Antibacterial Activity of Free Fatty Acids, Potassium Soap, and Fatty Acids Methyl Esters from VCO (Virgin Coconut Oil)

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Abstract. Virgin coconut oil is edible oil obtained from fresh coconut meat (Cocos nucifera L.) and is widely used for health such as for alternative healthy diets, anti-bacterial, anti-virus and anti-fungal. This is because VCO contains a lot of MCFA (Medium-Chain Fatty Acids) such as lauric acid (65.84%), myristic acid (13.16%) and caprylic acid (7.08%). The antibacterial ability of MCFAs is one of the most studied, but free fatty acids from the hydrolysis of VCO have not been done. The objective of the research is to derivatize VCO and determine the potential antibacterial activity of the product. The results of the research are as follows. (1) K-soap (solid, white, melting point 46-124 °C). (2) Free fatty acids (liquid, colorless, boiling point 266-296 °C, density 0.93 g.mL⁻¹, refractive index 1.44, viscosity 27.18 cP, acid value 184.54, saponification value 296.59 and ester value 112.05). (3) Fatty acid methyl ester (liquid, colorless, boiling point 260-262 °C, density 0.89 g.mL⁻¹, refractive index 1.43, viscosity 3.36 cP, acid value 0.33, saponification value 268.6 and ester value 268.27). VCO and its derivatives at concentrations of 2 and 1% are active as antibacterial agents against Escherichia coli and Staphylococcus aureus.

1. Introduction
After careful observed, the use of natural resources as raw materials for chemicals is generally used for food, textiles, building material, energy (fuel), and drugs or cosmetics. Natural resources are classified as biological natural resources (i.e. carbohydrates, lignocellulose, and triglycerides) and non-biological natural resources (i.e. minerals, petroleum, and natural gas). As one of the abundant renewable resources, triglycerides are used as raw materials in making soap [1], free fatty acids [2], foodstuffs, and biodiesel [3].

Triglycerides can be divided into two based on their sources, namely vegetable oil and fish oil. Chemical or enzymatic engineering of oils, especially edible vegetable oils can produce a variety of fine chemicals that are beneficial to human life. The benefits of making fine chemicals from edible vegetable oil can avoid products that are toxic and safe to use. One of the most abundant edible vegetable oils in Indonesia is VCO.

VCO is a processed natural oil obtained from a fresh and ripe coconut core. Recent research shows that VCO has anti-inflammatory activity, which protects the skin by increasing its endurance function [4]. VCO has medium-chain fatty acids and is rich in saturated fatty acids, such as lauric acid which has antibacterial activity [2][5][6]. Other studies report that VCO inhibits the growth of
Staphylococcus aureus by destructive mechanisms against bacterial cell walls and increases the ability of the cell's immune system [7].

Lauric acid (C₁₂) is a saturated fatty acid that most inhibits the growth of Gram-positive organisms [8]. Lauric acid at a concentration of 5% can inhibit the growth of bacteria S. aureus, B. cereus, S. Typhimurium and E. Coli [9]. The antibacterial properties of free fatty acids are used by many organisms to defend against parasitic or pathogenic bacteria, with the main target being the cell membrane, disrupting the electron transport chain, and oxidative phosphorylation [10].

In the context of antibacterial activity, for oil and fatty acids there are various results. For example tamarin oil (Tamarindus indica), free fatty acids are antibacterial against E. coli and S. Aureus [11] while the oil is inactive [12]. Coconut oil (Cocos nucifera), palm oil (Elaeis guineensis), babassu (Attalea speciosa), murumuru (Astrocaryum murumuru), tucuma (Astrocaryum vulgare), and Cuphea oil (Cuphea ignea) do not show effects on bacteria but their hydrolysis results are active against C perfringens, E. cecorum, L. monocytogenes, and S. Aureus [5]. Antibacterial agents can be applied in medicine, agriculture, food preservation, and the use of conventional antibiotics [10]. In addition to hydrolysis with acids, oils can be hydrolyzed with bases to obtain their salts which are useful as an antibacterial soap, such as potassium laurate.

Potassium laurate has high antibacterial activity against S. mutans and has great potential as an antibacterial agent [13]. Potassium laurate also shows antifungal activity against P. pinophilum which is a medium-chain fatty acid salt [14]. Lauric salts from magnesium, calcium, and manganese are effective in sterilizing the bacteria S. aureus and P. acnes while the cobalt, nickel and copper salts from lauric can sterilize the bacteria S. aureus, P. acnes and S. Epidermidis [15]. Sodium lauryl ether sulfate and sodium lauryl sulfate as a synthetic detergent can remove biofilms from S. Aureus [16].

