Profile of Bioactive Compounds and Antioxidant Capacity of Indonesian Cocoa Powder: A Case of Food Processing Authentication

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Abstract: One aim of food authentication for cocoa powder is to prove the manufacturing process. Cocoa powder is one of the derivative products of cacao resulting from crushing and refining cocoa cake. Cocoa cake is processed further through alkalization and/or grinded to become cocoa powder. The major bioactive compounds found in cocoa powder are polyphenols and methylxanthines that potential to be sources of antioxidants. The objectives of this research are to identify bioactive compounds and antioxidant capacity of Indonesian cocoa powder from different sources for identifying the manufacturing process of alkalization. Profile of bioactive compounds was identified by using HPLC-UV. DPPH and FRAP method was used for the quantification of antioxidant capacity. Folin-Ciocalteau method was used for total phenolic content. The total phenolic content of Indonesian cocoa powder ranged from 14.80 – 79.93 mg (GAE/g). Antioxidant capacity using DPPH method ranged from 91.67 – 362.24 (µmol TE/g) and FRAP method ranged from 249.16 – 1000.95 (µmol Fe^{2+}/g). The average content of theobromine in cocoa powder ranged from 1.89 – 3.14 (mg/g) respectively. For caffeine content, ranged from 0.12 – 0.46 (mg/g). Levels of (+)-catechin in 9 samples ranged from 0.04 – 1.10 (mg/g) respectively. Whereas the average content of (-)-epicatechin ranged from 0.04 – 4.68 (mg/g). Strong positive correlation with Pearson test was established between total phenolic content and antioxidant capacity with (R^2 = 0.99) for DPPH and (R^2 = 0.97) for FRAP. Higher total phenolic content indicates higher antioxidant capacity. Analysis of PCA divides the sample based on similarity of chemical characteristics. The right quadrant on PCA analysis was a group of natural cocoa powder, illustrates the similarity of color and higher content of theobromine, (-)-epicatechin, caffeine, and fat content. The left quadrant was a group of alkalized cocoa powder, illustrates the similarity of higher pH and (+)-catechin content of Indonesian cocoa powder.

1 INTRODUCTION

One of the largest agricultural commodity in Indonesia is cocoa. In 2014, Indonesian cocoa production reached 728,414 tonnes with the largest production found in Celebes approximately 484,387 tonnes. Cocoa widely used by the food industry as raw materials in the field of confectionary and non-confectionary. Cocoa derivative products include cocoa paste, cocoa butter, cocoa cake, cocoa powder, and chocolate products such as dark chocolate, milk chocolate, and white chocolate. One of the cocoa derivative products are widely used in the food industry is cocoa powder.

A number of studies have been reported on the benefits of cocoa powder to human health (Cooper *et al.,* 2008; Ramljak *et al.*, 2005). Polyphenol compounds contained in cocoa powder has potential as an antioxidant that can significantly contribute to human health (Abbe and Ismail 2010). The polyphenol content of the cocoa powder has a high correlation to the antioxidant capacity. Antioxidant capacity of cocoa per serving is greater than green tea or black tea (Joli'c *et al.* 2011). The main polyphenol compounds in cocoa are flavan-3-ol, anthocyanine and procyanidins where (+)-catechin and (-)-epicatechin are the monomers of flavanol component (Weisburger 2001). In vitro studies have shown that the polyphenol content in cocoa powder can inhibit

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reactive species such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid; ABTS) and superoxide radicals. The polyphenol content of cocoa powder also can inhibit the lipid peroxidation and chelate free pro-oxidant metal ions (Fe^{2+}, Cu).

Cocoa products are also rich in methylxanthine compounds such as caffeine, theobromine, and theophylline (Rios *et al.,* 2003). Theophylline is found in very small quantities in cocoa products and derivatives (Franco *et al.,* 2013). Methylxanthine compounds contribute to the bitter taste of the cocoa product. According to Pinilla *et al*., (2015), the combination of caffeine and theobromine in cocoa has benefited as antitumor, anti-inflammatory and protective action from cardiovascular disease.

