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Cocoa Powder Antioxidant Activity Test Using Cyclic Voltammetry and Differential Pulse Voltammetry Methods

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Abstract

The main antioxidant compounds contained in cocoa are polyphenols, including flavonoids such as epicatechin, catechin, and procyanidin. This study aims to determine the antioxidant activity of three samples of cocoa powder using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a free radical by electroanalytical methods, namely cyclic voltammetry (CV) and differential pulse voltammetry (DPV). From the CV information obtained, the first anodic peak from DPPH appears at a potential of 0.33 V with an anodic current of 2.75 A/cm^2, while the first anodic peak at DPPH after adding the chocolate sample which has the highest antioxidant activity appears at a potential of 0.43 V with an anodic current of 4.60 A/cm^2. From the DPV information obtained, the anodic peak of DPPH appears at a potential of 0.19 V at an anodic current of 2.11 mA, while the anodic peak at DPPH after adding the chocolate sample which has the highest antioxidant activity appears at a potential of 0.02 V at an anodic current of 1.97 mA. The results show that the electroanalytical method has the potential to analyze the antioxidant activity of cocoa powder samples.

Keywords: antioxidant activity, cocoa powder, cyclic voltammetry, differential pulse voltammetry

1. Introduction

Free radicals are reactive molecules that can trigger oxidative damage to DNA, proteins, lipids, can cause mutations and are carcinogenic [1]. This oxidative damage can lead to various degenerative diseases such as coronary heart disease, cancer and other degenerative diseases [2]. This radical is very reactive and always tries to find an electron pair so that its condition is stable [3].

However, this free radical reaction can generally be inhibited by certain antioxidants, both natural and synthetic [4]. Antioxidants are compounds that delay, control or inhibit the auto-oxidation process [5]. Antioxidants can protect cells by fighting free radicals. The trick is to complement the lack of electrons possessed by free radicals and then inhibit the occurrence of a chain reaction from the formation of free radicals which can cause oxidative stress that causes cell damage [6]. Natural antioxidants can be obtained from fruits, spices, tea, leaves, seeds, enzymes and proteins [7].

Cocoa beans are one of the main sources of polyphenols, especially (-)-epicatechins, which have been reported to have antioxidant capacity [8]. Catechins and epicatechins are reported to be potential candidates for counteracting free radicals present in the body [9]. Chocolate is a food product made from cocoa mass, cocoa butter and sugar. The compound components contained in chocolate have health benefits, and chocolate itself is sometimes referred to as a functional food. These functional components are flavonoid compounds as antioxidants which are known to be able to reduce free radical production due to oxidation processes, lower blood pressure and lower LDL cholesterol [10]. According to Afoakwa, the main antioxidant compounds contained in cocoa are polyphenols, including flavonoids such as epicatechin, catechin and procyanidin [11].

In general, to test the antioxidant activity of cocoa using the spectrophotometric method with the compound 1,1-diphenyl-2-picrylhydrazyl (DPPH) has been done by various researchers [12–17]. Even though this method is

relatively simple, this spectrophotometric method is not feasible because it takes a long time when applied manually to a large number of samples [18]. In addition, electrochemical testing of the antioxidant activity of cocoa (particularly cocoa powder) is still very limited.

The test results reported by Suliasih *et al.* [19], showed that by using the electroanalytical method, namely CV and DPV, it was possible to determine the antioxidant activity in local honey samples up to 89.36%. From the test results it appears that the electroanalytical method has the potential to test antioxidant activity. Therefore, this study aims to determine the antioxidant activity of three cocoa powders using electroanalytic methods with cyclic voltammetry (CV) and differential pulse voltammetry (DPV).

2. Materials and Method

The materials used in this study were 70% ethanol and 96% ethanol which functioned as a solvent, 3 samples of cocoa powder purchased from online shops with various brands, namely: Vanhouten (S1); Bensdorp (S2); Roman (S3), ascorbic acid as a comparison, 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a radical compound, and tetrabutylammonium perchlorate (TBAP) as a supporting electrolyte.

