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# Physical and Chemical Characteristic of Virgin Coconut Oil under Mix Culture Fermentation Technique

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**Abstract.** This research focus on applying of mixed culture fermentation methods to produce high quality of VCO. The culture was mixed by combining several microorganisms that play a role in this VCO fermentation are yeast *Saccharomyces cerevisiae*, *Lactobacillus plantarum* bacteria, and *Rhizopus* sp. Research activities were conducted in two stages, the first stage is the production of VCO by fermentation using mix culture. The products were analyzed will consist of a large calculation of oil yield, physical properties analysis (moisture content and refractive index), chemical sift analysis (acid number, iodine number and peroxide number) and organoleptic test. Results showed that of all combination SL, SR, LR, and SLR have appropriate value compared with SNI and APCC standards.

**Keywords:** characteristic, fermentation, mixed culture, quality, Virgin Coconut Oil

## 1. Introduction

Oils and fats are important components in human food sources. The quality, stability and nutritional content of oils and fats are an important factor in food processing technology, Baltork, Torbati and Damirchi (2016). Virgin Coconut Oil (VCO) is one of the most important food oils in the world as a source of dietary fat. VCO has capability to promote health effect due to its antioxidant properties and medium-chain fatty acid (MFA) content, Hee, Tan, Rahman, Smith, and Chong (2017). Marina, Che Man and Amin (2009) proved that VCO has good chemical properties due to its high phenolic content. In the food industry, the presence of Virgin Coconut Oil is more widely used as cooking oil, substitutes for buttermilk and cheese and ice cream, Hamsi (2015) and Bawalan (2006).

Recently, there are some improvements in VCO production technology VCO. Extraction process is one of important step to produce high quality VCO product. There are several methods for extraction, such as with fresh-dry processes (using high pressure and low) and fresh-wet processes (fermentation and centrifugation), Gerard and Dumancas (2016). Extraction techniques with wet processes are capable of maintaining active components such as tocotrienols, polyphenols and tocopherols capable of processing antioxidant content, Hamsi (2015).



VCO extraction technique using fermentation method has been widely applied by using *Saccharomyces cerevisiae*. Based on the physicochemical analysis, this method proved able to provide the highest value of iodine number, the lowest free fatty acid and very low water content, Mansor (2012). High Iodine values indicate fewer saturated fatty acid, and this is very good for health, Marina, Che Man, Nazimah and Amin (2009). Besides that, lower water content will prolong the shelf life of the product, Gerard and Dumancas (2016). In addition, the free fatty acid content and the lowest moisture content will probably avoid oil from rancidity.

VCO production process by fermentation method can also be done with the help of *Lactobacillus plantarum* NDRI strain 184, Satheesh and Prasad (2014). The use of *L. plantarum* in the fermentation process is due to its ability to have immune system and probiotic benefits. Yeast (inoculum) tempe or a collection of mold or mold spores that can form fine threads. Tempe contains at least three species of mold ie *Rhizopus oligosporus*, *Rhizopus oryzae*, *Rhizopus stolonifer*. These microbes have the ability to produce protease enzymes and lipases that can hydrolyze the oil with the support of high water content. During fermentation *Rhizopus oligosporus* synthesizes more protease enzymes, while *Rhizopus oryzae* synthesizes more amylase enzymes, Anshori (1992).

Until now the fermentation method uses only one type of yeast or bacteria, whereas the role of each type of microorganisms can provide different characteristics on the quality of VCO product. Due to there is lack information of performance of mix-culture fermentation, this research become potential technique for applying of mixed culture methods to produce high quality of VCO. In particular, this study was conducted to determine the effect of mixed culture on the value of physical and chemical characteristic of VCO products.

