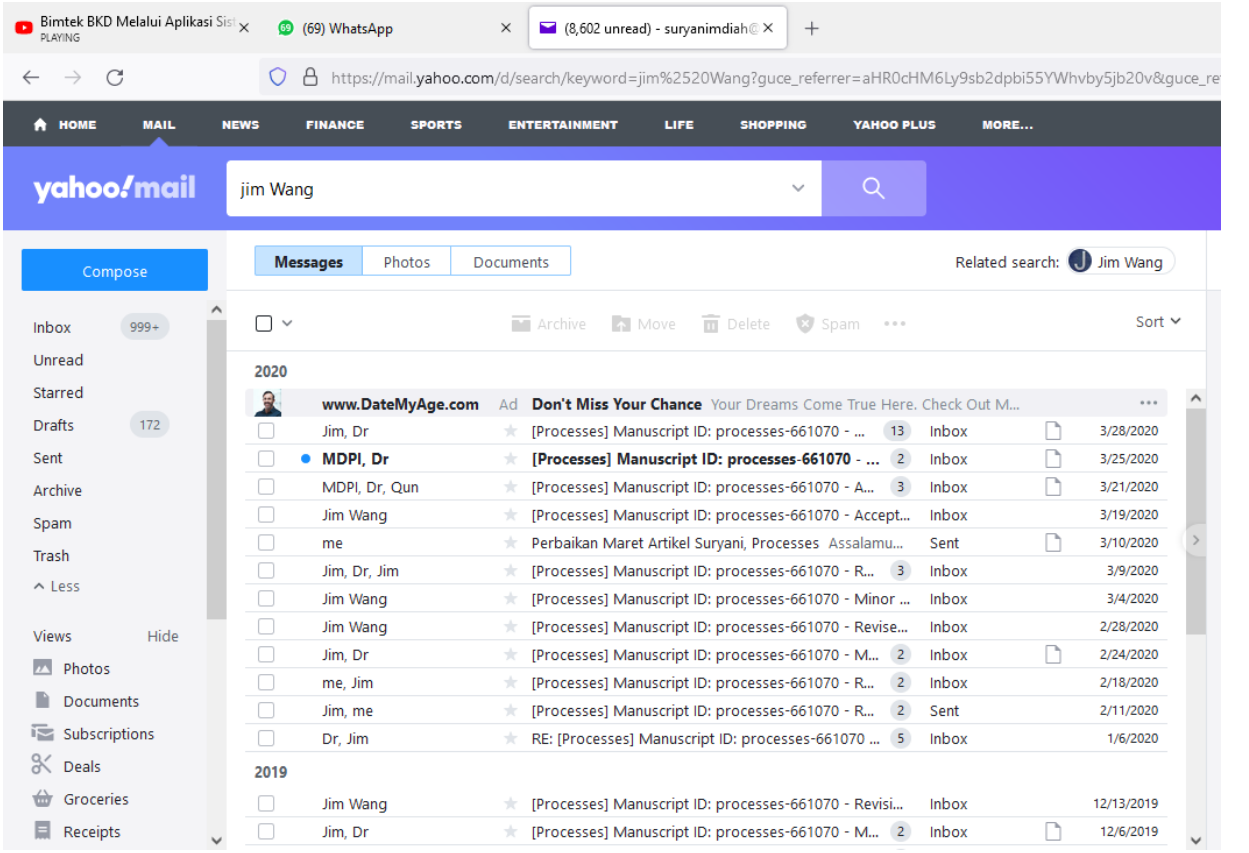
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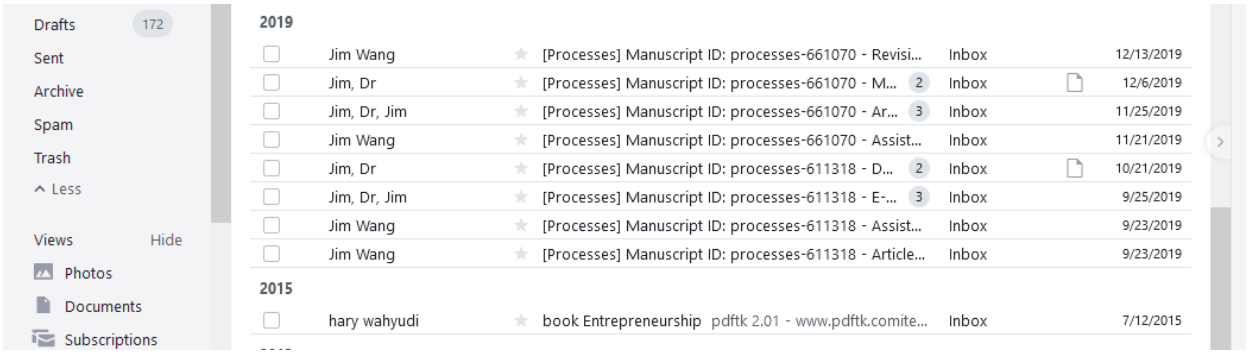
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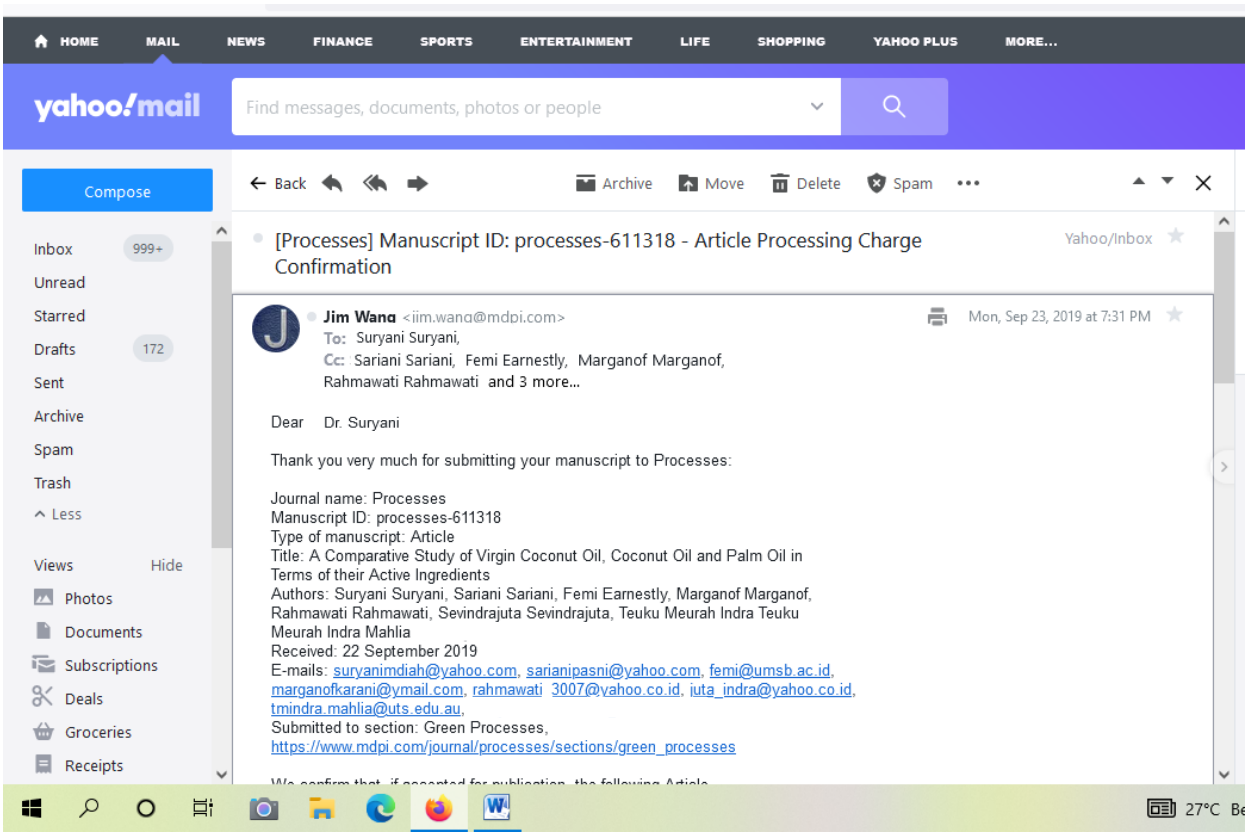
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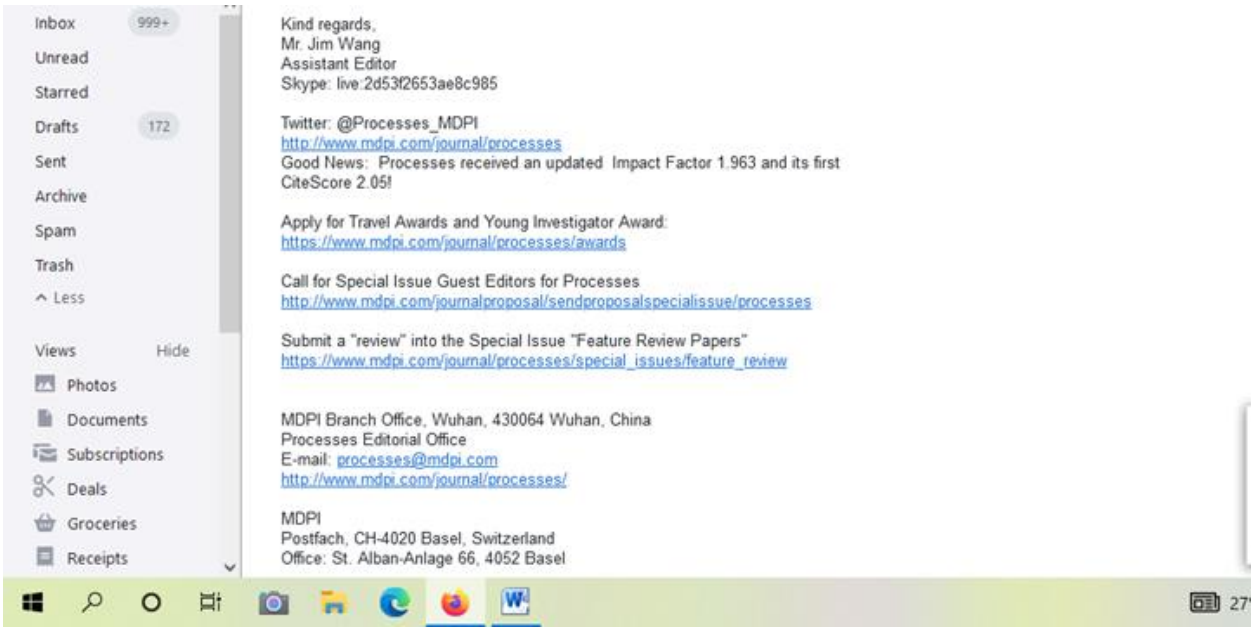
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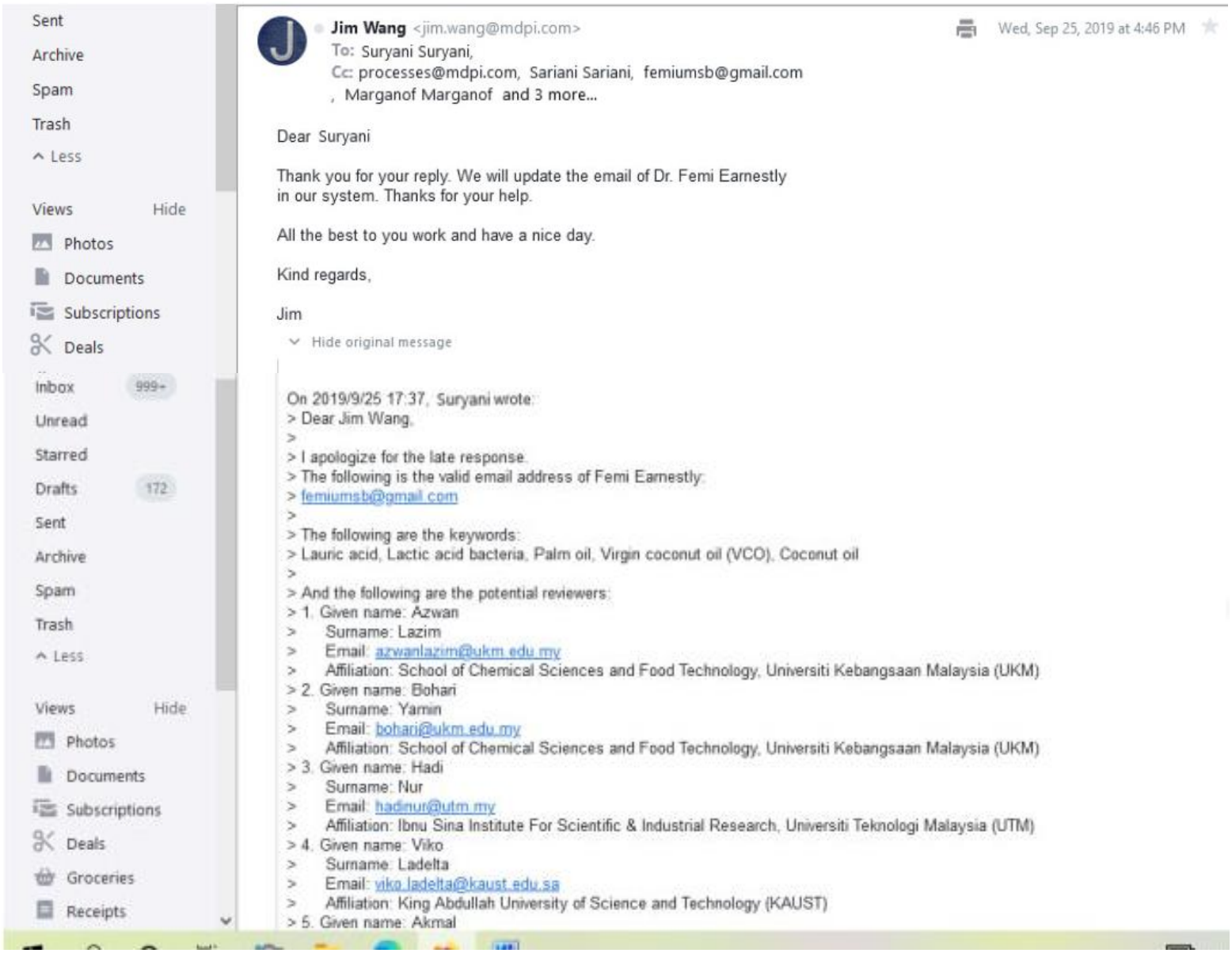
