THE PROCESSING AND ANALYSIS OF THE POLYPHENOLS CONTENT OF COCOA BEAN (*THEOBROMA COCOA* L) AND THE DEVELOPMENT AS FUNCTIONAL FOODS

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ABSTRACT

Production and quality of cocoa in South Sulawesi have been decreasing that causes it falls below the industrial standard. The quality of cacao bean is mainly determined by aroma and flavour produced from fermentation process of wet cacao bean before drying. In this research processing of various cacao bean without fermentation to polyphenols-rich cacao powder and polyphenols (catechin) analysis using HPLC have been carried out.

The experimental results showed that total polyphenols content of cacao bean (raw bean) was between 82.14 – 126.67 mg GAE/g powder. Cacao samples coded PLPK, BLKK and SWK (without boiling pretreatment) have polyphenols content of 113, 119.78, and 126.67 mg/g powder respectively. Whereas the polyphenols content of cacao sample coded PLP, SW and BLK (with blanching pretreatment) were 110.76, 113.75 and 82.14 mg GAE/g powder respectively. NP-HPLC analysis result showed that cacao bean from Palopo, Bulukumba and Siwa have high polyphenols content ranging from 149.38 mg/g to 367.21 mg/g powder.

Heat pretreatment decreases polyphenols content of cacao bean and it shows that polyphenols is not heat resistant. Polyphenols - rich cacao bean can be obtained without fermentation and heat treatment. Cacao bean from Bulukumba, Siwa and Palopo have high economic value when processed as polyphenols - rich health food products.

Keywords: *Cacao bean, Polyphenols, Catechin.*

1. INTRODUCTION

Cacao is an export comodity which contributes to increase state revenue. Indonesia is one of major cacao exporter after Ivory Coast (38.3%) and Ghana (20.2%) with percentage of 13.6%. International demand for cacao is always increasing. Until the year 2011, ICCO (International Cocoa Organization) estimates that global cocoa production will reach 4.05 million tons, while consumption will reach 4.1 million tons, so there will be a deficit of about 50 thousand tons per year. This condition is a good opportunity for Indonesia to become a major producer of cocoa world.

South Sulawesi as the main cocoa-producing area has contributed 70% of national production of cocoa beans. In 2003 the volume of exports of cocoa products are as follows: 258,545.994 tons of cocoa beans, 4281.627 tons cocoa butter, 2290.120 tons cocoa cake, 4187.076 tons of cocoa powder and 557.500 tons cocoa liquor (Sulawesi Plantation Office, 2003). Although the production of Indonesian cocoa beans is increasing significantly, but the quality is very low and varied. This is reflected in the price of Indonesian cocoa beans in the world market that is relatively low and charged discounted price compared to similar products from other producing countries. Indonesian cocoa quality, especially from South Sulawesi and West Sulawesi are lower compared to that cocoa from Ivory Coast and Ghana. The main causes of poor quality cocoa beans is due to the fermentation process that is not perfect or not fermented at all.

Unfermented cocoa beans contain a variety of compounds called polyphenols, approximately 60% of total polyphenols in cocoa beans (raw cocoa beans) are monomers flavanols (epicatechin and catechin) and pro-sianidin oligomers (dimers and dekamer) with varying concentrations. According to Osakabe *et al*. (1998), concentrations of epicatechin in cocoa seed extract is estimated 7-10 times higher than that of cocoa liquor. The content of polyphenols and flavonoids in cocoa /chocolate products depends on several factors such as: varieties of cocoa beans, post-harvest handling, fermentation, drying and roasting.

Fermentation and roasting process of cocoa bean was caused the decreased of polyphenols in the process of fermentation and roasting leads to reduced cocoa flavanol content, while processing cocoa with alkali (alkalization process) can reduce content of flavanol and procyianidin (L. Stahl, *et al* 2009).

Amin Ismail., *et al* (2010) reported that the ethanol extract of unfermented cocoa beans from South Sulawesi showed the highest antioxidant activity compared with extracts of cocoa beans from Malaysia,

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Ghana, Cote d'Ivoire (CD). This is due to the presence of the main components in the form of high polyphenols epicatechin compound. Giving extract polyphenols in test animals was able to reduce the level of plasma glucose and stimulate insulin secretion in patients with diabetes mellitus type 2 (Amin *et al* (2010). Epidemiological data indicate 50% of stroke and heart attack in the elderly can be reduced if consuming cocoa products that are rich in polyphenols or flavanols (Buijsse and others 2006). Therefore, the aim of this study is focused on the production process of cocoa powder rich in polyphenols from cocoa beans without fermentation and its development as a health product.

