

A Comparative Analysis of Phenolic Compound Quality in Virgin Coconut Oil

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Abstract. Virgin coconut oil (VCO) is derived from fresh coconut fruit and offers numerous health benefits, such as preventing heart diseases, improving digestion, reducing fat accumulation, and more. VCO contains medium-chain fatty acids that are easily digested and metabolized by the body, preventing fat buildup. Additionally, it is rich in antioxidants like tocopherols and beta-carotene, which help prevent premature aging and maintain vitality. The phenolic content in VCO can be influenced by the processing method and the age of the coconuts used. Therefore, this research examined the impact of processing methods on the quality of phenolic content as a product of the pharmaceutical industry. The largest group of phenolic compounds includes tocopherols, sterols, and flavonoids. Phenolic compounds in coconuts are primarily located in the coconut fruit's epidermis. VCO produced from coconuts with the epidermis or coconut fruit's outermost layer contains a higher concentration of phenolic compounds compared to VCO produced from coconuts without the epidermis. The research involved defining and refining the problem, formulating hypotheses or tentative answers, drawing conclusions, and conducting meticulous testing of all conclusions to determine their alignment with the hypotheses. In this study, the researchers compared the quality and phenolic compound content of virgin coconut oil (VCO) by sampling from several small and medium enterprises in various regions, specifically in the Island of Sulawesi. The findings revealed that each graph represents the percentage of five samples, depicting various contents, such as water content, free fatty acids, peroxide value, phenolic compounds, and antioxidant activity.

Keywords: VCO, Phenolic Compounds, Antioxidants.

1 Introduction

Virgin coconut oil (VCO) is coconut oil produced from fresh coconut material by extracting the oil from the kernel through controlled heating or even without any heating process. VCO offers numerous health benefits, including the prevention of heart diseases, improved digestion, reduced fat content, and more. Phenolic compounds are chemical compounds with one or more hydroxyl groups attached to an aromatic ring (Vermeris & Nicholson, 2006). These compounds are commonly found in various parts of plants, such as stems, leaves, flowers, and fruits. Phenolic compounds are also produced by plants in response to environmental stress and serve as protection against UV-B rays and cell damage, safeguarding DNA from dimerization [1].

Another definition of virgin coconut oil (VCO) is coconut oil obtained from fresh coconuts and processed mechanically or naturally, with or without heating, without purification, or the addition of chemicals. This definition has led to various interpretations and understandings of VCO production methods among manufacturers. VCO can be produced using several methods, including chemical, physical, and enzymatic processes. The chemical method involves precipitating proteins in coconut milk at the isoelectric point, where the pH of fresh coconut milk is lowered from 6 to 4 using acid. Meanwhile, the physical method breaks the emulsion system of coconut milk by adding coconut oil to increase the surface tension between protein and water, causing the oil embedded in the emulsion system to separate and merge with the added oil. The enzymatic method, on the other hand, uses protease enzymes to break down peptide chains of proteins into simpler molecules. Several methods are commonly employed and considered by the community (producers) to be more efficient and effective for producing VCO following standards. These methods include Spontaneous Fermentation, Baiting with Oil, Fermentation with Starter Addition, Centrifugation Method, Minimal Heating Method, and Cooling with Centrifugation Method. Virgin coconut oil

contains various types of fatty acids, which make up the largest part of its chemical composition among all fats and oils. In addition to major fatty acids, it also contains minor components in the form of phenolic compounds [2].

Small and medium enterprises (SMEs) are economic activities that involve the processing of raw materials, semi-finished goods, and/or finished products into higher-value items for commercial use. These activities typically require investments ranging from 200 million rupiahs (200,000,000 IDR) to 10 billion rupiahs (10,000,000,000 IDR), excluding the cost of land and business premises. SMEs can be undertaken by individuals, households, or organizations, to produce goods or services for commercial purposes, having a sales value of at least one billion rupiahs but less than 50 billion rupiahs.