The process of preparing cocoa powder extract was carried out as done by Tamrin *et al.* [13]. First dissolving 1 gram of cocoa powder sample in 25 mL of 70% ethanol solution, then macerating for 2 hours at 50 °C in a 150 rpm orbital shaker. The results of maceration are filtered with filter paper. Then a 2.5 µM ascorbic acid solution was prepared. Later, 1 mL of cocoa extract filtrate and ascorbic acid solution were taken to be tested for CV and DPV.

The electrochemical testing process was carried out by preparing a solution of 250 µM DPPH in 96% ethanol and 0.1 M TBAP, then adding 1 mL of the chocolate sample filtrate. As a comparison, 1 mL of ascorbic acid was added to the DPPH solution. All solutions to be tested were incubated for 30 minutes. Then stir the solution manually for 10 seconds before testing.

Measurements were made using a three-electrode system, with the working electrode being GCE, the counter electrode being Pt plate, and the reference electrode being Ag/AgCl. CV analysis was performed with a scan rate of 50 mV/s over a potential range of −0.3 to 1.0 V for 3 cycles. By recording the current at the first oxidation peak in the DPPH solution (control) before and after adding the cocoa powder sample filtrate, the percent inhibition can be calculated with Eq. 1 as shown below.

$$\%Inhibition = \left|\frac{I^{O}_{pa} - I^{S}_{pa}}{I^{O}} x\ 100\%\right| \quad (1)$$

Where I^{0}_{pa} is the first oxidation peak current at DPPH before adding antioxidants (samples) and I^{s}_{pa} is the first oxidation peak current at DPPH after adding antioxidants.

Meanwhile, DPV was performed with a scan rate of 10 mV/s, pulse amplitude of 50 mV, and pulse width of 50 ms. Based on research conducted by Siaka [20], antioxidant activity with the DPV method can be determined using Eq. 2 as follows:

$$I = \frac{I_{pa0} - I_{pas}}{I_{pa0}} x\ 100 \quad (2)$$

Where I_{pa0} is the intensity of the DPPH oxidation current before adding antioxidants and I_{pas} is the intensity of the DPPH oxidation current after adding antioxidants.