2. METODOLOGI

2.1 Material and Equipment

The materials used in this study is the fruit of the cocoa from cocoa production center in South Sulawesi (Bulukumba, Palopo and Shiva), ethanol, methanol, acetone, hexane, Folin - Ciocalteus reagent (Merck), catechins (Siqma Chemicals). The main equipment for the analysis of polyphenols is Spektrofotmeter UV-VIS and HPLC (Shimadzu), sieving and fat press machine tool.

2.2. Cocoa Bean Sample Processing

Riped Cocoa pods were peeled, seeds were removed and washed with water to remove the pulp attached to the seed. cocoa beans were then boiled with hot water at a temperature of 80 ° C (blanching) for 10 minutes for inactivate the polyphenol oxidase enzyme (PPO). As a control was the same sample of cocoa beans without blanching. Cocoa beans were sun dried until it reaches 5% seed moisture content. The outer cocoa seed skin was removed to obtain cocoa beans without skin (nibs). Nibs were cracked then blended until smooth, cocoa powder was pressed with high pressure to approximately 10% fat remaining. Cocoa powder was crushed and blended back into powder and sieved to obtain cocoa powder with a size of 100 mesh.

2.3. Extraction and Cocoa Total Polyphenols Determination.

Fat content of cocoa powder samples were separated by addition of 200 ml of solvent hexane and stored for 24 hours, then centrifuged at 2,500 rpm for 10 minutes. This method was repeated 3 times with hexane solvent so that a total of 600 mL of solvent used. 1 gram of fat-free cocoa powder was extracted three times with acetone: water (7:3 v/v) and then centrifuged at 2,500 rpm for 10 minutes. Total extracts of acetone-water as much as 60 mL was diluted with acetone - water into a 100 mL flask. Total polyphenols content of cocoa is determined by the modified Folin-Calciteau method. A total of 1 mL acetone-water extract incorporated into 50 mL flask, 20 mL distilled water and 2 mL of Folin-Calciteau reagent was added, then shaken and allowed to settled for 5 minutes, then 20 mL of Na2CO3 15% was added and filled up to the mark, incubated at room temperature for 90 minutes. The absorbance was measured at a wavelength of 750 nm. Catechins are used to generate a standard calibration curve at various concentrations (5 ppm - 60 ppm). Total polyphenols content expressed in mg catechin equivalent per gram of cocoa powder (mg CE/g cocoa powder).

2.4. Identification of Cocoa Catechins using HPLC method.

A total of 1 gram of fat-free cocoa powder is extracted with acetone: water (70:30) at room temperature with shaking. Centrifugation was done at 2,500 rpm for 20 minutes the filtrate was taken while the precipitate or residue was discarded. The filtrate was saturated with NaCl, the bottom layer was removed and the upper layer (acetone: water) was taken and concentrated by rotary evaporator to dryness. Polyphenol extract powder was added with 15 mL of water and washed with chloroform 4 times. The aqueous phase was taken and filtered with a 0.45 um filter paper. The filtrate was diluted with methanol or water to 25 mL for the catechin content analysis by NP HPLC method (Nelson and Sharpless, 2003). Type column Shim Pack VP-ODS with a size of 250 x 4.6 mm, detector UV systems, mobile phase mixture of methanol: water 70: 30 with a flow rate of 1mL / min were used. Catechin content was determined by UV detector system at a wavelength of 280 nm with catechins as standard.

3. RESULT AND DISCUSSION

3.1. Total Polyphenols Content

Polyphenols content of cocoa or cocoa derivative products depend on cocoa plant varieties (genetic, agronomical, and others), and a number of factors related to post-harvest processing. In previous studies it has been reported that the fermentation of cocoa beans for 5-7 days can produce cocoa beans with a distinctive aroma of chocolate accompanied with good physical characteristics such as fine grain leather, thin and round seed shape. However, the content of polyphenols in cocoa bean fermentation results showed a significant reduction compared to the polyphenol content of unfermented cocoa beans. Decreased content of polyphenols from cocoa beans during fermentation due to the diffusion of the compound out of pieces of seed through the release of water molecules and then oxidation and condensation reactions of polyphenolic compounds catalyzed by the enzyme polyphenol oxidase. Preparation of polyphenols rich cocoa powder from various samples are shown in Figure 4.2, while the total polyphenols cocoa powder from various samples are shown in Table 4.1.

