil and blondo layers (VCO dregs). This LAB will be present when VCO is made through traditional fermentation process or fermentation using the existing bacteria in the air[36].

2. Materials and Methods

2.1. Materials:

The samples were three types of virgin coconut oils, with different methods of extraction/fermentation), coconut oil (derived from heating coconut milk), and consumer-grade palm oil .

2.1.1. Chemical Material:

For Lauric Acid Analysis, -hexane (p.a), CHCl₃, Aquadest, reagent for sample preparation, namely saturated NaCl, Na₂SO₄ anhydrate, BF₃, Methanol, and N₂gas to stop the oxidation occurred were used. The internal of fatty acid standard. Whereas for Acid Number analysis (%FFA), 95%Alcohol (pa), blue bromothymol indicator, phenolphthalein indicator, HCl 0.5 N Standard Solution, and KOH Standard Solution were used. KOH ethanolic, HCl 0.5 N standard solution was taken for Saponification Number Analysis, and Potassium Iodide was used for Peroxide Number Analysis. Meanwhile MRSA (deMann Rogosa Sharpe Agar) p.a + 0.5% CaCO₃ media was used for Isolation of Lactaid Acid Bacteria and in order to increase their amount using MRS media. The materials used for isolation bacteriocin, are MRSB media (Merck), Lactobacillus plantarum M0, Ammonium Sulfate (NH4) 2SO4, Phosphate buffer, DEAE cellulose, butanol, SDS stacking gel and markers.

2.1.2. Instruments:

The instrument used where ordinary laboratory glassware such as petri dish, Erlenmeyer, test tube, and beaker glass, where all of them made from Pyrex. In addition, Gas Chromatography GC GC-MS Shimadzu QP 2010S (Shimadzu Corp, Kyoto, Japan) was set for lauric acid analysis and Autocklav Yamata SN 21 for sterilization and Laminar Flow as the working media for isolating the bacteria and for performing antimicrobial analysis.

2.2.1. The standardization of VCO

2.2.1.1. The determination of pH

The pH of the sample is determined using the pH meter in accordance with the applicable general procedure.

2.2.1.2. The analysis of fatty acid sample using GC-MS

Prior to injecting the sample into GC-MS instrument, the oil sample was prepared by setting 50 gram of VCO sample, and adding 400 μ L of NaOH Metanolic . This mixture was vortexed and heated at 50° C for 10 minutes. After undergoing the cooling process, 1mL CH₃COOH, 1mL distilled water, and 1mL n-hexane were added respectively. Then this mixture was vortexed and cooled for several minutes where two layers were formed as the result.

About 1 μ L at the top layer was taken as the sample to be injected and analyzed in GC-MS: Shimadzu QP2010 which was equipped with capillary colum of (30m) x 0,25 mm ID; 0,25 im (interspersed by

DB5MS. Japan), by injecting the sample into the capillary colum. The carrier gas was helium, where the injector and detector temperature was set at 280° C. The injection was performed using the split mode (1:30). The column temperature was programmed to change from 50° C to 280 ° C at a rate of 5° C per minute. Fatty acid ethyl esters were separated at the constant pressure (100kPa), and the peak was identified through the comparison of mass spectrum with mass spectral as the database (Internal Standard). The compound identification was as regards to the comparison of its mass spectrum with NIST Mass Spectral Library 2008.

2.2.1.3. The determination of water content

A porcelain dish along with a loose-fitting cover were cleaned, and dried in the dryer oven at 105°C for 1 hour. Then they were taken using a pair of clamps and inserted into desiccators where the lid was detached. Soon after the dish has cooled, the lid was tightened and weighed. A 2 gram sample was weighed into the dish and undergone another eight hours's dry-heating in a hot-air oven at 105°C, until reaching a constant weight. Another cooling process in desiccators was conducted for 30 minutes before determining its water level.

2.2.1.4. The determination of acid number

5 grams of the sample was weighed into a 300mL Erlenmeyer flask, then added 25 mL neutral alcohol, after that the flask was connected to an upright condenser, and boiled for 30 minutes. After cooling down, the sample was titrated with NaOH 0.1 M using pp indicator. The volume of NaOH titrer was recorded.

2.2.1.5. The determination of Iod number

0.5 grams of oil was weighted in a covered Erlenmeyer flask, then 10 mL chloroform was added and shaken. Then 15 mL Wij's solution was added and shaken slowly for another 30 minutes where the Erlenmeyer continued to be covered. The lid and inner wall of Erlenmeyer have been washed with 50 mL distilled water (initially heated and cooled). Next step was titration with 0,1 N Tio (Na₂S₂O₃) until the color changed into light brown, and 2mL of 1% starch was used as indicator. The titration was continuously conducted until the dark blue color disappeared.

2.2.1.6. The isolation of lactic acid bacteria

The oil sample was diluted utilizing saline solution with dilution up to 10^{-7} . Then this sample was planted on the dish containing MRSA + 0,5 % CaCO₃ selective media, and incubated overnight at 37°C. The growth was observed.

2.2.1.7. The molecular identification

Initially, the identification is began with isolating genomic DNA of lactic acid bacteria, then, continued by amplying 16S rRNA gen. In order to determine the size of its DNA, this gen is analyzed using Electrophoresis gel, and ended with sequencing.

