COMPARISON OF ANTIOXIDANT ACTIVITY AND CATECHIN CONTENT BETWEEN YEAST FERMENTED COCOA BEAN AND NON FERMENTED COCOA BEAN

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ABSTRACT

Background: The government regulation required that in 2012, cacao bean must be fermented firstly before it being exported. This government regulation of course should be supported by scientific data especially concerning on efficacy and the content of fermented cacao bean. The fermentation was known could alter the content of a chemical property in cocoa bean especially catechins which it was the main antioxidant compound. It meaned that the antioxidant activity of extracts of cocoa bean could be affected too.
Objective: The research of the comparison of antioxidant activity and catechins content between non-fermented cacao bean with yeast fermented cacao bean has been done.
Method : Antioxidant Activity and Catechin Content Between Yeast Fermented Cacao Bean and Non Fermented Cacao Bean
Outcome Measured : -
Result: The results showed that the fermentation process could raise the chemical content of ethanol-soluble from extract of cocoa beans. The antioxidant activity of extract of fermented cocoa was higher than non-fermented. % IC_{50} of extract of fermented cocoa was achieved at a concentration 6.865 µg/ml. while the non-fermented achieved at a concentration 9.839 µg/ml. The fermented cocoa bean were known contain catechins for 2329.63 $\pm$ 14.618 µg/ml and non-fermented cocoa bean amounted to 2258.781 $\pm$ 31.625 µg/ml.
Conclusion : The fermented cocoa bean were known contain catechins for 2329.63 $\pm$ 14.618 µg/ml and non-fermented cocoa bean amounted to 2258.781 $\pm$ 31.625 µg/ml.

Keyword : cacao bean, yeast fermentation, catechin, antioxidant.

INTRODUCTION

Cocoa bean is one of export commodity of Indonesia plantation which has high value. Even, the cacao including is one of Indonesia's main product. This export value will increased to China, associated with the ACFTA (Asean China Free trade Area). Indonesia is the world's third largest cocoa exporter after Ivory Coast and Ghana (Askindo data, 2005).
Based on regulation, starting in 2012 The Indonesia Government obliging that cocoa bean which would be exported must be fermented firstly. This regulation was set to increase the export price of cacao bean Indonesian farmers. As reported earlier that the fermented cacao having the higher value compared with non-fermented cacao. at this

time, Indonesian cacao beans that are produced by indonesian farmer plantations still valued very low. this is because the cocoa which are sold is dominated by non fermented cacao, so it taste is not good enogh, not pure from contaminant such as insect, fungi, or mycotoxin (Wahyudi, 2008).

Cocoa beans are known to contain polyphenol compounds consisting of anthocyanin, leucoantocyanin, catechin, epicatechin monomer (flavanols), procyanidin molecules (polymers form) and polyphenol complex. The process of fermentation of cocoa beans was able to add the power of color, taste and flavour of cocoa, bitter and astringent taste reduction, and improvement of the physical appearance of cocoa beans (Susanto, 2004). Bitter and astringent taste in cocoa beans before be fermented is resulted from catechins which contained in seed. But besides that, catechin and epicatechin together procyanidin in cocoa beans is useful as a natural antioxidant compounds that can reduce free radicals and inhibiting the oxidation of enzymes such as lipooksigenase. Based on research, cocoa beans have higher antioxidant activity than the tea and red wine (Hii et al, 2009). Besides catechin, Flavan-3-ol in cocoa beans is also beneficial to the cardiovascular system, decreased blood pressure, platelet aggregation, and lower levels of LDL cholesterol oxidation (Hurst et al, 2011).

Most of natural flavonoids in general are always shaped flavonoid glycosides, which it bound with sugar group (Markham, 1988). The processing of the cocoa beans after harvest could be possible to give effect to the content of chemical compounds as well as the activity of cocoa beans. The fermentation process could make flavonoid glycosides lose the sugar group so that turned into an aglycone. The Analysis of QSAR (Quantitative Structure Activity Relationship) have proven that an aglycon of flavonoids were known to have activity much larger than the glycoside form (Melo, 2010 **;** Sanbongi et al, 1998). In some crops such as tea, fermentation was also able to convert a compound of catechins into theaflavins and thearubigin, with the change of catechins of course would affect the antioxidant power of these compounds (Sahlin, 1999).

