Antioxidant activity of Virgin Coconut Oil and Virgin coconut Oil Emulsion

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ABSTRACT
Virgin Coconut Oil (VCO) contains high medium-chain fatty acids that are functional to improve the health or as supplement. This study aim of research was to determine the antioxidant activity of Virgin Coconut Oil and Virgin Coconut Oil Emulsion with different sweetener. Antioxidant activity of Virgin Coconut Oil was due mainly of Phenolic compound. The major phenolic acids were ferulic acid and p-coumaric acid. Emulsion was more palatable than oil form, because oily taste is not convenient for the consumer or patient, so the sweetener. Virgin Coconut oil emulsion contain of VCO, Xanthan gum, orange juice and different sweetener. Sorbitol, Honey, Gukose as sweetener. The Antioxidant activity of the Virgin Coconut Oil and Virgin Coconut Oil Emulsion were measured by β-carotene bleaching method. The result showed that all sample Virgin Coconut Oil, Virgin Coconut Oil emulsion with honey, with sorbitol, sith glucose, emulsion without sweetener have an antioxidant activity with Antioxidant activity (AA) 56.2%, 63.2%, 70.5%; 37.1%. 57.1% respectively and Quarsetin as a control was 92.5%. These data confirmed that the VCO Emulsion with sorbitol as sweetener has the highest activity of antioxidant.

INTRODUCTION
Coconut (Cocos nucifera L.) is a plant with high economic value for the people of Indonesia. Virgin coconut oil is the most popular of coconut products and still being developed. (Lim et al., 2014). VCO virgin coconut oil is produced from fresh coconut which is produced without high heating, so that the important content in coconut oil can be maintained (Aladin et al., 2016). The chemical components of fatty acids contained in VCO are medium and short-chain saturated fatty acids, medium and short-chain. The samples were used VCO (Virgin Coconut Oil) and VCO emulsion which was the result of the research formulation by Wiyani et al. All other chemicals used were analytical grade. Saturated fatty acids are easily digested and absorbed in the body. The saturated fatty acid compounds are lauric acid (41-52%), myristic fatty acid (13-19%), palmitic fatty acid (7.5-10.5%), caprylic fatty acid (5-10%), Capric fat (4-5.8%), stearic fatty acid (1-3%). In health terms, saturated fatty acids are better known as Medium Chain Fatty Acid (MCFA). Meanwhile, unsaturated fatty acids consist of oleic acid (omega 9) (5-8%), linoleic acid (omega 6) (1-2-5%), and palmitoleic acid (1.3%). While the composition of virgin coconut oil includes ± 66% oil, 6-7% protein from dry weight, 48% water, 5% crude fiber, ± 2% ash content. Apart from fatty acids, several other chemical components that are known to be contained in virgin coconut oil are sterols, vitamin E, and the polyphenol fraction (phenolic acid). The major phenolic acids detected were ferulic acid and p-coumaric acid (Marina, 2009).

VCO is available in the form of clear oil. This oil preparation certainly causes discomfort when consumed orally. Organoleptic test showed the consumer not convenient because the oily taste in the mouth (Wiyani, 2013). Therefore it is necessary to modify the virgin coconut oil form into an oral emulsion form which more palatable for oral administration.

Wiyani et al (2016-2017) have been tested various VCO (Virgin Coconut Oil) emulsion formulations using tween 80 and span 80 as emulsifier/emulgator. Natural Emulsifying agent such as Xanthan gum can produce VCO emulsion too. VCO emulsion preparations with various sweeteners are used as an alternative to overcome the unpleasant taste problem of VCO. (Wiyani et al 2016-2017).

The β-carotene bleaching method is a method for measuring antioxidant activity (AA) in inhibiting lipid peroxidation. This method is based on the ability of antioxidants to prevent and inhibit the fading of the carotene orange color due to the oxidation of peroxide radicals formed in the linoleic acid oxidation reaction (Pustaka). We evaluated the ability of the antioxidant of the VCO and VCO emulsion in various sweeteners with β-carotene bleaching method.

