The comparison characteristics of fermented Virgin Coconut Oil (VCO) on kefir addition

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Abstract. Indonesia is the largest coconut producing country in the world. They are many products from coconut that have high economic value, one of them is VCO. No research has been found in making VCO by kefir fermentation, so the purpose of this research is to describe the comparison of characteristics VCO produced on kefir addition based on organoleptic, water content, and free fatty acid tests. This research was carried out using two replications of the three old coconuts obtained 1.26 L thick coconut milk, then divided into three samples, fermented 48 hours with different treatments (0% kefir for control; 3.5%; 6% kefir). The results of this study obtained the best VCO characteristics in the composition of 3.5% kefir addition, colorless, normal taste, and aroma of coconut aroma, with water content of 0.0028% and a free fatty acid content of 0.23%. The VCO produced are in accordance with SNI VCO 7381: 2008. Further research needs to be conducted to produce an optimal VCO randement with a range of 1-3.5% kefir addition.

1. Introduction
Nowadays, there are many culinary and snacks with a frying process, and not a few sellers use cooking oil to blacken. However, there are still many people who are not aware of the dangers of consuming such foods often, such as causing cholesterol, heart disease, and stroke due to high levels of Low-Density Lipoprotein (LDL) in the body [1]. The solution, reduce and avoid fried foods or switch to more healthy VCO, VCO is coconut oil obtained from coconut meat (Cocos nucifera L.) processed from coconut milk squeezing with or without the addition of water, with or without heating and not through purification with chemicals that are safe for consumption [2]. The VCO contains polyphenol compounds that can reduce LDL levels in the body. Not only for cooking, but it also turns out that VCO is rich in benefits and has been applied in the world of pharmacy [3], cosmetics, and food, which is why the demand for VCO is increasing [4].

In addition, Indonesia is the largest coconut producing country in the world. Unfortunately, it is still exported as ordinary coconut oil. Though the price of VCO can reach three times that of ordinary coconut oil, it has the potential to develop the Indonesian economy. Although it is quite expensive, we can make it easily, cheaply and can be made at home, one of them is by fermentation method [5]. No research has been found in making VCO with addition kefir. Whereas lactic acid bacteria in kefir are able to produce protease enzymes [6], these protease enzymes can break down coconut milk emulsions...
by hydrolyzing peptide bonds in proteins into simpler compounds, so that oil can separate from water and protein [7].

VCO was superior with fatty acid content compared to ordinary coconut oil, water content, the color that was according to standard, and longer shelf life [8]. Then Fauziyah has applied a worksheet to the analysis of free fatty acid levels in VCO products that are circulating in the market, but there are shortcomings, VCO samples should be made by themselves so that it can improve students’ knowledge, creativity and ability to create [9]. Based on the previous description, it is necessary to conduct research to determine the comparison of the characteristics of fermented VCO with the addition of kefir based on organoleptic, water content, and free fatty acid levels.

2. Materials and methods
This type of research is experimental research. This research was conducted in February 2020 at the Integrated Laboratory of Chemistry Education at UIN Sunan Gunung Djati, Bandung.

2.1. The instrument and materials
The instrument needed in making VCO are containers, clear plastic, measuring cups, funnels, mixers, pans, and stoves. While the tools needed for the test in this study are burette, clamp, static boss head, Erlenmeyer, measuring flask, measuring cup, chemical beaker, analytical balance, porcelain cup, dropper, spatula and stirring rod. The ingredients used in making VCO are, three old coconuts (purchased at a local market in Cicalengka, Bandung), prime kefir and boiled water, while the material used in the test of free fatty acid levels was ethanol 96% (ROFA Merck), solids of oxalic acid 0.1 N, 0.1 N NaOH solution (ROFA Merck), PP indicators and aquadest. In this research, the treatment is the method and kefir starter concentration.