The fermentation process which was usually done on the cocoa bean was through a natural fermentation process by means of deposited in containers with sun dried until the seventh day (Aikpokpodion et al, 2010). The impact of the addition of yeast on the cocoa beans in order to help speed up the fermentation process, certainly it would have a significant influence on the catechin content and also antioxidant activity from cocoa beans (Siregar, 2010; Susanto, 1994).

The purpose of this study is produce data the extent of the influence of fermentation with yeast on catechin content and antioxidant activity of cocoa beans compared with non fermented cocoa beans.

From this study, expected that the implications of fermentation with yeast to the catechin content and antioxidant activity in cocoa beans could be known. So that farmers are able to improve the quality and price of his products with a very simple way. Not only pay attention to the taste of cocoa, but also the content of catechins in cocoa beans, because catechins are primary antioxidants in cocoa.

The existence of new regulation which require the cocoa bean exported should be fermented, must be supported by scientific data that states the advantages of fermented cocoa beans compared with non fermented cocoa beans

MATERIAL AND METHOD

Material

Materials used in this study were cocoa beans (*Theobroma cacao* L.), compound DPPH (1,1-diphenyl-2-picrylhydrazyl), catechin hydrate compounds were obtained from Sigma Aldrich, ethanol 95%, the yeast Saccharomyces cereviceae, ascorbic acid, n-hexane, ethyl acetate, chloroform, methanol, toluene, acetonitrile pro HPLC, aquabidest, $AlCl_3$, plate Thin Layer Chromatography (GF_{254}), NH_4OH 1%, HCl 2N, Mayer reagent, Dragendorf reagent (bismuth subnitrat and mercury(II) Cl_2), Lieberman-Burchard reagent, $FeCl_3$, gelatin solution 1%, Mg powder, amyl alcohol, vanillin 10% in H_2SO_4, KOH 5%.

Methode

This study was held in the Pharmacy Laboratory of MIPA Faculty UNISBA. This research was done through several stages consisting of the preparation of cocoa beans, processing, phytochemical screening, evaluation standard parameters botanicals and extracts, extraction, fractionation, assay of antioxidant activity and assay of catechins content. Every stages were conducted on both material fermented and non fermented cocoa beans.

The cocoa (Theobroma cacao L.) were obtained from Plantation Rajamandala (PT, Plantation Nusantara VIII) Bandung. Cocoa bean processing was done in two ways, the first was cocoa beans fermented for 7 days with the addition of yeast, and then dried with artificial drying cupboard. The Second, cocoa beans were dried directly without going through the process of fermentation. Both dry beans, both it resulted from fermented and non fermented, subsequently they were made become coarse powder and stored in sealed containers for further research stage. Against both cocoa beans is then performed phytochemical screening includes qualitative analysis alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids, and quinones by using specific reagents. The evaluation of standard parameters botanicals and extracts covering specific parameters such as macroscopic analysis, level of content soluble in water, level of content soluble in ethanol and non specific parameters such as the determination of drying shrinkage, total ash, acid insoluble ash content and weight type. Each parameter was done in accordance with the method contained in the WHO guidelines for quality control (WHO, 1998).

Coarse powder of cocoa beans subsequently macerated with 70% ethanol for 3 days. The extract obtained is then hydrolyzed with HCl 1 N. Then extract were concentrated with a rotary evaporator to further divided into two parts, where the first part used for test the antioxidant activity, while the second part to measure the content of catechins.

The antioxidant activity of the extract was measured by the method of reduction of DPPH free radicals with vitamin C as a comparison. Then IC_{50} (inhibitory concentration to eliminate 50% of concentration of DPPH) were calculated so that be known the antioxidant activity of cacao. The reduction process of free radical DPPH is measured using a UV-VIS spectrophotometer at a wavelength maximum for DPPH.

As for the determination of catechin content, extract firstly fractionated by liquid-liquid extraction method and then fraction obtained was monitored by thin layer chromatography. Fraction containing catechins, then was used for establish the amount of catechins using HPLC using a linear regression curve.

RESULT AND DISCUSSION

Macroscopic assay

The macroscopic test results showed that the fermented cocoa was more darker, and fragrant, while the non fermented cocoa beans pale and less smelly. The different colour between fermented cocoa extract and non fermented cocoa extract can be seen in figure 1.

(a) (b)

Figure 1. (a) non fermented cocoa, (b) fermented cocoa

Screening Phytochemistry

Data analysis phytochemical screening can be seen in the table 1.