Studies on VCO from coconut oil in Indonesia and its derivatives as antibacterial have never been reported. This study aims to investigate the antibacterial activity of VCO and its derivatives (K-soap, FA, and FAME) against Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria and examine the relationship of polarity with their antibacterial activity.

2. Experimental Procedure

2.1. Chemicals, equipment, and instrumentations

2.1.1. Chemicals.
VCO is obtained from markets that are sold online at Tokopedia - Indonesia under the Sofia brand name. All reagents and solvents (i.e. potassium hydroxide, sodium chloride, hydrochloric acid, methanol, anhydrous magnesium sulfate, ethanol, n-hexane, chloroform, oxalic acid, and sodium carbonate) were obtained from Merck and the quality of the analysis.

2.1.2. Equipments and instrumentations.
A set of Iwaki reflux equipment, Pyrex titration tool, Thermo Scientific heater and stirrer, DA-20D Ulvac vacuum pump, Memmert oven, Kokusan H-103N Centrifuge, Capillary-Viscosimeter Schott Gerate, Refractometer Abbe, Iwaki Pycnometer, Hot plate HP-30S HP Shimadzu, Fischer Scientific Melting Point Apparatus, IR-prestige 21 Shimadzu and GCMS-QP2010S Shimadzu.

2.2. Experiment

2.2.1. Saponification reaction.
Amount 20g of VCO and 100mL of 3M potassium hydroxide solution was refluxed at 80°C for 3 h. The mixture is added with 100mL saturated sodium chloride solution and stirred until a precipitate is obtained. The formed product is filtered and washed with distilled water (2 x 100mL), then dried at 80°C.
2.2.2. Soap acidification.  
The synthesized K-soap (10 g) of VCO was added with 40 mL of distilled water and added drop by drop of hydrochloric acid 1 M until the emulsion used completely. The mixture is separated, filtered, and purified with distilled water until the remaining is not acidic. After that, centrifuged at 3000 rpm for 10 minutes and the upper layer taken.

2.2.3. trans-Esterification reaction.  
A total of 20 g of VCO, 9 mL of methanol, and 0.2 g of potassium hydroxide were refluxed at 60 °C for 4 hours, then cooled, and allowed to stand for 24 hours then obtained two layers. The two layers are separated, accommodated in different containers and tested for acidity with litmus paper. The top layer is washed with warm distilled water and then dried with anhydrous magnesium sulfate.

2.2.4. Characterization and identifications.  
The characterization of VCO and its derivatives includes: (1) visual state and color, (2) boiling point is determined by boiling point equipment, (3) melting point by melting point apparatus, (4) density with a pycnometer, (5) viscosity with Capillary-Viscosimeter tubes, (6) refractive index with an Abbe refractometer, (7) saponification value, acid value, and esters value by the method in Indonesian National Standards [17], and (8) solubility test with n-hexane, chloroform, ethanol, methanol, and water. Identification of VCO and its derivatives includes: (1) IR spectra conducted by Shimadzu FT-IR, and (2) GC-MS conducted by GC-MS Shimadzu QP2010S device.

2.2.5. Antibacterial activity [18].  
2.2.5.1 Preparation NA (Nutrient Agar) Media. 5 g of NA was dissolved in 250 mL of distilled water and heated until clear. Furthermore, this solution is heated to a temperature of 115°C for 15 minutes, and then stored at room temperature for 1 x 24 h.

2.2.5.2 Preparation NB (Nutrient Broth) Media. Bacto peptone (0.1 g) and beef extract (0.06 g) were dissolved in 20 mL distilled water and separated into 3 mL test tubes. The test tubes were heated at 115°C for 15 minutes and stored at room temperature for 1 x 24 h.

2.2.5.3 Inoculation. The S. aureus and E. coli bacteria were transferred to NB media, dissolved, and then incubated for 1 x 24 h at 37°C. The media was standardized with 0.5 McFarland and applied to NA.

2.2.5.4 Planting samples and testing activities. Sample (20 µL) were input into holes in NA with a diameter of 0.6 mm and stored at 37°C for 1 x 24 h. Finally, samples observed and the inhibition zone diameter is calculated.

