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The antioxidant activity of virgin coconut oil using the FRAP, ABTS, CUPRAC, and DPPH methods

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Abstract
Virgin Coconut Oil (VCO) is a type of vegetable oil that can be beneficial for health because it contains antioxidants. VCO can prevent and help treat certain diseases such as cancer, coronary heart disease, diabetes, gout, and other degenerative diseases. This research aims to find out the antioxidant potential contained in virgin coconut oil. This research used 4 methods, that were the FRAP, ABTS, CUPRAC, and DPPH methods. The results of this research showed that the antioxidant activity value (IC_{50}) in virgin coconut oil was 10.78 µg/mL for the FRAP method, 43.5986 µg/mL for ABTS, 25.53 µg/mL for CUPRAC, and 93 µg for DPPH. 9177 µg/mL. From the research results it can be concluded that Virgin coconut oil has antioxidant activity which is characterized by a very strong average IC_{50} value.

Keywords: Virgin coconut oil, antioxidants, FRAP, ABTS, CUPRAC, DPPH

Introduction

Coconut (*Cocos nucifera* L.) is a plant that has high economic value for Indonesian people. One of the coconut products that is currently developing and in demand is virgin coconut oil (VCO) (Lim *et al*., 2014) [5]. VCO can help in treating certain diseases such as cancer, coronary heart disease, diabetes, gout, and other degenerative diseases. Apart from that, VCO can also help slow down the aging process and act as an antibacterial and antiviral (Alamsyah. 2005) [1]. The results of previous research using VCO in mice showed that VCO could reduce levels of total cholesterol, triglycerides, phospholipids, Low-Density Lipoprotein (LDL), and Very Low Lipoprotein (VLDL) and increase High-Density Lipoprotein in serum and tissues (Nevin *et al*., 2004) [9].

VCO ability to overcome various diseases is thought to be due to its fatty acid content, especially lauric acid. Lauric acid in the human body is converted into a monoglyceride compound, that was monolaurin. Monolaurin is a compound that has antiviral, antibacterial, and antifungal properties. In its mechanism, monolaurin can damage the lipid membrane (the covering layer of virused) including HIV, influenza, and several other virused. Several types of bacteria such as Staphylococcus aureus, and Helicobacter pylori (Bacteria that cause stomach ulcers) are reported to be killed by the compound monolaurin, a saturated fatty acid with a C carbon chain (Rindengan *et al*., 2006) [13].

Apart from fatty acids, other ingredients in VCO that have antioxidant activity are tocopherol and β-carotene. Tocopherol and β-carotene compounds can trap free radicals and can reduce cholesterol in the blood. Apart from that, β-carotene has also been reported as a stimulator of enzymes that destroy carcinogens/cancer-causing substances (Muis, 2014) [8]. Antioxidants are substances that inhibit oxidation reactions caused by free radicals which can cause damage to unsaturated fatty acids, cell wall membranes, blood vessels, DNA bases, and lipid tissues, causing disease (Widyastuti *et al*., 2010) [17]. Free radicals in normal amounts are beneficial for health, for example, fighting inflammation, killing bacteria, and controlling smooth muscle tone in blood vessels and organs in the body. Meanwhile, excessive amounts can cause oxidative stress. This situation can cause oxidative damage starting from the cell, tissue, and organ levels, which accelerates the process of disease emergence. Therefore, antioxidants are needed to delay or inhibit oxidation reactions by free radicals (Rahardja *et al*., 2006) [11]. Based on the background above, further research is needed regarding the antioxidant activity contained in virgin coconut oil.

Methodology

Alat dan Bahan

This research used several tools include the measuring flasks (Pyrex), cuvettes (Quartz), beaker glass (Pyrex) and UV-visible spectrophotometer (Shimadzu). The materials used in this research are Virgin coconut oil (Ayu VCO®), Ethanol (Smart lab), Methanol (Smart lab), Vitamin C Smart lab), Neocupproine ethanolic (Aldrich), Ammonium acetate buffer (Smart lab), Fecl3 (Merck), HCL (Merck), TPTZ Solution (Aldrich), Trichloroacetic acid (Merck), and DPPH (TCI).