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>
> The following are the keywords:
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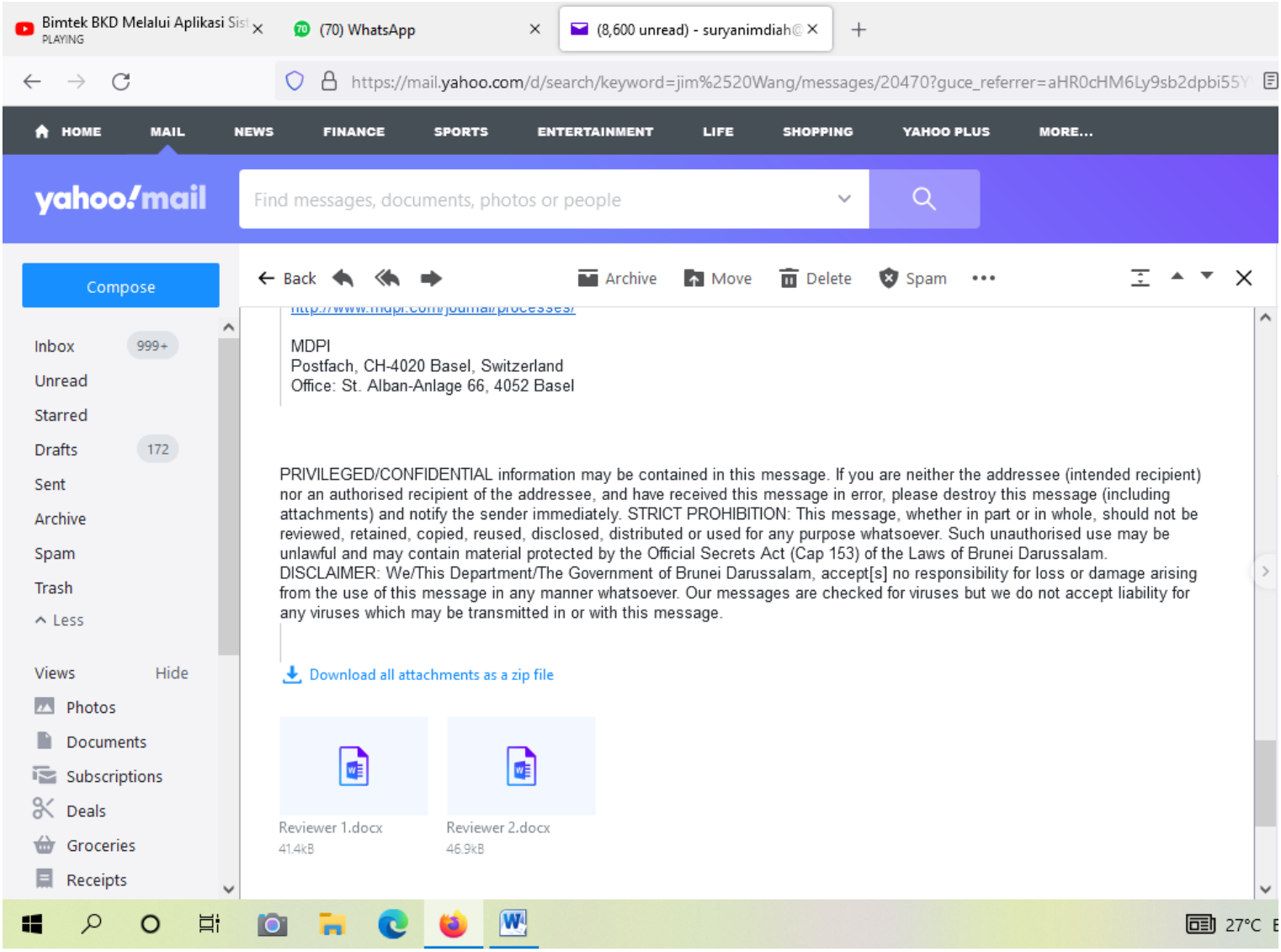
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A Comparative Study of Virgin Coconut Oil, Coconut Oil and Palm Oil in Terms of their Active Ingredients