Tabel 4.1 Polyphenols Content (gram sampel fat free cocoa powder)

No	Sampel Code	Total Polyphenols (mg/g powder)	Remarks
1	PLP 1	111.51	Blanching
2	PLP2	110.09	Blanching
3	PLP3	119.78	Without Blanching
4	SW1	82.14	Blanching
5	SW2	126.67	Without Blanching
6	BLK1	113.75	Blanching
7	BLK2	120.14	Without Blanching
8	BLK	184.27 to 101.92 188,69 to 91.76	Fermented cocoa bean (Pirman, 2008)

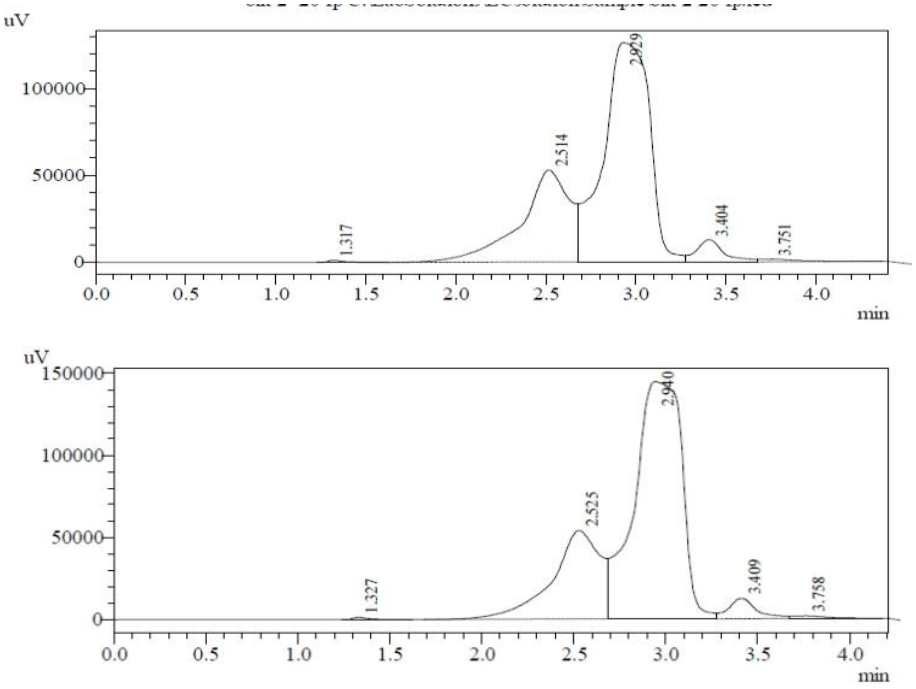
Total polyphenols content of cocoa beans samples (raw cocoa bean) ranged between 82-126 mg / g cocoa powder. Samples cocoa PLP3, BLK2 and SW2 without boiling treatment had higher levels of polyphenols at 113 mg / g powder, 119.78 mg / g powder and 126.67 mg / g powder respectively. In contrast levels of cocoa polyphenols sample code PLP1, PLP2, SW1, BLK1) with successive boiling treatment 111.51 mg / g powder, 110 mg / g powder, 113.75 mg / g powder and 82.14 mg / g powder respectively. Heat treatment (boiling) for 10 minutes causes reduction of polyphenols levels in the sample. This suggests that the cocoa polyphenols compounds are not resistant to heat. Another experimental result showed that the content of total flavonoids of cocoa beans samples (raw cocoa bean) from the Bulukumba and Palopo were quite high ranging from 171.880 to 180.567 mg / g cocoa powder.

Cocoa samples Palopo (CK4 code) without boiling have a highest total polyphenols and flavonoid (180.567 mg / gram samples of cocoa powder and 59.512 mg / g powder samples). Test showed that the antioxidant content of total polyphenols and total flavonoids was positively correlated with antioxidant activity (Pirman, 2011). Therefore, to obtain a cocoa powder with high polyphenols content of the proposed method involves the processing of cocoa beans without fermentation process, boiling and roasting. Osakabe et al. (1998) reported that cocoa extracts obtained from cocoa liquor showed potent antioxidant activity. High antioxidant capacity of cocoa powder due to the existant of phenolic compounds in the form of monomers, dimers and trimers mainly catechins and epicatechin compounds (Ismail and Abbe Maleyki, 2010). The quantity of phenolic compounds and flavonoids determine antioxidant activity of cocoa beans and cocoa products.