2 Materials and Methods

2.1 Materials

2.1.1 Antioxidant Activity Test

The materials used in this study included a solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and virgin coconut oil. The equipment employed in this research consisted of as following.

- a. Stirrer
- b. Micropipette
- c. 100 mL volumetric flask
- d. 100 mL Erlenmeyer flask
- e. Test tubes
- f. 100 mL measuring glass
- g. Vortex
- h. UV-Vis Spectrophotometer

2.1.2 Phenolic Compound Test

The materials utilized in this study encompassed a 0.07% bipyridine solution, 0.2% FeCl₃, and toluene. The tools used in this research were as follows.

- a. Stirrer
- b. Micropipette
- c. 100 mL volumetric flask
- d. 100 mL Erlenmeyer flask
- e. Test tubes
- f. 100 mL measuring glass
- g. Vortex
- h. UV-Vis Spectrophotometer

3 Methods

The type of this study was experimental research [3]. In experimental research, researchers formulate a minimum of 2 (two) hypotheses that describe cause-and-effect relationships among the variables involved. The variables under investigation include independent and dependent variables, which have been clearly defined by the researchers since the beginning of the study.

3.1 Testing Parameters

- a. Phenolic compounds
- b. Antioxidant activity
- c. Moisture content
- d. Peroxide value
- e. Free fatty acid (FFA)

The testing of virgin coconut oil (VCO) was conducted at the Integrated Laboratory of Sam Ratulangi University and the Standardization and Industrial Service Center (Indonesian: *Balai Standardisasi dan Pelayanan Jasa Industri [BSPJI]*) of Manado.