Consumption of Indonesian cocoa powder is expected to increase in the years 2016-2020 with an average growth of 1.17% per year (Pusdatin, 2016). The increasing consumption of Indonesian cocoa powder not supported yet with the information about the profile of bioactive compounds and antioxidant capacity contained. Since the level of bioactive compounds and positive effects of polyphenol, methylxanthines in cocoa powder are affected by alkalization process in the manufacturing and limited information about it, the objectives of this research are to identify bioactive compounds and antioxidant capacity of Indonesian cocoa powder from different sources for identifying the manufacturing process of alkalization.

2 MATERIAL AND METHODS

2.1 Material

The main materials were cocoa powder samples produced by PT. Ceres Industrial Company Bandung, PT. Bumitangerang Mesindotama Tangerang, household Industry KSU "Guyub Santoso" Blitar, Aneka Food "Kopkar Sekar" Jember, Big Tree Farm Bali, Tanjung Subur Farmer Group Padang, West Sumatra, and bulk cocoa powder from Makassar, South Sulawesi. Chemicals needed are acetone 80% (Mallinckrodt, USA), reagent Folin-Ciocalteu 50% (Merck, Germany), n-hexane, Na_2CO_3 20%, glacial acetic acid (Merck, Germany), gallic acid solution, standard trolox (Sigma, Switzerland), standard $FeSO_4.6H_2O$, distilled water, a solution of 300 mM acetate buffer pH 3.6; TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCL, the solution $FeCl_3 \cdot 6H_2O$ 20mm, 10^4 mM DPPH reagent, methanol (Merck, Germany), theobromine standard (Sigma, USA), caffeine standard (Sigma, USA), (+)-catechin standard (Fluka 22110), (-)-epicatechin standard (Sigma E1753), metanol HPLC *grade* (Merck, Germany) water HPLC *grade* (Merck, Germany).

2.2 Extraction Preparation

All samples were prepared for analysis as described by Brcanovic *et al.,* (2013) with slight modifications. A mass of 2 g of cocoa powder was defatted with hexane by using Soxhlet apparatus and the residue was dried in oven 105 ^{0}C 30 minutes. Defatted cocoa powder (0.15 g) was then extracted with acetone/ water/ acetic acid (70.29.5.0.5 by volume) using a sonicator (37 ° C, 10 min) and then centrifuged (1500 rpm, 10 min). The resulting supernatant was decanted to a 10-mL volumetric flask and diluted with solvent to the mark. The resulting supernatant here after referred as extract of cocoa powder.

2.3 Fat, Color, and pH Analysis

Total fat of cocoa powder was determined by Soxhlet apparatus. The color of cocoa powders was measured by using Chromameter with Hunter Lab output notation. the Hunter L scale measures degree of lightness 0 (black) to 100 (light), the Hunter a scale measures red to green with true red equal to +100 and true green equal to -100, and the Hunter b scale measures yellow to blue with true yellow equal to +100 and true blue equal to -100. Analysis of pH was conducted by using pH meter. 1 part of cocoa powder dissolves with 10 part of water.

2.4 Total Phenolic Content (TPC)

The total phenolic content was determined by using Folin-Ciocalteu method with gallic acid as standard according to Miller *et al.,* (2008). 1000 ppm of gallic acid was prepared and diluted to concentrations ranging from 50-600 ppm to create a standard curve. For each analysis, 0.5 ml solution of gallic acid or extracts of cocoa powder was added to 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent 50% then vortexed. After that, 1 ml of 20% Na_2CO_3 was added and incubated in the dark at room temperature for 30 min. Total polyphenols were measured from the absorbance at 755 nm. Total phenolic content expressed in milligrams of gallic acid equivalents per gram defatted cocoa powder.

2.5 DPPH (1,1-diphenyl-2-picrylhydrazyl) Assay

Antioxidant capacity with DPPH method was determined by using a method described by Brcanovic *et al.,* (2013). DPPH reagent (10^{-4} mol/L) was dissolved using methanol. 0.5 ml of extracts cocoa powder was added to 4.5 ml of reagent DPPH then vortexed. The solution then performed incubation for 30 minutes in a dark room. The color change of solution was measured using UV-Vis spectrophotometry at 520 nm. Trolox standard curve ranging from 50-400 µM was used to calculate the final result of DPPH. Antioxidant capacity of DPPH method was expressed in micromoles Trolox equivalents (TE) per gram defatted cocoa powder.

