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Abstract— Using various culture to produce Virgin Coconut Oil (VCO) by applied fermentation methods are important aspect impacted on characteristic of VCO. The objective of this research is to investigate the physico-chemical properties of VCO produced by various culture. The cultures were used in fermentation methods are *Saccharomyces cerevisiae, Lactobacillus plantarum,* and *Rhizopus oligosporus*. Research activities were conducted in two stages; the first stage was the production of VCO by fermentation at 35ºC for 24 hours. Then, physico-chemical properties of products were analyzed using standart methods. Results showed that moisture content, acid number and iodine number of VCO produced using *Saccharomyces cerevisiae* lower than others. Density, viscosity and peroxide number has almost the same value. Nevertheless, all of physico-chemical properties conform to the Asian and Pacific Coconut Community (APCC) and Standar Nasional Indonesia for VCO product.

Keywords— **culture, fermentation, physico-chemical properties, quality, Virgin Coconut Oil.**

INTRODUCTION

Virgin coconut oil (VCO) is well known as functional food that provides health and nutritional benefits. It provide over and beyond basic nutrients (Aziz et al 2014). VCO has been recognized in health food markets and has high dramatic growth in the international market (Rohyami et al, 2017; Marina et al 2009). The emerging application of VCO in the health area strongly influenced by medium-chain fatty acids (MCFA) content such as lauric, myristic, palmitic, capric, stearic, oleic, and linoleic acids wich are easily digestible. For instance lauric acid, a medium chain fatty acid component in VCO becomes potential use as anti-obesity treatment as it increases energy expenditure, directly absorbed and burnt as energy in the liver. As a results thus leading to weight loss (Mansor et al 2012). Furthermore VCO has multipurpose nutrient supplement such as vitamins, amino acids, antioxidants, antimicrobial, and antiviral compounds (Ghani et al 2018).

There are few several extraction methods to extract coconut oil, such as cold and hot extraction processes. The hot extractions carried out by pressing and followed by heating at high temperature that may reduce the useful micronutrient. In cold process, extraction of coconut oil takes place through destabilization of coconut milk emulsion without heating. This process could retain quality of VCO. Cold process can be done by fermentation, chilling and thawing, or centrifugation, and enzymatic treatment (Agarwal and Bosco, 2017; Handayani et al, 2009). The difference extraction technique will influence oil quality and oil storage stability (Harni and Putri, 2014). Extraction method, fruit maturity, fermentation time, pH, culture concentration are important variables impacted on oil recovery yield and physico-chemical characteristics of the extracted virgin coconut oil (Firdaus, 2015).

The fermentation methods can use various types of microbial cultures such as yeast, bacteria and mold where each type of microbe used has a different performance on characteristics of VCO (Asiah et al, 2018). The microbial strain used in fermentation is the main choice for carrying out amylolytic, proteolytic, and lipolytic activities. This enzyme is needed to hydrolyze the components of protein, carbohydrates, and fats to make coconut emulsions become unstable, so that the oil component can be separated. The culture starter commonly used in the production of VCO is *Lactobacillus plantarum* (Marina, 2009), *Rhizopus oligosporus* (Djajasoepena et al, 2011), and *Saccharomyces cerevisiae* (Isworo, 2013).

Physico-chemical properties of VCO such as moisture content, fatty acid content, free fatty acid content, iodine value, peroxide value, saponification value, and viscosity becomes very important to measure quality and monitor deterioration of virgin coconut oil (VCO) during storage where it is influenced by moisture, temperature, and the presence of microorganisms (Dimzon et al, 2011). The

physico-chemical properties of VCO have been standardized by the Asian and Pacific Coconut Community (APCC). However, until now still less information and studies focusing on looking at and comparing the performance of *Saccharomyces cerevisiae, Lactobacillus plantarum*, and *Rhizopus oligosporus* used in processing VCO by fermentation methods. Where the performance can be seen by comparative study physico-chemical properties VCO produced using various culture.

MATERIALS AND METHODS

A. Material Preparation

The coconuts that will be used in processing VCO are coconuts aged 11-13 months, characterized by brown coir and newly harvested or stored for 1-4 weeks in a dry place with a temperature of 25-28°C. Coconut meat that has been shredded was added with water (50°C) with a ratio of coconut meat and water was 1: 2. Then, pressing process was done by using filter cloth to get the coconut milk. Coconut milk that has been obtained was then put into a transparent container and left for 2 hours until 3 layers are formed where the top layer is cream, the middle layer is skimmed, and the bottom layer is the sediment. Then the cream was separated for the next process. The cream that has been obtained was then divided into 3 parts. After that it is mixed with each starter of microorganisms as much as 5% (v/v) then stirred until homogeneous and poured into a transparent container. Then the mixture was fermented at 35°C for 24 hours until formed 3 layers where the top layer is oil, the middle layer is the protein, and the bottom layer is water. The oil obtained was then separated from protein and water.