3.2 Experiment Design and Construction

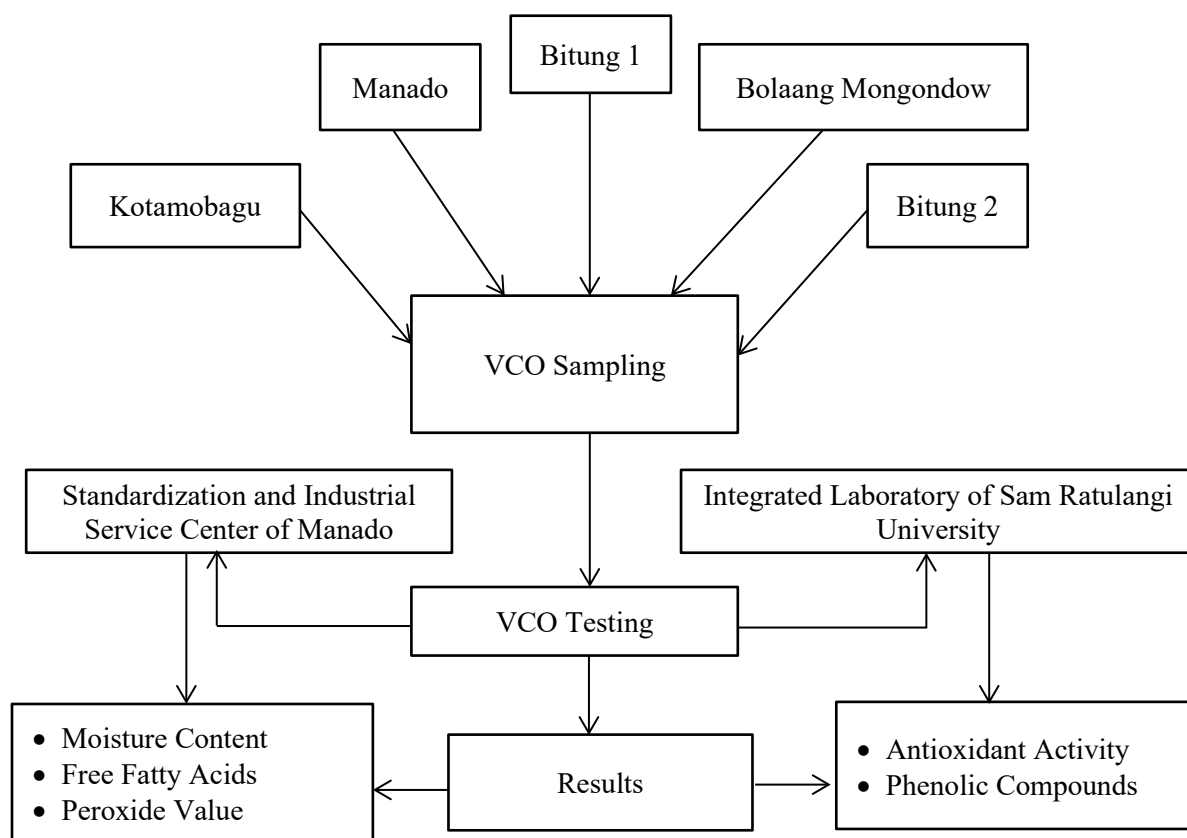


Figure 1. Experimental Design and Construction Chart

4 Results and Discussion

4.1 Results

4.1.1 Sampling of Virgin Coconut Oil (VCO)

At this stage, virgin coconut oil (VCO) samples for testing were collected from small and medium enterprises in various regencies and cities in North Sulawesi, including Manado, Bitung, Bolaang Mongondow, and Kotamobagu.

4.1.2 Methods Used in VCO Production

a. Heating Method

The gradual heating method involves extracting the flesh from fresh coconuts, grating it, and then adding water and squeezing it. The obtained coconut milk is allowed to settle until two layers form (cream and water). The cream is separated and then heated (cooked) at a temperature below 90°C until oil and white sediments form. The oil is filtered to separate it from the white sediments. The oil is then heated again for 10 hours at 65°C and filtered to obtain virgin coconut oil (VCO).

b. Baiting Method

The baiting method involves extracting the flesh from fresh coconuts, grating it, and then adding water and squeezing it. The obtained coconut milk is allowed to settle until two layers form (cream and water). The cream is separated. Baiting oil is added to the cream in a 1:3 ratio and mixed thoroughly. It is left to sit for 10-12 hours. The result is the formation of three layers: oil, white sediments, and water. The VCO is then carefully separated by filtering.

c. Fermentation Method

The fermentation method involves extracting the flesh from fresh coconuts, grating it, and then adding water and squeezing it. The coconut milk obtained (with or without the addition of a starter) is then left to ferment for 18-24 hours. After that, the formed components are separated or filtered.

d. Centrifugation Method

The centrifugation method begins with grated coconut flesh mixed with water, which is then squeezed and filtered to produce coconut milk. The coconut milk is collected in a container, and the subsequent process involves centrifuging the coconut milk to obtain three layers: an oil layer, white sediments (protein), and water. These three layers are components within the coconut milk separated due to differences in density. The uppermost layer, which consists of oil, is the virgin coconut oil (VCO) product.

4.1.3 Methods for Determining Moisture Content, Free Fatty Acids, Peroxide Value, Antioxidant Activity, and Phenolic Compounds

a. Moisture Content

Moisture content in oil can be determined using the oven method. In the oven method, approximately 5 g of the sample is weighed into a crucible and placed in an oven at 105°C for about 4-5 hours until a constant weight is achieved. The sample is then removed, placed in a desiccator for approximately 15 minutes, and subsequently weighed.

$$\text{Moisture Content} = \frac{A - B}{A} \times 100\%$$

Where:

A = the initial weight of the sample (oil)

B = the weight of the sample (oil) after heating

b. Free Fatty Acids (FFA)

The determination of free fatty acids (Mehlenbacher, as cited in Sudarmadji *et al.*, 1981) involves weighing approximately 5 g of the sample in a 250 ml Erlenmeyer flask. The sample is added 50 ml of hot 96% neutral ethanol and 2 ml of phenolphthalein indicator. The sample is titrated with 0.05 N NaOH solution that has been standardized until a pink color is achieved and remains for 30 minutes. Free fatty acids are expressed as %FFA.

