Similarity result of_Impact of Virgin Coconut Oil (VCO) on Probiotic Lactobacillus delbrueckii subsp. Bulgaricus

by Miksusanti Salbi

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Impact of Virgin Coconut Oil (VCO) on Probiotic *Lactobacillus delbrueckii subsp. Bulgaricus*

Miksusanti[1, a)], Herlina[2, b)], Budi Untari[2,c)], Dasril Basir[1, d)], Indah Solehah[2,e)], Ulfi[2, f)]

[1]Department of Chemistry, Faculty of Mathematics and Natural Science, Indonesia.
[2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sriwijaya, Inderalaya Ogan Ilir, Postal Code: 30662, Indonesia.

[a)]Corresponding Author : miksusanti@unsri.ac.id;
b)rinaafdil@gmail.com;
[c)]untaribudi@yahoo.com;
[d)]debasril_chem@yahoo.com;
[e)]indahsolihah26614@gmail.com;
[f)]ulfiaziz@gmail.com

Abstract. VCO is a functional food that is often consumed as an alternative in improving health. In the body there are probiotic bacteria which are normal micro flora of the intestine, an example of probiotic bacteria is *L. bulgaricus*. This research was aimed to see the effect of VCO on the activity of *L. bulgaricus* probiotic bacteria in vitro. The growth of *L. bulgaricus* was counting by total plate count method. Diffusion method was used to determine the antibacterial activity of *L. bulgaricus* metabolites against *E. coli*. Minimum Inhibitory Concentration (MIC) was determined using the dilution method. VCO was meet the standards for specific gravity (0.9149), refractive index (1.4546) and water content (0%). The fatty acid component was tested using GCMS with the highest concentration, was lauric acid (17.92%). The VCO concentration used in the bacterial growth test was 1%, 5%, 10%, 15%. The best concentration of bacteria was at concentration of 1%. There was a significant difference in the number of probiotic bacteria in the concentration of 15% with negative and positive controls. The number of bacteria at the four concentrations were 150.8 x 10^{12}, 111.4 x 10^{12}, 109.25 x 10^{12}, 55.667 x 10^{12}, respectively. Antibacterial activity tested of *L. bulgaricus* metabolites with three concentrations (100%, 75%, 25 %) gave weak-moderate result. In the antibacterial activity test on *L. bulgaricus* metabolites that were not treated with VCO produced a significant difference. Testing the Minimum Inhibitory Concentration (MIC) on *L. bulgaricus* metabolites that had been treated with VCO and not treated resulted in different MIC. The MIC on untreated metabolites was 4.69%, while the MIC on treated metabolites was 9.38%.

INTRODUCTION

In Indonesia, various types of food products that are claimed to have benefits in maintaining and improving health are rapidly developing, commonly known as functional food. Functional food is processed food that contains one or more functional components based on scientific studies that have certain physiological functions and are proven to be harmless and beneficial to health.

Virgin Coconut Oil (VCO) contains Medium Chain Fatty Acids (MCFA). Lauric acid was the largest component of VCO. VCO can be used as antibacterial, antiviral and antifungal. Lauric acid contained in VCO can inhibit the growth of pathogenic bacteria such as *Staphylococcus aureus*, Streptococcus species, *Escherichia coli* and probiotic bacteria *Lactobacillus sp*. with the inhibition zone respectively 7.50 mm, 7.00 mm, 1.50 mm, 8.00 mm at a concentration of 30% [1]

Apart from lauric acid, VCO also contains unsaturated fatty acids, namely oleic acid which is a component of functional food [1]. MRS media combined with synthetic compounds from oleic acid can increase the growth of *Lactobacillus delbrueckii lactis* LKT. The highest OD (Optical Density) value produced was 0.8 with a concentration of 0.1 mM. In contrast, the combination of lauric acid and MRS medium (deMann Rogosa Sharpe) decreased the growth of *L. delbrueckii subsp. lactis* LKT which is characterized by a decrease in the OD (Optical Density) value.

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Some oils that contain oleic acid such as olive oil, linseed oil, Turnip rape oil can also increase the growth of *L. delbrueckii subsp. lactis* LKT with the highest concentration of 1% [2].

Probiotics are a component of functional food. Probiotics are microbes that can live and develop in the intestine, and can benefit their host either directly or indirectly from the results of their metabolites. Probiotics can change the micro ecology of the gut in such a way that those who consume them are healthy [3].