3. Results and Discussion

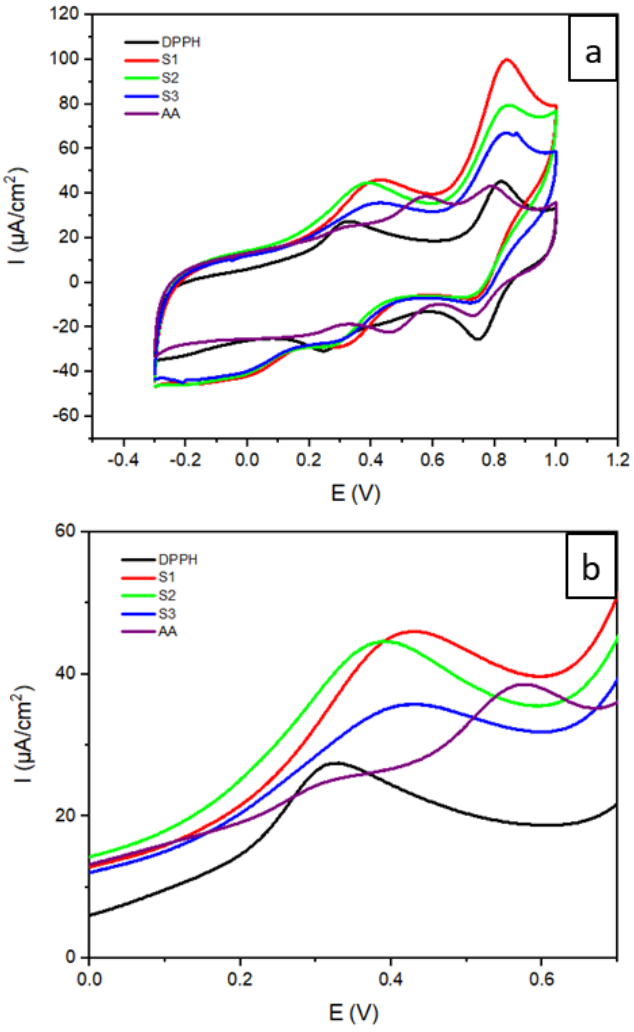

Figure 1. (a) Voltammogram of DPPH radical inhibition by cocoa powder, (b) More detailed voltammogram 1a.

Based on Fig. 1 it is known that all the tested cocoa powder samples have antioxidant activity with different levels. CV measurement was carried out based on the increase in anodic current in DPPH solution after adding

brown filtrate. It is noted that the anodic peak 1 of DPPH occurs at a potential of 0.33 V with an anodic current of 2.75 A/cm^2, while the anodic peak 1 of DPPH after each brown filtrate is added occurs at an even higher anodic current as shown in Table 1. This indicates that in the reaction process that occurs, antioxidants present in chocolate donate hydrogen to the DPPH radical so that it will be reduced to DPPH-H [21]. The higher anodic current produced after DPPH was added to the brown filtrate indicated that many DPPPH-H species were formed, in other words chocolate had succeeded in reducing or counteracting DPPH radicals by forming DPPH-H species. The higher the anodic current generated, the more DPPH-H species are formed.

It was found that the current is directly proportional to the concentration of the solution. Therefore, the inhibition value can be calculated by the difference in current intensity at the DPPH peak before and after adding the chocolate filtrate. The results of calculating the percentage of inhibition are listed in Table 1, which if sorted from those with the highest to the lowest inhibition values are as follows: S1 > S2 > S3, meaning that sample-1 has the highest antioxidant activity of 67.27%. Ascorbic acid as a comparison in this study produced an inhibition percentage of 68.85%, this shows that the inhibition percentage found in chocolate is not less high than the inhibition percentage contained in ascorbic acid.

Table 1. Percent inhibition of cocoa powder samples.

Sample Code*	$E1_{pa}$ (V)	Is_{pa} [I(A/cm^2)]	Percent Inhibition (%)
S1	0.43	4.60	67.27
S2	0.39	4.47	62.54
S3	0.42	3.58	30.18
AA (comparison)	0.57	3.85	68.85

*S1 Vanhouten, S2 Bensdorp, S3 Roman, AA ascorbic acid

The CV voltammogram results were then compared with the DPV voltammogram results. From the DPV voltammogram (Fig. 2), it can be seen that the current intensity decreased after each brown filtrate was added to the DPPH solution. It was noted that the anodic peak of DPPH occurred at a potential of 0.19 V at an anodic current (I_{pa}) of 2.11 mA, while the anodic peak of DPPH decreased after each brown filtrate was added as shown in Table 2. According to Siaka [20], This decrease in current intensity reflects the trapping or inhibition of radicals by an antioxidant.