Antioxidant Measurement

Measurement of antioxidants in virgin coconut oil is done using 4 methods, that were:

Antioxidant Measurement using the FRAP Method

A total of 2 mL of sample was weighed and dissolved in 10 mL of methanol in a 10 mL volumetric flask until it reached the limit to obtain a concentration of 1000 µg/ml (Stock solution). Then each 0.125 is taken; 0.25; 0.50; 1; and 2 mL of the stock solution into a 10 mL volumetric flask to obtain concentrations of 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, and 400 µg/ml. 1 mL sample solution was taken and 1 mL phosphate buffer (0.2 M pH 6.6) and 1 mL potassium ferricyanide 1% were added, then incubated at 50 ℃ for 20 minutes. After incubation, 1 mL of trichloroacetic acid was added and homogenized for 10 minutes, then centrifuged at 3000 rpm for 10 minutes. Take 1 of the top layer of the solution mL then add 1 mL of distilled water and 0.5 FeCl3 0.1%. The solution was left for 10 minutes and the absorbance at the maximum wavelength was measured, the work was done in a dark place (Syarif *et al*., 2015) [16].

Measurement of Antioxidants using the ABTS Method

Pipette a stock sample solution of 1000 µg/mL with each concentration of 10 µg/mL; 20 µg/mL; 30 µg/mL; 40 µg/mL; 50 µg/mL or 0.05 mL; 0.1 mL; 0.15 mL; 0.2 mL; 0.25 mL into a 5 mL volumetric flask. Then the volume was increased to the limit with methanol pa. From each concentration, 0.1 mL of sample solution was pipetted plus 2 mL of ABTS stock solution. Next, it was incubated for 6 minutes and the absorbance was measured using UV-visible spectrophotometer at the maximum wavelength (Rahimah *et al*., 2015) [12].

Antioxidant Measurement using the CUPRAC Method

Pipet the sample stock solution in amounts of 100 µL, 200 µL, 300 µL, 400 µL, 500 µL. Then 200 µL Cuprac was added and filled with 1 mL ethanol to obtain concentrations of 10, 20, 30, 40, and 50 ppm. The mixture was incubated for 10 minutes at 30 ℃, then the absorbance of the solution was measured using a UV-visible spectrophotometer at the maximum wavelength (Haeria *et al*., 2018) [3].

Antioxidant Measurement using the DPPH Method

Pipette 0.1 mL; 0.2 mL; 0.3 mL; 0.4 mL; and 0.5 mL of 1000 ppm stock solution. Then added 1 ml of DPPH solution (Concentration 200 ppm) at each concentration and added methanol to the mark (5 mL volumetric flask). Concentrations of 20, 40, 60, 80, and 100 ppm were obtained. Incubated for 30 minutes then absorbance was measured using a UV-visible spectrophotometer at the maximum wave- length (Karadita, 2011) [4].

Determination of % Inhibition

Determination of the percent of free radicals by compounds that have antioxidant activity using UV-visible spectrophotometer so that the percent value of free radicals is known, which is calculated using the following formula (Molyneux., 2004) [7].

$$\% \text{ Free Radical Inhibition} = \left(\frac{A\ blank - A\ sample}{A\ blank}\right) X\ 100\ \%$$

Description:
A blank = absorbance that does not contain the sample
A sample = sample absorbance

Determination of IC_{50} Value

IC_{50} (Inhibitor Concentration) is the concentration of the sample solution needed to inhibit 50% of free radicals. The IC_{50} value of the test sample is obtained from the calculation results which are substituted into the calibration curve regression equation y= ax + b, where the y value is substituted for 50 (Silalahi *et al*., 2018) [15]. Specifically, a compound is said to be a very strong antioxidant if the IC_{50} value is less than 50 µg/mL, strong if the IC_{50} is 50,100 µg/mL, moderate if the IC_{50} value is 100-150 µg/mL, and weak if the IC_{50} is 151-200 µg/mL. mL (Molyneux., 2004) [7]. The following is the regression equation obtained from the calibration curve used to find out the IC_{50} value:

$$y = ax + b$$

Note:
y = percentage of radical scavenging activity (y = 50)
a = slope/gradient;
x = sample volume/ IC_{50} (ml)
b = intercept/regression coefficient

Results and Discussion

Antioxidant Activity with the FRAP Method

The FRAP method was chosen because it has the advantage of providing fast and easy results and showing antioxidants in the matrix complex (Maulana *et al*., 2019) [6]. The maximum wavelength was measured using a UV-visible spectrophotometer at a wavelength of 400-800 nm so that the maximum absorption wavelength was obtained at 716 nm with an absorbance of 0.186 which can be seen in Figure 1. This is by previous research that the wavelength for FRAP measurements is 716.20 nm (Maulana *et al*., 2019) [6].