Saccharomyces cerevisiae, Lactobacillus plantarum, and *Rhizopus oligosporus* inoculums were prepared as follows: *Saccharomyces cerevisiae* was rejuvenated in Potato Dextrose Agar (PDA) media by means of 11.7 grams of PDA dissolved in 1 L of distilled water. *Lactobacillus plantarum* and *Rhizopus oligosporus* were rejuvenated on Nutrient Agar (NA) media by 2.4 grams of Nutrient Broth (NB) and 4.5 grams to be dissolved in 300 mL of distilled water. Then the three inoculums were inserted into the test tube and tilted to form a slant. The culture medium was sterilized first (at 121°C for 15 minutes) and incubated at 35°C for 24 hours (Barlina, 2004). After that, suspension was made by adding 0.085% NaCl until homogeneous.

B. Physico-chemical Analysis

Water Content Analysis refers to Oseni et al (2017), density analysis method refers to Fachry et al (2006), viscosity analysis method refers to (Aini and Tjahjani, 2013). While Acid Numbers, Iod Numbers and Peroxide Numbers refers to BSN 1988.

Acid Numbers (BSN, 1998)

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

Iod Numbers (BSN, 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 was 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 $Na_2S_2O_3$ 0,1 N.

Peroxide Numbers (BSN, 1998)

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 $Na_2S_2O_3$ 0.01 N

RESULTS AND DISCUSSIONS

The physico-chemical characteristics were tested for moisture content, density, viscosity, acid number, iodine number and peroxide number of VCO samples produced using *Saccharomyces cerevisiae, Lactobacillus plantarum*, and *Rhizopus oligosporus* as showed in Table 1.

Moisture content

Moisture content is one of the quality parameter that affects the level of oil resistance to damage. The presence of water in oil can cause the hydrolysis process where it potentially causes oil damage which is characterized by the presence of rancidity in oil. The higher water content of an oil, the greater likelihood of the hydrolysis process occurring (Raharja et al, 2008). Based on Table 1, the data from the average calculation of water content between VCO samples produced using *Saccharomyces cerevisiae, Lactobacillus plantarum,* and *Rhizopus oligosporus* cultures were 0.02%, 0.05% and 0.04% respectively. The three samples did not differ significantly ($p > 0.05$). Research conducted by Fadlana (2006), VCO which was produced by mechanic method, has a higher water content compared to VCO which is processed by fermentation method. Moisture content of VCO by mechanical method has a moisture content of 0.181%. This shows that VCO which is processed by fermentation method has a lower water content compared to other methods such as mechanic.

The moisture content of VCO produced using *Saccharomyces cerevisiae, Lactobacillus plantarum*, and *Rhizopus oligosporus* has lower than standard water content. In other words, the water content of the three samples still meets the requirements of quality standards according to SNI (2008) and APCC (2009), which is a maximum of 0.2%. It can be concluded that the use of different types of microbes does not affect the VCO water content values produced.

Table 1. Physicochemical characteristics of VCO Produced by Using *Saccharomyces cerevisiae, Lactobacillus plantarum,* and *Rhizopus oligosporus*

Physicochemical characteristics	Culture			Standart	
	Saccharomyces cerevisiae	*Lactobacillus plantarum*	*Rhizopus oligosporus*	SNI (2008)	APCC (2009)
Water Content (%)	0,0265 ± 0,0016	0,0503 ± 0,0082	0,0359 ± 0,0004	Max.0,2	Max.0,2
Density (gram/ml)	0,9156 ± 0,0001	0,9154 ± 0,0001	0,9153 ± 0,0004	-	0,915-0,920
Viscosity (cP)	6,4911 ± 0,0079[a]	6,4026 ± 0,0105[b]	6,4016 ± 0,0074[b]	-	-
Acid Numbers (mg KOH/ g fat)	0,23 ± 0,0141	0,24 ± 0,0212	0,26 ± 0,0141	Max.0,2	Max.0,2
Iod Numbers (Wijs)	7,74 ± 0,1555	8,16 ± 0,0353	7,76 ± 0,0070	4,1-11,0	4,1-11,0
Peroxide Numbers (mEq O_2/kg)	0	0	0	Max.0,2	Max.3
Indeks Bias (^{o})	1,454	1,454	1,454	-	1,4480-1,4492

Density

Density is one of the physical properties possessed by a substance. VCO density produced from *Saccharomyces cerevisiae, Lactobacillus plantarum*, and *Rhizopus oligosporus* respectively have almost the same average value of 0.915gram/ml (Table 1). The use of different types of microbes does not affect the density value of the VCO produced.