$$\%FFA = \frac{\text{ml NaOH} \times N \times \text{Molecular Weight of Fatty Acids}}{\text{Sample Weight} \times 1000} \times 100\%$$

c. Peroxide Value

The determination of peroxide value involves the weighing of approximately 5 g of the sample into a covered 250 ml Erlenmeyer flask. Then, 30 ml of acetic acid-chloroform solution (3:2) is added to the sample. The solution is shaken until all the material dissolves, followed by the addition of 0.5 ml of saturated potassium iodide solution. It is allowed to stand for 1 minute, with occasional shaking, and then 30 ml of distilled water is added. The sample is titrated with 0.01 N Na₂S₂O₃ solution until the yellow color nearly disappears. After that, 0.5 ml of 1% starch solution is added, and the titration continues until the blue color in the solution begins to fade. The peroxide value is expressed in milliequivalents of peroxide per 1000 g of the sample.

$$\text{Peroxide Value} = \frac{\text{ml Na}_2\text{S}_2\text{O}_3 \times N \times 100}{\text{Sample Weight}}$$

d. Antioxidant Activity

The antioxidant activity being tested is the capability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals at various concentrations. Each 0.5 ml of extracted virgin coconut oil (VCO) at 1000 µg/ml is mixed with 1.5 ml of DPPH solution and vortexed for 2 minutes. The solution changes from purple to yellow, indicating the efficiency of free radical scavenging. This is further observed in the last 5 minutes

before reaching 30 minutes at a wavelength of 517 nm using a UV-Vis spectrophotometer. The free radical scavenging activity is calculated as a percentage reduction in DPPH color using the following formula.

$$1 - \frac{\text{Sample Absorbance}}{\text{Control Absorbance}} \times 100\%$$

e. Tocopherols (Phenolics)

1 ml of virgin coconut oil (VCO) is weighed, and 5 ml of toluene is added to a 25 ml Erlenmeyer flask. Then, 3.5 ml of 0.07% bipyridine solution is added, and the mixture is vortexed for 2 minutes. Subsequently, the solution is adjusted to 10 ml with 95% ethanol, and measurement is conducted at a wavelength of λ 520 nm.

4.2 Discussion

4.2.1 Test Results for Moisture Content, Free Fatty Acids (FFA), Peroxide Value, Antioxidant Activity, and Phenolic Compounds

Table 1. Results of Analysis of Average Moisture Content, FFA, and Peroxide Value in the Samples of Virgin Coconut Oil (VCO)

No.	Samples	Moisture Content (%)	Free Fatty Acid (FFA) (%)	Peroxide Value (meq/kg)
1.	V1	0.15	0.08	0.99
2.	V2	0.14	0.14	0.26
3.	V3	0.15	0.12	0.37
4.	V4	0.15	0.2	0.49
5.	V5	0.12	0.09	0.79

Source: Research Results, 2022

- V1: VCO from Kotamobagu
- V2: VCO from Manado
- V3: VCO from Bitung 1
- V4: VCO from Bolaang Mongondow
- V5: VCO from Bitung 2

4.2.2 Moisture Content

Based on the analysis results of moisture content presented in Table 1 and Figure 2, it is evident that the VCO samples have varying moisture content. The lowest moisture content is found in sample V5, at 0.12%, followed by V2 at 0.14%, and then V3 and V4 with the same moisture content of 0.15%. According to the Indonesian National Standard (SNI:7381, 2008), all VCO samples meet the quality standard requirement for moisture content, which is a maximum of 0.2%.