2.6 FRAP (Ferric Reduction Antioxidant Power) Assay

FRAP assay was measured by using methods of Benzie and Strain (1996). FRAP reagent was prepared from 2.5 ml of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40mM hydrochloric acid with 2.5 mL of 20 mM iron (III) chloride and 25 ml of 300 mM acetate buffers at pH 3.6. The FRAP reagent was prepared fresh daily and warmed to 37 °C in a water bath. 200 µL of cocoa powder extract was added to 1.3 ml of FRAP reagent and allowed to react 30 minutes in 37 °C water bath. The absorbance of the reaction mixture was recorded at 593 nm using UV-Vis spectrophotometer. The standard curve was constructed using ferrous sulfate with concentrations ranging from 50-400 µM and the result was expressed in micromol ferrous equivalent per gram defatted cocoa powder.

2.7 Analysis of Individual Bioactive Compounds using HPLC

Individual bioactive compounds were determined using an RP-HPLC (Shimadzu SPD-20A). Determination of individual phenolic compounds ((+)-catechin and (-)-epicatechin)) and methylxanthines (theobromine and caffeine) was performed according to the method described by Ramli *et al.,* (2001). Separation was performed with C18 (150 mm ID x 4.6 mm, 5 m) column, at flow rate 1 mL/min and an injection volume of 20 µL. Detection was performed by scanning at 280 nm. Isocratic elution was used in this method with mobile phase Methanol: water: acetic acid (20: 79: 1) at runtime 17 min.

The standard mixture used as a standard for calibration curve. 50 mg of each standard consisting of theobromine, (+)-catechin, caffeine and (-)-epicatechin was weighed then dissolved using mobile phase in a 25 ml volumetric flask. Mix standard curve has five points using a dilution series ranging from 10 - 160 ppm. Extracts of cocoa powder prior to use was filtered by using PTFE 0.45 µm microfilter to prevent any impurities. Identification was carried out by comparing the retention times with standards. Quantitative determination of individual bioactive compounds in the sample was done using calibration lines of standard curves.

2.8 Statistical Analysis

All determination was carried out in two replication and each replication was measured in duplicates. Data were subjected to one-way analysis of variance (ANOVA) and the level of significance of ($p < 0.05$) using SPSS version programs. The Duncan's Multiple Range Test (DMRT) was used to separate the means. A significant difference was considered at level ($p < 0.05$). Correlations between each analysis were conducted by Pearson test. Principal component analysis (PCA) was used to make the results more easily interpretable.

3 RESULTS AND DISCUSSION

3.1 Characteristics of Total Fat, pH, and Color of Cocoa Powder

Cocoa powder is divided into two types, natural cocoa powder and alkalized cocoa powder. Dutch process or alkalization process performed by washing cocoa powder with a solution of potassium which aims to neutralize the acidity of the cocoa beans, relieve astringent flavor, and initiate the reaction between pigment cocoa with alkali due to the presence of oxygen and heat that caused a reddish brown color to a dark color or often called dark cocoa (Dyer 2003). The darker the cocoa powder and the higher the pH contained in cocoa powder indicated a high degree of alkalization. Characteristics of pH, color and fat content of Indonesian cocoa powder is presented in Table 1.

The powders range from pH 4.90 for sample 8, to pH 8.10 for sample 4. Cocoa powders have been grouped by pH ranges, described by Miller *et al.,* (2008) into lightly alkalized (pH 6.5-7.2), medium alkalized (pH 7.21-7.60), and heavily alkalized (pH 7.61). Total fat ranged from 11.27 – 37.57 %. Total

fat contained by alkalized cocoa powder was higher than natural cocoa powder samples. Color measurement of cocoa powder by Chromameter with Hunter Lab notation scale shows L scale value decrease on alkalized cocoa powder. The results show that natural cocoa powders a bright color appearance and alkalized cocoa powder has a darker color. 1, 2, 3, 4 and 5 has a darker color and higher pH (> 7) than the other samples. This indicates those samples had undergone a process of alkalization.