Compared to the research conducted by Raharja et al (2008) processing VCO using the cream coconut freezing method, it was found that the density value that was not much different was 0.91517gram/ml. Similarly, the research conducted by Sari et al (2010), processing VCO using the enzymatic method (using pineapple hump extract) has a density value of 0.915-0.920grams/ml. Moeksin et al (2008), also conducted an enzymatic processing of VCO processing with the addition of papain, and obtained a density value that changes with the percentage of addition of papain. The VCO density value ranges from 0.900-0.920 grams/ml. It was stated that the change in density was influenced by the percentage of papain addition, because it was influenced by enzyme activity in breaking down the protein bonds that bind the coconut oil. According to Diyah et al (2010), that the specific gravity of oil produced enzymatically (using fruit skin and papaya seeds) is slightly higher than that produced by heating.

Thus, based on the data of the average value of the density that has been mentioned, it can be concluded that the density of VCO produced by fermentation and enzymatic methods (with the addition of enzymes), meets the quality standards set by the APCC, which ranges from 0.915-0.920 grams/ml.

Viscosity

VCO samples using *Saccharomyces cerevisiae* have greater viscosity values than VCO produced by *Lactobacillus plantarum* and *Rhizopus oligosporus*. From the three samples, there were significant differences ($p < 0.05$). Then, from the results of further tests, the samples that had a significantly different effect on the viscosity values produced were VCO samples using *Saccharomyces cerevisiae*. This is presumably because the value of the density of VCO using *Saccharomyces cerevisisae* was also greater than the density of VCO using *Lactobacillus plantarum and Rhizopus oligsporus*. According to Sutiah et al (2008), that oil which has a large viscosity value due to its density (density) is also large, so that the friction that occurs between the layers in the oil also becomes greater. The viscosity in the liquid is caused by the presence of friction in the layers in the liquid, so that the greater the friction that occurs, the greater the viscosity.

Acid Numbers

Acid numbers are numbers that indicate the amount of free fatty acids contained in oil or fat which are usually associated with the hydrolysis of oil or fat. Hydrolysis of oil or fat will produce free fatty acids. The existence of free fatty acids is usually used as an initial indicator of damage to oil or fat because it is easily oxidized. Coconut oil based on its fatty acid content is classified as lauric acid oil (Raharja et al, 2008). The amount of free fatty acid in the

sample was indicated by the acid number which is usually expressed as the number of milligrams of KOH needed to neutralize the free fatty acids contained in 1 gram of oil or fat. VCO samples produced using *Saccharomyces cerevisiae, Lactobacillus plantarum*, and *Rhizopus oligosporus* have acidic values ranging from 0.23-0.26 mg KOH / g fat (Table 1). This value meets the standards set by SNI (2008) and APCC (2009). The low acid number in the three samples is probably due to the low water content. Low water content can prevent oil hydrolysis.

Iod number

Iodine value is defined as the number of grams of iodine absorbed by 100 grams of oil. The amount of iodine absorbed shows the degree of unsaturation of oil, the more iodine is absorbed, the more the double bonds or the more unsaturated the oil or fat is. Iodine states a measure of oil unsaturation and is related to the content of non-saturated fatty acids in oil. VCO samples produced by *Saccharomyces cerevisiae, Lactobacillus plantarum,* and *Rhizopus oligosporus* have iodine values ranging from 7.74-8.16 Wijs (Table 1). This value meets the standards set by SNI (2008) and APCC (2009). VCO contains more saturated fatty acids compared to unsaturated fatty acids. The most saturated fatty acids found in VCO are lauric acid around 43.0 - 53.0%.

Peroksida numbers

Peroxide numbers are important parameters that can be used as a reference to determine the degree of oil damage. Peroxide is formed because unsaturated fatty acids can bind oxygen to their double bonds, the process is known as the oxidation process (Raharja et al, 2008). The peroxide compounds of VCO samples produced using *Saccharomyces cerevisiae, Lactobacillus plantarum*, and *Rhizopus oligospores* were not detected (Table 1). This has met the requirements set by SNI (2008) and APCC (2009). This is presumably because the packaging process was carried out by inserting a sample of VCO until it overflows, then tightly closed to prevent the presence of oxygen trapped in the packaging. The absence of oxygen in the packaging will prevent oxidation reactions.

CONCLUSIONS

Different culture influence different moisture content, acid number and iodine number. However the value of density, viscosity and peroxide number has no significant different for each culture. The physicochemical test results of VCO using *Saccharomyces cerevisiae, Lactobacillus plantarum*, and *Rhizopus oligosporus* have physicochemical properties conform to the Asian and Pacific Coconut Community (APCC) and Standar Nasional Indonesia.

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