2.2.1.8. Antimicrobial analysis

The antimicrobial analysis was carried out using agar-disk method, which has been modified using a disk made from filter paper. It was assumed that 1 unit of antimicrobial activity was defined as 1 mm² inhibited zone area or "halo" zone. It began with testing bacteria grown on NA media (Merck) of 1.5% agar concentration at 37°C. Incubated overnight, then 1 dose of testing bacteria was moved into test tubes containing 10 mL sterile distilled water. After that, the cells numbers was conformed to Mc Farland 0.5, which was estimated to be $10^6 - 10^7$ CFU mL⁻¹ number of cells. Toothpicks wrapped with cotton and sterilized were dipped into testing bacteria (more or less 100 µL). Left to dry, then took the filter paper which has been sterilized and perforated like a disk with 80 mm diameter, dipped it into LAB isolate, and stuck onto the solid media surface in petri dish which has been smeared by testing bacteria. Incubated for the period of 3 x 24 hours, and observed until the clear zone or "halo" zone was formed indicating the occurrence of the growth inhibition of pathogenic bacteria by lactic acid bacteria. Finally the diameter of clear zone is measured.

3. Results and Discussion

3.1. . Composistion and properties VCO, coconut Oil, and palm oil.

From the research conducted on virgin coconut oil (VCO), coconut oil and palm oil, there are differences from the three. Virgin Coconut Oil contains lauric acid (53.70% - 54.06%), and stearic acid (2.65% - 12.10%), and lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus paracasei*). and Coconut oil contains very little lauric acid (and stearic and palmitic acids. While palm oil contains only palmitic acid . Because of the presence of lactic acid bacteria containing bacteriocin, VCO *is* characterized as *a* antimicrobial, in contrast to coconut and palm oils.

As shown in Table 1 below, the lauric acid content of VCO is the highest with 54.06%. In contrast, coconut oil contains only 2.81%, and palm oil none (0%). Table 1 shows that there are two types of fatty acid contents in VCO; 54.06% lauric acid and 12.06% stearic acid, and none (0%) palmitic acid. The absence of palmitic acid is because VCO is not made of palm oil. Palmitic acid is found in palm oil. The lauric acid content of VCO is considered high compared to what has been obtained through this research [12]. This is because VCO is processed without heating, or through fermentation. Thus the fatty acid carbon bonds are not broken, in other words, the fatty acid is in the form of medium chain triglycerides, particularly lauric acid. On the other hand, coconut oil made from cooked coconut milk has low lauric acid content, i.e. 2.81%, containing 2.65% saturated acids, due to the production process involving heating. Conversely, palm oil has absolutely no lauric acid content, whereas its palmitic acid is high; 2.28% compared to VCO and coconut oil.

Table 1. The Result: Composition and properties of virgin coconut oil, coconut oil and

palm oil

Type of the Oil	Fatty acids	%	Acid number	Saponification number	%FFA	Iodin number	рН	Water content %
VCO(A)	Lauric Acid (C12:0)	54.06	1.01	348.00	0.26	5.32	6.50	0,11
	Fammal	-						

	Acid							
	Stearat	12.03						
	Acid(C18:0)							
VCO(B)	Lauric Acid	53.90	1.03	345.70	0,25	5.24	6.40	0,12
	(C12:0)							
	Palmitat	-						
	Acid							
	(C16:0)							
	Stearat Acid	12.01						
	(C18:0)							
VCO(C)	Louria Acid	52 70	1.02	246.64	0.26	5.25	6 50	0.11
VCO(C)	(C12.0)	55.70	1.02	340,04	0.20	5.25	0.50	0,11
	Palmitat							
	Acid							
	(C16:0)							
	Stearat Acid	11.9						
	(C18:0)							
Coconut	Lauric Acid	2.81	0.39	269.62	0.28	7.02	6.90	0.11
oil	(C12:0)							
	Palmitat	2.31						
	Acid							
	<u>(C16:0)</u>	• / -						
	Stearat Acid	2.65						
	(C18:0)							
Palm	Lauric Acid	0.45	0.39	204.00	0.51	51.00	6.60	0.09
Oil 1	(C12.0)	0.45	0.59	204.00	0.51	51.00	0.00	0.09
	Palmitat	2.88						
	Acid	2.00						
	(C16:0)							
	Stearat Acid	_	•					
	(C18:0)							
Palm	Lauric Acid	-	0.39	203.02	0.73	49.71	6.50	0.09
Oil 2	(C12:0)							
	Palmitat	24.42						
	Acid							
	<u>(C16:0)</u>							
	Stearat Acid	-						
	(C18:0)							

From Table 1. above it can be said that VCO has an acid number of 1.0165 which is higher than coconut milk (acid number of 0.39695), and palm oil (acid number of 0.39645). VCO water content is similar to palm oil, which is 0.11% whereas coconut oil processed through heating has a lower water content of 0.10%. However, the water content of the three samples still meet the limit standard of cooking oil 2177 SNI 3741 2013. In other words, the smaller number of the three samples' water content simplifies the heating process so the three samples can be used as biodiesels, in accordance with .

The analysis result of % FFA contains of 0.264 VC), 0.281 coconut oil, and 0.51 palm oil. It shows that VCO heating process is much better than other oil samples according to the study of

The VCO saponification number is 348.003 whereas coconut milk has a saponification number of 269.6266 and palm oil 204.0045. The high saponification number reflects the number of the fatty acid molecules. The bigger the saponification number, the smaller the molecules, or consisting of smaller fatty acid molecules or shorter chain, and vice versa. Hence, the higher saponification number of the VCO is because it consists of medium chain triglyceride fatty acids [14], [42]. The higher saponification number compared to palm and coconut oils mean that the saponification occuring in VCO is greater, even though still within tolerable limits.