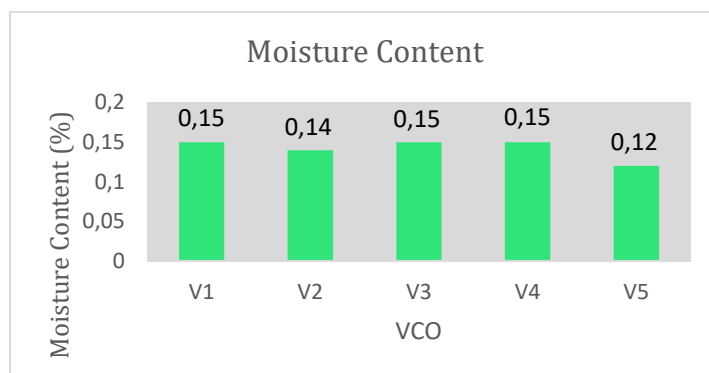


Figure 2. The Graph of Moisture Content

4.2.3 Free Fatty Acids (FFA)

Based on the analysis of free fatty acids (FFA) content presented in Table 1 and Figure 3, it is observed that the samples of VCO have varying levels of free fatty acids. The highest FFA content is found in sample V4, at 0.2%, followed by V2 at 0.14%, V3 at 0.12%, and V5 at 0.09%. The lowest is found in sample V1, which is 0.08%. According to the Indonesian National Standard (SNI:7381, 2008), all FFA content in the VCO samples meets the quality standard requirement, which is a maximum of 2%.

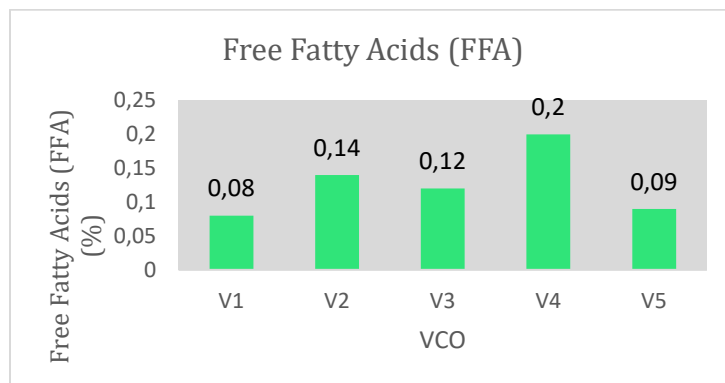


Figure 3. The Graph of Free Fatty Acid (FFA)

4.2.4 Peroxide Value

Peroxide value is a critical parameter for assessing the extent of damage in oils and fats. Peroxide compounds form as a result of oxidative reactions, which occur when oxygen comes into contact with oil and fat. Based on the analysis results of the peroxide value presented in Table 1 and Figure 4, it is evident that the five VCO samples exhibit varying peroxide value levels. Sample V1 has the highest peroxide value at 0.99 meq/kg, followed by V5 at 0.79 meq/kg. Next is V1 with 0.49 meq/kg, followed by V3 with 0.37 meq/kg. The lowest value is found in sample V2 at 0.26 meq/kg. All VCO samples meet the quality standard requirements outlined in the Indonesian National Standard (SNI:7381, 2008), which specifies a maximum peroxide value of 2 meq/kg.

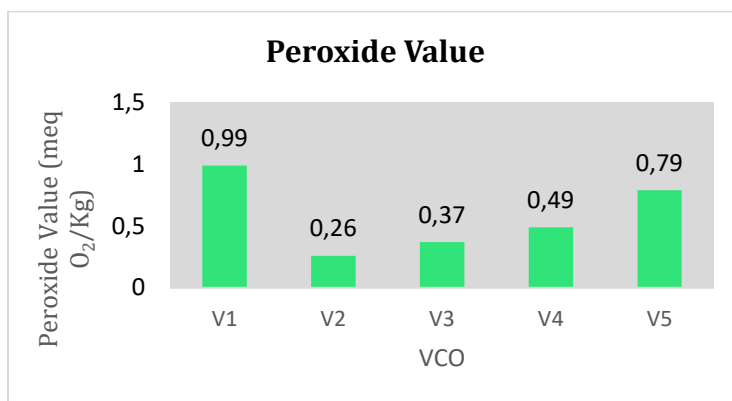


Figure 4. The Graph of Peroxide Value

4.2.5 Phenolic Compounds

Virgin coconut oil (VCO) has the potential to prevent and assist in the treatment of specific diseases, such as cancer, coronary heart disease, diabetes, gout, cholesterol-related issues, and various degenerative diseases. Furthermore, it can aid in the digestion of food and the absorption of nutrients. Research findings have indicated that the medical and health benefits of VCO are attributed to phenolic compounds, which are minor constituents within VCO.