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Abstract: Global petroleum-based fuel reserves are declining, resulting in the necessity to find alternatives, one of which is biofuel derived from sustainable sources. One of such fuels, i.e. biodiesel, can be produced from palm oil and coconut oil, however, another alternative source of fuel is Virgin Coconut Oil (VCO). This research aims to study the unique factors of VCO compared to coconut oil and palm oil to determine whether VCO can be used as biodiesel. Psychochemistry (Iodium umber, peroxide, % FFA) is analyzed using a general method. 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. 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.* Compare with VCO is based on having a high content of Lauric Acid, 54%, and LAB content. Nevertheless, based on the content of their water level, free fatty acid, and Iodium number, these three kinds of oil are applicable to be used as biodiesel.

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

1. Introduction

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

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

plant-derived energy sources could reduce negative environmental impacts on the environment, especially reducing carbon dioxide emissions to the atmosphere [6, 7]. Several materials from the tropical biodiversity have also been tried for energy storage materials, which can replace batteries in 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.

Several possible alternatives for biodiesel include the oil of the coconut, normally used for cooking, which originated from heated coconut milk and known as *coconut oil*, and the cooking oil made from the oil of the palm kernel, known as *palm oil* [2-5]. In addition, recently the oil of the coconut processed without heating has 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]

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 [14], and is useful for for helping lose weight in terms of metabolism. VCO contains medium chain triglycerides, which is initially digested or processed in the body from carbohydrates that can cut back hunger. Thus, it causes people to consume less carbohydrates, which eventually reduce body weight [18]. VCO also affects the healing of ovariectomy [19], and can be used as an antioxidant [20]. VCO can also reduce blood pressure [10]. In addition, VCO can also be used for skincare [22], as an external drug such as wound medicine, and can function as a probiotic [23].

VCO and palm oil have different characteristics with different function. VCO gravitates more towards medicine, probiotic, and cosmetics, whereas palm oil characteristics quite suitable to be converted to diesel fuel [24]. 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 [25-27]. The chemical composition of VCO has been studied in [28] 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

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.

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.

Nevertheless, there have been many studies concerning palm oil as an alternative for biodiesel [2-4]. In the meantime, the palm oil biomass and its fatty acid have been studied, besides the possibilty of the palm oil to be used for dietary purposes [23].

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), $CHCl_3$, Aquadest, reagent for sample preparation, namely
104 saturated NaCl, Na_2SO_4 anhydrate, BF_3, Methanol, and N_2gas to stop the oxidation occurred were
105 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% $CaCO_3$ 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

122 Prior to injecting the sample into GC-MS instrument, the oil sample was prepared by setting 50
123 gram of VCO sample, and adding 400 µL of NaOH Metanolic . This mixture was vortexed and
124 heated at 50^0 C for 10 minutes. After undergoing the cooling process, 1mL CH_3COOH, 1mL distilled
125 water, and 1mL n-hexane were added respectively. Then this mixture was vortexed and cooled for
126 several minutes where two layers were formed as the result.
127 About 1 µL 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 ìm
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 280^0 C. The injection was
131 performed using the split mode (1:30). The column temperature was programmed to change from
132 50^0 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

137 A porcelain dish along with a loose-fitting cover were cleaned, and dried in the dryer oven at
138 105^0 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

oven at 105°C, until reaching a constant weight. Another cooling process in desiccators was conducted for 30 minutes before determining its water level.

2.2.4. 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.

2.2.5. 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 ($Na_2S_2O_3$) 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.

2.2.6. 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 % $CaCO_3$ selective media, and incubated overnight at 37°C. The growth was observed.

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.

2.2.8. 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 mm^2 inhibited zone area or "halo" zone[23]. It began with testing bacteria grown on NA media (Merck) of 1.5% agar concentration at 37^0C. 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 occurence 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 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 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.

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.

Table 1. The Result of Standardization and Ingredient sample.

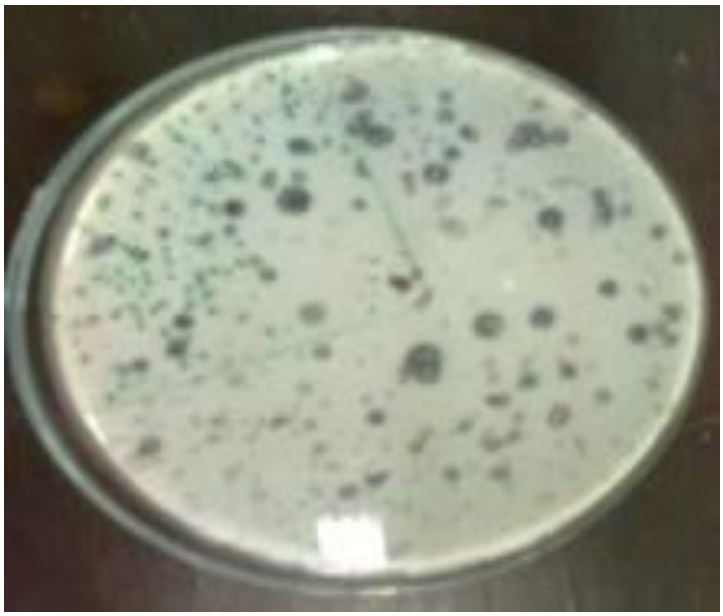
Type of the Oil	**Type of Fatty acid**	**%**	**Acid number**	**Saponification number**	**%FFA**	**Iodin number**	**pH**	**Water content %**
VCO(A)	Lauric Acid	54.06	1.0165	348.003	0.264	5.3287	6.5	0,11
	Palmitat Acid	-						
	Stearat Acid	12.03						
VCO(B)	Lauric Acid	53.9	1.0322	345.705	0,258	5.2431	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 oil	Lauric Acid	**2.81**	0.39695	*269.6266*	*0.281*	*7.023*	6.9	0.11
	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

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

211

212 *3.2. Result of LactidAcid Bacteria Isolation*

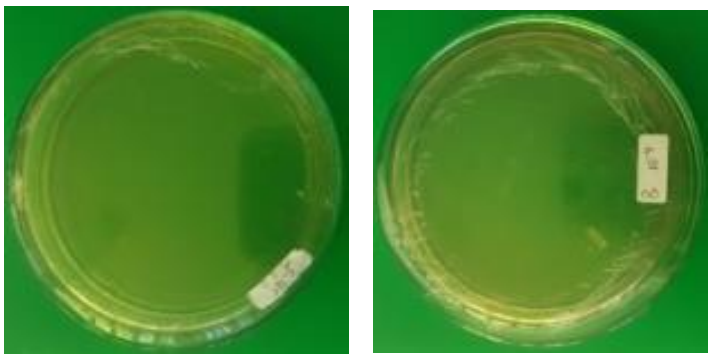
213 Figure 1, below presents the isolated result of lactic acid bacteria onto VCO, coconut oil, and
214 palm oil. It is shown that VCO contains lactic acid bacteria, as when it was being isolated using
215 MRSA + $CaCO_3$ selective media, the lactic acid bacteria colony which can grow in "Halo" area is
216 found. This area is a clear zone where it can produce lactic acid neutralising CaCO3. It is in line with
217 [5], and as an additional, the results in Figure 2 and 3 points out the identification of lactic acid
218 bacteria using 16S rDNA which turn out to be *Lacobacillus plantarum* and *Lactobacillus paracasei* as
219 mentioned in [6], [7], and [8].



220

221 **Figure 1.** Lactid acid bacteria isolate grown in MRS + 0,5% $CaCO_3$ media

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



225

***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.

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%

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTT
GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATA
ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTAT
CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC
CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA
AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTA
AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACA
GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCT
GTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT
GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGG
CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTAC
GCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATC
AGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA
TCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTA
AGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAAT
CGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAAC
ACCCAAAGTC

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

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
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 sequence	276	276	100%	3e-71	100%	EU249147.1
Lactobacillus rhamnosus 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
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 rhamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
Lactobacillus rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1
Lactobacillus 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 23S	274	274	99%	1e-70	100%	KU954559.1
Lactobacillus 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
Lactobacillus paracasei strain FS3 16S ribosomal RNA gene, partial sequence	274	274	99%	1e-70	100%	KU315064.1
Lactobacillus rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

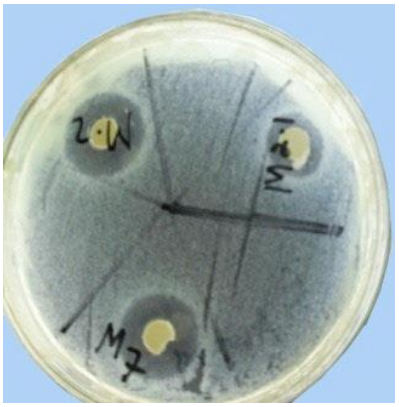
Figure 4. The result of molecular identification of lactid acid bacteria showing *Lactobacillus paracasei.*

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 [20], 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.	*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

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]

4. Conclusions

Compared to coconut oil and palm 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, all three kinds of oil are applicable to be used as biodiesels. 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.

Author Contributions

Conceptualization: Suryani Suryani and Sariani Sariani; Resources: Marganof Marganof, and Suryani Suryani, 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 Indra 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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394 Pathogenic Bacteria Secretion od Chronic Suppurative Otitis Media Patients, *Rasayan*
395 *J.Chem.***2018 ,** Vol 11, No 3, pp 1139-1143.

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 of English language and style required
( ) Moderate English changes required
( ) English language and style are fine/minor spell check required
( ) I don't feel qualified to judge about the English language and style

	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include 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 supported by the results?	( )	(x)	( )	( )

Comments and Suggestions for Authors

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

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

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*], Sariani[2], Femi Earnestly[3], Marganof [4] , Rahmawati[4], Sevindrajuta[4], Teuku Meurah Indra Mahlia[5]

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 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. ." **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.”

processes

MDPI

Article

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

Suryani Suryani[1,*], Sariani Sariani[2], Femi Earnestly[3], Marganof Marganof [4] , Rahmawati[5], Sevindrajuta[6],and TeukuMeurah Indra Mahlia[7]

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ABSTRACT

Virgin Coconut Oil (VCO) is very nutritious to the human health because it contains of Lauric Acid and Lactic Acid Bacteria (LAB). The aims of this research is to study the uniqueness factors of VCO compared to coconut oil and palm oil. Lauric Acid content was analyzed by Chromatographic Gas method. Isolation of LAB ~~ia~~ was conducted by dilution method using MRSA + 0.5% CaCO3 media. In addition, the macromolecular identification was conducted by 16S rRNA. ~~The results obtained shows that the content of Lauric Acid is considered high; 41% – 54.5% for VCO, where 0% for coconut oil, and 0. 11% forpalm oil.~~VCO

. The VCO also contains LAB, namely *Lac.plantarum* and *Lactobacillus paracasei* , which has the ability to 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.* Compare to VCO, coconut oil and palm oil do not have above mentioned bacteria.It is concluded that the uniqueness of VCO is that it has a high content of Lauric Acid, 54% and contains LAB.