3.2. Result of LactidAcid Bacteria Isolation

. Figure 1, below presents the isolated result of lactic acid bacteria onto VCO, coconut oil, and palm oil. It is shown that VCO contains lactic acid bacteria, as when it was being isolated using MRSA + CaCO₃ selective media, the lactic acid bacteria colony which can grow in "Halo" area is found. This area is a clear zone where it can produce lactic acid neutralising CaCO3. It is in line with, and as an additional, the results in Figure 2 and 3 points out the identification of lactic acid bacteria using 16S rDNA which turn out to be *Lacobacillus plantarum* and *Lactobacillus paracasei* as mentioned in, , and.



Figure 1. Lactid acid bacteria isolate grown in MRS + 0,5% CaCO₃ media

It can be seen, in Figure 1, there is a clear area, in the middle there is a white dot which is a colony of lactic acid bacteria present in VCO oil. This is proof that VCO contains lactic acid bacteria.[29], [32]



Figure 2. Result of lactid acid bacteria isolation from coconut oil and from palm oil with no evidence of growth

Meanwhile, in Figure 2, is a petri dish filled with coconut oil and palm oil grown on the same media. No visible growth of lactic acid bacteria. This proves that lactic acid bacteria do not exist in coconut oil and palm oil. This is in accordance with[8], [35] [43]. But not so [35], which only uses MRSA media, without the addition of CaCO3. But growing colonies are not in the "Hello" area

3.3. Molecular Identification of Lactid Acid Bacteria

The molecular identification of lactic acid bacteria produces *Lactobacillus plantarum* as shown in the data below Figure 3

-	
	>CONTIQ_M16.4_1440bp_Lactobacillus plantarum_100%
l	GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAA
l	GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATA
l	ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTAT
l	CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC
l	CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
l	CACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGA
l	AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA
l	GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG
l	AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACA
l	GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCT
l	GTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT
l	GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGG
l	CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTAC
l	GCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA
l	GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATC
l	AGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA
l	TCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGG
l	AGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAAT
I	CGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAAC
l	ACCCAAAGTC
L	

Figure 3. Lactid acid bacteria gene sequence from isolation of Lactobacillus plantarum.

11 AV	Aligoments 🗊 Download – GenBank Graphics, Distance tree of results.									
	Description	Max score	Total score	Query cover	E value	Ident	Accession			
1	Bacterium strain A2 16S ribosomal RNA gene, partial sequence	276	276	100%	3e-71	100%	KX268350.1			
-	Ladobacillus paracasei strain HD1.7.16S ribosomal RNA gene, partial seguence	276	276	100%	3e-71	100%	EU249147.1			
3	Lactobacillus rhamnosus strain L156.4.16S ribosomal RNA gene, partial sequence	274	274	99%	1e-70	100%	KX644947.1			
	Ladobacillus casei strain LC5, complete genome	274	1372	99%	1e-70	100%	CP017065.1			
3	Ladobacillus rhamnosus strain 4B15, complete genome	274	1372	99%	1e-70	100%	CP021426.1			
1	Ladobacilius paracasei strain IIA, complete genome	274	1372	99%	1e-70	100%	CP014985.1			
3	Ladobacillus mamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1			
3	Ladobadilus rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1			
3	Ladobacillus rhamnosus strain BFE5264, complete genome	274	1372	99%	1e-70	100%	CP014201.1			
3	Lactobacillus casel strain K1C 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S	274	274	99%	1e-70	100%	KU954559.1			
3	Ladobacillus rhamnosus strain LRB, complete genome	274	1372	99%	1e-70	100%	CP016823.1			
1	Ladobacillus rhamnosus gene for 16S ribosomal RNA, partial sequence, strain: NGRI 0110	274	274	99%	1e-70	100%	LC177236.1			
3	Lactobacillus paracasei strain FS3 16S ribosomal RNA gene, partial seguence	274	274	99%	1e-70	100%	KU315064.1			
1	Ladobacillus rhamnosus strain ASCC 290 genome	274	274	9996	1e-70	100%	CP014645.1			

Figure 4. The result of molecular identification of lactid acid bacteria showing Lactobacillus paracasei.

3.4. Result of Anti Microbial Analysis.

The antimicrobial ability of VCO is also analyzed below. Antimicrobial analysis was performed with *Lactobacillus paracasei* and *Lactobacillus plantarum* lactid acid bacteria onto testing bacteria; *Pseudomonas*

aeruginase, Klebsiella, Staphilococcus aureus, Staphilococcus epidermidis, Proteus, from [31][35], Eschericia coli, Listeria monocytogenes, Bacillus cereus, and Salmonella typhosa.

As seen in Figure 4 below, clear zones show that the growth of pathogenic bacteria was inhibited by *Lactobacillus plantarum* and *Lactobacilus paracasei*.





Result of antimicrobial analysis of *Lactobacillus* plantarum Lactid Acid Bacteria onto *E, coli* testing bacteria

Result of antimicrobial analysis of *Lactobacillus`plantarum* Lactid Acid Bacteria onto *S.aureus* testing bacteria

Figure 4. Result Antimicrobial analysis of *Lactobacillus*`*plantarum* Lactid Acid Bacteria onto *E, coli and S. aureustesting* bacteria

As illustrated in Figure 4, VCO has the antimicrobial ability derived from two types of lactic acid bacteria against nine testing bacteria. It is seen that these lactic acid bacteria have a good ability to kill pathogenic bacteria; *Listeria monocytogenes*, and *E. coli*, as stated in [8], where the antimicrobial ability of *Lacobacillus plantarum* is found to be most effective against *Listeria monocytogenes*, *E. coli* and *Bacillus subtilis* testing bacteria.