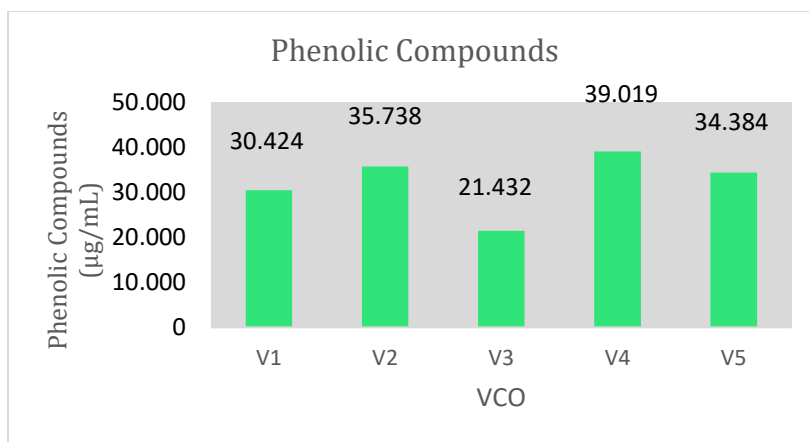


Figure 5. The Graph of Phenolic Compounds

Natural plant antioxidants typically consist of phenolic compounds, including flavonoids, tocopherols, and organic acids. One of the objectives of this study was to determine the phenolic compound content in various virgin coconut oil (VCO) samples produced by small and medium-sized enterprises (SMEs) from different regencies and cities in North Sulawesi.

Based on the research findings and phenolic compound content analysis shown in Figure 5, the five VCO samples have varying levels of phenolic compound content. The highest content is found in sample V4 at 39.19 µg/ml, followed by V5 at 34.38 µg/ml, V2 at 35.73 µg/ml, and V1 at 30.42 µg/ml. The lowest content is found in sample V3 at 21.43 µg/ml. The phenolic compound content in VCO is influenced by several factors, including the coconut harvesting age, coconut variety, growing location, and VCO processing methods and techniques.

The VCO samples used in this research were collected from different regencies and cities, with varying coconut harvesting ages, resulting in different phenolic compound contents. Sample V3 has the lowest phenolic compound content because, as obtained from the information, during the processing, the coconut fruit's epidermis is removed from the coconut flesh (paring). Research results indicated that phenolic compounds are most abundant in the flesh portion closest to the coconut fruit's epidermis.

The coconut fruit's epidermis refers to the outer surface of coconut flesh removed during the processing of coconut flesh into flour, coconut milk, oil, and other coconut products, with a thickness of approximately 2mm. This part consists of coconut flesh (endosperm) and coconut fruit's epidermis (brown skin). Research findings have shown that the total phenolic compound content in oil from coconut fruit's epidermis is around 0.15 - 0.2%. In addition, this part contains antioxidant and photoprotective compounds, making it beneficial for health and capable of preserving the quality of the resulting oil [4]. Other research results have also indicated that the phenolic compound content in VCO made from coconuts with epidermis is three times higher than VCO from coconuts without epidermis because phenolic compounds in coconuts are primarily found in the epidermis. VCO from coconuts with epidermis contains 75.92 mg/kg of phenolic compounds, while VCO from coconuts without epidermis contains 23.87 mg/kg of phenolic compounds [5].

4.2.6 Antioxidant Activity

Antioxidant compounds exhibit their free radical scavenging activity by reacting with peroxy radicals before these radicals can interact with long-chain unsaturated fatty acids [6]. In this study, we assessed the antioxidant activity using the DPPH method, a widely recognized approach for estimating the antioxidant capacity of various compounds. The DPPH method is renowned for its simplicity, speed, sensitivity, and multiple advantages, such as cost-effectiveness, ease of use, stability (remaining in a monomeric state in solution), and oxidation resistance [7]. The DPPH method relies on the reduction in absorbance due to a color change in the solution. Initially, the purple solution turns yellow as the DPPH radical is captured by the antioxidant, releasing a hydrogen atom to form a stable DPPH-H.