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

1.Introduction

The tropical biodiversity has been contributed to so many industrial products including pharmaceutical, biofuel and as well as energy storage materials. Some of sources from tropical biodiversity have been converted to pharmaceutical product such as drugs [1]. Where some other

such as *Sterculiafoetida*, *Jatropha curcas*, *Calophylluminophyllum*and*Reutealistrisperma*have been converted to biodiesel to power internal combustion engine [2-5]. By using bioenergy such as biodiesel could reduce negative environmental impact on the environment especially by reducing carbon dioxide to atmosphere [6, 7]. Several of materials from tropical biodiversity have also been used for energy storage material than can replace batteries in the future [8-10]. This can help to store a significant amount of solar energy from tropical country which usually has plenty of sunrays[11]. Anotherexceptional plant from tropical biodiversity that has been used for many purposes is coconut, however, in this research the author attempts to studycoconut oil and their active ingredients.

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

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

2. Materials and Methods

Materials:

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

Chemical Material:

For **Lauric Acid Analysis**, -hexan (p.a), $CHCl_3$, Aquadest, reagent for sample preparation namely saturated NaCl, Na_2SO_4 anhidrat, BF_3, , Methanol, and N_2gas to stop the oxidation occured were used. The internal and exeternal of fatty acid standard. Whereas for Acid Number analysis (%FFA), 95%Alcohol (pa), blue bromtimol indicator, phenolphtalein indicator, HCl 0.5 N Standard Solution,

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% $CaCO_3$ media was used for Isolation of Lactid Acid Bacteria, and in order to increase their amount using MRS media.

Instruments:

The instrument taken were ordinary laboratory glassware like 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.

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

Methods:

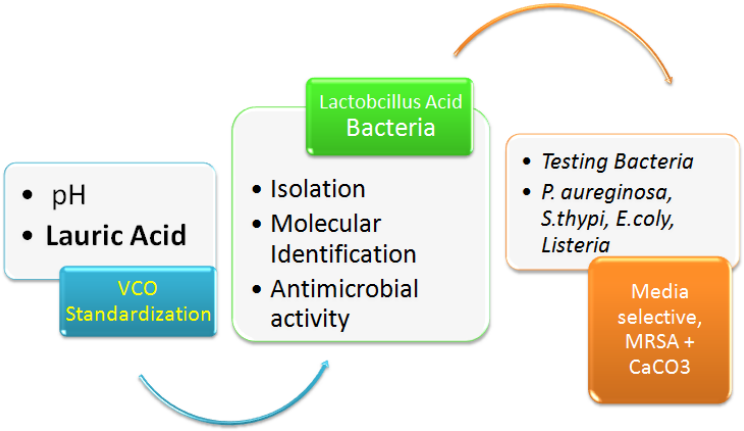


Figure2. Method of a comparativestudy of virgin coconut oil, coconut oil and palm oil in terms of their active ingredients

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 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 palm oil.

Shown inTable 1. below that lauric acid ingredients of VCO is the highest which is 54.06%. In contrast, the lauric acid ingredients of coconut oil is 2.81%, and none for palm oil. Table 1. Presents that there are two types of fatty acid content in VCO; 54.06% lauric acid and 12.06% stearat acid, and none for palmitic acid. The absence of this palmitic acid is because VCO is not formed by palm oil. It is based on the fact that the highest content of palmitic acid will exist in the oil originally from palm oil. The 54.06% of lauric acid content in VCO is considered high, compared to what has been obtained through this research [1]. It is because VCO is processed without heating or by fermentation. Thus the fatty acid carbon bonds are not broken, in other words, the fatty acid is included into Medium Chain Trigliserida, particularly the lauric acid. For coconut oil made from cooked coconut milk,apparently the lauric acid content is low which is 2.81%, containing 2.65% stearat 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.

Table 1. The Result of Standardization and Ingridient sample

Type of the oil	Type of the fatty acid	%	Acid number	Saponification number	%FFA	Iodin number	pH
VCO	Lauric Acid	54.06	1.0165	348.003	0.264	5.3287	6.5
	Palmitat Acid	-					
	Stearat Acid	12.03					
Coconut oil	Lauric Acid	**2.81**	0.39695	269.6266	0.281	7.023	6.9
	Palmitat Acid	2.31					
	Stearat Acid	2.65					
Palm Oil 1	Lauric Acid	**0.45**	0.39645	204.0045	0.515	51.0042	6,6
	Palmitat Acid	2.88					
	Stearat Acid	-					
Palm Oil 2	Lauric Acid	-	0.39645	203.02595	0.733	49.71675	6.5
	Palmitat Acid	24.42					
	Stearat Acid	-					

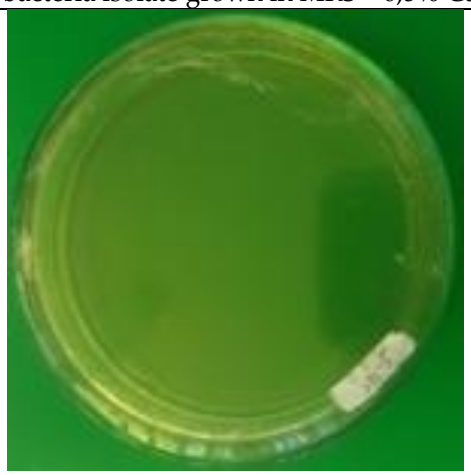
From Table 1. above it can be said that VCO acid number is 1.0165 which is higher than coconut milk acid number in 0.39695, and palm oil acid number in 0.39645 [2]. It is as a result of the acid number showing that KOH and NaOH amount can neutralize the free fatty acid. It means that VCO acid number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

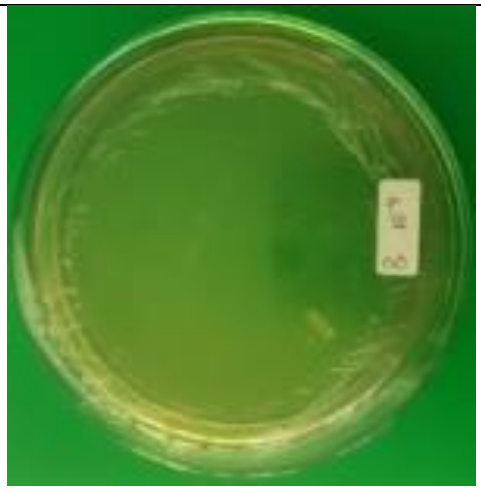
VCO saponification number is 348.003 whereas coconut milk saponification number is 269.6266 which is higher than palm oil saponification number; 204.0045 due to saponification number that shows the number of fatty acid molecule. The bigger the saponification number, the smaller the molecule, or consisting 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].

3.2. Result of LactidAcid Bacteria Isolation

149 Table 2. below presents the isolated result of lactic acid bacteria onto VCO, coconut oil, and
150 palm oil. It is shown that VCO contains lactic acid bacteria, as when it was being isolated using
151 MRSA + $CaCO_3$ selective media, lactic acid bacteria colony which can grow in "Halo" area is found.
152 This area is a clear zone where it can produce lactic acid neutralising CaCO3. It is in line with [5], and
153 as an additional, the results in Figure 2 and 3 points out the identification of lactic acid bacteria using
154 16S rDNA which turn out to be *Lacobacillus plantarum* and *Lactobacillus paracasei* as mentioned in [6],
155 [7], and [8].
156
157
158
159

160 **Table 2. Result of LactidAcid Bacteria Isolation**

Sample	Result
VCO	 Lactid acid bacteria isolate grown in MRS + 0,5% $CaCO_3$ media
coconut oil	 Result of lactid acid bacteria isolation from coconut oil with no evidence of growth

Palm oil	

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

3.3. Molecular Identification of Lactid Acid Bacteria

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.

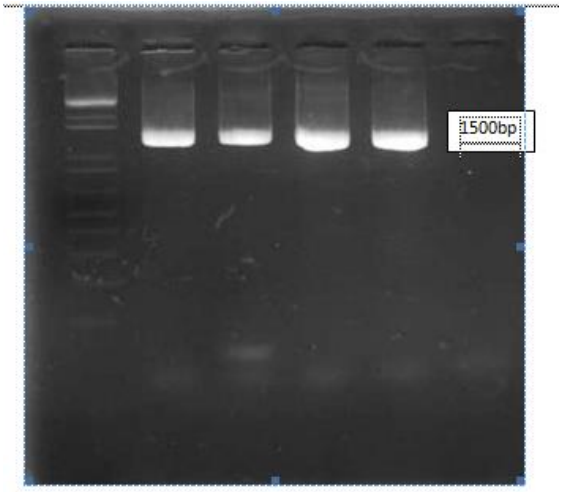


Figure3.Electrophoresis result on 4 isolates

>CONTIQ_M16.4_1440bp_*Lactobacillus plantarum*_100%

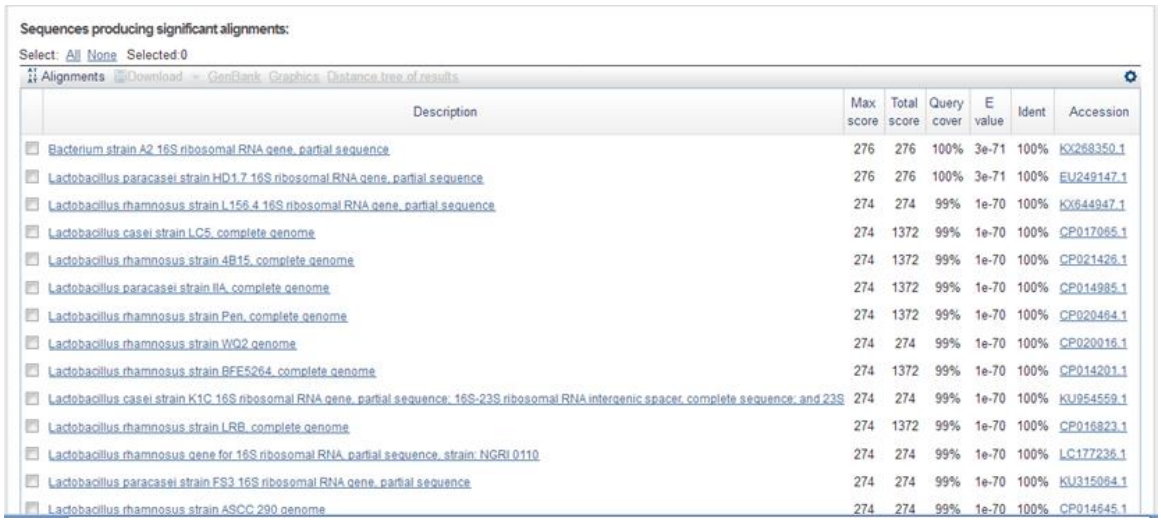
GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTT
GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATA
ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTAT
CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC
CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA
AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTA
AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACA
GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCT
GTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT
GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGG
CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTAC
GCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATC
AGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA
TCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTA
AGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAAT
CGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAAC
ACCCAAAGTC