	Table 2. Antimicrobial activity analysis of LAB in the form of clear zone diameter (mm)								
No	Testino Basteria	Lactobacillus	Lactobacillus						
INO.	Testing Bucieriu	plantarum	paracasei						
1.	Escherichia coli	16	16						
2.	ListeriaMonocitogenes	17	18						
3.	BacillusSubstiliss	15	11						
4.	SalmonellaTyphyphosa	12	11						
5.	StaphillococcusAureus	11	11						
6.	Pseudomonas aeruginase	17	14						
7.	Klebsiella	13	12						
8.	Staphilococcus epidermidis	13	12						
9.	Proteus,	14	13						

In Table 2 it can be seen that the lactic acid bacteria present in the VCO are able to inhibit the growth of pathogenic bacteria as *StaphillococcusAureus*, *Pseudomonas aeruginase*, *Klebsiella*, *Staphilococcus epidermidis*, and *Proteus*, in accordance with[36]

4. Conclusions

. Compared with coconut oil and palm oil, virgin coconut oil (VCO)has a higher content of Lauric Acid and lactic acid bacteria. Nevertheless, based on the content of water, free fatty acid, and Iodine number, . VCO has other features in the field of health because it has lactic acid bacteria that can kill pathogens, characterizing it as a antimicrobial, in contrast to coconut and palm oil. The lauric acid content of the VCO is the highest (54.06%) compared to the lauric acid content of coconut oil (0.45%), and palm oil (0%). Having a high acid number; 1.10165, and high saponification number and Iodine number demonstrate the characteristic of VCO, as it contains lauric acid with small molecules which are medium chain triglycerides (MCT). In the future, a more specialized assessment needs to be done, in the economic field to examine whether VCO is more appropriate as a fuel alternative or as an antimicrobial.

Contribution Author.

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Article

A Comparative Study of Virgin Coconut Oil, Coconut Oil and Palm Oil in Terms of Their Active Ingredients

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ABSTRACT: This research aims to study the unique factors of virgin coconut oil (VCO) compared with coconut oil (i.e., coconut oil processed through heating the coconut milk and palm oil sold on the market). Its novelty is that it (VCO) contains lactic acid bacteria and bacteriocin. Lauric acid content was analyzed by the Chromatographic Gas method. Isolation of lactic acid bacteria (LAB) was conducted by the dilution method using MRSA + 0.5% CaCO3 media. Iodium number, peroxide, and %FFA were analyzed using a general method, and isolation bacteriocin by the deposition method using ammonium sulfate. In addition, macromolecular identification was conducted by 16S rRNA. VCO was distinguished by a higher content of lauric acid (C12:0) 41%-54.5% as compared with 0% coconut and 0,1% palm oil, respectively. The VCO also contains LAB, namely Lactobacillus plantarum and Lactobacillus paracasei, and can inhibit the growth of pathogenic bacteria, such as Pseudomonas aeruginosa, Klebsiella, Staphylococcus aureus, S. epidermidis, Proteus, Escherichia coli, Listeria monocytogenes, Bacillus cereus, Salmonella typhosa and bacteriocin. Comparison with VCO is based on having a high content of lauric acid, 54%, and LAB content. The difference between VCO and coconut oil and palm oil is fatty acids. In VCO there are lauric acid and stearic acid, namely lauric acid VCO (A) 54.06%, VCO (B) 53.9% and VCO (C) 53.7%. The content of stearic acid VCO (A) is 12.03%, VCO (B) 12.01% and VCO (C) 11.9%. Coconut oil contains a little lauric acid, which is 2.81%, stearic acid 2.65% and palmitic acid 2.31%. Palm oil can be said to have very little lauric acid, namely in palm oil 1, 0.45%, and even in palm oil 2, 0%; in turn, palmitic acid palm oil 1 has 2.88% and palm oil 2 palmitic acid has 24.42% .

Keywords: bacteriocin; lactic acid bacteria (LAB); lauric acid; virgin coconut oil (VCO)

1. Introduction

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MDPI

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Comment [MDPI3]: Please carefully check the accuracy of names and affiliations.

Please complete the authors name, if possible.

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The 1st, 2nd, 4th, 5th, and 6th author's name only one word, so we have added to become 2 words; first and last name. ex: Suryani, Suryani; Sariani, Sariani: Marganof, Marganof; Rahmawati, Rahmawati; Sevindrajuta, Sevindrajuta.

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Virgin coconut oil (VCO) can be made through several methods, such as by fermenting coconut milk [1–3] and by adding microbes (*Lactobacillus fermentum* and *Lactobacillus plantarum*) as starter cultures [4,5]. VCO can also be produced through centrifugation [6] and microwave processes [7], and by fermentation without the addition of microbes as a starter [8]. This oil is called virgin oil because it is made without any heating [9].

VCO has been used widely because it is believed to have benefits compared to coconut oil, made through a heating process, and palm oil. VCO is useful against microbes, bacteria and viruses [10], and is useful for helping one lose weight in terms of metabolism. VCO contains medium chain triglycerides [11,12], which is initially digested or processed in the body from carbohydrates that can cut back hunger [13]. Thus, it causes people to consume less carbohydrates, which eventually reduces body weight [14,15]. VCO also affects the healing after an ovariectomy [16] and can be used as an antioxidant [17]. VCO can also reduce blood pressure. In addition, VCO can also be used for skincare [18–20], as an external drug, such as wound medicine, and can function as a probiotic [4,21–23].