The result of phytochemical screening showed that the cocoa beans fermented with non fermented cocoa beans contain the same metabolite compounds. The data also showed that in both of cocoa beans still contained flavonoid. This showed that in both of cocoa beans still contained catechins which was the main antioxidant compounds in cocoa beans, which means catechin in fermented cocoa beans was not damaged and remained actives as an antioxidant.

Quality Parameter

Data analysis of the determination of the quality parameters of the botanical and the extract can be seen in the table 2.

Table1. The phytochemical screening result of botanical and extract of fermented and non fermented cocoa bean

Classes of Coumpound	Fermentated		Unfermentated	
	Cocoa bean	Extract of cocoa bean	Cocoa bean	Extract of cocoa bean
Alkaloid	✓	✓	✓	✓
Poliphenol	✓	✓	✓	✓
Flavonoid	✓	✓	✓	✓
Tannin	✓	✓	✓	✓
Condenced tannin	✓	✓	✓	✓
Quinon	✓	✓	✓	✓
Monoterpen and sesquiterpen	✓	✓	✓	✓
Triterpenoid	✓	✓	✓	✓
Steroid	-	-	-	-
Saponin	-	-	-	-

Analysis of standard parameters botanicals and extracts showed that the percentage of water-soluble extractable matter on cocoa extract of fermented slightly larger than non fermented extract, even nearly the same. While data analysis of ethanol-soluble extractable matter showed that content in fermented extract much greater than nonfermented. This was likely to occur due to non-fermented extract contained more glycoside compounds than aglycone such as flavonoids, terpenoids, steroids and alkaloids that were bound with sugar group. This glycoside compounds tended to be more polar so more soluble in water. The fermentation process made the bond of sugar become separated to form the aglycone which tend to be less polar than glycoside form, so it was more soluble in ethanol which was the universal solvent.

Table 2. The results of the determination of standard parameters botanicals and extracts cocoa bean

Standard Quality	Fermented		Non Fermented		Quality Standard (%)
	Cocoa Bean (%)	Extract (%)	Cocoa Bean (%)	Extract (%)	
Water-soluble extractable matter	9,968 ± 2,631	52,382 ± 1,742	10,283 ± 2,932	50,139 ± 0,880	> 14
Ethanol-soluble extractable matter	11,682 ± 5,608	45,819 ± 9,865	14,453 ± 4,270	42,903 ± 7,234	> 4,5
Water and volatile matter	5,575 ± 0,298	-	7,430 ± 0,407	-	-
Total Ash	3,452 ± 1,311	-	3,427 ± 2,099	-	< 4
Acid-Insoluble Ash	0,890 ± 0,623	-	0,723 ± 0,458	-	< 1
Spesific Gravity	-	1,277 ± 0,055 g/ml	-	1,281 ± 0,071 g/ml	-

Water and volatile matter determination of fermented cocoa beans were lower than non fermented cocoa beans. This shows that there were effect of fermentation process on the content of volatile compounds in cocoa beans. This may occur because in the cocoa containing compounds oil and fat. The presence of yeast that helped the process of fermentation, making oils and fats which had a long chain turned into short-chain compounds that have volatile character.

Determination of Antioxidant Activity

IC_{50} at Cocoa Bean Extract

Data of vitamin C concentrations that be needed to muffle DPPH can be seen in the following table 3.

The Increasing of concentration of vitamin C in muffle of DPPH provide linear regression equation y = 6.123 x + 41.54 with with a correlation coefficient (r) was 0.997. From the linear regression equation was known that the concentration of vitamin C which produced IC_{50} was at 1.382 µg/ml.

Extract of fermented cocoa beans provided a linear regression equation y = 0.437 x + 47.26, with a correlation coefficient of 0.993. From the linear regression equatio. It was known that the IC_{50} was of 6.27 µg/ml. The data of analysis of the concentration of fermented cocoa extract against the muffle of DPPH can be seen in the table 4.