Figure 4. Lactid acid bacteria resulted from isolation; *Lactobacillus plantarum*

4. Bac4

Consensus :

CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACC
CGAAGCCGGTGGCGTAACCCTTTTAGGGAGCGAGCCGTCTAAGGTGGGACAAATGATTAG
GGTGAAGTCGTAACAAGGTAGCCGTAA



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
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 sequence	276	276	100%	3e-71	100%	EU249147.1
Lactobacillus rhamnosus 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
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 rhamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
Lactobacillus rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1
Lactobacillus 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 23S	274	274	99%	1e-70	100%	KU954559.1
Lactobacillus 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
Lactobacillus paracasei strain FS3 16S ribosomal RNA gene, partial sequence	274	274	99%	1e-70	100%	KU315064.1
Lactobacillus rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

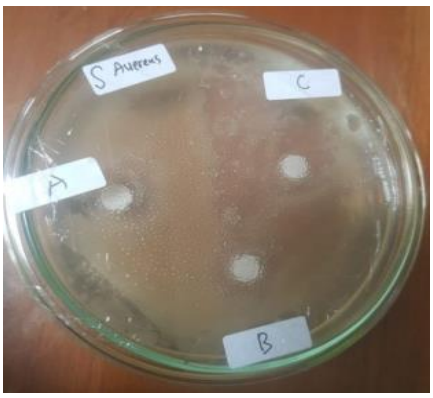
Figure 5. The result of molecular identification on lactid acid bacteria showing *Lactobacillus paracasei.*

3.4. Result of Anti Microbial Analysis.

Anti microbial analysis was oerformed between *Lactobacillus paracasei* lactid acid bacteria, and *Lactobacillus plantarum* onto testing bacteria; *Pseudomonas aeruginase, Klebsiella, Staphilococcus aureus, Staphilococcus epidermidis, Proteus,* from *[24], Eschericia coli, Listeria monocytogenes, Bacillus cereus, and Salmonella typhosa.*Lactid Acid Bacteria which can inhibit the growth of testing or pathogenic bacteria is pointed ut in

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

Illustrated in Table 3 below, here 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 [9], where the antimicrobial ability of *Lacobacillus plantarum* is the best rather than *Listeria monocytogenes, E.coli* and *Bacillus sbtillis* testing bacteria.

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

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.

Contribution Author.

Conceptualization: Suryani Suryani and Sariani Sariani;Resources: Marganof Marganof, and Suryani Suryani, 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.

Funding:

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

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ABSTRACT

Virgin Coconut Oil (VCO) is very nutritious to human health because it contains Lauric Acid and Lactic Acid Bacteria (LAB). The aims of this research are to study the unique factors of VCO compared to coconut oil and palm oil. Lauric Acid content was analysed by the Chromatographic Gas method. Isolation of LABiawas conducted by the dilution method using MRSA + 0.5% CaCO3 media. In addition, macromolecular identification was conducted by 16S rRNA. 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. The VCO also contains LAB, namely *Lac.plantarum* and *Lactobacillus paracasei*, which has the ability to 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.* Compare to VCO, coconut oil and palm oil do not have above mentioned bacteria. It is concluded that the uniqueness of the VCO is that it has a high content of Lauric Acid, 54% and contains LAB.

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

1. Introduction

The tropical biodiversity has been contributed to so many industrial products including pharmaceutical, biofuel and as well as energy storage materials. Some of the sources from tropical biodiversity have been converted to pharmaceutical product such as drugs [1]. Where some other such as *Sterculiafoetida*, *Jatropha curcas, Calophylluminophyllum*and*Reutealistrisperma*have been converted to biodiesel to power internal combustion engine [2-5]. By using bioenergy such as biodiesel could reduce negative environmental impact on the environment, especially by reducing

carbon dioxide to atmosphere [6, 7]. Several of materials from tropical biodiversity have also been used for energy storage material than can replace batteries in the future [8-10]. This can help to store a significant amount of solar energy from tropical country which usually has plenty of sunrays [11]. Another exceptional plant from tropical biodiversity that has been used for many purposes is coconut, however, in this research the author attempts to study coconut oil and their active ingredients.

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

The VCO has been used widely by many people because people believe that the VCO has some benefits compared to original coconut oil made from cooked coconut milk and palm oil. The VCO is useful for anti-microbial, or antibacterial and antiviral [14], and is useful for losing weight or helping obese people in terms of metabolism. It is due to that VCO contains Medium Chain Trigliserida which is initially digested or processed in the body out from carbohydrates that can cut back hunger. Thus, it causes obese people to consume less carbohydrates, which eventually reduce their weight [18]. By consuming the VCO, it also affects the healing of ovariectomy [19], and can also be used as an antioxidant [20]. The VCO can also reduce blood pressure [10]. In addition, VCO can also be used for skincare [22], as an external drug such as wound medicine, and can function as a probiotic [23]. Both VCO and palm oil have their own characteristics with different function. VCO characteristics are more to medicine, probiotic, and cosmetics, whereas palm oil characteristics quite suitable to be converted to diesel fuel [24]. 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 [25-27]. The chemical composition of VCO has been studied in [28] 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

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.

2. Materials and Methods

Materials:

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

Chemical Material:

For **Lauric Acid Analysis**, -hexane (p.a), $CHCl_3$, Aquadest, reagent for sample preparation, namely saturated NaCl, Na_2SO_4 anhydrate, BF_3, Methanol, and N_2gas to stop the oxidation occurred were used. The internal and external 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 + 0.5% $CaCO_3$ media was used for Isolation of Lactaid Acid Bacteria and in order to increase their amount using MRS media.

Instruments:

The instrument has taken where ordinary laboratory glassware like a 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.

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

Methods:

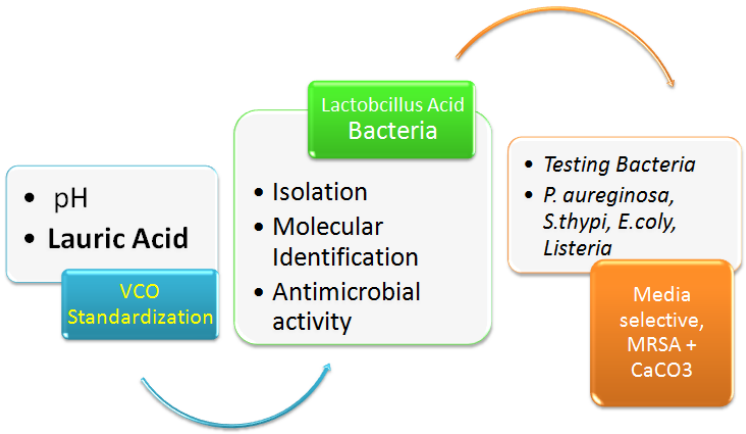


Figure 2. Method of a comparative study of virgin coconut oil, coconut oil and palm oil in terms of their active ingredients.

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 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.

Presents that there are two types of fatty acid content in VCO; 54.06% lauric acid and 12.06% stearat acid, and none for palmitic acid. The absence of this palmitic acid is because VCO is not formed by palm oil. It is based on the fact that the highest content of palmitic acid will exist in the oil originally from palm oil. The 54.06% of lauric acid content in the VCO is considered high, compared to what has been obtained through this research [12]. It is because VCO is processed without heating or by fermentation. Thus the fatty acid, carbon bonds are not broken, in other words, the fatty acid is 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.

Table 1. The Result of Standardization and Ingredient sample

Type of the oil	Type of the fatty acid	%	Acid number	Saponification number	%FFA	Iodin number	pH
VCO	Lauric Acid	54.06	1.0165	348.003	0.264	5.3287	6.5
	Palmitat Acid	-					
	Stearat Acid	12.03					
Coconut oil	Lauric Acid	**2.81**	0.39695	269.6266	0.281	7.023	6.9
	Palmitat Acid	2.31					
	Stearat Acid	2.65					
Palm Oil 1	Lauric Acid	**0.45**	0.39645	204.0045	0.515	51.0042	6,6
	Palmitat Acid	2.88					
	Stearat Acid	-					
Palm Oil 2	Lauric Acid	-	0.39645	203.02595	0.733	49.71675	6.5
	Palmitat Acid	24.42					
	Stearat Acid	-					

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.