3.2 Total Phenolic Content

Analysis of total phenolic in cocoa powder uses Folin-Ciocalteau method. The total phenolic content based on data obtained in Table 2 ranging from 14.80 - 79.93 mg Gallic Acid Equivalent per gram of defatted cocoa powder dry basis. Results of total phenolic content were supported by research from Ramli *et al.,* (2001) that was 20 - 62 (mg GAE / g) and Miller *et al.,* (2008) that was 7 - 63 (mg GAE/g). The total phenolic content of samples from the various regions and brands in Indonesia have significant differences value ($P < 0.05$). The highest value of TPC was shown by sample 9 from West Sumatra (0.37 ± 79.93 mg GAE / g) and the lowest value was shown by sample 4 from Tangerang (14.80 ± 0.23 mg GAE/g). Alkalized cocoa powder samples (1,2,3,4,5) have a lower value of TPC compared with natural cocoa powder samples (6,7,8,9). According to the study of Miller *et al.,* (2008), alkalization process could damage the flavanol compounds, resulting in the decrease of TPC on cocoa powder. Beside alkalization, TPC of cocoa powder can be influenced by the processing involved in the production of cocoa powder including fermentation, drying, and roasting. These treatments affect the content of polyphenols in cocoa powder (Thomas-Barberan *et al.,* 2012).

Table 1: Characteristics of total fat, pH, and color of Indonesian cocoa powder.

Sample	Total fat[1] (%)	pH[2]	Color identification			Category
			L	a	b	
1	16.38 ± 1.22	7.13 ± 0.11	30.47	+8.34	+9.35	Alkalized
2	17.01 ± 2.71	7.16 ± 0.03	30.05	+8.59	+9.38	Alkalized
3	13.43 ± 1.72	7.13 ± 0.05	31.66	+8.40	+8.70	Alkalized
4	11.27 ± 0.35	8.10 ± 0.02	24.65	+5.41	+5.69	Alkalized
5	15.52 ± 0.07	7.44 ± 0.06	21.98	+6.09	+5.59	Alkalized
6	27.84 ± 1.30	6.15 ± 0.05	34.98	+7.79	+8.96	Natural
7	29.22 ± 0.41	5.16 ± 0.07	34.32	+8.28	+8.63	Natural
8	37.57 ± 0.31	4.90 ± 0.03	23.83	+5.52	+5.86	Natural
9	22.84 ± 0.42	5.71 ± 0.10	37.29	+8.39	+9.57	Natural

[1]Values listed in the column is the mean ± SE; n = 2
[2]Values listed in the column is the mean ± SD; n = 3

Table 2: Total phenolic and antioxidant capacity of Indonesian cocoa powder.

Sample	Production Origin	Total Phenolic Content (mg GAE/g)	DPPH (mikromol TE/g)	FRAP (mikromol Fe^{2+}/g)	T* (mg/g)	Caff* (mg/g)	C* (mg/g)	EC* (mg/g)
1	Bandung	36.90 ± 0.22^{d}	183.96 ± 2.38^{d}	540.68 ± 2.78^{d}	1.99 ± 0.01^{a}	$0.22 \pm 0.15^{b,c}$	1.10 ± 0.02^{e}	1.88 ± 0.03^{d}
2	Bandung	34.19 ± 0.40^{c}	158.74 ± 1.75^{c}	473.00 ± 2.85^{c}	2.51 ± 0.21^{b}	0.27 ± 0.19^{c}	1.06 ± 0.06^{e}	$1.97 \pm 0.02^{d,e}$
3	Tangerang	38.32 ± 0.20^{e}	185.33 ± 1.26^{d}	555.55 ± 5.84^{d}	2.39 ± 0.09^{b}	$0.16 \pm 0.11^{a,b}$	0.73 ± 0.00^{d}	0.77 ± 0.01^{b}
4	Tangerang	14.80 ± 0.23^{a}	91.67 ± 3.05^{a}	249.16 ± 2.45^{a}	1.89 ± 0.16^{ac}	0.12 ± 0.09^{a}	1.07 ± 0.06^{e}	0.04 ± 0.01^{a}
5	Blitar	20.93 ± 0.53^{b}	119.10 ± 2.20^{b}	367.02 ± 17.13^{b}	$2.69 \pm 0.15^{b,c,d}$	0.26 ± 0.18^{c}	0.81 ± 0.02^{d}	0.08 ± 0.01^{a}
6	Jember	67.76 ± 0.20^{g}	310.14 ± 5.93^{f}	826.97 ± 20.89^{f}	$2.89 \pm 0.00^{c,d}$	0.46 ± 0.32^{d}	0.23 ± 0.01^{b}	4.68 ± 0.01^{g}
7	Bali	70.47 ± 1.13^{h}	346.85 ± 4.29^{g}	1000.95 ± 18.19^{h}	2.97 ± 0.14^{d}	0.42 ± 0.29^{d}	0.38 ± 0.00^{c}	2.42 ± 0.13^{f}
8	South Celebes	61.91 ± 0.58^{f}	296.39 ± 4.15^{e}	682.27 ± 0.34^{e}	2.43 ± 0.07^{b}	0.43 ± 0.31^{d}	0.04 ± 0.00^{a}	1.65 ± 0.02^{c}
9	West Sumatera	79.93 ± 0.37^{i}	362.24 ± 3.10^{h}	934.32 ± 30.49^{g}	3.08 ± 0.01^{d}	$0.17 \pm 0.12^{a,b}$	0.43 ± 0.01^{c}	2.09 ± 0.00^{e}