MATERIALS AND METHODS
Materials and reagents
VCO emulsion formulation
Preparation o of the VCO based on the Wiyani et al (2016-2017). The emulsion is made by mixing VCO, orange juice, and sweetener (honey, sorbitol, and glucose) in a ratio of 10: 86: 4 and Xanthan gum as emulsifier 0.75 mL. The emulsion was made with a homogenizer at 15,000 rpm for 4 minutes.

Antioxidant analysis by using the β-carotene bleaching method
The antioxidant activity of the samples was determined using the β-carotene bleaching method. In this study, 1 mg β-carotene dissolved in 1 mL chloroform was added 30 mg of linoleic acid and 200 mg of tween 80 and homogenized. After chloroform evaporation, 60 mL of distilled water was added and the mixture was emulsified for 4 minutes in the vortex until a β-carotene - linoleic acid emulsion was used as a control. Transferred 2 mL from the mixture, each added 1 mL of distilled water, 1 mL
of the test sample (500 ppm), and 1 mL of comparator quercetin (50 ppm) into the cuvette. Then, it was incubated at 50°C on a hot plate. And measured at wavelength 495.35 nm.

The second emulsion was also prepared and used as blank to zero the spectrophotometer. This emulsion consist of 10 mL of distilled water, 5 mg linoleic acid and 33 mg of tween 80. The absorbance data were recorded at intervals of 15 minutes to 120 minutes by using a UV-Vis spectrophotometer. The percentage inhibition was calculated according to the following formula (Jayaprakash et al. 2001, p. 286):

\[
\% \text{AA} = 100 \left[ 1 - \left( \frac{A_{0} - A_{t}}{A_{0}} \right) \right]
\]

Where : % AA : % antioxidant activity, A0 and At: is the absorbance of the sample at 0 minutes and t=120 minutes, A0 and A: is the absorbance of the control 0 minutes and t=120 minutes.

RESULTS AND DISCUSSION

The antioxidant activity was measured by using the β-carotene bleaching method. This method is proper to determine the antioxidant activity of lipophilic compounds such as essential oils (VCO). (Mita et al., 2015). The measurements of control quercetin, and samples used a UV-Vis spectrophotometer at the maximum wavelength of β-carotene about 400-600 nm which is the wavelength range of the carotenoid class compounds used as an antioxidant activity indicator. Measurements for 0-120 minutes and the wavelength were measured every 15 minutes time interval to see the rate of inhibition of β-carotene bleaching in control, quercetine, and VCO (Virgin Coconut Oil) samples and VCO emulsion. The mechanism of antioxidant action can be seen from how much antioxidants can slow the bleaching or fading of the orange color of β-carotene (Salah et al., 2014).

The result obtained the absorbance of control quercetin, VCO (Virgin Coconut Oil) products and VCO emulsion which were measured for 120 minutes with interval time every 15-minute (Table 1).

<table>
<thead>
<tr>
<th>Minute</th>
<th>Average Value of Absorbance (495.35 nm)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>0.783</td>
</tr>
<tr>
<td>15</td>
<td>0.615</td>
</tr>
<tr>
<td>30</td>
<td>0.504</td>
</tr>
<tr>
<td>45</td>
<td>0.402</td>
</tr>
<tr>
<td>60</td>
<td>0.310</td>
</tr>
<tr>
<td>75</td>
<td>0.217</td>
</tr>
<tr>
<td>90</td>
<td>0.126</td>
</tr>
<tr>
<td>105</td>
<td>0.036</td>
</tr>
<tr>
<td>120</td>
<td>0.004</td>
</tr>
<tr>
<td>(0-120)</td>
<td>0.864</td>
</tr>
</tbody>
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The absorbance was analyzed and collected % antioxidant activity (% AA) of quercetin, pure VCO samples, and VCO emulsions with various sweeteners that stated the percentage of the ability of pure VCO samples and VCO emulsions as antioxidants (Figure 1).