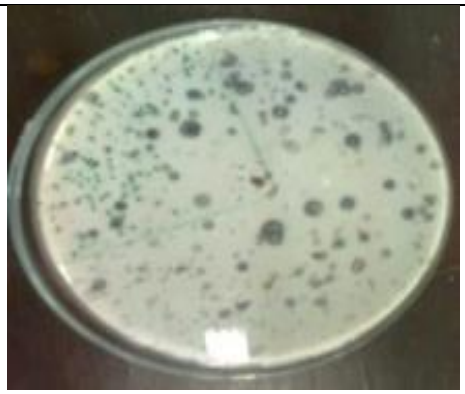
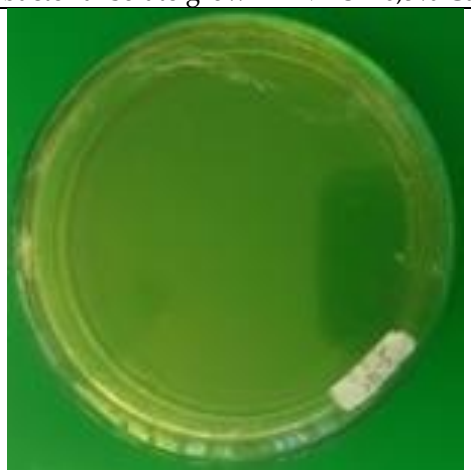
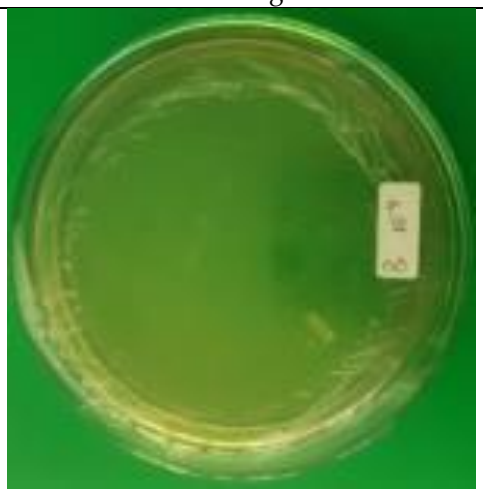
The VCO saponification number is 348.003 whereas coconut milk saponification number is 269.6266 which is higher than the palm oil saponification number; 204.0045 due to saponification number that shows the number of the fatty acid molecule. The bigger the saponification number, the smaller the molecule, 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].

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 + $CaCO_3$ 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].

Table 2. Result of LactidAcid Bacteria Isolation

Sample	Result
VCO	 Lactid acid bacteria isolate grown in MRS + 0,5% $CaCO_3$ media
coconut oil	 Result of lactid acid bacteria isolation from coconut oil with no evidence of growth
Palm oil	 lactid acid bacteria isolation obtained from palm oil with no evidence of growth

3.3. Molecular Identification of Lactid Acid Bacteria

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.

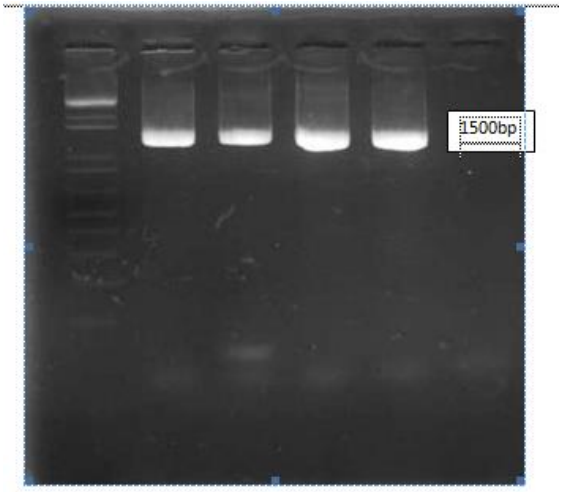


Figure 3. Electrophoresis result on 4 isolates.

>CONTIQ_M16.4_1440bp_*Lactobacillus plantarum*_100%

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTT
GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATA
ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTAT
CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC
CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA
AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTA
AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGAC
GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCT
GTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT
GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGG
CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTAC
GCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATC
AGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA
TCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTA
AGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAAT
CGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAAC
ACCCAAAGTC

Figure 4. Lactid acid bacteria resulted from isolation; *Lactobacillus plantarum*.

168

4. Bac4

Consensus :

CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACC
CGAAGCCGGTGGCGTAACCCTTTTAGGGAGCGAGCCGTCTAAGGTGGGACAAATGATTAG
GGTGAAGTCGTAACAAGGTAGCCGTAA

169

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
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 sequence	276	276	100%	3e-71	100%	EU249147.1
Lactobacillus rhamnosus 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
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 rhamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
Lactobacillus rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1
Lactobacillus 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 23S	274	274	99%	1e-70	100%	KU954559.1
Lactobacillus 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
Lactobacillus paracasei strain FS3 16S ribosomal RNA gene, partial sequence	274	274	99%	1e-70	100%	KU315064.1
Lactobacillus rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

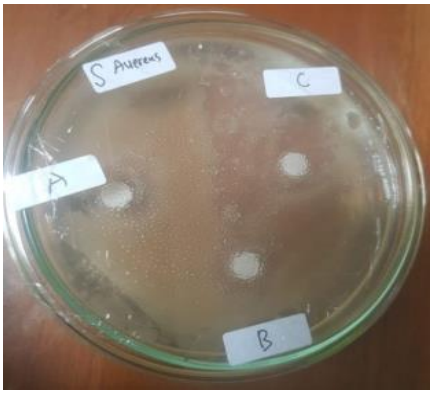
170 **Figure 5**. The result of molecular identification of lactid acid bacteria showing *Lactobacillus paracasei.*

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

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.

Table 3. Antimicrobial activity analysis of LAB in 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

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 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 lauric acid with small molecules which are Medium Chain Triglycerides (MCT), where initially will be digested in order to reduce weight.

Contribution Author.

Conceptualization: Suryani Suryani and Sariani Sariani; Resources: Marganof Marganof, and Suryani Suryani, 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 Indra Mahlia and Suryani Suryani; Writing – review and editing: Suryani Suryani, Rahmawati, Femi Earnestly, Sariani Sariani and Wahyu Caesarendra; Project administration: Sevindrajuta, and Rahmawati.

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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% $CaCO_3$ 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 palm oils ~~(0% and 0.11%~~, 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), $CHCl_3$ and Aquadest reagent for sample preparation, namely saturated NaCl, Na_2SO_4 anhydrate, BF_3, methanol and N_2gas 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% $CaCO_3$ 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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used for isolation bacteriocin were MRSB media (Merck), *Lactobacillus plantarum* M0, ammonium sulfate (NH_4) $2SO_4$, 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 µL of NaOH Metanolic. This mixture was vortexed and heated at 50 °C for 10 min. After undergoing the cooling process, 1 mL CH_3COOH, 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 µL 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 ìm (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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N Tio ($Na_2S_2O_3$) 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% $CaCO_3$ 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 mm^2 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.

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

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

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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 + $CaCO_3$ 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 $CaCO_3$. 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.

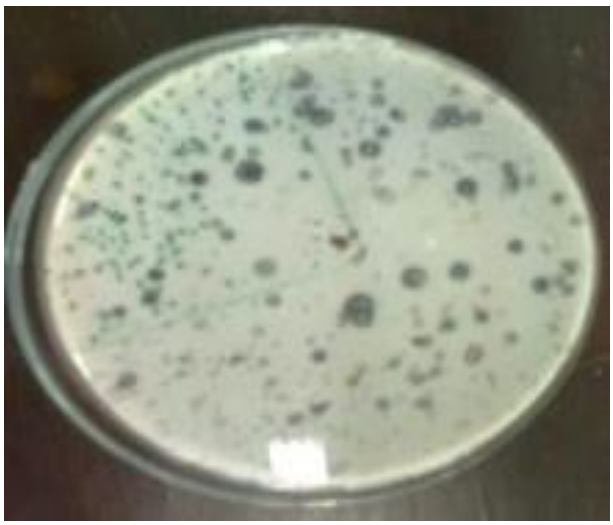


Figure 1. Lactid acid bacteria isolate grown in MRS + 0.5% $CaCO_3$ media.

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.

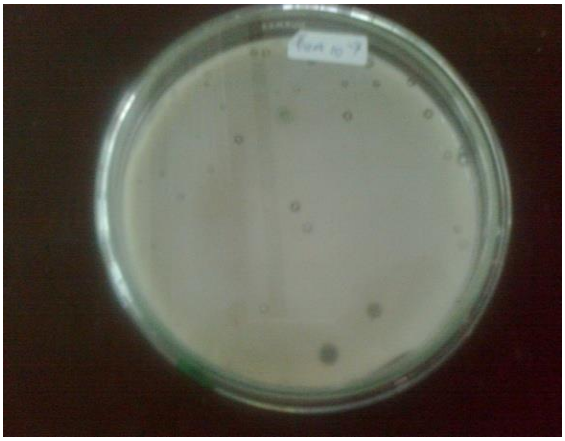


Figure 2. Result of lactid acid bacteria isolation from coconut oil and from palm oil with no evidence of growth.