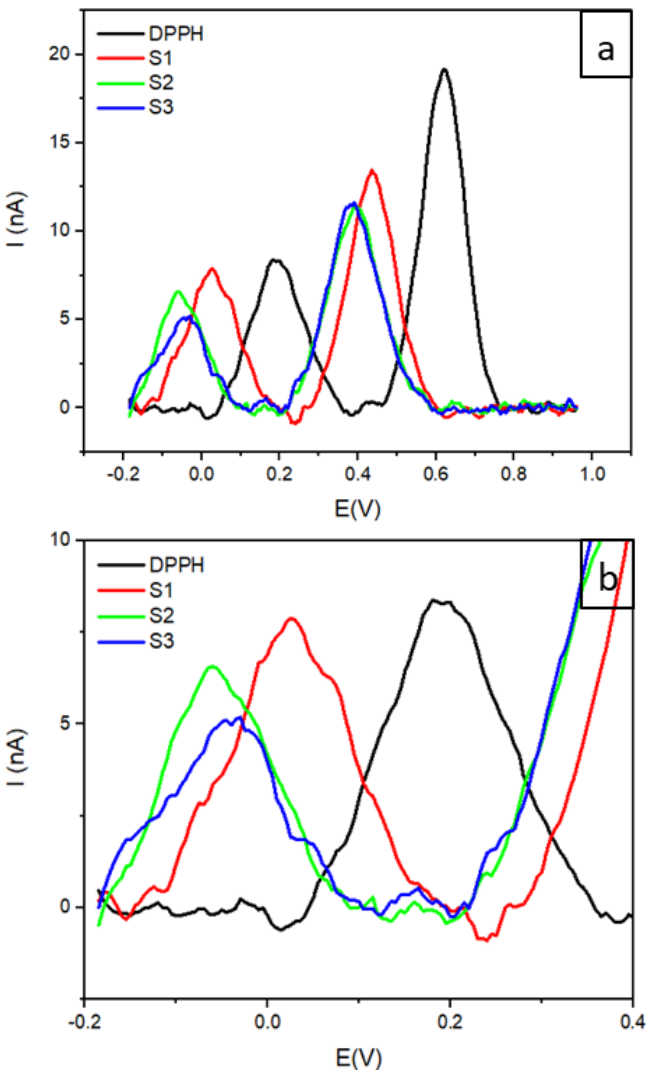

Figure 2. (a) DPV voltammogram of DPPH radical inhibition by cocoa powder, (b) More detailed voltammogram 2a.

Table 2. Value I sample of cocoa powder.

Sample Code*	E (V)	I_{pas} (mA)	Value I
S1	0.02	1.97	6.63
S2	-0.06	1.74	17.53
S3	-0.04	1.43	32.22

*S1 Vanhouten, S2 Bensdorp, S3 Roman

From the data generated, antioxidant activity can be determined by calculating the I value of the decrease or difference in current intensity at the DPPH peak. The calculation results are listed in Table 2, which if sorted from those with the highest I values to the lowest are as follows: S3 > S2 > S1, meaning that sample-3 has the highest antioxidant activity. This data is different from the data obtained using CV. However, based on data from the results of the two methods, it can be estimated that

electrochemical tests can be used to determine the antioxidant activity of cocoa powder samples.

The advantage of the CV technique is that it is a forceful and popular electrochemical technique commonly employed to investigate the reduction and oxidation processes of molecular species. CV is also invaluable to study electron transfer-initiated chemical reactions, which includes catalysis [22]. Meanwhile, the DPV technique has the advantages of simplicity, rapidity, and sensitivity [23]. The results of the work done by Suliasih *et al.* [19] shows that the electroanalytical method could be potentially utilized for antioxidant analysis of local honey samples, this method also proved to have the potential to test the antioxidant activity of cocoa powder samples. Further research is needed to evaluate the determination of antioxidant activity using CV and DPV tests in order to obtain even better data trends. It is also advisable to test antioxidant activity using other methods or instruments so that it can be clearly confirmed the peaks that appear in the CV and DPV data from antioxidants or what compounds make the samples used can be said to have antioxidant activity.

4. Conclusion

From the CV data obtained, sample-1 had the highest antioxidant activity when compared to other samples. Meanwhile, from the DPV data, sample-3 had the highest antioxidant activity. Based on the results of the electrochemical data, this study provides evidence that the electrochemical method is a potential method for analyzing antioxidant activity in cocoa powder samples. However, further research is needed using other methods or instruments to evaluate the determination of antioxidant activity in order to obtain better data trends and the peaks that appear in the CV and DPV data can be clearly confirmed from which antioxidants or compounds.

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