We evaluated the antioxidant activity of VCO by measuring its ability to scavenge DPPH radicals. VCO samples collected from various regencies and cities in North Sulawesi exhibited varying antioxidant activities. Figure 6 illustrates that the highest antioxidant activity was observed in sample V4 at 97.93%, followed by sample V2 at 97.13%. Sample V5 and V1 exhibited antioxidant activities of 96.98% and 95.52%, respectively, while the lowest

antioxidant activity was found in sample V3 at 20.56%. The differences in antioxidant activity among the samples were generally not very significant, except for sample V3, which displayed a highly significant difference compared to the other VCO samples.

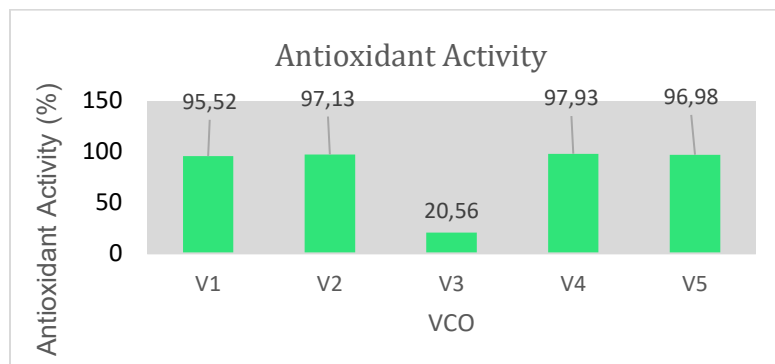


Figure 6. The Graph of Antioxidant Activity

The differences observed are influenced by the fact that the samples have the lowest phenolic compound content (see Figure 5).

Natural antioxidants derived from plants generally consist of phenolic compounds, including flavonoids, tocopherols, and organic acids. Coconut oil contains various components, such as tocopherols and β -carotene, which are commonly found in almost all vegetable oils, including VCO, and are classified as natural antioxidants. Therefore, the lower the phenolic compound content is, the lower the antioxidant activity will be [2], [4], [8], [9].

One effective way to inhibit lipid oxidation is through the use of antioxidants. Antioxidants are compounds that can be employed to protect food items by slowing down damage, rancidity, or color changes caused by oxidation. Antioxidants play a role in inhibiting oxidation through various mechanisms, including controlling substrates (oxygen and lipids), managing peroxidation (reactive oxygen species and catalytic metals), and controlling free radicals. The most effective antioxidants perform their function by interrupting the chain reaction of free radicals in lipid oxidation.

5 Conclusion

- a. Virgin coconut oil (VCO) from various regions/small and medium enterprises (SMEs) was processed using the cold method (cold process), specifically through spontaneous fermentation. Based on the test results, the VCO samples exhibited different qualities, but all still met the quality standards based on SNI:7381, 2008, especially regarding moisture content, peroxide value, and free fatty acids (FFA). The phenolic compound content in the VCO samples varied, with the highest found in sample V4 at 39.19 $\mu\text{g/ml}$ and the lowest found in sample V3 at 20.56 $\mu\text{g/ml}$. These differences can be influenced by several factors, including coconut harvesting age, coconut variety, growing location, and processing methods and techniques. The antioxidant activities of the VCO samples also varied, with the highest in sample V4 at 97.93% and the lowest found in sample V3 at 20.56%. The differences in antioxidant activity among the samples were not very significant, except for sample V3, which showed a highly significant difference from the other samples, likely due to differences in phenolic compound content.
- b. Based on the phenolic compound content and antioxidant activity, it can be recommended that all samples except for sample V3 should be used as raw materials or additives for pharmaceutical products.

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