## 2. Methodology

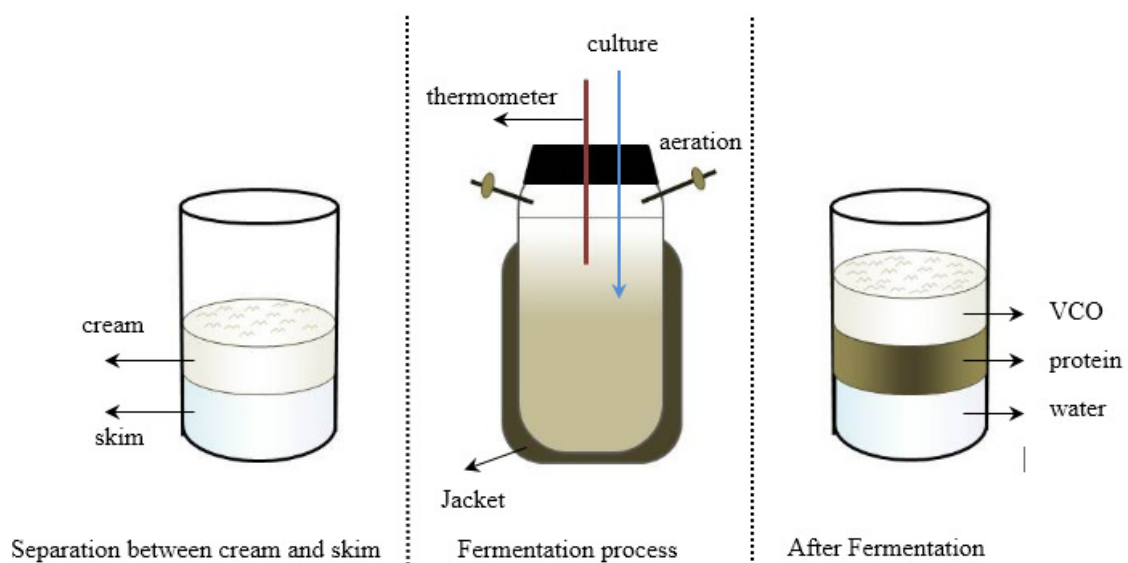
### 2.1. VCO Production

Coconut meat that has been separated from the shell was shredded. The result of grated coconut then added hot water (70°C) with a ratio of 2:1. Furthermore, the process of extortion and screening. The coconut milk then put in a large jar and let stand for 2-3 hours, until it separates into two layers (cream and skim). The top layer of cream then separated and added 15% pure or mixed cultures. In each ml of culture there are  $1 \times 10^6$  cells.ml<sup>-1</sup>. In a mixed culture the percentage is divided equally to meet the amount of inoculum of 15%. Next cream was put in a closed bottle for curing for 20, 24, and 28 hours. The results of curing will produce 3 layers of VCO, protein, and water. Finally, oil was separated by centrifuge and analyzed physical and chemical characteristics. The procedure presented in Figure 1.

The research is planned to be done by varying the type of inoculum in the fermentation process of single culture and mix culture. The number of trials is presented in Table 1. Each experiment performed 2x replications to minimize errors. The combination of cultures consist of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* (SL), *Saccharomyces cerevisiae* and *Rhizopus Oligosporus* (SR), *Lactobacillus plantarum* and *Rhizopus Oligosporus* (LR) and *Saccharomyces cerevisiae*, *Lactobacillus plantarum* and *Rhizopus Oligosporus* (SLR).

**Table 1.** Mix Cultures Formulation For Producing VCO

Culture	Formulation (%)			
	SL	SR	LR	SLR
<i>Saccaromyces cerevisiae</i>	2.5	2.5	-	1.67
<i>Lactobacillus plantarum</i>	2.5	-	2.5	1.67
<i>Rhizopus Oligosporus</i>	-	2.5	2.5	1.67

**Figure 1.** Schem of fermentation unit

## 2.2. Physical and Chemical Analysis

### 2.2.1. Water Content Test refer to Oseni, Fernando, Coorey, Gold and Jayasena (2017)

Determination of moisture content is done by weighing 20 g sample and heated at oven with temperature  $110 \pm 5$  °C until no bubbles or water is evaporated. Next enter the sample into the desiccator and then weigh it and calculated by using the formula:

$$\text{Moisture (\%)} = ((\text{start-weight-weight end}) / (\text{initial weight})) \times 100\% \quad (1)$$

### 2.2.2. Refractive index refer to Ketaren (1986)

Refractive index testing was performed by using abbe refractometer which has been equipped with temperature regulator which set at temperature 25°C. The refractive index value at a given temperature is obtained by calculation:

$$N = C / V_p \quad (2)$$

where:

$n$  = Index of Bias

$C$  = Light Speed in a vacuum (value of 0.78)

$V_p$  = Fast light ripple on medium

### 2.2.3. Acid Numbers refer to Badan Standarisasi Nasional (1998)

The sample weighed 2 grams into a 200 ml Erlenmeyer flask, then added 50 ml of Alcohol (Ethanol Absolute). The following is heated in a water bath at 90°C, stirring for 10 minutes. Then the sample is titrated with PP 1% indicator in Ethanol until a pink change occurs.

