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Effect of Time Variation on Chlorophyll Concentration in Cocoa Leaf Extraction using the UAE (Ultrasonic Assisted Extraction) Method

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Abstract

Until now, Cocoa (*Theobroma cacao* L.) plantation is a sector that has been continuously cultivated. Cocoa leaves contain chlorophyll, which is an important pigment in photosynthesis as sunlight absorber. The aim of this study is to determine the effect of extraction time to the concentration of chlorophyll in cocoa leaves. The extraction time was varied from 5 to 25 min with constant variables, namely power and ratio of raw materials. In this study, Ultrasonic Assisted Extraction (UAE) method was used due to its effectiveness. The concentration of obtained chlorophyll was determined by using UV-Vis spectrophotometry. The optimum extraction time was 15 min producing concentration of chlorophyll a of 9.39 mg/L, chlorophyll b of 4.78 mg/L, and total chlorophyll of 14.16 mg/L.

Keywords: *Chlorophyll, Cocoa Leaves, UV-Vis Spectrophotometry, Ultrasonic Assisted Extraction*

1 Introduction

Cocoa (*Theobroma cacao* L.) is one of the plantation sectors that is beneficial for the national economy. Cocoa industry plays an important role in regional development and agro-industry [1]. Indonesia is one of cocoa producer country with a production of 720,862 tons ranking two in the world [2]. Cocoa leaves have good potential for the community, especially cocoa farmers to take advantage of existing opportunities [3].

Chlorophyll has high antioxidant content. Photosynthesis is carried out in chlorophyll, where the pigment is found in green plants, photosynthetic bacteria, and algae [4]. Two types of known chlorophyll are chlorophyll a, and b [5]. The green color of chlorophyll has long been used as a natural colorant in various food and non-food products [6]. Chlorophyll in daily life can be used as antibiotics, disinfectants and dietary supplements, such as ointments, pharmaceutical treatments, can repair cells, and accelerate cell growth, where chlorophyll has been researched and can be used as a source of pigment in cosmetics [7].

The choice of extraction method will determine the success of extraction process. Several known extraction methods are maceration, Soxhlet, reflux, steam distillation, MAE (Microwave Assisted Extraction), and UAE (Ultrasonic Assisted Extraction). The maceration method requires a longer extraction time, but can be used on a small or large scale [8]. Soxhlet method is not suitable to be applied on a large scale due to the high solvent boiling point [9]. Reflux method requires a lot of solvent, and steam distillation has compounded whose contents are also distilled [10]. MAE method could damage the desired bioactive compound if the extraction temperature and time is too high. Therefore, to minimize damage, the extraction time should be adjusted [11].

The UAE method is an alternative method, which is constantly developed to optimize the extraction process. The use of ultrasonic equipment will result in a more effective and faster extraction process, because the amount of solvent required, extraction time and temperature can be reduced [12]. So, the advantage of the UAE method is that the results obtained are more concentrated extracts, and prevent the loss or evaporation of compounds that have low boiling points [13]. Therefore, research on the extraction

of chlorophyll from cocoa leaves using the UAE method has a greater potential to be developed [14].

However, to date, there is no detailed report on the extraction of chlorophyll from cocoa leaves. So, the purpose of this study was to extract chlorophyll from cocoa leaves using the UAE method. The use of the UAE method is expected to efficiently extract chlorophyll from cocoa leaves. Several extraction parameters were varied to obtain optimum conditions.

2 Methods

2.1 Sample Preparation

The selected cocoa leaves are cleaned by rinsing with water to remove dirt, soil, or other foreign materials. Cocoa leaves are dried without sunlight (only aerated). This is done because, if exposed to light with high intensity and for a long time, there will be a change in the color of the leaves [15]. After drying, then the cocoa leaves are cut into small pieces and blended so that it is easy to sieve, so that the raw material is obtained with a size of 0.250 m. The ingredient:solvent ratio is 0.06 g/mL and the power is 180 watts. Greenish scale of cocoa leaves using BWD (Leaf Color Chart). The BWD used is with four panels, and for the greenish scale of cocoa leaves it is on a scale of 2.

2.2 Extraction Process

The Ultrasonic Assisted Extraction setup was assembled according to Figure 1, after that the raw materials are put into a glass beaker with the addition of solvent (ethanol) which is put into a glass beaker. The extraction time ranged from 5 to 25 min. The extraction mixture was filtered using filter paper and rinsed with distilled water, then the filtrate was obtained.