Figure 1 showed the % Antioxidant Activity (% AA) of pure VCO samples and VCO emulsion against β-carotene degradation by comparing the absorbance value with the absorbance of negative control and quercetin. % The antioxidant activity of 56.2% pure VCO and 57.1% unsweetened VCO emulsion did not experience much difference, so it can be said that VCO either in pure or emulsion form does not affect the antioxidant content contained in it. Whereas in VCO emulsion with various sweeteners and unsweetened variations there was an increase in% AA where VCO emulsion with honey sweetener obtained a % AA percentage of 70.5%, with sorbitol was 63.2%, with glucose 37.1% although the highest % AA was indicated by the addition of quercetin 92.5% which is clear is one compound that is proven to have antioxidant effects. The antioxidant activity of CO emulsion with honey sweetener increased in which honey has a function as a peroxide inhibition. The bioactive components in honey are potential as antioxidants, such as flavonoids and melanoidins. Also, the addition of honey to beverages produces peroxide inhibitory activity, presumably because honey contains phenolic components and organic acids such as citric acid and cinnamic acid which have potential as antioxidants. These organic acids are thought to have a synergistic effect on phenolic antioxidants so that they can increase their antioxidant activity (Septiana et al., 2010). In the research of Septiana et al. (2009) also said that the content of antioxidant compounds in honey-sweetened beverages was more able to prevent oxidation of fatty acids than antioxidants in drinks sweetened with coconut sugar, although the content of phenolic compounds in drinks sweetened with coconut sugar was higher. This is presumably because the antioxidant activity in honey is higher than the antioxidants in coconut sugar. Besides having phenolic compounds as primary antioxidants, honey also has organic acids such as citric acid (Belitz and Grosch, 1999) which can play a role in inhibiting oxidation reactions by binding to metals (Gordon, 1990) (Quoted from the journal Septiana et al., 2009).
oxidation rate under acidic conditions and lipid oxidation is inhibited. Then if the higher the sorbitol concentration is added, the droplet size will be bigger. The droplet size can affect the rate of lipid oxidation. The smaller the droplet size, the larger the surface area that more oil will be in contact with the water phase, consequently the rate of lipid oxidation will be faster. Also, according to Jakson (1995) sorbitol will increase the activity of antioxidants in ingredients due to the ability of sorbitol as a humectant that can bind water in the material, further water cannot be evaporated during heating and minimizes loss of nutrients, especially antioxidants during heating. Apart from functioning as a humectant in maintaining moisture stability, sorbitol can protect components that are firmly bound in the material (Syafutri et al., 2010).

The decrease levels of γ antioxidant activity occur in the VCO emulsion with glucose sweeteners, where is a monosaccharide group and is classified as reducing sugar. High levels of reducing sugar in food are characterized by a sweet taste. Thus, the sweeter the taste of a product, the higher the reducing sugar content. Reducing sugar is a sugar that can reduce oxidizing compounds, in other words, this sugar itself undergoes oxidation (Ameljya, 2018). According to Potter and Hotchkiss (1998) sugars that have free aldehydes and ketones are known as reducing sugars. All monosaccharides are reducing sugars. When two or more monosaccharides are bonded together via their aldehyde and ketone groups, these reducing groups are not free and sugars are not reducing. According to Lehninger (1982) states that monosaccharides (glucose/fructose) immediately reduce oxidizing compounds, such as feriansiades, hydrogen peroxide, or copper ion (Cu++). In this reaction, the sugar is oxidized to the carbonyl group and the oxidizing compound is reduced. Glucose and other sugars that can reduce oxidizing compounds are called reducing sugars (Zulfahmi and Dwi Nirmagustina, 2012).

The AA power of the β-carotene bleaching method is classified into three levels strong antioxidants (> 70%), intermediate (40-70%), and weak (<40%) (Hassimotto et al., 2005).

The antioxidant activity of quercetin is the highest compared to pure VCO samples and VCO emulsions. However, these samples pure VCO sample and VCO emulsion showed the antioxidant activity with the intermediate antioxidant category (40-70%), and the orange VCO emulsion sample with glucose sweeter is classified in the weak category of antioxidants (<40%).

REFERENCES