### 2.2.4. Iod Numbers refer to Badan Standarisasi Nasional (1998)

The sample was weighed as much as 0.1 g using Erlenmeyer 250 ml, then added 15 ml of chloroform and 25 ml of Wij's solution. This solution is stored in the dark room for 1 hour. The next addition was KI 20 ml and dissolved 150 ml of aquadest. Lastly add 1% starch solution 2 drops and titrated with  $\text{Na}_2\text{S}_2\text{O}_3$  0,1 N. Created blank as previous work step.

### 2.2.5. Peroxide Numbers refer to Handayani and Enjarlis (2016)

The sample was weighed 1 gram using 250 ml Erlenmeyer flask, then added 6 ml of glacial acetic acid mixture with chloroform. The samples were shaken until dissolved completely and added 0.1 ml KI saturated with left for 2 minutes in dark space while in shake. Next add 2 ml of boiling water, and finally titrated with  $\text{Na}_2\text{S}_2\text{O}_3$  0.01 N, made the same blank as before.

## 3. Result and Discussion

### 3.1. Water content

Water content is an important parameter that determines the quality of VCO. Amount of water in the oil allow hydrolysis reaction that contribute to the formation of free fatty acids. Oxidized free fatty acids will cause rancidity in VCO, Raghavendra and Raghavarao (2010). In addition, high level water content causes bacterial contamination that is able to hydrolyze fat molecules, Raharja and Dwiyuni (2008). The average water content produced SL, SR, LR, and SLR in the range of 0.02 - 0.03% (Table 2). These results showed that all of samples have appropriate value compared with the standards of SNI and APCC. Based on the analysis of variance, the water content produced by the four samples did not have a significant difference ( $p > 0.05$ ). This explains that combination of various culture does not affect the water content of VCO.

**Table 2.** Water content of vco at various mix cultures

Mix cultures	Water content (%)	SNI Standart	APCC Standart
SL	$0.02 \pm 0.000^b$	$\leq 0.2 \%$	(0.1 -0.5) %
SR	$0.025 \pm 0.007^{ab}$		
LR	$0.03 \pm 0.007^a$		
SLR	$0.035 \pm 0.000^{ab}$		

### 3.2. Viscosity

Viscosity is the nature of the fluid associated with flowing resistance where there is a rapid flow that occurs in small viscosity and slow flow occurs at large viscosity, Apriani (2013). The attractive force between molecules in a liquid produces high viscosity. The viscosity values can be influenced by temperature, size and molecular weight, and molecular shape. The results showed that the viscosity values of four samples in the range 5.75 - 6.45 cp. The highest viscosity is obtained by VCO SLR sample with a value of 6.45 cp and the lowest viscosity obtained by the VCO SR sample. The analysis of variance showed that the viscosity of four samples differed significantly ( $p < 0.05$ ). It is suspected that there is still protein dissolved in oil samples. This is in line with statement of Herlina, Astryaningsih, Windrati and Nurhayati (2018) that an increase in viscosity in oil can be caused by high soluble solid content. The viscosity value can also be affected by the presence of fatty acids. The high viscosity value when oil sample has a double bond on more fatty acids while the viscosity value will be low if the oil sample contains a lot of saturated fatty acids. This shows that the low viscosity value in the sample SL, SR, LR and SLR is due to VCO sample contains 90% saturated fatty acids with medium chains (medium chain fatty acids) and 10% unsaturated fatty acids in the form of oleic fatty acids and linoleate so that the viscosity of the VCO becomes lower Azizah, Wulandari and Suradi (2015). Thus, it is known that the saturated fatty acids contained in VCO can also affect the value of acid numbers, iodine numbers, and the peroxide number produced. Besides being used as a parameter in physicochemical testing, viscosity can also be used as an early detection of a product's forgery. In sensory parameters, viscosity can also be used as a parameter to analyze the after taste and texture (such as oily) of the VCO produced.

### 3.3. Density

Table 3 showed that all samples have the same density value, which is  $0.91 \text{ g. mL}^{-1}$ . Based on the results of the analysis of variance, the resulting density does not have a significant difference ( $p > 0.05$ ). This is suspected, combination of various microorganisms does not change the composition of the oil so that the density of the oil in the sample does not change.