2.2. Manufacture and test of VCO characteristics
The three shredded old coconuts, added with water as much as 2.25 L, squeezed with coconut milk cloth left 2 hours to obtain 1.26 L thick coconut milk, then mixed in for 30 minutes. Then it was divided into three samples, fermented 48 hours at room temperature with different treatment (0% kefir for control; 3.5% kefir; for the addition of 6% kefir was heated with low heat until oil formed). After the oil is formed, filtering was conducted three times with filter paper until the oil colorless and then transferred in a bottle. Then performed organoleptic tests, including color, taste, and odor. The water content was measured by the oven method. Roast porcelain cups at 105°C for 1 hour, chill in 30 minutes desiccator, weigh the cup mass, then weigh 3 grams of sample in a cup than in oven 105°C for 1 hour, chill in a desiccator, then weighed and duplicated. The free fatty acid (FFA) levels test carried out by the acid-base titration method. First, a 0.1 N oxalic acid solution and 0.1 N NaOH solution was prepared, then standardized the NaOH solution. Prepared 15 g of sample in Erlenmeyer, then added 25 mL of 96% ethanol and three drops of PP indicator, then titrated with NaOH solution until it is pink and duplicated. The data obtained is then analyzed using the formula [2]:

\[
\text{water content formula} = \frac{\text{sample mass} - \text{mass of sample after drying}}{\text{sample mass}} \times 100\% \quad (1)
\]

\[
\text{FFA formula} = \frac{\text{volume NaOH} \times \text{Normalitas NaOH} \times \text{Mr of lauric acid}}{\text{sample mass} \times 1000} \times 100\% \quad (2)
\]

3. Results and discussion

3.1. Synthesis of VCO
In the process of synthesis VCO, the grating process is done first to facilitate the fat extraction process because the smaller the particle size, the more surface area to contact with water. The water used is boiled water to make it more sterile. Adding water to the grated coconut serves to lure coconut milk in the coconut so it can easily come out [10]. The process of settling for 2 hours is done to separate thick coconut milk with water, and this separation aims to reduce the water content of coconut milk that will
be taken the oil, thereby reducing the possibility of oxidation reactions that cause the oil to be rancid easily [11]. Furthermore, the stirring process serves to break the protein bonds in the coconut milk so that the oil can separate. The addition of kefir is done because, in kefir, there are lactic acid bacteria that contain the enzyme protease, which can increase the hydrolysis reaction so that the oil can be separated from water and protein.

Figure 1. Fermentation after 24 hours (a) control 0% kefir, (b) 3.5% kefir, (c) 6% kefir.

In the control sample or the addition of 0% kefir, oil has formed quite a lot in 24 hours, in contrast to the addition of 3.5% kefir, a little oil is formed. While at the addition of 6% kefir, oil formed very little after 48 hours (optimal fermentation time) [12]. Based on the many volumes of oil produced, that the most oil amendment is at the addition of 0% kefir. The higher the concentration of the starter added, the less volume of VCO produced. This explained that starters who have proteolytic enzymes could hydrolyze peptide bonds in the substrate (thick coconut milk), the protein contained in old coconut is not proportional to the amount of LAB contained in the starter, so as to reduce the volume of VCO and make the VCO color a little murky [11]. So that the sample with a 6% kefir starter, in order to speed up the reaction and increase the volume of oil to be tested, then the heating process is carried out.

Meanwhile, the VCO formed by 3.5% kefir fermentation occurs because the protein contained in old coconut can be said to be comparable or perhaps more than the amount of BAL in the starter. Besides fermentation, it causes the release of alcohol, organic acids, and CO₂, through the breakdown of glucose in coconut milk and destabilization of coconut milk emulsion [13]. The decomposition of the protein is then continued by proteolytic enzymes that can break the peptide bonds in the protein so that it is degraded into amino acids, and then the oil will come out and clump together.

3.2. Organoleptic test
The color organoleptic results in this research have fulfilled the SNI requirements that have been set, namely colorless (clear) to pale yellow [14]. The strongest aroma of coconut aroma is produced by VCO without the addition of kefir and heating because the ingredients are 100% pure coconut. At the addition of 3.5% kefir, the aroma of fermented acid was smelled, but only briefly, the distinctive aroma of coconut in this sample was still smelled. In contrast to the VCO that is given a heating treatment, the heat energy given will dissolve and empty the pigments of coconut fruit so that the VCO oil produced becomes yellowish, the resulting coconut aroma is not too strong, and the resulting VCO taste feels normal to coconut oil, but a little rough.

Figure 2. A:0%, B:+3.5%, C:+6%.