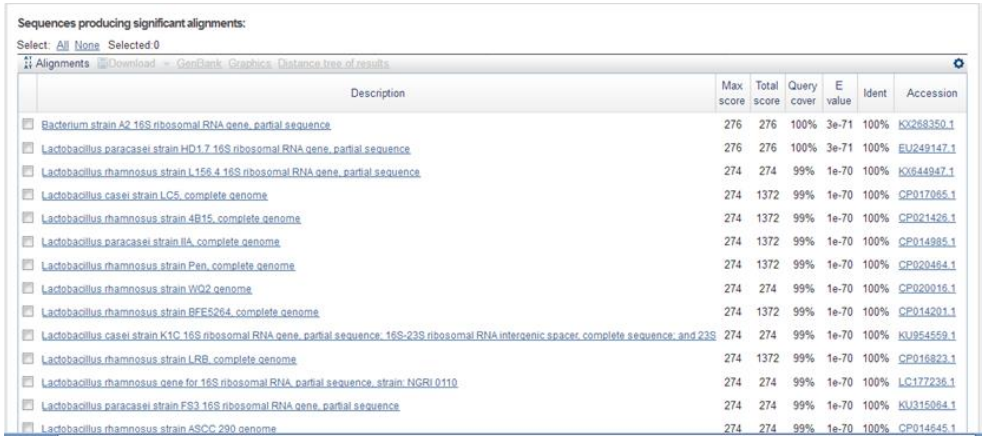
3.3. Molecular Identification of Lactid Acid Bacteria

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%

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTT
GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATA
ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTAT
CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC
CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA
AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTA
AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACA
GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCT
GTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT
GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGG
CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTAC
GCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATC
AGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA
TCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTA
AGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAAT
CGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAAC
ACCCAAAGTC

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



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
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 sequence	276	276	100%	3e-71	100%	EU249147.1
Lactobacillus rhamnosus 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
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 rhamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
Lactobacillus rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1
Lactobacillus 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 23S	274	274	99%	1e-70	100%	KU954559.1
Lactobacillus 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
Lactobacillus paracasei strain FS3 16S ribosomal RNA gene, partial sequence	274	274	99%	1e-70	100%	KU315064.1
Lactobacillus rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

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

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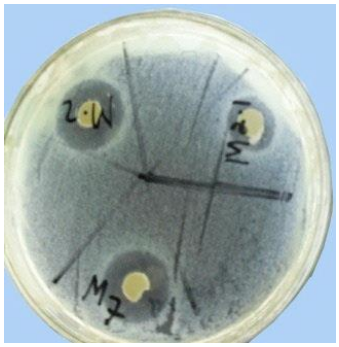
3.4. Result of Antimicrobial Analysis

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 54 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].

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

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

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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 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), $CHCl_3$, Aquadest, reagent for sample preparation, namely saturated NaCl, Na_2SO_4 anhydrate, BF_3, Methanol, and N_2gas 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% $CaCO_3$ 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 μL of NaOH Metanolic . This mixture was vortexed and heated at 50^0 C for 10 minutes. After undergoing the cooling process, 1mL CH_3COOH, 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 μL 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 ìm (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 280^0 C. The injection was performed using the split mode (1:30). The column temperature was programmed to change from 50^0 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 105^0 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 ($Na_2S_2O_3$) 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 % $CaCO_3$ 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 mm^2 inhibited zone area or "halo" zone. It began with testing bacteria grown on NA media (Merck) of 1.5% agar concentration at 37^0C. 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 occurence 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* characterize*d* as *a* antimicrobial, in contrast to coconut and palm oil*s*.

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	pH	Water content %
VCO(A)	Lauric Acid (C12:0)	54.06	1.01	348.00	0.26	5.32	6.50	0,11
	Palmitat	-						

	Acid							
	Stearat Acid(C18:0)	12.03						
VCO(B)	Lauric Acid (C12:0)	53.90	1.03	345.70	0,25	5.24	6.40	0,12
	Palmitat Acid (C16:0)	-						
	Stearat Acid (C18:0)	12.01						
VCO(C)	Lauric Acid (C12:0)	53.70	1.02	346,64	0.26	5.25	6.50	0,11
	Palmitat Acid (C16:0)	-						
	Stearat Acid (C18:0)	11.9						
Coconut oil	Lauric Acid (C12:0)	**2.81**	0.39	269.62	0.28	7.02	6.90	0.11
	Palmitat Acid (C16:0)	2.31						
	Stearat Acid (C18:0)	2.65						
Palm Oil 1	Lauric Acid (C12:0)	**0.45**	0.39	204.00	0.51	51.00	6.60	0.09
	Palmitat Acid (C16:0)	2.88						
	Stearat Acid (C18:0)	-						
Palm Oil 2	Lauric Acid (C12:0)	-	0.39	203.02	0.73	49.71	6.50	0.09
	Palmitat Acid (C16:0)	24.42						
	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 + $CaCO_3$ 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.

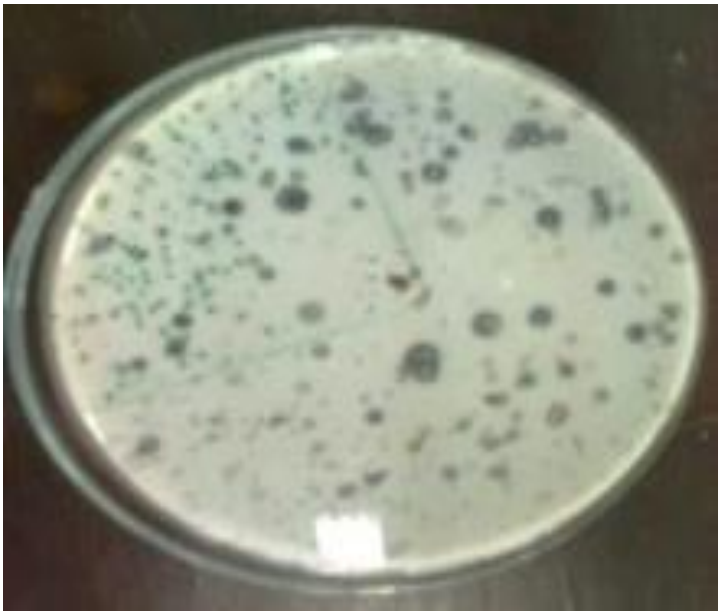


Figure 1. Lactid acid bacteria isolate grown in MRS + 0,5% $CaCO_3$ media

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]

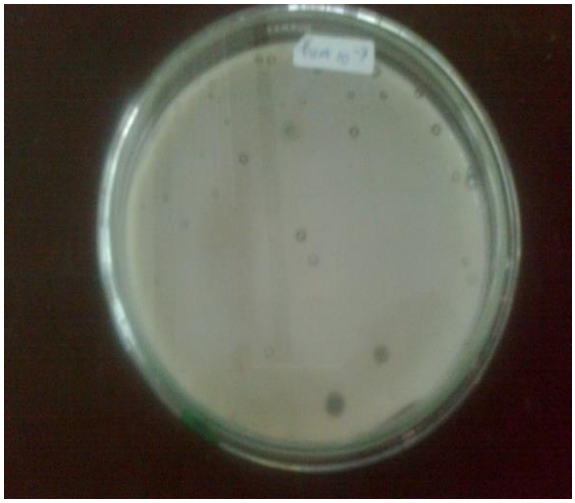


Figure 2. Result of lactid acid bacteria isolation from coconut oil and from palm oil with no evidence of growth

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

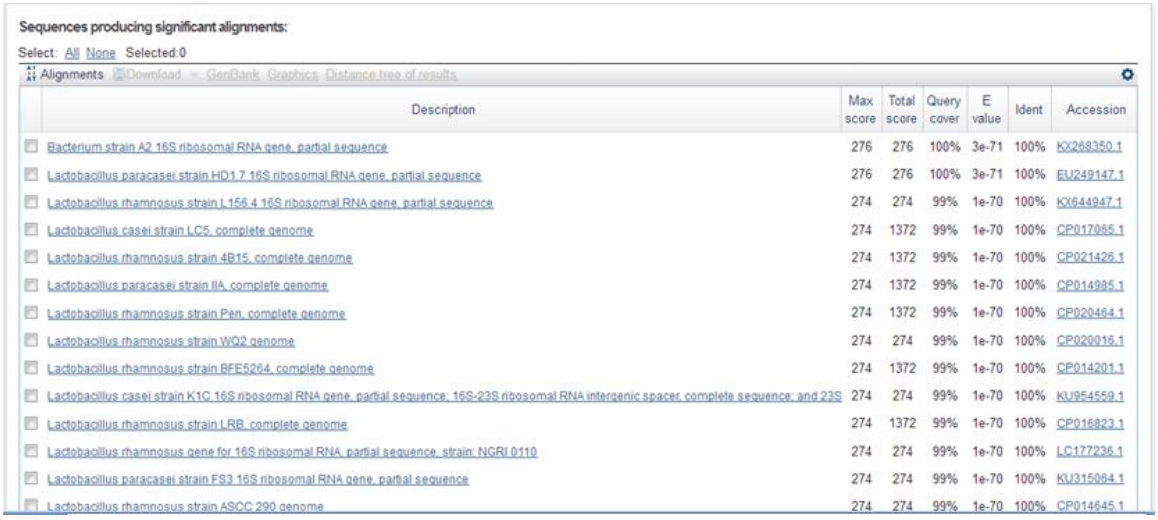
3.3. Molecular Identification of Lactid Acid Bacteria

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%

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTT
GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATA
ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTAT
CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC
CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA
AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTA
AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACA
GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCT
GTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT
GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGG
CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTAC
GCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATC
AGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA
TCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTA
AGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAAT
CGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAAC
ACCCAAAGTC

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



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
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 sequence	276	276	100%	3e-71	100%	EU249147.1
Lactobacillus rhamnosus 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
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 rhamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
Lactobacillus rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1
Lactobacillus 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 23S	274	274	99%	1e-70	100%	KU954559.1
Lactobacillus 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
Lactobacillus paracasei strain FS3 16S ribosomal RNA gene, partial sequence	274	274	99%	1e-70	100%	KU315064.1
Lactobacillus rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

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

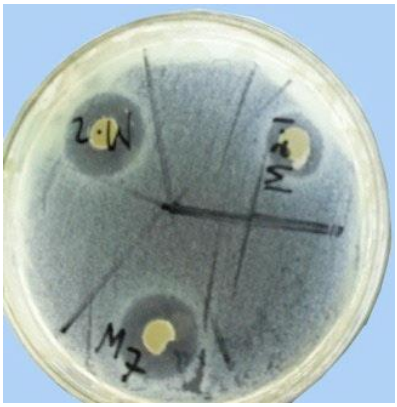
3.4. Result of Anti Microbial Analysis.

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.	*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

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.

Conceptualization: Suryani Suryani and Sariani Sariani; Resources: Marganof Marganof, and Suryani Suryani, 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 Indra Mahlia and Suryani Suryani; Writing – review and editing: Suryani Suryani, Rahmawati, Femi Earnestly, Sariani Sariani and Wahyu Caesarendra; Project administration: Sevindrajuta, and Rahmawati.