The ultrasonic waves propagate to the extract through the medium that is passed. The propagation of this wave will cause vibration, during the extraction process the vibration will provide intensive stirring. After the vibration during the extraction process, there will be a double effect in the form of damaged cell walls so that during the penetration process the solvent can easily enter the material or extract sample. In addition to causing a double effect, vibration also produces local heating of the liquid so that it can increase the diffusion of the extract [16].

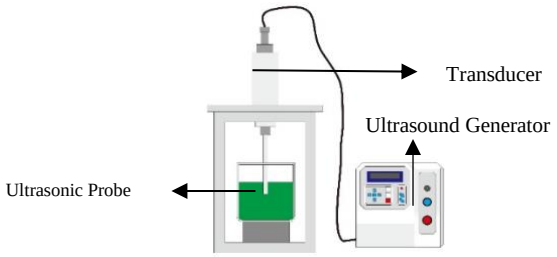

Figure 1. Ultrasonic Assisted Extraction Design

The type of ultrasonic instrument used in this study is a Probe Sonicator type. In Figure 1, the specifications of the tools contained in the ultrasonic tool are several parts, including the ultrasonic probe which is used to transmit ultrasonic waves during the extraction process. An ultrasound generator is used to control the waveform and extraction time, and a transducer is used to connect the probe to the ultrasound generator [17].

2.3 Chlorophyll Analysis

The sample that has been obtained from the extraction process is put into a 10 mL volumetric flask as much as 1 mL. Add ethanol to the mark, then shaken, and put into a test tube, and do the same for each cycle. Visible spectrophotometer was used to find the absorbance of solutions with wavelengths of 663 nm and 645 nm. Ethanol was used as a blank, because ethanol is a solvent without analyte in this treatment [18]. After that, the absorbance of each run was obtained and the concentrations of chlorophyll a and chlorophyll b were calculated.

Calculations for the chlorophyll concentration were carried out using the equation. The equation obtained from the experimental results, then formulated by Arnon (1949) using the substitution method, so that the following formula is obtained:

a. Chlorophyll a = 12,7 (A.663) – 2,69 (A.645) mg/L.
b. Chlorophyll b = 22,9 (A.645) – 4,68 (A.663) mg/L.
c. Chlorophyll total = 8,02 (A.663) + 20,2 (A.645) mg/L.

3 Result and Discussion

3.1 Chlorophyll Analysis Absorbance Test

Chlorophyll is the main pigment found in the thylakoid membrane of chloroplasts. Pigments that can absorb solar radiation and can release electrons in photochemical processes are pigments that play an important role in photosynthesis, so they can convert light energy into chemical energy [19]. Chlorophyll is found on the surface of the leaves and stems, namely in the spongy layer

below the cuticle. In a previous study, cocoa leaves had chlorophyll a content of 2.35 mg/L and a chlorophyll b content of 1.68 mg/L.

In the absorbance test of chlorophyll solution using visible light spectrophotometer. Absorbance is the amount of light absorbed by a material. UV-Vis spectrophotometer was used to test the absorbance to determine the pigment concentration. Chlorophyll analysis was measured at a wavelength of 645 and 663 nm, where at a wavelength of 600 nm is the red area. The use of these wavelengths was made because the observed extract was green, so with absorbance at wavelengths of 645 and 663 nm it was possible to determine the chlorophyll content contained in the extract [20]. The use of wavelengths of 645 and 663 nm is based on an experiment conducted by Mckinney (1941), where Mckinney looks for the absorbance value of chlorophyll at each wavelength (425 – 680 nm), the largest absorbance values are known at wavelengths of 645 nm and 663 nm. Chlorophyll a is at a wavelength of 663 nm and the largest absorbance value of chlorophyll b is at a wavelength of 645 nm.

This measurement uses a blank solution in the form of ethanol. This blank was used to determine the absorbance of the solution and for calibration purposes as a comparison solution in the analysis. In the calculations that have been carried out, the absorbance values at wavelengths of 645 and 663 nm get the same results with the largest results at 15 min. The largest chlorophyll concentration was also found at 15 min. From these results it can be interpreted that with wavelengths of 645 and 663 nm, with increasing time until it reaches the optimum limit, it will produce a large chlorophyll concentration according to the data in table 1.