3. Result and Discussion

The data of physico-chemicals properties from the results of VCO experiments and their derivatives are presented in the Figure 1 and Table 1. Based on visual observations, it appears that there is a change from VCO which was initially colorless and liquid to be solid and white. This shows that the lathering reaction was successful and produced a new product, fatty acid potassium salts (K-soap) from VCO. K-soap from VCO is synthesized to produce a liquid and colorless product which indicates that a new product has been formed. From the results of the acidification process, it can be seen that the synthesis of soap into fatty acids has been successful. Visual changes from the trans-esterification process do not show changes in state and color. Therefore, it is necessary to have other parameters such as boiling point, density, viscosity, and refractive index.

The trans-esterification product has been successfully characterized by a boiling point value, refractive index, viscosity, and density different from the initial product. The difference in the physical properties of a compound is influenced by intermolecular forces and molecular weights [19]. VCO has a higher molecular weight (calculated as C_{42}H_{64}O_{23}) than its methyl ester (calculated as C_{13}H_{26}O_{2}) so breaking bonds requires more energy. In the solubility test, the order of the nature of the polarity from low to high, namely VCO, VCO methyl ester, VCO fatty acid, and VCO K-soap.
Figure 1. Physical performance of VCO and its derivatives

Table 1. Characterization of physicochemical properties of VCO and its derivatives

| Properties                  | VCO   | K-soap | Fatty acid | Methyl ester |
|------------------------------|-------|--------|------------|--------------|
| State                        | Liquid| Solid  | Liquid     | Liquid       |
| Color                        | Colorless| White  | Colorless  | Colorless    |
| Boiling point (°C)           | 274–336| 266–296| 260–262    |              |
| Melting point (°C)           |        | 46–124 |            |              |
| Density (g.mL⁻¹)             | 0.93  | 0.93   | 0.89       |              |
| Viscosity (cP)               | 45.99 | 27.18  | 3.36       |              |
| Refractive index             | 1.45  | 1.44   | 1.43       |              |
| Saponification value, SV (mg KOH/g sample) | 320.92| 296.59 | 268.6      |              |
| Acid value, AV (mg KOH/g sample) | 0.93  | 184.54 | 0.33       |              |
| Ester value (SV – AV)        | 319.99| 112.05 | 268.27     |              |
| Solubility in:               |       |        |            |              |
| n-hexane                     | soluble| insoluble| soluble   | soluble      |
| chloroform                   | soluble| insoluble| soluble   | soluble      |
| ethanol                      | soluble| emulsified| soluble | soluble     |
| methanol                     | insoluble| emulsified| soluble | soluble     |
| water                        | insoluble| emulsified| insoluble| insoluble    |

The IR spectrophotometric analysis of VCO and its derivatives, the results of the IR spectra are listed in Figure 2, 3, 4, and Figure 5. The characteristic bands in this IR spectra that distinguish functional groups from each substance are listed in Table 2. Based on the results of the interpretation of the IR spectra (Table 3) there are several typical absorption bands. VCO has an absorption area at wavenumbers 2852.72, 2922.16 and 2954.95 cm⁻¹ with strong intensity which indicates the vibration strain of the C-H alkane bond and wavenumber 1745.58 cm⁻¹ with strong and sharp intensity which indicates the presence of strain vibration C=O as ester. The synthesized potassium soap has a wavenumber of 1573.91 cm⁻¹ with a strong and sharp intensity which indicates the vibrations of stretching asymmetric C=O bonds as carboxylic salts. The synthesized fatty acid has a wavenumber of 1697.36 cm⁻¹ with strong and sharp intensity which shows the vibration stretching of the C=O bond as carboxylic acid. The synthesized methyl ester has a wavenumber of 1743.65 cm⁻¹ with a strong and sharp intensity which indicates the vibration stretching of the C=O bond as an ester. VCO derivative was successfully synthesized based on the results of IR spectra interpretation.
Figure 2. Infra-Red spectrum of VCO

Figure 3. Infra-Red spectrum of fatty acids potassium salt (K-soaps) from VCO
Figure 4. Infra-Red spectrum of free fatty acids (FFAs) from VCO