One of the bacteria that acts as a probiotic is Lactic Acid Bacteria (LAB). *Lactobacillus delbrueckii subsp. bulgaricus* (*L. bulgaricus*) is a Lactic Acid Bacteria (LAB) which is often used for daily food such as yogurt and fermented milk. Some of the active metabolites produced by lactic acid bacteria are lactic acid, ethanol, hydroperoxide and bacteriocin. The metabolites produced by these bacteria are compounds that can be used as antibacterial. *L. bulgaricus* metabolites can inhibit pathogenic bacteria such as *E. coli* and *Staphylococcus aureus* with an inhibition zone of 2.4 cm and 1.95 cm respectively [4,5].

Lactobacillus sp. can coexist with VCO, which states that the yield of *Lactobacillus plantarum* encapsulation with dextrin coating and the addition of two percent VCO gives good results, weighing 20.425 g (51.06 percent). Meanwhile, the viability after encapsulation was relatively better than all VCO concentrations, namely the addition of one percent VCO with an average log value of 9.645 CFU/ g. At a temperature of 4 ℃ storage for two weeks, it can still meet the minimum requirements for probiotics, namely 10^6CFU /g [6]

From 11 colonies of lactic acid bacteria isolated from 7 types of VCO in Padang can inhibit the growth of pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus and Bacillus subtilis*). A total of 11 colonies of lactic acid bacteria were bacteria of the *Lactobacillus genus*, including *Lactobacillus plantarum* and *Lactobacillus sakei.* This shows that some *Lactobacillus* species can still inhibit pathogenic bacteria even though they live in a VCO environment [7].

The viability of *L. bulgaricus* in VCO stored at room temperature for 24 days continued to decline. The highest number of cells was achieved on the 0th day of storage with the number of cells reaching 1.65×10^{12} and the number of the lowest cells on the 24th day with the number of bacteria 5.33×10^{1}.The decrease in number was due to the death of *L. bulgaricus* bacteria during storage [8].

Virgin coconut oil is antibacterial because of the fatty acid content in it, this oil can inhibit the probiotic bacteria *Lactobacillus sp*. Because of the lauric acid content in it. Besides lauric acid, VCO also has oleic acid compound molecules which can stimulate the growth of *Lactobacillus delbrueckii subsp. lactis* LKT. Based on the description above, it is necessary to conduct research on the effect of VCO on the growth activity of *L. bulgaricus* probiotic bacteria by counting colonies using a total plate count. In addition, it is also necessary to conduct research on MIC (Minimum Inhibitory Concentration) of *L. bulgaricus* metabolites against pathogenic bacteria (*E. coli*) using the liquid dilution method, as well as testing the fatty acid content of VCO using GCMS and characterizing VCO [1].

MATERIALS AND METHOD

Chemicals

The materials used are virgin coconut oil (PT. CocoFarma), cotton, n-hexane (MERCK®), 0.5 M methanolic NaOH (MERCK®), CH_3COOH (MERCK®), aquadest (MyerWaterExpert), MRSB (MERCK®). , MRSA (MERCK®), NA (MERCK®), NB (MERCK®), $CaCO_3$ (MERCK®), amoxicillin (DANKOS), DMSO (MERCK®), H_2SO_4 (MERCK®), *L. delbrueckii subsp. bulgaricus, Escherichia coli.* The sample or VCO (Virgin Coconut Oil) was obtained from PT. CocoFarma.

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Microorganisms and Growth Media

Lactobacillus delbrueckii subsp. bulgaricus **FNCC-0041** strain used in this study were obtained by purchasing from Centre Laboratory of Gajah Mada University. The standard lactobacillus strains were maintained on "de Man and Sharpe "(MRSA) medium, having the following composition (% w/v): yeast extract 0,4; beef extract 0.8; glucose anhydrous 2.0; peptone 1.0; sodium acetate trihydrate 0.5; triammonium citrate 0.2; manganese sulfate tetra hydrate 0.005; manganesium sulfate heptahydrate 0.02, tween (80) 0.1; dipotassium hydrate phosphate 0.2, agar 1,5; final pH (at 37^0C) was adjusted to 6.2±0.2 [5].

Physical Properties of VCO

Specific Gravity

Virgin coconut oil is filtered using filter paper, cooled to 30°C, and put into a pycnometer until it overflows and try not to form air on the pycnometer and cover the pycnometer. Clean the oil that has overflowed and stuck to the outside pycnometer. Next, immerse the pycnometer in a water bath with a temperature of 30° C and let stand for 30 minutes. Remove the pycnometer from the water bath, clean and dry the pycnometer carefully. Weigh the pycnometer and its contents. The weight of oil is the difference between the weight of the pycnometer and its contents minus the weight of the pycnometer empty. The density of oil at 30° C can be calculated using the following formula [10].

$$\text{Specific Gravity} = \frac{(pycnometer + oil\ weig\quad) - weig\quad of\ the\ empty\ pinmeter}{volume\ of\ oil\ at\ a\ temperature\ of\ 30\ ℃} \tag{1}$$