Table 1. Data Absorbance and Chlorophyll Test Concentration

Absorbance		Concentration		Total Chlorophyll (mg/L)
645 nm	663 nm	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)	
0,23	0,55	6,38	2,60	8,98

3.2 Graph Analysis of Chlorophyll a Concentration

Chlorophyll analysis using curves in expert design. In Figure 2, the results show that the graph of the concentration of chlorophyll a is affected by time. The optimum concentration of chlorophyll a occurred at 15 min. The response of the concentration of chlorophyll a indicates that the greater the time used, the concentration of chlorophyll a will increase. This result is in accordance with the statement stated in the research of Yuliantari et al., (2017) that the extraction yield will increase followed by an increase in extraction time until it reaches the optimum limit [21]. This happens, because the increase in extraction time can result in a longer contact between the material and the solvent [22]. The longer the use of extraction time and the optimum limit is exceeded, it will result in changes in the structure of the compound, because the oxidation process takes place, so that less extract is produced [23], so that at the 25th min the chlorophyll concentration decreases.

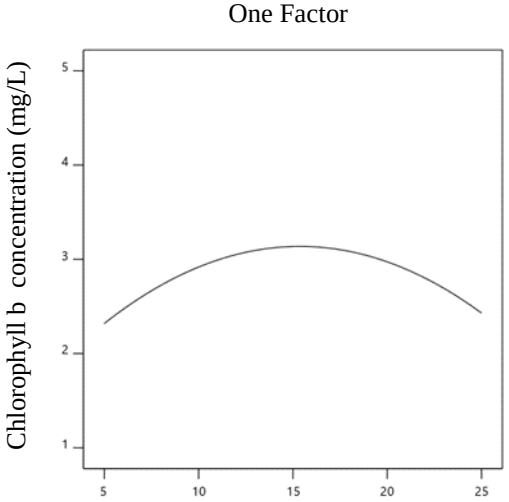

Figure 2. Effect of Time (minutes) on Chlorophyll b Concentration

3.3 Graph Analysis of Total Chlorophyll Concentration

Based on Figure 3, the results show that the graph of the total chlorophyll concentration is affected by time. The total chlorophyll concentration occurred at the optimum time of 15 min. Time that exceeds 15 min will decrease the chlorophyll concentration. This is because the equilibrium point has been reached between the solvent and the solute. The response of total chlorophyll concentration indicates that the greater the time used, the total chlorophyll concentration will increase. These results are consistent, that the longer the use of extraction time and the optimum limit is exceeded, it will change the compound structure, because of the oxidation process, so that less extract is produced [24]. This was caused by the oxidation of the extracted bioactive compounds, but if the time used was too little the active compounds will not being fully extracted [25].

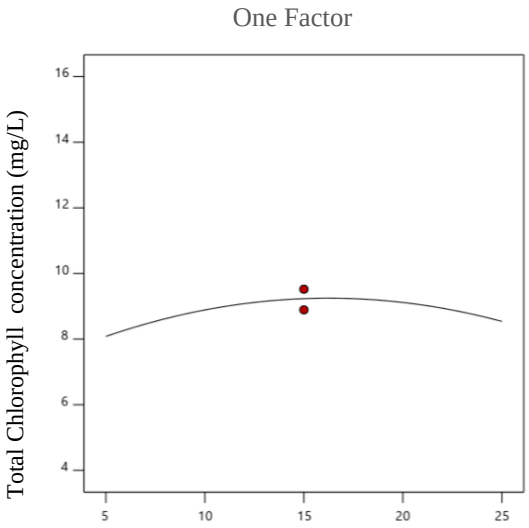

Figure 3. Effect of Time (minutes) on Total Chlorophyll Concentration

4 Conclusion

The results of chlorophyll extraction in cocoa leaves are influenced by time, the longer the extraction, the results will increase until it reaches the optimal limit. The results of the extraction of chlorophyll from cocoa leaves with the UAE method yielded a fairly good time. The highest yield of cocoa leaf extraction occurred at optimum conditions with a time of 15 min, which resulted in a total chlorophyll concentration of 8.98 mg/L. If the concentration of chlorophyll produced is the highest, the operating conditions that occur are the extraction time, an increase in the extraction time can cause the contact between the material and the solvent to be longer, and the extraction concentration will increase followed by an increased extraction time until it reaches the optimal limit.

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