Figure 5. Infra red spectrum of fatty acids methyl esters (FAMEs) from VCO
Table 2. Typical bands functional groups on IR spectra of VCO and its derivatives

| Typical absorption band | VCO | K-soaps | FFAs | FAMEs | Remarks |
|-------------------------|-----|---------|------|-------|---------|
| C=O stretching          | 1732.08 (sharp, strong) | 1573.91 (sharpless, strong) | 1697.36 (sharp, strong) | 1743.65 (sharp, strong) | Electron polarizability cause weakness of the C = O bond, shifts to the lower wave number |
| O-H stretching          | not appear | not appear | 3100 – 2600 (very broad, medium) | not appear | The very broad band characterizes O-H stretches with H bonds in a carboxylic acid |
| O-K stretching          | 3207.62 (sharp, strong) | not appear | Not appear | not appear | |

GC-MS analysis of the FAMEs of VCO trans-esterification results with MeOH/KOH produced 9 components, (Figure 6). The peak with the largest area (peak 4, t. 25,913 minutes), from the mass spectrum analysis indicates it is a lauric acid methyl ester. Therefore, it is proven that lauric acid is the most component in VCO. The overall composition of the fatty acids making up the VCO identified as the fatty acid methyl esters are listed in Table 3. GC data shows that VCO consists of 9 fatty acids which are shown by the appearance of 9 peaks on the chromatogram. In the MS results, it can be seen that the synthesized VCO have the main content, namely lauric acid.

Based on the results of the experiment, the synthesized fatty acids have a higher acid value than VCO which shows that the fatty acids have been successfully synthesized from VCO. However, the synthesized methyl esters have lower acid value than VCO caused by free fatty acids in VCO which have formed fatty acid methyl esters through esterification with a potassium hydroxide catalyst. VCO has the highest saponification value showing that the triglyceride content in VCO is the highest compared to the synthesized fatty acids and methyl esters. For ester value, VCO shows the highest number because VCO contains a fairly high ester.

Figure 6. GC-MS Chromatogram of fatty acids methyl ester from VCO
Table 3. Fatty acids content in VCO

| Peak | Retention time (second) | Area% as relative content (%) | Fatty acid (that identified as FAMEs) | Fatty acids symbol |
|------|-------------------------|------------------------------|--------------------------------------|-------------------|
| 1    | 7.052                   | 0.35                         | hexanoic acid (caproic acid)          | C6:0              |
| 2    | 13.891                  | 7.08                         | octanoic acid (caprylic acid)         | C8:0              |
| 3    | 20.224                  | 5.53                         | decanoic acid (capric acid)           | C10:0             |
| 4    | 25.913                  | 65.84                        | dodecanoic acid (lauric acid)         | C12:0             |
| 5    | 30.688                  | 13.16                        | tetradecanoic acid (myristic acid)    | C14:0             |
| 6    | 35.035                  | 4.16                         | octadecanoic acid (stearic acid)      | C18:0             |
| 7    | 38.425                  | 0.37                         | octadeca-9,12-dienoic acid (linoleic acid) | C18:2           |
| 8    | 38.565                  | 2.55                         | hexadeca-9-enoic acid (palmitoleic acid) | C16:1           |
| 9    | 38.988                  | 0.96                         | tetracosanoic acid (lignoceric acid)  | C24:0             |

Analysis of mass spectrometry by EI-MS (electron impact mass spectrometry) for each peak of the nine peaks of GC results obtained by mass spectra as shown in Figure 7 to Figure 15. The appropriate content of fatty acids from their fatty acid methyl esters as listed in Table 3, obtained from interpretation of each mass spectrum from nine peaks, and subsequently refered to WILEY229.LIB.

Figure 7. Mass spectra (EI-MS) of peak 1 (methyl hexanoate)

Figure 8. Mass spectra (EI-MS) of peak 2 (methyl octanoate)
Figure 9. Mass spectra (EI-MS) of peak 3 (methyl decanoate)

Figure 10. Mass spectra (EI-MS) of peak 4 (methyl dodecanoate)

Figure 11. Mass spectra (EI-MS) of peak 5 (methyl tetradecanoate)
Figure 12. Mass spectra (EI-MS) of peak 6 (methyl octadecanoate)

Figure 13. Mass spectra (EI-MS) of peak 7 (methyl 9,12-octadecadienoate)