The organoleptic characteristics of VCO that have been made, are presented in the following table 1.
Table 1. Organoleptic VCO results from 3 treatments.

| Treatment Fermentation | Color         | Smell            | Taste          |
|------------------------|---------------|------------------|----------------|
| 0% kefir               | No color, Clear | Typical strong coconut | Coconut oil |
| 3.5% kefir             | No color, Clear | Typical coconut | Coconut oil |
| 6% + Warming up        | A little yellow | Weak coconut aroma | Coconut oil, a little rough |

3.3. Water content test

In this water content test has the principle that the loss of weight at 105 °C heating is considered as the water content contained in the VCO sample. Based on statistical data processing for the results of the analysis of the water content of the three samples in accordance with SNI which has a maximum value of 0.2%, it appears that the treatment with the heating method produces water content with the smallest percentage of 0.0013%, this is clear because the heating method weighing VCO test water content evaporates water quickly, so that the water content in VCO is reduced and lower than the treatment without heating. Low water content is very desirable to increase the life of VCO so that the shelf life of VCO is more durable, because with a low water content can prevent oxidation and rancidity. However, high heating process can also change the structure of oil and oil color the less good [15].

![Figure 3. The process of the oven.](image)

The water content characteristics of VCO that have been made, are presented in the following table 2.

Table 2. VCO water content results from 3 treatments.

| Treatment Fermentation | Deuteronomy | Water content (%) | Average (%) |
|------------------------|-------------|-------------------|-------------|
| 0% kefir               | 1           | 0.0035            | 0.0031      |
|                        | 2           | 0.0027            |             |
| 3.5% kefir             | 1           | 0.0034            | 0.0028      |
|                        | 2           | 0.0022            |             |
| 6% kefir + Warming up  | 1           | 0.0016            | 0.0013      |
|                        | 2           | 0.0010            |             |

3.4. Free fatty acid test

To test the levels of fatty acids in VCO (calculated as lauric acid) using the alkalimetry method where the principle of the method used is the neutralization reaction due to a reaction between hydrogen ions originating from acids originating from oil with hydroxide ions originating from bases used in the titer. An acid-base titration is carried out with the principle of dissolving fats/oils in certain organic solvents (96% neutral alcohol) followed by basic spinning to form a pink color (NaOH) [15].
Based on the results of statistical data processing for free fatty acid tests showed that the fermentation method with the addition of 3.5% kefir produced the lowest free fatty acids compared to the control sample and with the heating process. This is the same as previous studies that produce superior VCO with low free fatty acid levels by fermentation method but using the papain enzyme, which also contains a protease enzyme [16].

The characteristics of FFA from VCO that have been made, are presented in the following table 3.

| Treatment Fermentation | Deuteronomy | V. titration (mL) | FFA content (%) | Average (%) |
|------------------------|-------------|------------------|----------------|------------|
| 0% kefir               | 1           | 1,8              | 0,264          | 0,25       |
|                        | 2           | 1,6              | 0,234          |            |
| 3,5% kefir             | 1           | 1,7              | 0,249          | 0,23       |
|                        | 2           | 1,5              | 0,220          |            |
| 6% kefir + Warming up  | 1           | 3,2              | 0,469          | 0,47       |
|                        | 2           | 3,2              | 0,469          |            |

Then, in contrast to the VCO, which is made by the heating process produces the highest levels of fatty acids, this is because the heating process causes the triglyceride hydrolysis reaction to increasing resulting in high free fatty acids as indicated by the acid number [17]. The more frequent the process of heating, the higher levels of free fatty acids in the oil, and will increase levels of Low-Density Lipoprotein (LDL) in the blood, which is bad cholesterol. VCO with a fatty acid content of 0.23% can be said in accordance with SNI VCO 7381: 2008, which is a maximum of 0.2% because the difference of 0.03% is considered not to have a significant effect, not exceeding 0.05%.