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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% $CaCO_3$ 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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Please complete the authors name, if possible.

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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), $CHCl_3$ and Aquadest reagent for sample preparation, namely saturated NaCl, Na_2SO_4 anhydrate, BF_3, methanol and N_2gas 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% $CaCO_3$ 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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used for isolation bacteriocin were MRSB media (Merck), *Lactobacillus plantarum* M0, ammonium sulfate (NH_4) $2SO_4$, 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 µL of NaOH Metanolic. This mixture was vortexed and heated at 50 °C for 10 min. After undergoing the cooling process, 1 mL CH_3COOH, 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 µL 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 ìm (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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N Tio ($Na_2S_2O_3$) 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% $CaCO_3$ 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 mm^2 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.

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

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

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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 + $CaCO_3$ 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 $CaCO_3$. 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.

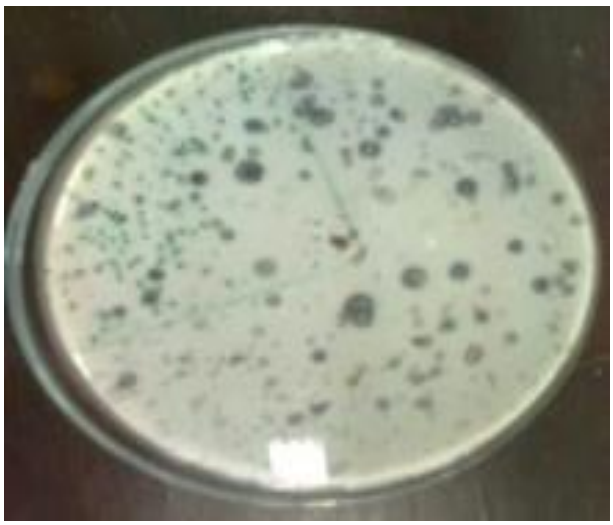


Figure 1. Lactid acid bacteria isolate grown in MRS + 0.5% $CaCO_3$ media.

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.

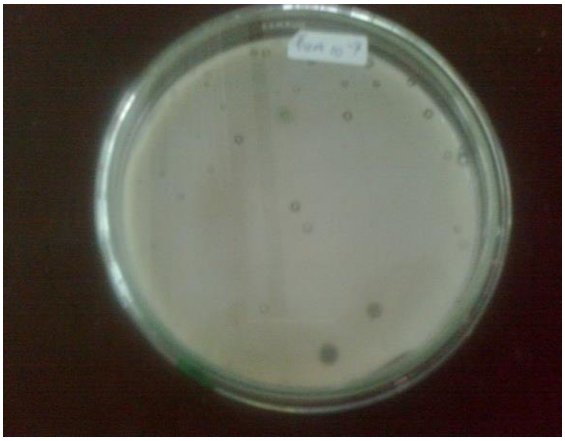


Figure 2. Result of lactid acid bacteria isolation from coconut oil and from palm oil with no evidence of growth.

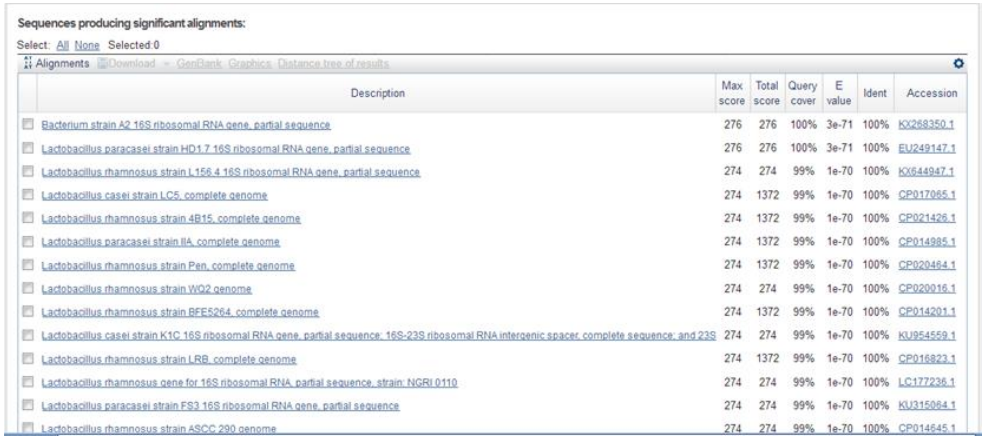
3.3. Molecular Identification of Lactid Acid Bacteria

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%

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTT
GCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATA
ACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTAT
CACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC
CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTC
CACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTA
AAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTA
AGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACA
GTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCT
GTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGAT
GAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGG
CCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTAC
GCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACA
GGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATC
AGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAA
TCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTA
AGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAAT
CGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAAC
ACCCAAAGTC

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



Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
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 sequence	276	276	100%	3e-71	100%	EU249147.1
Lactobacillus rhamnosus 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
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 rhamnosus strain Pen, complete genome	274	1372	99%	1e-70	100%	CP020464.1
Lactobacillus rhamnosus strain WQ2 genome	274	274	99%	1e-70	100%	CP020016.1
Lactobacillus 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 23S	274	274	99%	1e-70	100%	KU954559.1
Lactobacillus 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
Lactobacillus paracasei strain FS3 16S ribosomal RNA gene, partial sequence	274	274	99%	1e-70	100%	KU315064.1
Lactobacillus rhamnosus strain ASCC 290 genome	274	274	99%	1e-70	100%	CP014645.1

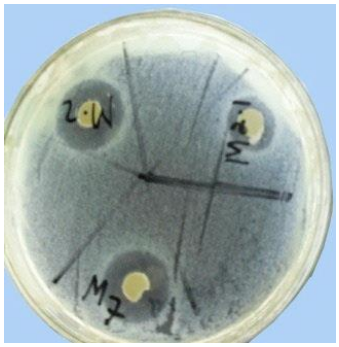
Figure 4. The result of molecular identification of lactid acid bacteria showing *Lactobacillus paracasei*.

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].

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

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

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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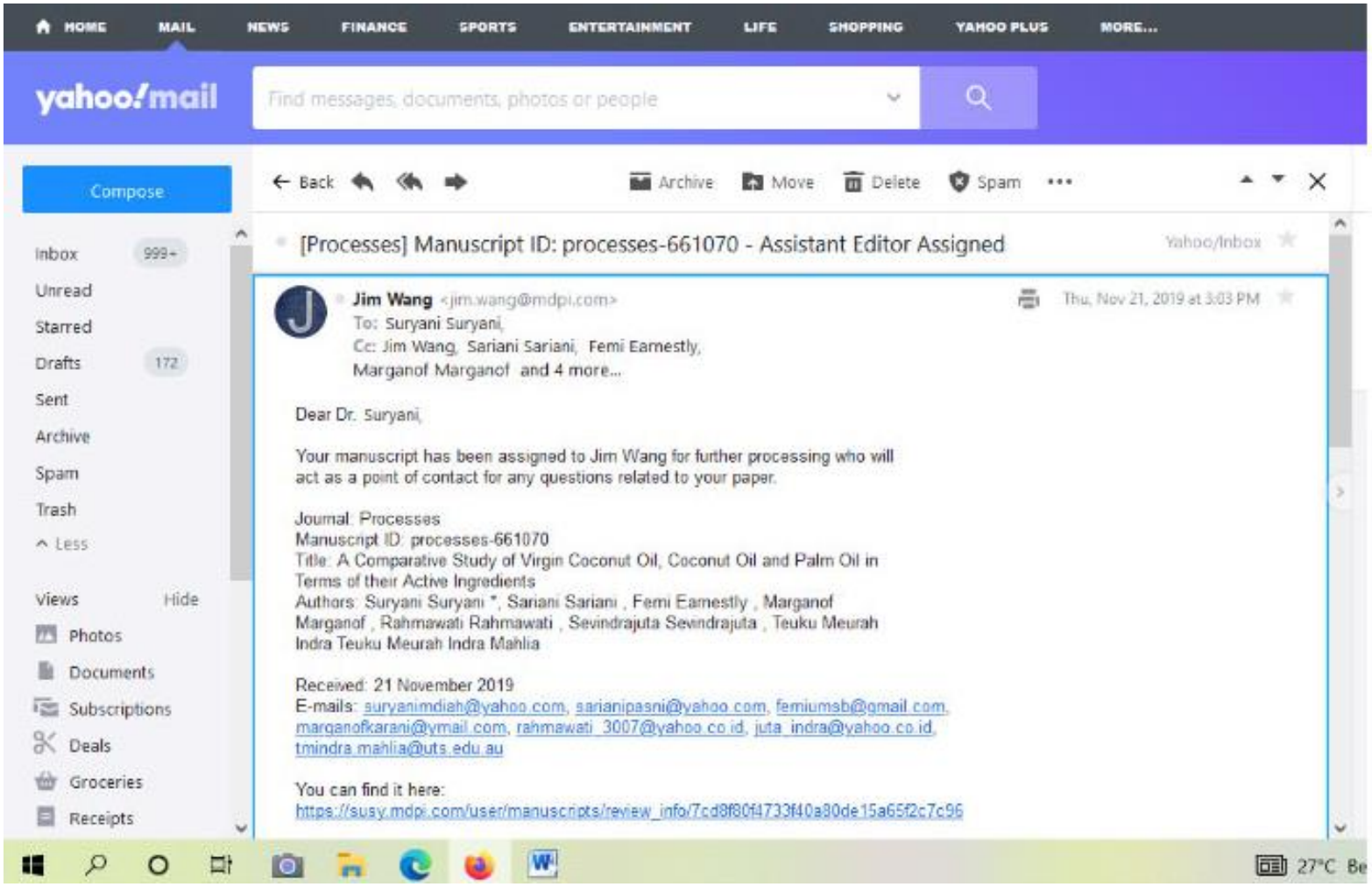
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