Refractive Index

The instrument used in this test is an abbe refractometer equipped with a temperature control. The upper and lower prisms are cleaned with alcohol until dry. The VCO that has been prepared is dropped on the lower prism surface then cover the two parts of the prism and tighten the screw. The test is carried out at a temperature of 25^oC for oil. Temperature readings can be seen on a thermometer. The refractometer is moved back and forth until you see a field of view which is divided into two parts, namely dark and light fields. The visible boundary area is usually not sharp and colorless. The boundary line is adjusted by turning the adjusting tool so that it falls exactly on the cross line. At this position the refractive index is read on the scale. The refractive index at a certain temperature can be obtained by the following calculations [11]

$$\text{Refractive index} = \frac{C}{Vp} \tag{2}$$

Moisture Content

Samples were weighed ± 3 grams using a scale bottle. The samples were heated in an oven at 105 ° C for 3 hours. Cooled the sample in a desiccator for 30 minutes. Weighed the weigh bottle. Heating and weighing the sample is repeated until a constant weight is obtained using the following formula [12].

$$\%water\ content = \frac{(initial\ weight - final\ weigh\ )}{initial\ weigh} \times 100\ \% \tag{3}$$

Analysis of VCO with Gas Chromatography –Mass Spectrometry (GC-MS)

Sample of 50 mL of virgin coconut oil, added with 400 µL methanolic NaOH, then centrifuged and heated at 50 °C for 10 minutes. After that it is cooled, 1 mL CH_3COOH is added, then 1 mL of distilled water is added then 1 mL of

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n-hexane is added then centrifuged, the solution is allowed to stand for a few minutes and two layers will be formed. The top layer of 1 μL will be used for analysis on a gas chromatograph [13].

Analysis of the content of virgin coconut oil using GC-MS. GC-2010 with a flame ionization detector (FID) under the following operating conditions: Rtx-5MS column with a length of 30 m, a diameter of 0.25 mm, a film thickness of 0.25 μ; The column temperature is from 80 ° C to 300 ° C with a running temperature of 5 ° C / min; Detector temperature 280 ° C; injection temperature 250 ° C; helium carrier gas; gas flow rate of 3 mL / minute and gas pressure of 12 kPa [10].

TABLE 1. The treatment group for the growth test of *L. bulgaricus*

No.	Treatment	Information
1	Control (+)	Probiotic
2	Control (-)	1% DMSO solvent
3	Test solution 1	VCO 1 %
4	Test solution 2	VCO 5%
5	Test solution 3	VCO 10 %
6	Test solution 4	VCO 15 %

Determination The Number of *L. bulgaricus* by Total Plate Count (TPC) method

Testing of bacterial activity was carried out by the total plate count method with MRS agar media. Making a positive control group, namely bacteria probiotic *L. bulgaricus* was put in a 10 mL volumetric flask and added NaCl 0.9% ad 10 mL, making a negative control by means of 1 mL probiotic added with 1 mL 10% DMSO and then 10 mL ad NaCl, the test group was made by 1 mL. Probiotic bacteria plus VCO in various concentrations which have been dissolved in DMSO and ad NaCl 0.9% 10 mL. after that, a 10-1 to 10-8 dilution is carried out. Plating dilution of 10-5 to 10-7 was performed in duplicate on a petri dish filled with MRSA. Samples and control treatment were incubated at 37 °C for 48 hours in reverse position. The calculation of the number of probiotic bacterial colonies was expressed using colony forming units (CFU) [14]

Antibacterial Activity and MIC Metabolite Test of *L. bulgaricus*

Sterilization of tools and materials is used to prevent contamination during testing. In testing the growth and antibacterial activity of probiotics, the tools and materials used must be sterile. All tools you want to use are washed and dried. Glassware and media are sterilized in an autoclave at a temperature of 121 ° C, a pressure of 1 atm, for 15 minutes. Ose needles and tweezers are sterilized by burning them over a bunsen fire.

TABLE 2. Antibacterial activity treatment groups

No.	Treatment	Information
1	Test hole 1	Methabolite100%
2	Test hole 2	Methabolite75%
3	Test hole 3	Methabolite25%

Antibacterial activity testing was carried out with paper discs. The test microbe used was *L. bulgaricus*. The first, second and third disc papers contained the concentration series of *L. bulgaricus* metabolites which were 100%, 75%, 25% (v/v), respectively. Preparation of 100%, 75% and 25% metabolites was carried out with multilevel dilution, namely 7.5 mL of ad metabolite 10 mL of distilled water to make a concentration of 75% then to make a concentration of 25% put metabolites 3.33 mL of 75% concentration into a 10 mL volumetric flask add aquadest 10 mL [9].