**Table 3.** Density value of vco at various mix cultures

Mix cultures	Density ( $\text{g.cm}^{-3}$ )	SNI Standart	APCC Standart
SL	$0.9 \pm 0.000$	-	$(0.915-0.920) \text{ g.cm}^{-3}$
SR	$0.9 \pm 0.011$		
LR	$0.91 \pm 0.000$		
SLR	$0.91 \pm 0.004$		

### 3.4. Acid Number

Acid number is one of the parameters that determine the quality of oil product. Acid number indicate how much free fatty acid is contained in VCO due to the hydrolysis process. The higher acid value, the higher level of damage to the oil, Wildan, Hartati and Widayat (2013). Free fatty acids that are formed will become more reactive to oxidative factors such as light and temperature so that the oil becomes more easily or rapidly damaged. Table 4 showed that Acid number of VCO appropriate with APCC standards with values less than  $6 \text{ mg KOH.g}^{-1}$ . The four VCO have an acid number between 0.26-0.3  $\text{mg KOH / g}$ . The highest acid number is found in VCO SL samples with a concentration of  $0.3 \text{ mg KOH. g}^{-1}$ (oil) and the lowest acid number is found in SR and SLR oil samples. The results of the variance test showed that

mixing the culture did not significantly affect the resulting acid number ( $p > 0.05$ ). The low acid value values in the SL, SR, LR, and SLR samples showed that all four VCO samples were of good quality, because the oil became more resistant to rancidity.

**Table 4.** Acid number value of vco at various mix cultures

Mix cultures	Acid Number (mg KOH/g)	SNI Standart	APCC Standart
SL	$0.3 \pm 0.021^a$	-	Max 6 (mg KOH.g <sup>-1</sup> )
SR	$0.26 \pm 0.007^b$		
LR	$0.28 \pm 0.021^{ab}$		
SLR	$0.26 \pm 0.014^{ab}$		

### 3.5. Iod number

Iod numbers indicate the quality of oil based on unsaturated fatty acids present in the sample, Awolu, Obafaye and Ayodele (2013). The high level of unsaturation oil causes oil become more easily oxidized Herlina, Astryaningsih, Windrati and Nurhayati (2018). The highest iodine number obtained by VCO SL sample is 8.4 g iod. 100g<sup>-1</sup> while the lowest iod number is 7.73 g iod. 100g<sup>-1</sup> for SLR samples. The amount of iodine number obtained in this study is in accordance with the standards determined by either SNI or APCC (Table 5). Based on the results of the variance test, the resulting iodine number showed significant results ( $p < 0.05$ ). The obtained iodine number is quite low, this shows that the VCO sample has more saturated fatty acids and less unsaturated fatty acids so that the oil becomes not easily damaged by oxidation, Sukandar, Hermanto and Silvia (2009).

**Table 5.** Iod number value of vco at various mix cultures

Mix cultures	Iod Number (g iod/100 g)	SNI Standart	APCC Standart
SL	$8.4 \pm 0.148^a$	4.10-11.00 (g iod.100g <sup>-1</sup> )	4.10-11.00 (g iod.100g <sup>-1</sup> )
SR	$8.34 \pm 0.064^a$		
LR	$8.02 \pm 0.091^b$		
SLR	$7.73 \pm 0.028^c$		

### 3.6. Peroxide Numbers

Peroxide number is one of parameter used in determining the degree of damage to oil and is used as an initial indicator of rancidity, Susanto (2012). Peroxide numbers from all four samples showed the same result, which was 0.00 meq / kg of oil. This explains that VCO peroxide numbers appropriate with the standard criteria set by SNI (Maks. 0.2 meq.kg of oil<sup>-1</sup>) and APCC (Maks. 0.3 meq.kg of oil<sup>-1</sup>). The amount of the peroxide number can be affected by high water content. This is because, water content in oil can act as a precursor for peroxide enzymes to oxidize unsaturated fatty acids so that peroxide is formed, besides that saturated fatty acids that undergo oxidation will form methyl ketones which cause rancidity in oil, Raharja and DwiYuni (2005). This is in line with Isworo (2013) study, which states that the high peroxide number can be caused by the high water content contained in the sample. The low peroxide value obtained because oil samples were not stored so that the oil sample had not had a reaction with oxygen. In addition, the oil storage process is carried out by filling the VCO sample until it was full so that the oxygen contained in the bottle can be minimized and the oxidation reaction process can be inhibited.

#### 4. Conclusion

Production of VCO using mix cultures resulted physical and chemical properties of SL, SR, LR, and SLR have appropriate criteria with SNI and APCC standards. Further research is needed on methods to maximize protein clumping and separation methods in the separation of oil contained in water and protein. Besides that, it is necessary to analyze the fatty acid composition using HPLC.

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