VCO and palm oil have different characteristics with different functions [24–26]. VCO gravitates more towards medicine, probiotics and cosmetics, whereas palm oil characteristics are quite suitable to be converted to diesel fuel. Other vegetable oils than palm oil have also been proven to be economical and can be converted to biodiesel production at a large scale [27–29]. The chemical composition of VCO has been studied in including the iodine number, the saponification number, the amount of free fatty acids (%FFA), viscosity and color. This chemical composition needs to be examined prior to consumption in order to follow Asian and Pacific Coconut Community (APCC) standardization, because VCO is originally made from fresh coconut milk [30]

VCO has been known for its high lauric acid content, which is between 46.36% and 48.42% [31] Processed from coconut milk, which is high in carbohydrates and proteins, the fermentation process of VCO results in lactic acid bacteria (LAB) [32–35]. LAB from VCO have been isolated and their antimicrobial ability also has been studied [36]. This atypical microbial ability exists because LAB contains bacteriocin, which can kill pathogenic bacteria. Further, bacteriocin from *Lactobacillus plantarum* [34,35,37,38] has been isolated as well. Thus, the aim of the research was comparison of the psychochemical parameters and contents of the fatty acids (lauric, palmitic and stearic) of coconut oil, palm oil and VCO as indicators of the characteristics of each oil. The antimicrobial ability of VCO was also analyzed.

The newest fact referring to VCO, which is yet to be acknowledged, is the presence of lactic acid bacteria (LAB) in the oil and blondo layers (VCO dregs). This LAB will be present when VCO is made through traditional fermentation processes or fermentation using the existing bacteria in the air [36].

2. Materials and Methods

2.1. Materials

The samples were three types of virgin coconut oils, with different methods of extraction/fermentation, coconut oil derived from heating coconut milk and consumer-grade palm oil.

2.1.1. Chemical Material

For lauric acid analysis, -hexane (p.a), CHCl₃ and Aquadest reagent for sample preparation, namely saturated NaCl, Na₂SO₄ anhydrate, BF₃, methanol and N₂gas to stop the oxidation occurring, were used. The internal fatty acid standard was used. Whereas, for acid number analysis (%FFA), 95% alcohol (pa), blue bromothymol indicator, phenolphthalein indicator, HCl 0.5 N standard solution and KOH standard solution were used. KOH ethanolic, HCl 0.5 N standard solution was taken for saponification number analysis, and potassium iodide was used for peroxide number analysis. Meanwhile MRSA (deMann Rogosa Sharpe Agar) p.a + 0.5% CaCO₃ media was used for isolation of lactic acid bacteria and in order to increase their amount using MRS media. The materials

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Comment [MDPI11]: Citation

format has been corrected. Replace "[21], [22], [23], [4]" here, please confrim

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used for isolation bacteriocin were MRSB media (Merck), *Lactobacillus plantarum* M0, ammonium sulfate (NH4) 2SO4, phosphate buffer, DEAE cellulose, butanol, SDS stacking gel and markers.

2.1.2. Instruments

The instrument used where ordinary laboratory glassware, such as petri dishes, Erlenmeyer flasks, test tubes and beaker glass, all of them made by Pyrex. In addition, gas chromatography (GC) GC–MS Shimadzu QP 2010S (Shimadzu Corp, Kyoto, Japan) was set for lauric acid analysis and Autocklav Yamata SN 21 for sterilization and laminar flow as the working media for isolating the bacteria and for performing antimicrobial analysis.

2.2. The Standardization of the VCO

2.2.1. The Determination of pH

The pH of the sample is determined using the pH meter in accordance with the applicable general procedure.

2.2.2. The Analysis of Fatty Acid Sample Using GC-MS

Prior to injecting the sample into a GC–MS instrument, the oil sample was prepared by setting 50 g of VCO sample and adding 400 μ L of NaOH Metanolic. This mixture was vortexed and heated at 50 °C for 10 min. After undergoing the cooling process, 1 mL CH₃COOH, 1 mL distilled water and 1 mL n-hexane were added, respectively. Then this mixture was vortexed and cooled for several minutes where two layers were formed as the result.

About 1 μ L at the top layer was taken as the sample to be injected and analyzed in a GC–MS, Shimadzu QP2010 which was equipped with capillary column of (30 m) × 0.25 mm ID; 0.25 im (interspersed by DB5MS. Japan), by injecting the sample into the capillary column. The carrier gas was helium, where the injector and detector temperatures were set at 280 °C. The injection was performed using the split mode (1:30). The column temperature was programmed to change from 50 °C to 280 °C at a rate of 5 °C per minute. Fatty acid ethyl esters were separated at the constant pressure (100 kPa), and the peak was identified through the comparison of mass spectra with mass spectral as the database (internal standard). The compound identification was with regard to the comparison of its mass spectrum with the NIST Mass Spectral Library 2008.

2.2.3. The Determination of Water Content

Porcelain dishes along with loose-fitting covers were cleaned and dried in the dryer oven at 105 °C for 1 h. Then they were taken using a pair of clamps and inserted into desiccators where the lid was detached. Soon after the dish has cooled, the lid was tightened and weighed. A 2 g sample was weighed into the dish and undergone another eight hours dry-heating in a hot-air oven at 105 °C, until reaching a constant weight. Another cooling process in the desiccators was conducted for 30 min before determining its water level.

2.2.4. The Determination of Acid Number

A total of 5 g of the sample was weighed into a 300 mL Erlenmeyer flask, and then 25 mL neutral alcohol was added; after that the flask was connected to an upright condenser, and boiled for 30 min. After cooling down, the sample was titrated with NaOH 0.1 M using a pp indicator. The volume of the NaOH titer was recorded.