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The test solution was dropped onto disc paper as much as 0.2 µL. Before being put on the media containing the test bacteria, the disc containing the compound is allowed to dry, this is to find out if the solvent has evaporated. The tested bacterial culture was incubated in an incubator at 37 ° C for 24 hours and then observed the clear zone around the disc paper. The diameter of the inhibition zone is obtained by subtracting the overall diameter of the clear zone with disc paper [29].

Positive control used amoxicillin 0.003%. The preparation of 0.003% amoxicillin is carried out by dissolving 0.003 grams of pure amoxicillin and adding aquadest to a volume of 100 mL. Preparation of 10% DMSO solution was carried out by adding 10 mL of DMSO and added with distilled water to a volume of 100 mL. The use of DMSO is because DMSO is a solvent that can dissolve non-polar compounds and a concentration of 10% is used because at this concentration it does not inhibit bacteria[15].

TABLE 3. Minimun Inhibitory Concentration test for treatment group

No.	Treatment	Information
1	Hole 1	Metabolites 75%
2	Hole 2	Metabolites 37.5%
3	Hole 3	Metabolites 18.75%
4	Hole 4	Metabolites 9.38%
5	Hole 5	Metabolites 4.69%
6	Hole 6	Metabolites2.34%
7	Hole 7	Metabolites 1.17%
8	Hole 8	Metabolites 0.59%
9	Hole 9	Metabolites 0.29%
10	Hole 10	Metabolites 0.15%
11	Hole 11	Control (+) amoxicillin 0.003%
12	Hole 12	Control (-) NB without treatment

3 mL of NB media (nutrient broth) was dropped in each hole of the microplate 12 wells. 100% metabolites was dropped into hole 1 as much as 4.5 mL ad 6 mL nutrient broth so that in hole 1 there is a metabolite concentration of 75%. Then, a stratified dilution is carried out by inserting 3 mL of liquid in hole 1 into hole 2 and so on until the concentration becomes a concentration variation as shown in table 7. After gradual dilution, 100 µL of the *Escherichia coli* bacteria culture is added [7].

Hole 11 contains amoxicillin, which is positive control, while hole 12 contains NB without treatment, namely negative control. 12 well microplates were incubated at 37 ° C for 24 hours. The Minimum Inhibitory Concentration (MIC) value can be seen from the turbidity and translucency observations on the microplate well 12 that have been incubated. The hole which has lost its turbidity is determined as the KHM value [4].

ANALYSIS OF DATA

Data analysis of the effect of VCO on the growth of *L. bulgaricus*, comparison of antibacterial activity and comparison of MIC metabolites after and before adding VCO using SPSS 16.0 for windows. The data entered in the form of the concentration of the test solution, MIC value, and antibacterial activity. The data distribution normality test was carried out by using the Shapiro-Wilk analysis, the data were normally distributed if $p > 0.5$. If the data is normally distributed, the test is continued with one way ANOVA. If it shows a significant result, that is, there is a significant difference, then proceed with the Post Hoc test to determine the difference (significance between test groups) with $\alpha > 0.05$. Data that were not normally distributed were analyzed using the Kruskal-Wallis test followed by the Mann Whitney test.

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RESULTS AND DISCUSSION

The results of the organoleptic observations of VCO in this study were carried out by examining the color and smell of the VCO. The results of the observations can be seen in **Table 4.**

TABLE 4. Results of VCO Organoleptic Examination

Sample	Type of Observation	Result
VCO	Color	Colorless
	Smell	Specific smell of coconut, a little rancid
	Taste	Oil taste, tasteless

The smell produced by VCO is a specific smell of coconut. These results are in accordance with the Indonesian National Standard (SNI) which states that VCO has a distinctive coconut aroma and is not rancid. The rancid odor found in oil naturally can also occur due to the formation of very short acid chains due to the decomposition of the oil which can affect the aroma of the oil produced [16]. The taste check found that the VCO did not taste like oil in general. The results of color inspection are also in accordance with the Indonesian National Standard which states that the color of VCO is colorless.

Determination of the VCO refractive index using an abbe refractometer. The result of the VCO refractive index examination is 1.4546 at a temperature of 20 °C. The value of the VCO refractive index has met the quality requirements and is in accordance with the research of , which states that the VCO refractive index at a temperature of 40 °C ranges in the range of 1,448-1,450.