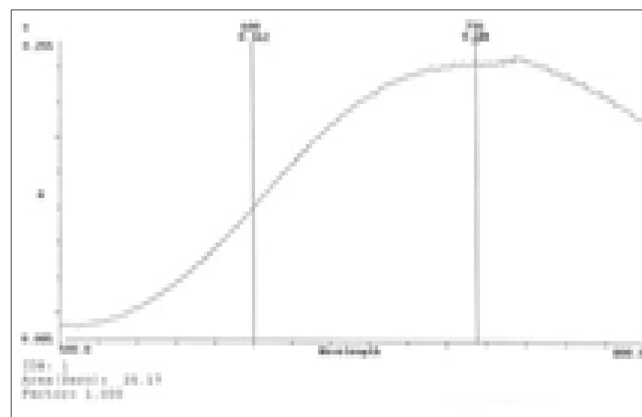

Fig 1: Absorbance curve of the FRAP method

Determining the operating time to reduce free radicals using spectrophotometry at 0-30 minutes, and calculating the absorbance every 1 minute. From the results of the operating time measurements, it can be seen that from 0-30 minutes the absorbance remains stable. The results of measuring the free radical scavenging activity of samples using the FRAP method at each concentration can be seen in the following table:

Table 1: Percent inhibition of FRAP method

No.	Concentration (µg/mL)	Average absorbance	% Inhibition
1	25	0,201	7,46%
2	50	0,203	8,37%
3	100	0,205	9,27%
4	200	0,208	10,58%
5	400	0,212	12,26%

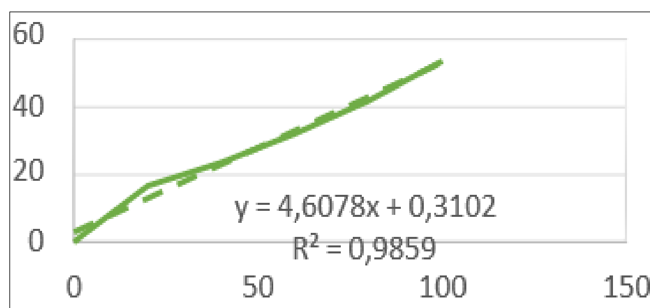

Fig 2: Calibration curve of the FRAP method.

From the research results, the calibration curve in Figure 2 was obtained and the IC_{50} value of virgin coconut oil using the FRAP method was 10.78 µg/ml. This shows that virgin coconut oil is classified as a very strong antioxidant. This is to previous research, that was the IC_{50} value for the FRAP method was 11.59 µg/mL (Wahyuni, 2015) [18].

Antioxidant Activity by ABTS Method

ABTS method was chosen because it has high sensitivity and a fast reaction (Rahimah *et al.,* 2015) [12]. The maximum wavelength was measured using a UV-visible spectrophotometer at a wavelength of 650-800 nm so that the maximum absorption wavelength was obtained at 732 nm with an absorbance of 0.802 which can be seen in Figure 3. This is to previous research that the wavelength for ABTS measurements is 750 nm (Rahimah *et al.,* 2015) [12].

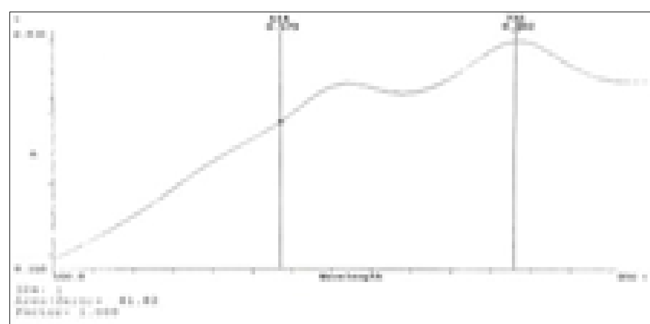

Fig 3: Absorbance curve of the ABTS method

The operating time used in this research is 6 minutes. The results of measuring the free radical scavenging activity of samples using the ABTS method at each concentration can be seen in the following table:

Table 2: Percent inhibition of the ABTS method

No.	Concentration (µg/mL)	Average absorbance	% Inhibition
1	10	0,547	31,7655%
2	20	0,528	43,1646%
3	30	0,508	36,6583%
4	40	0,4764	40,5985%
5	50	0,3647	54,5217%

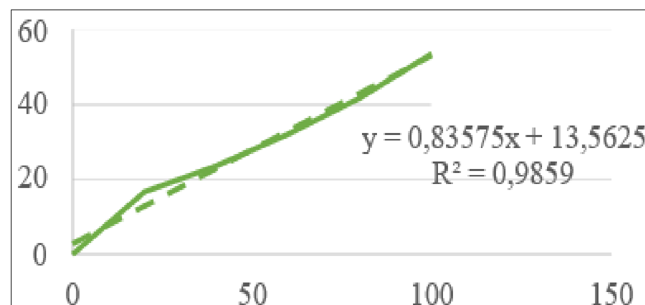

Fig 4: Calibration curve of the ABTS method.

From the research results, the calibration curve in Figure 4 was obtained and the IC_{50} value of virgin coconut oil using the ABTS method was 43.5986 µg/ml. This shows that virgin coconut oil is classified as a very strong antioxidant.

Antioxidant Activity with the CUPRAC Method

The CUPRAC method was chosen because it has the advantage of being a simple method, quite selective, stable, and sensitive to types of antioxidants, and can measure the ability of phenolic compounds in samples (Heria *et al*., 2018) [3]. The maximum wavelength was measured using a UV-visible spectrophotometer at a wavelength of 400-800 nm so that the maximum absorption wavelength was obtained at 440 nm with an absorbance of 0.218 which can be seen in Figure 5. This is by previous research that the wavelength for CUPRAC measurements is 450 nm (Heria *et al*., 2018) [3].

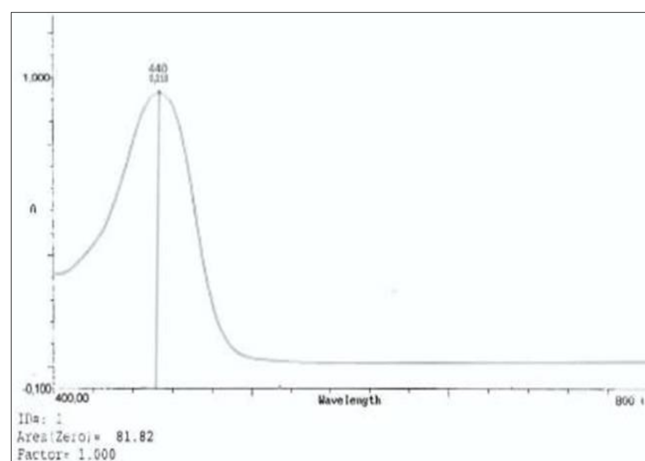

Fig 5: Absorbance curve of the CUPRAC method

Determining the operating time to reduce free radicals using spectrophotometry was done for 60 minutes and the absorbance was calculated every 1 minute. From the results of the operating time measurements, it can be seen that from the 29 minute to the 32 minute it remained stable with an absorbance of 0.309. The results of measuring the free radical scavenging activity of samples using the CUPRAC method at each concentration can be seen in the following table:

Table 3: Percent inhibition of the CUPRAC method

No.	Concentration (µg/mL)	average absorbance	% Inhibition
1	10	0,388	43,81%
2	20	0,466	53,21%
3	30	0,574	61,95%
4	40	0,650	66,46%
5	50	0,748	70,85%

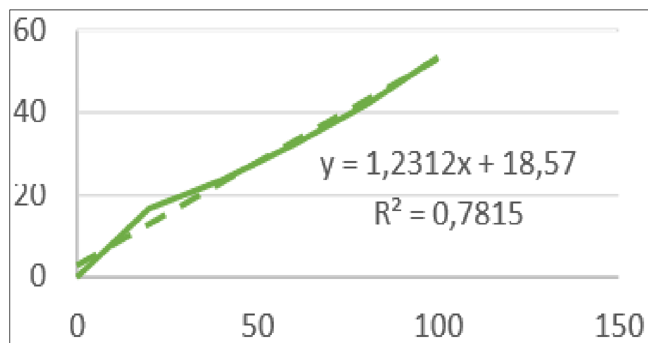

Fig 6: Calibration curve of the CUPRAC method

From the research results, the calibration curve in Figure 6 was obtained and the IC_{50} value of virgin coconut oil was used CUPRAC method was 25.53 µg/ml. This shows that virgin coconut oil is classified as a very strong antioxidant.