2.2.5. The Determination of Iodine Number

A total of 0.5 g of oil was weighted in a covered Erlenmeyer flask, then 10 mL chloroform was added and shaken. Then 15 mL Wij's solution was added and shaken slowly for another 30 min where the Erlenmeyer continued to be covered. The lid and inner wall of the Erlenmeyer were washed with 50 mL distilled water (initially heated and cooled). The next step was titration with 0.1

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Comment [MDPI13]: "2.2." and "2.2.1–2.2.8" have been renumbered. Please confirm.

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N Tio (Na₂S₂O₃) until the color changed into light brown, and 2 mL of 1% starch was used as indicator. The titration was continuously conducted until the dark blue color disappeared.

2.2.6. The Isolation of Lactic Acid Bacteria

The oil sample was diluted utilizing saline solution with dilution up to 10^{-7} . Then this sample was planted on the dish containing MRSA + 0.5% CaCO₃ selective media, and incubated overnight at 37 °C. The growth was observed.

2.2.7. The Molecular Identification

Initially, the identification began with isolating genomic DNA of lactic acid bacteria, then, continued by amplifying 16S rRNA gen. In order to determine the size of its DNA, this gen was analyzed using electrophoresis gel and ended with sequencing.

2.2.8. Antimicrobial Analysis

The antimicrobial analysis was carried out using the agar-disk method, which has been modified using a disk made from filter paper. It was assumed that 1 unit of antimicrobial activity was defined as the 1 mm² inhibited zone area or "halo" zone. It began with testing bacteria grown on NA media (Merck) of 1.5% agar concentration at 37 °C. Incubated overnight, 1 dose of testing bacteria was moved into test tubes containing 10 mL sterile distilled water. After that, the cell numbers were conformed to McFarland 0.5, which was estimated to be 10^{6} – 10^{7} CFU mL⁻¹. Toothpicks wrapped with cotton and sterilized were dipped into testing bacteria (more or less 100 µL). After being left to dry, we took the filter paper that was sterilized and perforated it like a disk with a 80 mm diameter, dipped it into LAB isolate, and stuck it onto the solid media surface in petri dishes, which were smeared by testing bacteria. Samples were incubated for the period of 3 × 24 h and observed until the clear zone or "halo" zone was formed, indicating the occurrence of the growth inhibition of pathogenic bacteria by lactic acid bacteria. Finally, the diameter of the clear zone was measured.

3. Results and Discussion

3.1. Composistion and Properties of VCO, Coconut Oil and Palm Oil

From the research conducted on virgin coconut oil (VCO), coconut oil and palm oil, differences among the three were found. Virgin coconut oil contains lauric acid (53.70%–54.06%), stearic acid (2.65%–12.10%) and lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus paracasei*). Coconut oil contains very little lauric acid (and stearic and palmitic acids), while palm oil contains only palmitic acid. Because of the presence of lactic acid bacteria containing bacteriocin, VCO is characterized as an antimicrobial, in contrast to coconut and palm oils.

As shown in Table 1 below, the lauric acid content of VCO is the highest with 54.06%. In contrast, coconut oil contains only 2.81% and palm oil none (0%). Table 1 shows that there are two types of fatty acid contents in VCO; 54.06% lauric acid and 12.06% stearic acid, and no (0%) palmitic acid. The absence of palmitic acid is because VCO is not made of palm oil. Palmitic acid is found in palm oil. The lauric acid content of VCO is considered high compared to what has been obtained through this research [12]. This is because VCO is processed without heating, or through fermentation. Thus, the fatty acid carbon bonds are not broken, in other words, the fatty acid is in the form of medium chain triglycerides, particularly lauric acid. On the other hand, coconut oil made from cooked coconut milk has a low lauric acid content, 2.81%, containing 2.65% saturated acids due to the production process involving heating. Conversely, palm oil has absolutely no lauric acid content, whereas its palmitic acid is high (2.28%) compared to VCO and coconut oil.

Type of the Oil	Fatty Acids	%	Acid Number	Saponification Number	%FFA	Iodine Number	рН	Water Content %
	Lauric Acid (C12:0)	54.06		348.00	0.26	5.32	6.50	
VCO (A)	Palmitic Acid	-	1.01					0.11
	Stearic Acid(C18:0)	12.03						
	Lauric Acid (C12:0)	53.90		345.70	0,25	5.24	6.40	0.12
VCO (B)	Palmitic Acid (C16:0)	-	1.03					
	Stearic Acid (C18:0)	12.01						
	Lauric Acid (C12:0)	53.70	1.02	346.64	0.26	5.25	6.50	0.11
VCO (C)	Palmitic Acid (C16:0)	-						
	Stearic Acid (C18:0)	11.9						
	Lauric Acid (C12:0)	2.81		269.62	0.28	7.02	6.90	
Coconut oil	Palmitic Acid (C16:0)	2.31	0.39					0.11
	Stearic Acid (C18:0)	2.65						
	Lauric Acid (C12:0)	0.45		204.00	0.51	51.00	6.60	
Palm Oil 1	Palmitic Acid (C16:0)	2.88	0.39					0.09
	Stearic Acid (C18:0)	-						
	Lauric Acid (C12:0)	-		203.02	0.73	49.71	6.50	
Palm Oil 2	Palmitic Acid (C16:0)	24.42	0.39					0.09
	Stearic Acid (C18:0)	-						

Table 1. The results: Composition and properties of virgin coconut oil, coconut oil and palm oil.