**T = theobromine, C = (+)-catechin, Caff = Caffeine, EC = (-)-epicatechin*
Results are the means of two ± standard deviation. Values accompanied by different letters in the same row statistically different ($p<0.05$)

Effect of fermentation of cocoa beans showed polyphenol content of fermented cocoa beans smaller than the unfermented cocoa beans (Prayoga *et al.,* 2013). Fermented cocoa beans decline in the polyphenol content due to oxidation, polymerization and proteins binding (Ramli *et al.,* 2006). Generally, processing of cocoa powder that has a higher temperature or longer time will reduce the levels of polyphenols in cocoa powder component as a result of chemical reactions such as acceleration of oxidation reactions (Bernaert *et al.,* 2012).

3.3 Antioxidant Capacity

The antioxidant capacity of Indonesian cocoa powder was evaluated by using DPPH and FRAP (Ferric Reducing Antioxidant Power) assay. The identification results of antioxidant capacity using the two methods are presented in Table 2. The results of DPPH antioxidant capacity stated by micromoles Trolox equivalent per gram of defatted cocoa powder dry basis, whereas for FRAP methods expressed by Fe^{2+} micromoles per gram of defatted cocoa powder dry basis.

The capacity of antioxidants in Indonesian cocoa powder depends on the number of hydroxyl groups which can inhibit the chain reaction of free radicals associated with hydrogen donors. High levels of antioxidant in cocoa powder increase the chances of hydroxyl and hydrogen donors of free radicals (Tamrin 2012). The antioxidant capacity of DPPH method ranged from 91.67 - 362.24 (mol TE / g). The results supported by research of Genovese *et al.,* (2009), which has an antioxidant value of 120 mol TE / g, but higher than the results reported by Brcanovic *et al.,* (2013) that was 11.65 - 32.01 (mol TE/g). Sample 4 had the lowest antioxidant capacity value (91.77 mol TE/g) and sample 9 had the highest antioxidant capacity of DPPH method (362.24 mol TE/g). All samples were significantly different at the 5% significance level except for sample 1 and 3.

The ability for reducing radical compounds is the latest antioxidant defense mechanisms. This mechanism is divided into electron transfer and hydrogen atom transfer (Oboh and Omoregie, 2011). FRAP method used in this research to calculate the ability to reduce Fe^{3+} to Fe^{2+}. FRAP methods have antioxidant capacity value ranging from 249.16 - 1000.95 (mol Fe^{2+} / g). Results of this study were lower than the results Oboh and Omoregie (2011) that was 1466.33 - 2097.12 (mol Fe 2+ / g), but higher than the Brcanovic *et al.,* (2013), 22:45 - 137.51 (mol Fe 2+ / g).