Antioxidant Activity with the DPPH Method

The DPPH method was chosen because it has the advantages of being easy to use, fast, quite thorough, good for use in organic solvents, and sensitive for testing antioxidant activity in plant extracts (Apak *et al.*, 2013) [2]. The maximum wavelength was measured using a UV- visible spectrophotometer at a wavelength of 400-800 nm so that the maximum absorption wavelength was obtained at 515 nm with an absorbance of 1,007 which can be seen in Figure 7. This is by previous research that the wavelength for measuring DPPH is between 515nm to 520 nm (Molyneux. 2004) [7].

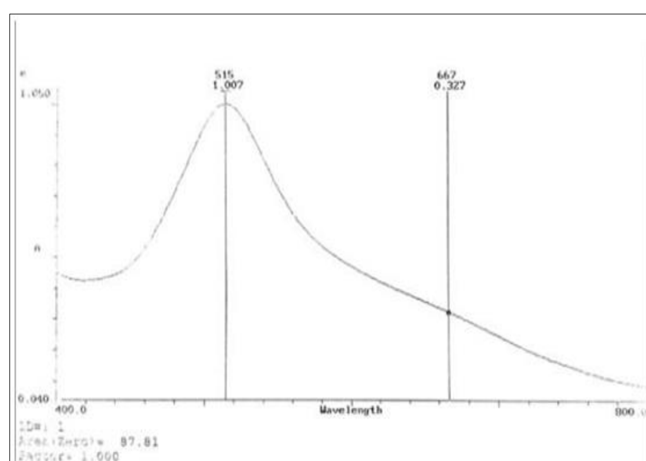

Fig 7: Absorbance curve of DPPH method

Determining the operating time to reduce free radicals using spectrophotometry is up to 60 minutes and the absorbance is calculated every 1 minute. From the results of the operating time measurements, it can be seen that from the 28th minute to the 31st minute it remains stable with an absorbance of 1.047. The results of measuring the free radical scavenging activity of samples using the DPPH method at each concentration can be seen in the following table:

Table 4: Percent inhibition of the DPPH method

No.	Concentration (µg/mL)	average absorbance	% Inhibition
1	20	0,839	16,68%
2	40	0,777	22,84%
3	60	0,684	32,07%
4	80	0,586	41,80%
5	100	0,468	53,52%

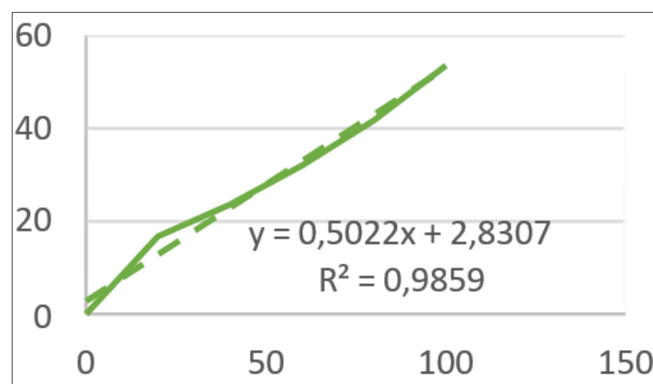

Fig 8: Calibration curve for DPPH method.

From the research results, the calibration curve in Figure 8 was obtained and the IC_{50} value of virgin coconut oil using the DPPH method was 93.9177 µg/ml. This shows that virgin coconut oil is classified as a strong antioxidant. This is by previous research, that was IC_{50} value for the DPPH method was 17.19 µg/mL (Pulung *et al.*, 2016) [10].

Conclusion

Virgin coconut oil has potential antioxidant activity as indicated by the IC_{50} value for the FRAP method 10.78 µg/mL, ABTS method 43.5986 µg/mL, CUPRAC method 25.53 µg/mL, and DPPH method 93.9177 µg/mL.

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