4. Conclusion
The results showed that the fermentation method, with the addition of 3.5% kefir, could reduce levels of VCO free fatty acids compared to the control. In the heating, the method produces the lowest VCO moisture content, while the best VCO characteristics in the composition of the addition of 3.5% kefir, colorless, normal taste and aroma of coconut aroma, with a moisture content of 0.0028% and a free fatty acid content of 0.23%. So the VCO product with the addition of 3.5% kefir can be said in accordance with SNI VCO 7381: 2008. Further research a test is needed to produce an optimal VCO with a concentration range of kefir addition of 1 - 3.5% with a maximum free fatty acid content of 0.20%

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References
[1] Satria, Purnomo T and Martini 2014 Pengembangan Lembar Kerja Siswa (LKS) Berorientasi Inkuiri untuk Meningkatkan Keterampilan Berpikir Kritis Siswa SMP Kelas IX Pada Tema
Virgin Coconut Oil (VCO) J. Biol. FMIPA UNESA 2 89–94

[2] Anon 2008 *Standar Nasional Indonesia (SNI). Minyak Kelapa Virgin (VCO) SNI 7381:2008. Badan Standardisasi Nasional*

[3] Intahphuak S, Khonsung P and Panthong A 2010 Anti-inflammatory, Analgesic, and Antipyretic Activities of Virgin Coconut Oil *Pharm. Biol.* 48 151–7

[4] Manisha D. and Shyamapada M 2011 Coconut (Cocos nucifera L. Arecales): In Health Promotion and Disease Prevention *Asian Pac. J. Trop. Med.* 4 241–7

[5] Fabian M D, Olivia E M B, Edward T C, Ian M S and Ian K D D 2007 Essential Quality Parameters of Commercial Virgin Coconut Oil *CORD* 23 71–80

[6] Ismawati D 2018 *Isolasi dan Karakterisasi Bakteri Asam Laktat (BAL) Proteolitik dari Kefir* (Universitas Lampung)

[7] Silaban R, Hutapea V, Manukkang R and Alexander I J 2016 Pembuatan Virgin Coconut Oil Melalui Kombinasi Teknik Fermentasi dan Enzimatis Menggunakan Getah Pepaya *J. Kim. FMIPA Univ. Negeri Medan* 55–64

[8] Mujdalipah S 2016 Pengaruh Ragi Tradisional Indonesia dalam Proses Fermentasi Santan terhadap Karakteristik Rendemen, Kadar Air, dan Kadar Asam Lemak Bebas Virgin Coconut Oil (VCO) *J. Fortech* 1 11–5

[9] Fauziyah D H 2019 *Penerapan Lembar Kerja Berbasis Masalah untuk Mengembangkan Kemampuan Berpikir Tingkat Tinggi pada Analisis Kadar Asam Lemak Bebas dalam VCO* (UIN Sunan Gunung Djati Bandung)

[10] Khoramnia A, Ebrahimpour A, Ghanbari R, Ajdari Z and Lai O 2013 Improvement of Medium Chain Fatty Acid Content and Antimicrobial Activity of Coconut Oil via Solid-State Fermentation Using a Malaysian Geotrichum candidum *J. Biomed Res. International* 2013 1–9

[11] Novitriani K, Nurpalah R and Kusmiati M 2016 Pengaruh Variasi Penambahan Konsentrasi Starter pada Karakterisasi Kimia Virgin Coconut Oil yang Berbahan Dasar Kelapa *J. Kesehat. Bakti Tunas Husada* 15 36–41

[12] Satheesh N and Prasad N B . 2014 Production of Virgin Coconut Oil by Induced Fermentation with Lactobacillus plantarum NDRI strain 184. *Croat. J. Food Technol. Biotechnol. Nutr.* 9 37–42

[13] Raghavendra S N and Raghavarao K S M S 2010 Effect of Different Treatments for the Destabilization of Coconut Milk Emulsion *J. Food Eng* 97 341–7

[14] Villarino B J, Dy L. M and Lizada C 2007 Descriptive Sensory Evaluation of Virgin Coconut Oil and Refined, Bleached and Deodorized Coconut Oil. *LWT-Food Sci. Technol.* 40 193–9

[15] Osawa C C, Goncalves and Ragazzi S 2007 Correlation between Free Fatty Acids of Vegetable Oils Evaluated by Rapid Tests and by Official Method. *J. Food Compos. Anal.* 20 523–8

[16] Mansor T S T, Che Man Y B, Shuhaimi M, Abdul Afik M J and Ku Nurul F K 2012 Physicochemical Properties of Virgin Coconut Oil Extracted from Different Processing Methods *J. Int. Food Res.* 19 3 837–845

[17] Kulkarni M G and Dalai A K 2006 Waste Cooking Oil-An Economical Source for Biodiesel: A Review *Ind. Eng. Chem. Res.*