3.4 Profile of Bioactive Compound

Identification of bioactive compounds of Indonesian cocoa powder using HPLC with UV detector using a wavelength of 280 nm. Methylxanthines and Phenolic compounds are identified. The HPLC chromatogram of bioactive compounds of cocoa powder and standard can be seen in figure 1. A retention time of bioactive compounds for theobromine, (+)-catechin, caffeine, and (-)-epicatechin were 3.3, 5.08, 8.5, and 11.8 minutes, respectively. The results of HPLC analysis are given in Table 2.

Theobromine content of Indonesian cocoa powder ranged from 1.89 – 3.14 (mg/g). These results lower than research from Lo Coco *et al.*, (2007) that was 4,6- 26 (mg/g). The caffeine content ranged from 0,12 – 0.46 (mg / g). The previous studies found the caffeine content 1.74 - 7:53 (mg/g) from Malaysian cocoa powder (Ramli *et al.,* 2001) and 0.15 - 1.42 from Croatian chocolate manufacture (Belscak *et al.,* 2009). Methylxanthine compound decreased during the fermentation process up to 30% of the initial methylxanthine content. The decreasing of methylxanthine is caused by the diffusion of alkaloids from cotyledon (Nigam and Singh, 2014). The concentration of methylxanthine decreased with the increasing of alkalization process, decreasing of the total theobromine content due to alkalization process reaches 20% (Li *et al*., 2012). This is consistent with the result that shows alkalized cocoa powder was lower in methylxanthine content than natural cocoa powder. The HPLC results show that theobromine compound is the highest bioactive compound of Indonesian cocoa powder. Consumption of theobromine from cocoa can significantly increase plasma HDL cholesterol, lowering LDL concentrations in plasma, providing cardiovascular protection and reducing the risk of coronary heart disease (Khan *et al.,* 2012).

Individual phenolic compounds identified in this study were (+)-catechin and (-)-epicatechin given in table 2. (+)-Catechin ranged from 0.04 to 1.10 (mg/g). This result is supported by the research of Belscak *et al.,* (2009) that found 0.04 to 0.33 (mg/g) and Brcanovic *et al.,* (2013) that found 0.03 to 0.18 (mg/g). The (-)-epicatechin content of Indonesian cocoa powder varied from 0.04 - 4.68 (mg/g). Ramli *et al.*, (2001) reported (-)-epicatechin content of Malaysian cocoa and chocolate product ranged from to 0.48 to 6.32 mg/g), but the result was greater than the results from Brcanovic *et al.,* (2013) that was 0.04 to 0.14 (mg/g).

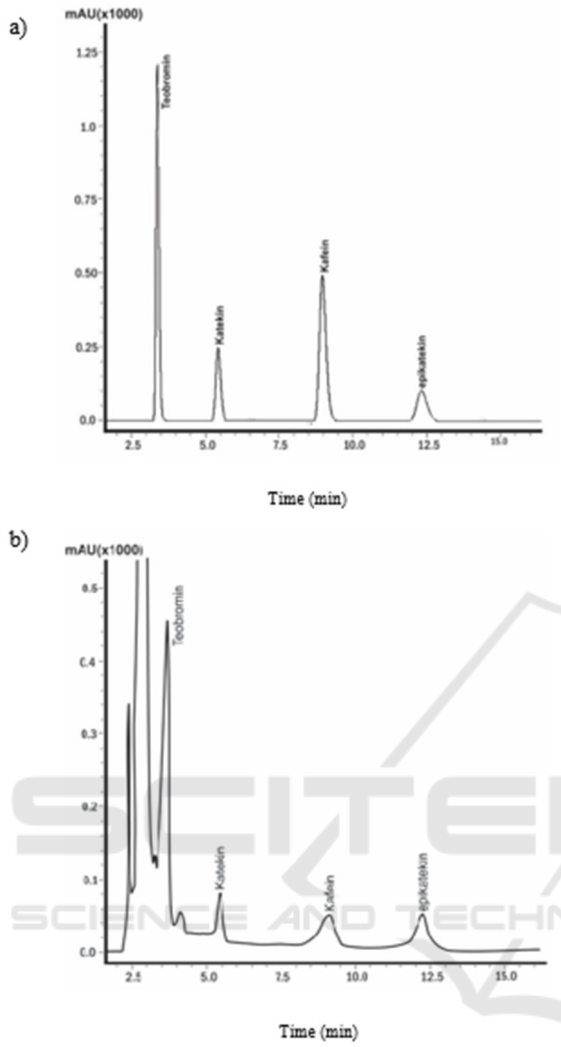

Figure 1: Chromatograms of: a) standard; b) cocoa powder sample. Analyzed using HPLC with UV detector at a wavelength of 280 nm.