Figure 14. Mass spectra (EI-MS) of peak 8 (methyl 11-octadecenoate)
Table 4. Test the VCO derivative antibacterial activity

| Bacteria     | Negative control (ethanol 60%) | K-soap 1% | K-soap 2% | Free fatty acids 1% | Free fatty acids 2% | Fatty acid methyl ester 1% | Fatty acid methyl ester 2% |
|--------------|---------------------------------|-----------|-----------|--------------------|--------------------|--------------------------|--------------------------|
| E. coli      | -                               | 8.85      | 10.9      | 11                 | 12.25              | -                        | 7.75                     |
| S. aureus    | -                               | 10        | 11.5      | 10.4               | 14.2               | -                        | 8.9                      |

Fatty acid potassium salt (K-soap), free fatty acids, and fatty acid methyl esters synthesized were tested for their antibacterial activity against *E. coli* bacteria as Gram-negative bacteria and *S. aureus* as Gram-positive bacteria with ethanol 60% as the solvent. The testing method used is the well diffusion method, shown by the formation of a clear zone around the wellbore. If the clear zone that forms around the wellbore increases showed the presence of antibacterial activity by the sample whose results are shown in Table 4. Based on the calculation of the clear zone diameter, it can be seen that fatty acids have the highest antibacterial activity compared to soap and methyl esters, the antibacterial activity of VCO derivatives more effective against Gram-positive bacteria (*S. aureus*). Medium-chain saturated fatty acids such as lauric acid have antibacterial activity[2] and are more active against Gram-positive bacteria than Gram-negative [10]. The fatty acids synthesized from VCO are more specific for Gram-positive bacteria as shown by the formation of a larger clear zone diameter than Gram-negative bacteria.

4. Conclusion

New products have been formed which are shown by the different values of the physicochemical properties and are supported by the IR spectra data of each product. The content of VCO fatty acids have been shown from the results of GC-MS. Of the three synthesized products (K-soap, free fatty acids, and fatty acid methyl esters from VCO) have antibacterial activity against Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*). This work proposes an increase in the study of antibacterial activity against VCO derivatives by testing in other bacteria such as *S. epidermidis*. VCO derivative products can potentially be a good fine chemical because they are produced from easily found substances that are not toxic.

References
[1] Danha G, Muzenda E and Maotsela T 2019 *Procedia Manuf.* 35 541
[2] Nguyen V T A, Le T D, Phan H N and Tran L B 2017 Journal of Lipids. 2017. 7170162
[3] Rodriguez G and Beckman E J 2020 Fluid Phase Equilib. 503 112303
[4] Varma S R, Sivaprasakam T O, Arumugam I, Dilip N, Raghuraman M, Pavan K B, Rafiq M and Paramesh R 2019 J. Tradit. Complement. Med. 9 5
[5] Hovorková P, Laloučková K and Skřivanová E 2018 Czech J. Anim. Sci. 3 119
[6] Nguyen T A V., Le T D, Phan H N and Tran L B 2018 Scientifica. 2018 9120942
[7] Cahya D, Tri C, Isrina S and Salasia O 2019 Heliyon 5 e02612
[8] Kabara J J, Swieczkowski D M, Conley A J and Truant J P 1972 Antimicrob. Agents Chemother. 2 23
[9] Odel F, Siswanta D and Nurwening E 2016 Procedia Chem. 18 132
[10] Desbois A P and Smith V J 2010 Appl Microbiol Biotechnol. 85 1629
[11] Sutrisno, Retnosari R and Marfu’ah S 2019 IOP Conf. Ser.: Earth Environ. Sci. 299 012004
[12] Sutrisno, Retnosari R, Marfu’ah S and Fajaroh F 2019 Key Engineering Materials vol 811 40
[13] Masuda M, Era M and Kawahara T 2015 Biocontrol Science. 20 209
[14] Ra M E, Akai S S, Anaka A T and Awahara T K 2015 Japan J. Food Eng. 16 99
[15] Yamamoto Y, Morikawa T, Kawai T and Nonomura Y 2017 ACS Omega 2 113
[16] Kawahara T, Takita M, Masunaga A and Morita H 2019 Int. J. Mol. Sci. 20 312
[17] Badan Standarisasi Nasional 1988 Standar Nasional Indonesia (SNI 01-3555-1998)
[18] Jorgensen J H and Ferraro M J 2009 Medical Microbiology. 49 1749
[19] Yang Y, Zhao Y, Xu M, Wu N, Yao Y, Du H, Liu H, Tu Y, Zhao Y, Xu M, Wu N, Yao Y, Du H, Liu H and Tu Y 2018 Food Hydrocolloids. 10 016