From Table 1 above it can be said that VCO has an acid number of 1.0165, which is higher than coconut milk (acid number of 0.39695) and palm oil (acid number of 0.39645). VCO water content is similar to palm oil, which is 0.11%, whereas coconut oil processed through heating has a lower water content of 0.10%. However, the water content of the three samples still meet the limit standard of cooking oil 2177 SNI 3741 2013. In other words, the smaller number of the three samples' water content simplifies the heating process so the three samples can be used as biodiesels, in accordance with current guidelines.

The analysis result of %FFA contains 0.264 VCO, 0.281 coconut oil and 0.51 palm oil. It shows that VCO heating process is much better than other oil samples according to the study of the VCO saponification number being 348.003, whereas coconut milk has a saponification number of 269.6266

Comment [C15]: In accordance with? A word or words seems to be missing here. "current guidelines" was inserted; please check if original meaning is retained.

Comment [A16]: Yes, it's right,. Thank you for your correction.

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and palm oil 204.0045. The high saponification number reflects the number of the fatty acid molecules. The bigger the saponification number, the smaller the molecules, or consisting of smaller fatty acid molecules or shorter chains, and vice versa. Hence, the higher saponification number of the VCO is because it consists of medium chain triglyceride fatty acids [14,42]. The higher saponification number compared to palm and coconut oils mean that the saponification occurring in VCO is greater, even though still within tolerable limits.

3.2. Result of Lactid Acid Bacteria Isolation

Figure 1 below presents the isolated result of lactic acid bacteria onto VCO, coconut oil and palm oil. It is shown that VCO contains lactic acid bacteria, as when it was being isolated using MRSA + CaCO₃ selective media, the lactic acid bacteria colony that can grow in the "Halo" area is found. This area is a clear zone where it can produce lactic acid, neutralizing CaCO₃. It is in line with, and as an addition to, the results in Figures 2 and 3, pointing out the identification of the lactic acid bacteria using 16S rDNA, which turned out to be *Lactobacillus plantarum* and *Lactobacillus paracasei* as mentioned.



Figure 1. Lactid acid bacteria isolate grown in MRS + 0.5% CaCO3 media.

It can be seen, in Figure 1, there is a clear area; in the middle there is a white dot that is a colony of lactic acid bacteria present in the VCO oil. This is proof that VCO contains lactic acid bacteria [29,32].

Meanwhile, in Figure 2, is a petri dish filled with coconut oil and palm oil grown on the same media. No visible growth of lactic acid bacteria was observed. This proves that lactic acid bacteria do not exist in coconut oil and palm oil. This is in accordance with [8,35,43] but not so in [35], who only uses MRSA media without the addition of CaCO3. But growing colonies are not in the "Halo" area.

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Comment [A17]: Yes, it's right. Thank you for your correction.

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Figure 2. Result of lactid acid bacteria isolation from coconut oil and from palm oil with no evidence of growth.

3.3. Molecular Identification of Lactid Acid Bacteria

The molecular identification of lactic acid bacteria produced *Lactobacillus plantarum*, as shown in the data below Figure 3.

_		_
	>CONTIQ_M16.4_1440bp_Lactobacillus plantarum_100%	-
	GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAA	Ć
	GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGGATA	ŧ
	ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTAT	Ę
	CACTTTTGGATGGTCCCGCGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGA	Ģ
	CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTC	ł
	CACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGA	ł
	AGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA	ų
	SCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCG	ł
	AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGAC	1
	GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTC	ļ
	GTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT	ļ
	GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGC	1
	CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTA	1
	GCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA	1
	SGI GGI GGA GGI TGI CGI CGI CGI CGI CGI CGI CGI CGGGI I GGGI I AGGI CCCCCAACGA GCGCAACCCI I A I I A I C	1
	AGTTGCCAGCATTAAGTTGGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAGGGTGGGGGATGACGTCACA	2
		1
	HOUTAATUTUTTAAAOUUATTUTUAGTTUGUATTUTAGUUGUTAUAUTUGUUTAUATUAAOTUGUTAGTAAT	
		1
	ACCOMMON	1

Figure 3. Lactid acid bacteria gene sequence from isolation of Lactobacillus plantarum.

11	Alignments Download - GenBank Graphics Distance tree of results						3
	Description	Max score	Total score	Query cover	E value	Ident	Accession
2	Bacterium strain A2 16S ribosomal RNA gene, partial seguence	276	276	100%	3e-71	100%	KX268350.1
2	Ladobacillus paracasei strain HD1.7.16S ribosomal RNA gene, partial seguence	276	276	100%	3e-71	100%	EU249147.1
2	Lactobacillus rhamnosus strain L156.4.16S ribosomal RNA gene, partial seguence,	274	274	99%	1e-70	100%	KX644947.1
	Lactobacillus casei strain LC5, complete genome	274	1372	99%	1e-70	100%	CP017065.1
1	Lactobacillus rhamnosus strain 4B15, complete genome	274	1372	99%	1e-70	100%	CP021426.1
1	Lactobacillus paracasei strain IIA, complete genome	274	1372	99%	1e-70	100%	CP014985.1
1	Lactobacillus mamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
1	Laclobacillus rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1
1	Ladobacillus rhamnosus strain BFE5264, complete genome	274	1372	99%	1e-70	100%	CP014201.1
1	Lactobacillus casel strain K1C 16S ribosomal RNA gene, partial sequence; 16S-23S ribosomal RNA intergenic spacer, complete sequence; and 23S	274	274	99%	1e-70	100%	KU954559.1
1	Lactobacillus rhamnosus strain LRB, complete genome	274	1372	99%	1e-70	100%	CP016823.1
	Lactobacillus mamnosus gene for 16S ribosomal RNA, partial sequence, strain: NGRI 0110	274	274	99%	1e-70	100%	LC177236.1
5	Ladobacillus paracasei strain FS3 16S ribosomal RNA gene, partial seguence	274	274	99%	1e-70	100%	KU315064.1
n	Ladobacillus rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

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Figure 4. The result of molecular identification of lactid acid bacteria showing *Lactobacillus paracasei*.