The (-)-epicatechin content is very influenced by the level of processing cocoa powder. Meng *et al*., (2009) reported the fermentation process can significantly reduce the epicatechin content up to 10-20 %. Sample 1,2,3,4,5 has a lower level of (-) – epicatechin, it assumed that those samples have undergone high-temperature of roasting. Roasting significantly affect the level of polyphenols. Roasting at higher temperatures induce the epimerization of (-)- epicatechin into (+) - catechin (Hurst *et al.,* 2011). This theory supported by the results that show the concentrations of (+) - catechin were higher in the alkalized samples (1, 2, 3, 4, 5). Alkalization process has been reported to reduce the content of (-)-epicatechin up to 98% and (+)-catechin up to 80% (Giacometti *et al.,* 2015). This is supported by data results that showed that the alkalized cocoa powder samples have a higher value of (+)-catechin and the lower value of (-) – epicatechin than natural cocoa powder.

Principal component analysis (PCA) was performed to classify samples based on the similarity of their chemical properties. Scatter plots analysis of PCA are illustrated in Figure 2. The components F1 and F2 represents the total diversity of 87.03% is considered quite describe the variance of the data structure. PCA results divided the alkalized and natural cocoa powder into left and right quadrant. Samples 6, 7, 8 and 9 (natural) are in the right quadrant and the alkalized samples are on the opposite sides. The right quadrant has a lighter color of cocoa powder and has similarity on high content of theobromine, (-) - epicatechin, caffeine, and total fat. These group of samples has high TPC and antioxidant capacity.

The left side of PCA analysis is identified of alkalized cocoa powder group. Samples 1, 2, 3, 4, 5 located in the left quadrant has the similar characteristics of higher pH and (+)-catechin content. These group of samples has low TPC and antioxidant capacity level. The pH value contained in the cocoa powder has a distant quadrant with total phenol and antioxidant capacity, it can be concluded that a higher pH value can reduce total phenolic content and antioxidant capacity.

Correlation between analysis was performed by Pearson correlation test. The result is shown in Table 3. TPC has a strong correlation with antioxidant capacity both DPPH and FRAP assay with the significance level ($p < 0.01$). These results confirm a relationship between their free radical scavenging and ferric reducing capacities with the concentration of phenolic compounds in cocoa powder. Therefore, the presence of phenolic compounds in the Indonesian cocoa powder samples contributes significantly to their antioxidant potential (Brcanovic *et al.*, 2013). Analysis of color and fat were also correlated ($p<0.05$) with TPC and antioxidant capacity. Strong negative correlation ($p< 0.01$) was also observed between pH, TPC, and antioxidant capacity. Higher pH contained by Indonesian cocoa powder decrease the level of TPC and antioxidant capacity.

Theobromine and (-)-epicatechin had no significant correlation at level ($p <0.05$) to TPC. Theobromine had a significant correlation at level ($p <0.01$) with antioxidant capacity. This is presumably due to the structure of theobromine that similar to uric acid which has a mechanism of secondary antioxidant (Azam *et al*., 2003). Caffeine compounds do not have