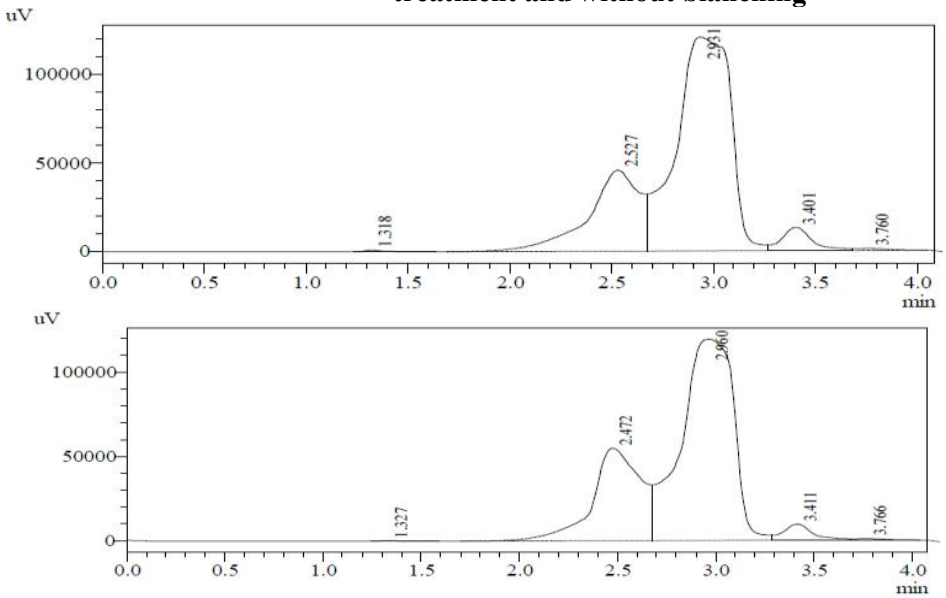
3.2. Profile of Cocoa Catechin Content

Cocoa catechin compounds can be identified using NP-HPLC method. Catechin chromatogram profile of various cocoa samples are shown in Figure 4.3 - 4.5. The chromatograms in Figure 4.3- 4.5 has different a retention time, area (area) and the peak heights. Peak with retention time 2.929, 2940, 2.931, 2.960, 2.939, 2.952, 2.921 and 2972 is catechin compounds and in accordance with the retention time standard

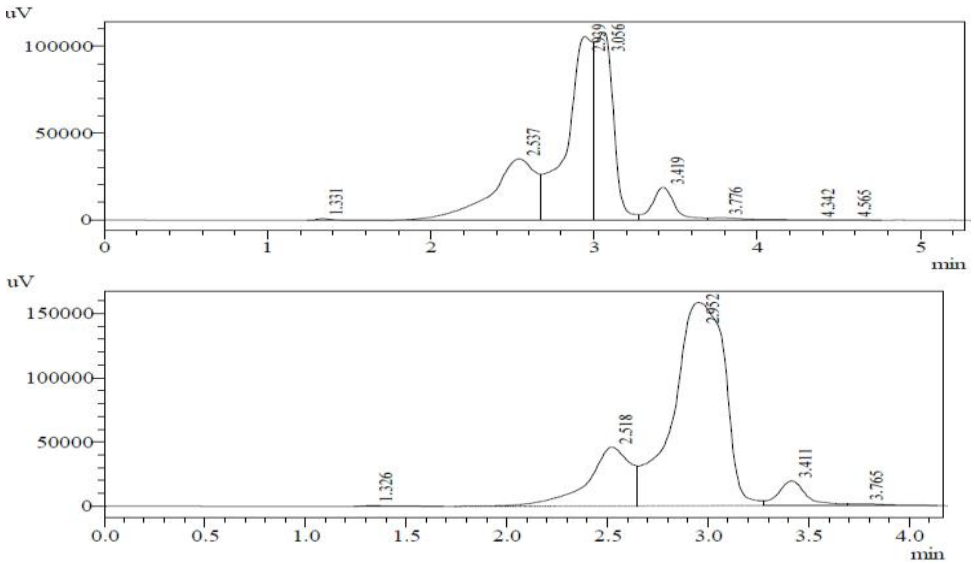
compounds (catechins). Results calculated with standard catechin showed that cocoa beans from different regions (Palopo, Bulukumba and Shiva) contain catechin compounds with varying levels of 149.38 mg / g cocoa powder up to 367.21 mg / g cocoa powder.



Gambar 4.3. NP - HPLC Chromatogram of catechin compounds of Bulukumba cocoa with boiling treatment and without blanching



Gambar 4.4. NP - HPLC Chromatogram of catechin compounds of Palopo cocoa with blanching treatment and without blanching



Gambar 4.5 NP - HPLC Chromatogram of catechin compounds of Siwa cocoa with blanching treatment and without blanching

Bioactive compounds (epicatechin and catechin) in cocoa has been identified by HPLC-DAD. According Osakabe et al. (2002), that epicatechin and catechin are the main components in cocoa powder. The content of cocoa beans epicatechin levels ranged between 270 - 1235 mg / 100 grams of cocoa beans. Sulawesi cocoa beans showed the highest levels of epicatechin followed cocoa Malaysia, Ghana, and Cote dIvoirian (Othman et al. 2010).

4. CONCLUSION

Based on the above results, it can be concluded that the unfermented cocoa beans from South Sulawesi (Bulukumba, Siwa and Palopo) shows high content of polyphenols, flavonoids (catechins) and thus potentially be developed into a product that has economic value as a health food product.

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