Determination of specific gravity using a pycnometer and then calculated using the specific gravity formula which can be seen in Appendix 10.The results of the examination and the weight of each treatment presented in **Table 5.**

TABLE 5. Determination of Specific Gravity

Sample	Empty pycnometer weight (g)	Pycnometer weight containing VCO (g)	VCO volume (mL)	Specific gravity
VCO	15,60	24,20	9,40	0,9149
	15,60	24,21	9,45	0,9111
	15,60	24,21	9,40	0,9149

The average density generated in this determination is 0.9136, which has met the codex standard for VCO with a range of 0.908-0.921. Determination of VCO moisture content is carried out by comparing the weight of VCO before oven and after oven using certain calculations. The results of VCO moisture content examination presented in **Table 6.**

TABLE 6. Determination of Water Content

Sample	Empty cup weight (g)	Weight of the cup containing VCO (before oven) (g)	Weight of the cup containing VCO (after oven) (g)	% Water content
VCO	39,07	42,07	42,07	0

The results of determining the percentage of water content in VCO are very good at 0%, where the percentage of water content allowed for VCO, is a maximum of 0.5% and meets the requirements of the Asean Pacific Coconut

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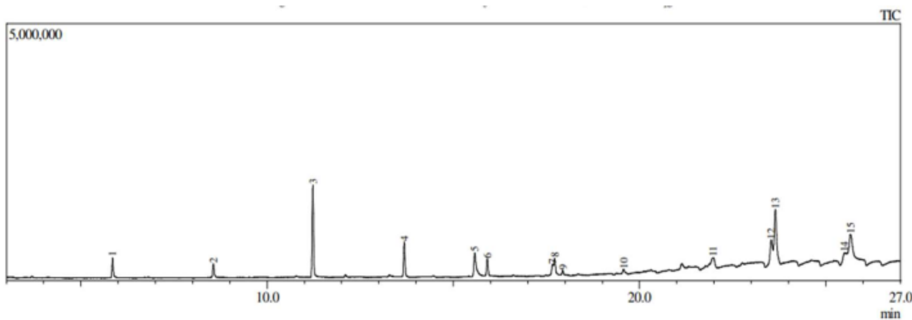
Community (APCC) with a range of 0, 1-0.5%. It is important to determine the water content in the oil because the water content in the oil can cause a hydrolysis reaction that causes the oil to smell rancid because the oil will react and turn into carboxylic acid compounds [16].

Analysis of VCO Fatty Acid Content by GC-MS

VCO analysis was carried out using GC-MS to determine the fatty acid content in it. On GC a chromatogram is obtained and on MS the spectrum is obtained. The results of the VCO chromatogram are shown in **Figure 1.**



Examination on GC shows 15 peaks produced on the chromatogram. This shows that there are 15 compounds contained in VCO and only 7 of them are fatty acids as shown in **Table 7.**

TABLE 7. Components of VCO fatty acids based on GC-MS

No.	Peak	Retention time (minutes)	Area(%)	Area(%)	Ssimilarity Index (%)
1	1	5,850	3,62	Caprylic acid	96
2	2	8,560	2,69	Capric acid	97
3	3	11,230	17,92	Lauric acid	97
4	4	13,685	6,88	Myristic acid	97
5	6	15,912	3,47	Palmitic acid	96
6	8	17,723	3,68	Oleat acid	94
7	9	17,941	0,81	Stearat acid	94

The retention time produced by the peaks of chemical compounds on the GC chromatogram varies. Retention time is the time spent by the solute in the column or it can be defined as the time spent by the test compound in the stationary and moving phases. The longer retention time depends on the interaction of the analyze with the stationary phase. The stronger the interaction, the longer the interaction time and the longer the compound's retention time.

Based on table 7, the peak that has the highest area or the highest concentration is lauric acid with a concentration or area of 17.92%, while the lowest concentration is owned by stearic acid with an area of0.81%. This is in accordance with the research of [13], which states that the highest compound component of VCO is lauric acid with a concentration of 50.49%.Other acids such as caprylic acid (3.62%), capric acid (2.69%), myristic acid (6.88%), palmitic acid (3.47%), oleic acid (3.68%) are almost have the same concentration. It's just that linoleic acid was not present in the VCO sample, even though in the research of [17], this compound was present in the VCO sample tested but with a low concentration of 1.5%.

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The concentration obtained for the fatty acid test on the VCO tested was not in accordance with the standard range in the AAPC (Asia Pacific Coconut Community). This is because during the trip virgin coconut oil was damaged due to the long delivery process. This can also be caused because the temperature during delivery increases and will speed up the time the formation of fatty acids and fatty acids will break down quickly [18,19].After analyzing the compounds, the fragmentation patterns of each fatty acid compound were analyzed. The fragmentation patterns of the components of the VCO constituent compounds, namely lauric acid and oleic acid, are as follows:

Before mixing with VCO, *L. bulgaricus* suspension was compared with Mc Farland standard 0.5. The use of Mc Farland standard 0.5 is in accordance with the number of bacteria 1.5 x 10^8 CFU / mL which have reached the requirements as a probiotic, namely the minimum requirement for total LAB is 10^6 CFU / mL and for the total LAB requirement in fermented milk is 1.0 x 10^8 CFU / mL [20].