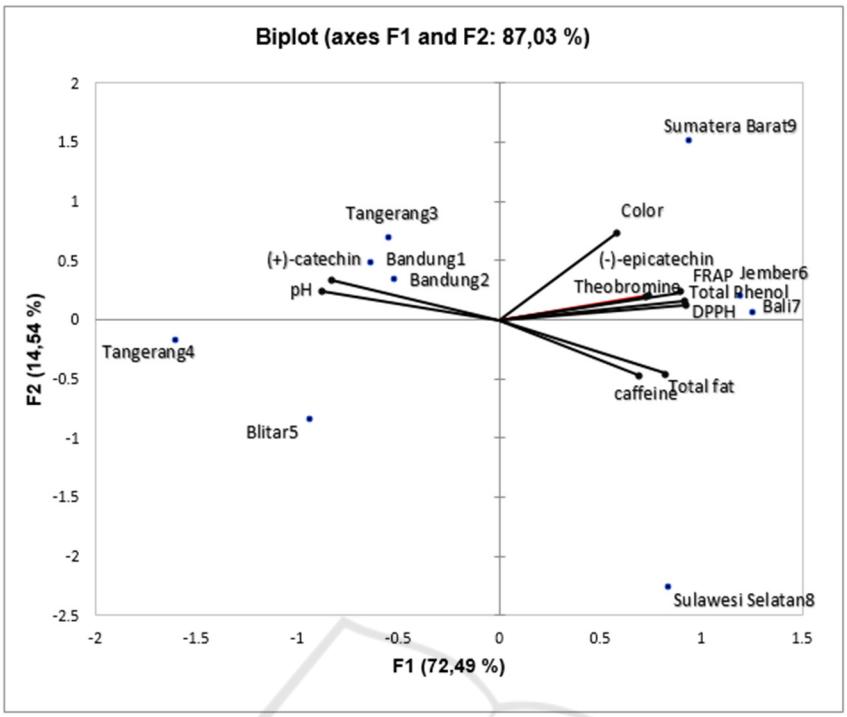

Figure 2: Scatter plots of the first two principal component vectors (F1 vs. F2) for 9 samples of Indonesian cocoa powder according to data of pH, total fat, color, TPC, DPPH, FRAP, and Individual bioactive compound.

Table 3: Pearson Correlation coefficient between pH, total fat, color, TPC, DPPH, FRAP, Theobromine, (+)- catechin, caffeine, and (-)-epicatechin.

	Total Phenols	DPPH	FRAP	T	C	Caf	EC	pH	Color	Total fat
TPC	1	0.996**	0.970**	0.745 *	-0.799**	0.552	0.727*	-0.898**	0.722*	0.774*
DPPH		1	0.977**	0.749 *	-0.811**	0.577	0.704*	-0.918**	0.693*	0.795*
FRAP			1	0.796**	-0.720*	0.555	0.719*	-0.863**	0.775*	0.703*
T				1	-0.615	0.524	0.538	-0.656	0.557	0.525
C					1	-0.708*	-0.522	-0.871**	-0.249	-0.889**
Caf						1	0.680*	-0.713*	0.126	0.845**
EC							1	-0.554	0.702*	0.582
pH								1	-0.391	-0.939**
Color									1	0.178
Total fat										1

*** significant at the level of the level of correlation 0:01*
** Correlation is significant at the level of 0:05*
T = theobromine, C = (+) - catechins, Caf = Caffeine, EC = (-) – epicatechin

a good positive correlation to total phenol and antioxidant capacity due to their small quantity found in cocoa powder samples. (+)–Catechin compound has a negative correlation with total phenol and antioxidant capacity. That statement can be described as the effect of epimerization from (-)-epicatechin to (+)–Catechin structure. According to Cooper *et al.,* (2008) epicatechin had a high correlation to total phenols but not for catechin compounds. There was a decreasing of polyphenol compounds due to a processing of cocoa powder, but the decline has different degrees between each polyphenolic compounds.

4 CONCLUSION

Profile of bioactive compounds and antioxidant capacity of Indonesian cocoa powder have different values. The total phenolic content ranged from 14.80-79.93 mg (GAE/g) on a dry basis. DPPH antioxidant capacity has a range of values 91.67 - 362.24 (mol TE/g) and FRAP method has a value range 249.16 - 1000.95 (mol Fe^{2+}/ g). The content of theobromine, caffeine, catechin, epicatechin ranged from 1.89 – 3.08 (mg / g); 0.12 - 0:46 (mg / g); 0.04 – 1.10 (mg/g); 0.04 - 4.68 (mg/g), respectively. The high positive correlation was found between TPC and antioxidant capacity both DPPH and FRAP. Total fat and color had a positive correlation with total phenol, however, pH had a strong negative correlation to total phenol. PCA analysis divides the samples based on similarity of chemical characteristics. Left quadrant was a group of alkalized cocoa powder and right quadrant was a group of natural cocoa powder.

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