3.4. Result of Antimicrobial Analysis

The antimicrobial ability of VCO is also analyzed below. Antimicrobial analysis was performed with *Lactobacillus paracasei* and *Lactobacillus plantarum* lactic acid bacteria onto the following testing bacteria: *Pseudomonas aeruginosa, Klebsiella, Staphylococcus aureus, Staphylococcus epidermidis, Proteus,* from [31,35], *Escherichia coli, Listeria monocytogenes, Bacillus cereus,* and *Salmonella typhosa*.

As seen in Figure 5 below, clear zones show that the growth of pathogenic bacteria was inhibited by *Lactobacillus plantarum* and *Lactobacillus paracasei*.



Result of antimicrobial analysis of *Lactobacillus plantarum* lactid acid bacteria onto *E. coli* testing bacteria.



Result of antimicrobial analysis of *Lactobacillus plantarum* lactid acid bacteria onto *S. aureus* testing bacteria

Figure 5. Results of the antimicrobial analysis of *Lactobacillus plantarum* lactid acid bacteria onto *E. coli* and *S. aureus* testing bacteria.

As illustrated in Figure 4, VCO has the antimicrobial ability derived from two types of lactic acid bacteria against nine testing bacteria. It is seen that these lactic acid bacteria have a good ability to kill pathogenic bacteria, e.g., *Listeria monocytogenes* and *E. coli*, as stated in [8], where the antimicrobial ability of *Lactobacillus plantarum* is found to be most effective against *Listeria monocytogenes*, *E.coli* and *Bacillus subtilis* testing bacteria.

In Table 2 it can be seen that the lactic acid bacteria present in the VCO are able to inhibit the growth of pathogenic bacteria as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella*, *Staphylococcus epidermidis*, and *Proteus*, in accordance with [36].

No.	Testing Bacteria	Lactobacillus plantarum	Lactobacillus paracasei
1.	Escherichia coli	16	16
2.	Listeria monocytogenes	17	<mark>18</mark>
3.	Bacillus substiliss	<mark>15</mark>	<mark>11</mark>
4.	Salmonella typhy	12	11
5.	Staphylococcus aureus	11	11
6.	Pseudomonas aeruginosa	17	14
7.	Klebsiella	<mark>13</mark>	<mark>12</mark>
8.	Staphylococcus epidermidis	13	12
9.	Proteus	14	13

Table 2. Antimicrobial activity analysis of LAB in the form of clear zone diameter (mm).

4. Conclusions

Comment [MDPI19]: Figure 4 is duplicated. Please renumber the figures and make sure the citation is before the figure

Comment [A20]: Yes, it's right. Thank you for your correction.

Comment [A21]: I have renumbered the related figure from (before) Figure 4 to (after) Figure 5.

Comment [M22]: Please check if the bold is necessary.

Comment [A23]: The bold is not necessary here, and I have corrected it.

Comment [A24]: I have corrected the species name, from 'Salmonella typhyphosa' to 'Salmonella typhy'.

Compared with coconut oil and palm oil, virgin coconut oil (VCO) has a higher content of lauric acid and lactic acid bacteria. Nevertheless, based on the content of water, free fatty acid and iodine number, VCO has other features in the field of health because it has lactic acid bacteria that can kill pathogens, characterizing it as an antimicrobial, in contrast to coconut and palm oil. The lauric acid content of the VCO is the highest (54.06%) compared to the lauric acid content of coconut oil (0.45%) and palm oil (0%). Having a high acid number, 1.10165, and high saponification number and iodine number demonstrate the characteristic of VCO, as it contains lauric acid with small molecules, which are medium-chain triglycerides (MCT). In the future, a more specialized assessment needs to be done, especially in the economic field to examine whether VCO is more appropriate as a fuel alternative or as an antimicrobial.

Author Contributions: Conceptualization: S.S. (Suryani Suryani) and S.S. (Sariani Sariani); resources: M.M., and S.S. (Suryani Suryani); methodology: S.S. (Suryani Suryani) and M.M.; software: S.S. (Suryani Suryani); validation: S.S. (Suryani Suryani), M.M.; formal analysis: S.S. (Suryani Suryani) and M.M.; writing—original draft preparation: S.S. (Sariani Sariani), T.M.I.M. and S.S. (Suryani Suryani); writing—review and editing: S.S. (Suryani Suryani), R.R., F.E., S.S. (Sariani Sariani) and A.F.; project administration: S.S. (Sevindrajuta Sevindrajuta) and R.R.

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Conflicts of Interest:

"The authors declare no conflict of interest."

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Comment [MDPI25]: Please confirm the authors' name first. Every author should be mentioned, please check and confirm.

Comment [A26]: Yes, it's right, Thank you

Comment [MDPI27]: The list has been merged into one paragraph. Please confirm.

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Comment [MDPI29]: Declare conflicts of interest or state "The authors declare no conflict of interest."

Comment [A30]: Yes, I have corrected, and written down that the authors declared no conflict of interest.

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Comment [A32]: Yes, it's right. Thank you for your correction

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