The growth test or viability test of *L. bulgaricus* bacteria uses several variations in the concentration of VCO (1%, 5%, 10% and 15%), this is because we want to see at which concentration VCO can inhibit or increase the growth and number of *L. bacteria. bulgaricus*. This test uses 2 replications. The calculation of the growth of *L. bulgaricus* bacteria (viability test) resulted from calculations using the pour plate method. The difference and growth seen can be seen in **Table 8.**

TABLE 8. Viability test for *L. bugaricus* bacteria with VCO

No	Concentration	Number of microbes (CFU / mL)
1	**1%**	150,8 x 10^{12}
2	**5%**	111,4 x 10^{12}
3	**10%**	109,25 x 10^{12}
4	**15%**	55,667 x 10^{12}
5	**(+)**	171,0 x 10^{12}
6	**(-)**	169,33 x 10^{12}

Based on the data above, the growth of *L. bulgaricus* bacteria decreased along with the increase in the VCO concentration. The highest number of *L. bulgaricus* bacteria is at a concentration of 1% with a total of 150.8 x 10^{12} followed by a concentration of 5% with the number of microbes as much as 111.4 x 10^{12}, a concentration of 10% as much as 109.25 x 10^{12} CFU / mL and a concentration 15% with the lowest number of microbes, namely 55.667 x 10^{12} CFU / mL. but still under positive control with the number of bacteria as much as 171.0 x 10^{12}. The number of *L. bulgaricus* at each VCO concentration was still under positive control and negative control.

This means that VCO with various concentrations affects the growth of *L. bulgaricus* bacteria, to be more precise reducing the number of *L. bulgaricus* bacteria along with the addition of the VCO concentration. This condition can occur because on the GC-MS examination to test the fatty acid content of VCO, the most abundant fatty acid or concentration is lauric acid with a concentration of 17.62% while the concentration of oleic acid which is expected to stimulate the growth *of L. bulgaricus* bacteria is only slightly ie 3.68%.

Lauric acid in VCO is antibacterial. Several medium chain fatty acids, especially lauric acid, are claimed to have properties bactericidal or able to inhibit the growth of pathogenic bacteria [23]. The mechanism of lauric acid as an antibacterial is to change to monolaurin and inactivate bacteria by dissolving the lipid components present in the bacterial cell membrane [24]. *L. bulgaricus* is a gram-positive bacteria which has a lipid component that can be damaged by lauric acid.

Apart from lauric acid, other fatty acids such as capric acid, caprylic acid, myristic acid, palmitic acid, stearic acid which are components of fatty acids can also inhibit bacteria. Fatty acids have been shown to have antibacterial and

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antifungal properties in inhibiting many microorganisms in plants and food. Gram negative is more resistant to fatty acids than gram positive in its antagonistic effect because gram-negative bacteria have a polysaccharide layer on their cell walls. *L. bulgaricus* is a gram-positive bacteria, which is why the number of *L. bulgaricus* bacteria decreases with increasing VCO concentration because fatty acids inhibit *L. bulgaricus* bacteria. The mechanism of fatty acids in inhibiting bacteria is by gaining access through the cell wall or outer membrane of bacteria and binding to the bonding electron carrier directly or into the inner membrane and causing the electron carrier to move apart or shift from the entire membrane [25].

The number of *L. bulgaricus* bacteria in positive control and negative control was not much different. The number of *L. bulgaricus* in positive controls containing *L. bulgaricus* alone was 171.0 x 10^{12} while the negative control was 169.33 x 10^{12}. It can be concluded that the negative control containing 10% DMSO did not affect the growth of L. bulgaricus bacteria because the number of bacteria was almost the same. This is consistent with research [26] which states that 10% DMSO does not cause inhibition in bacteria.

The analysis of *L. bulgaricus* growth data used SPSS 16.0. Previous data was tested for normality to find out normally distributed data which is an absolute requirement to be analyzed into one way ANOVA parametric statistics. The data is normally distributed because all data show a significance value> 0.05. Furthermore, looking at the homogeneity using the Test of Homogeneity of Variances on each data, the significance value is> 0.05 and it can be concluded that the group variants being compared homogeneous.

After the data was declared homogeneous, it was continued with one way ANOVA parametric statistical analysis. In this statistical analysis, if the data is not or the value is sig. less than 0.05 (<0.05) then there is a difference between groups whereas if the value is sig. > 0.05, there is no difference between groups. The results of the one way ANOVA statistical analysis stated that there was no difference between groups due to sig. which get> 0.05.

After being tested using one way ANOVA, then the Post Hoc LSD (Least Significant Difference) data analysis was carried out. This data analysis is used to see which variables have a significant difference, there is a difference in the concentration variation of 15% with positive control and negative control, which is marked with the sig value. which is <0.05. It means that VCO with a concentration of 15% has a significant effect on the growth of *L. bulgaricus* bacteria.

Antibacterial Activity Test of *L. bulgaricus* metabolites

Testing the antibacterial activity of *L. bulgaricus* metabolites using the paper disc method followed by the dilution method (micro dilution) to determine the MIC (Minimum Inhibitory Concentration) tested on pathogenic bacteria *E. coli*. The petri dishes used for this test were in the form of petri dishes with the best number of bacteria, namely petri dishes with 1% VCO concentration variations and positive control (*L. bugaricus* without treatment). The extraction of *L. bulgaricus* bacterial metabolites was carried out using the centrifugation method at a speed of 10,000 rpm and a temperature of 4 °C. This temperature is a storage temperature so that there is no bacterial growth and no protein denaturation occurs .

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Table 9. Antibacterial activity of metabolite from *L. bulgaricus* and VCO

Treatment	Concentration	Inhibition Zone (mm)	Criteria of Antibacterial Activity
Metabolite *L. bulgaricus* Without VCO	100%	6,77±0,97	moderate
	75%	5,47±1,01	moderate
	25%	4,37±0,20	weak
Metabolite *L. bulgaricus* with VCO	100%	5,23±1,54	moderate
	75%	4,0±1,80	moderate
	25%	3,63±1,56	moderate

The table above depicts the metabolites added with VCO decreased their antibacterial activity. It is characterized by a smaller diameter of the inhibition zone when compared to metabolites without treatment or metabolites that are not added with VCO. This decrease in antibacterial activity can occur because in the growth test of *L. bulgaricus* bacteria added by VCO, the number of bacteria is less than that of *L. bulgaricus* bacteria without treatment.

At a concentration of 100% *L. bulgaricus* metabolites with the treatment added with VCO or without VCO together resulted in an inhibitory response moderate. Whereas at a concentration of 50% the response to inhibition of the metabolites that were not added by VCO was moderate, while the metabolites added by VCO were weak. The inhibition response is in the form of inhibition zone category produced by *L. bulgaricus*, which states that the strength of the inhibition is weak if the resulting clear zone diameter is ≤5 mm. if the diameter of the clear zone is 5-10 mm, the strength of the inhibition is moderate [30]. The strength of the inhibition is strong if the diameter of the clear zone is 11-20 mm and the category of inhibition is very strong if the diameter of the clear zone is ≥ 20 mm.

Analysis of the antibacterial test data for *L. bulgaricus* metabolites against *E. coli* using SPSS 16.0. The data used were in the form of inhibition zone data for metabolite variation that was not added by VCO, the area of inhibition zone for metabolites added by VCO and the comparison of the two inhibition zones. Each data is tested for normality to find out normally distributed data which is an absolute requirement to be analyzed into one way ANOVA parametric statistics. The results obtained in the analysis of the metabolite inhibition zone area data that were not added by VCO showed that the data were normally distributed with a significance value> 0.05.

The homogeneity test was carried out after the normality test using the Test of Homogeneity of Variances on each data, obtained a significance number> 0.05 and it can be concluded that the group variants compared were homogeneous followed by one way parametric statistical analysis ANOVA stated that there were differences between groups where the sig . <0.05. If we look at the Post Hoc LSD (Least Significant Difference) test which is used to see which variables have a significant difference, there is a difference in variations in the metabolite concentration of 100% with a metabolite concentration of 25% which is indicated by the sig value. <0.05. Means that metabolites with a concentration of 100% differed significantly from the metabolite concentration of 25%.

The next data analysis is the analysis of metabolite data added with VCO. The normality test using the Test of Homogeneity of Variances shows that the data is normally distributed, the homogeneity test also shows that the compared data is homogeneous because it has a sig value. > 0.05. Furthermore, the data were analyzed using one-way ANOVA statistical analysis and the data showed that there was no difference between groups as indicated by the sig value. > 0.05. Comparison of added and without metabolite data analysis with VCO was analyzed using paired t-test. In the paired t-test the data showed that there was a significant difference between groups, namely the metabolite

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group that was treated with added VCO and not added with VCO, marked with a sig value < 0.05 ($p < 0.05$). This proves that the metabolites added by VCO have a significant difference with those that are not added with VCO or it can be said that VCO affects the antibacterial activity of *L. bulgaricus* metabolites by decreasing the diameter of the formed inhibition zone.

The Minimum Inhibitory Concentration (MIC) was determined after seeing the strength or resistance response produced in *L. bulgaricus* metabolites.MIC is determined using a microdilution. This test is carried out as a follow-up test of the antibacterial test because at a concentration of 25%, *L. bulgaricus* metabolites can still inhibit *E. coli* bacteria even with a weak inhibitory response. MIC test used positive control and negative control. The positive control used was amoxicillin with a concentration of 0.003%. This is based on research by [24], which states that the antibacterial activity of amoxicillin against *E. coli* at this concentration is intermediate. The results of the MICtest for *L. bulgaricus* metabolites can be seen in **Table 10.**

TABLE 10. MIC metabolites of *L. bulgaricus bacteria*

No	Concentration	Visible Growth of Bacteria	
		Plus VCO	
1	75%	-	-
2	37,5%	-	-
3	18,75%	-	-
4	9.38%	-	-
5	4,69%	+	-
6	2,34%	+	+
7	1,17%	+	+
8	0,59%	+	+
9	0,29%	+	+
10	0,15%	+	+

Information: (+) = Visible growth of bacteria , (-) = No growth of bacteria

The negative control used was nutrient broth without treatment which was then used to compare the turbidity in the microplate. In this test, the MIC of *L. bulgaricus* metabolites against *E. coli* was 9.38% in the metabolites added with VCO while the resulting MIC of the metabolites that were not added by VCO was 4.69%. This can be seen in the results obtained where the metabolites added with VCO the final limit of the concentration produced clear results at a concentration of 9.38%, while the metabolites that were not added with VCO were at a concentration of 4.69%. It can be concluded that the metabolites produced by *L. bulgaricus* which have been added with VCO will reduce their antibacterial activity to their MIC [25].

The antibacterial activity of *L. bulgaricus* metabolites was better than the positive control or amoxicillin with a concentration of 0.003%. It can be seen from the turbidity produced by amoxicillin showed more turbidity than *L. bulgaricus* metabolites. This is because the amount or concentration of amoxicillin used is small so that its activity is also small. Amoxicillin belongs to the beta-lactam group. The mechanism of action of the antibiotic amoxicillin itself by binding to penicillin binding proteins which inhibits the trans peptidation process (cross-linking process in cell wall synthesis), which leads to activation of autolytic enzymes in the bacterial cell wall. This process causes lysis of the cell wall, and thus, damages bacterial cells [26,27]

The antibacterial activity produced by *L. bulgaricus* metabolites is produced by compounds such as lactic acid, hydrogen peroxide, acetaldehyde, acetone, acetoin, diacetyl [25]. The mechanism of lactic acid in killing pathogenic bacteria is by damaging the bacterial extracellular membrane. In addition, lactic acid can also kill pathogenic bacteria by changing the membrane permeability of the cell membrane [26,27]. The mechanism of hydrogen peroxide in killing pathogenic bacteria is by oxidation of the components of pathogenic bacteria which causes irreversible damage to the

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bacterial cell membrane . Acetaldehyde works by inhibiting the division of *E. coli* (pathogenic bacteria). Acetone works almost the same as lactic acid, which disrupts the membrane permeability activity of pathogenic bacteria [28]. Acetone works by lowering the pH of the pathogenic bacteria medium [29]. Diacetyl has a mechanism of action similar to acetone, namely reducing the pH of the pathogenic bacteria medium [30].

CONCLUSIONS AND SUGGESTION

Characterization of VCO in the form of organoleptic (colorless, specific smell of coconut), specific gravity (0.9149), refractive index (1.4546) and moisture content (0%) produced by PT. CocoFarma meets codex and SNI standards. The fatty acid composition of virgin coconut oil based on GC-MS analysis shows the highest concentration of lauric acid (17.92%) followed by other fatty acids such as caprylic acid (3.62%), capric acid (2.69%), myristic (6.88%), palmitic acid (3.47%), oleic acid (3.68%) and stearic acid (0.81%). VCO with this chemical compound composition did decrease the growth of *L. bulgaricus* and antibacterial activity of *L. bulgaricus* metabolite invitro. According to this research, this VCO must be use below 10% to prevent the decreasing of probiotic *L. bulgaricus* performance invitro. Research must be continue to determine effect of this VCO to other kind of probiotic that exist in human gut. This research must be develop in vivo to ensure impact of this VCO on human probiotic.

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Similarity result of_Impact of Virgin Coconut Oil (VCO) on Probiotic Lactobacillus delbrueckii subsp. Bulgaricus

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