Table 3. Data of analysis of the decrease of DPPH levels by various concentrations of vitamin C

Concentration (µg/ml)	Data	
	Abs	% Inhibision
0	0,546	0
1	0,284	47,950
2	0,254	53,477
3	0,218	60,194
4	0,189	65,300
5	0,149	72,657

Tabel 4. Data of analysis of the decrease of DPPH levels by various concentrations of fermented cocoa bean extract

Concentration (µg/ml)	Data	
	Abs	% Inhibision
Zero	0,512	0
5	0,260	49,120
10	0,246	52,013
15	0,236	53,870
20	0,225	56,099
25	0,215	58,014

Extract from non fermented cocoa beans provided a linear regression equation y = 0.926 x + 40.77, with a correlation coefficient of 0.981. From the linear regression equation was known that the IC_{50} was of 9.968 µg/ml. The data of analysis of the concentration of fermented cocoa extract against the muffle of DPPH can be seen in the table 5.

Tabel 5. Data of analysis of the decrease of DPPH levels by various concentrations of non fermented cocoa bean extract

Concentration (µg/ml)	Data	
	Abs	% Inhibision
zero	0.499	0
5	0.270	45.909
10	0.257	48.689
15	0.220	55.971
20	0,206	58,772
25	0,179	64,033

From the data of analysis, it could be seen that the extract of fermented cocoa have more stronger antioxidant activity than the extract of non-fermented cocoa. From Table 6, it can be seen that when the antioxidant activity of the extract compared to the activity of

vitamin C, the data showed that the fermented cocoa extract has antioxidant power 0.201 time from the power of vitamin C. Whereas the non fermented cocoa extract has antioxidant power just 0.141 time from the power of vitamin C, it less strong than fermented cocoa extract.

Tabel 6. The analysis data of IC_{50} from fermented cocoa extract and non fermented cocoa extract compared with IC_{50} of vitamin C

	vit c	Fermented cocoa extract	Non Fermented cocoa extract
Replication 1	1,382	6,270	9,968
Replication 2	1,383	7,460	9,710
mean	1,382	6,865	9,839
SD	0,001	0,841	0,182
Compare with vit C	1,000	0.201	0,141

Determination of Total Cathecin

Monitoring of Catechin Content in Fractions

Based on the results of the TLC monitoring (Figure 2), the chromatogram showed that cocoa beans fermented and non-fermented give the result that almost similar in every fraction. The difference was seen only in the ethyl acetate fraction which was the fraction of semi-polar.

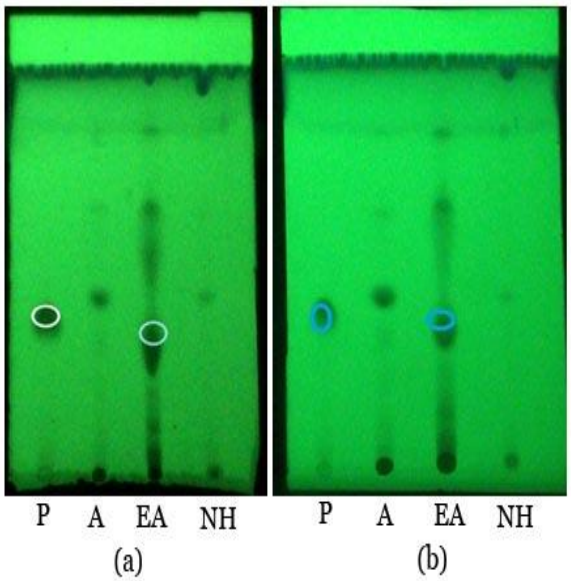

Figure 2. Chromatogram of fermented cocoa beans (a) and non fermented cocoa beans (b)

note :

P : Standard (Katekin) A : water fraction

EA : ethyl acetat fraction NH : n hexane fraction

The chromatogram of fermented cocoa beans showed that the ethyl acetate fraction containing more many spots pigment than the non-fermented cocoa beans This shows that the fermentation process gave the influence on semi-polar compounds, wherein the fermentation process was able to increase the amount of a chemical compound semi-polar. On this chromatogram, it can be seen that the catechin were a semi-polar substance and were in the ethyl acetate fraction. Therefore, the fraction of ethyl acetate was then used to determine of total catechins.

Analysis of The Levels of Catechins

Assay of catechins content by the HPLC method, resulted the linear regression equation y = 12783x + 58319 with a correlation coefficient r was 0.999. The data analysis of the Assay results could be seen in the following table 6.

Tabel 6. Data of analysis of catechin content in fermented cocoa and non fermented cocoa

Sample	Fermented cocoa extract (µg/ml)	Non Fermented cocoa extract (µg/ml)
1	2339,976	2281,141
2	2319,303	2236,420
mean	2329,639	2258,781
SD	14,618	31,623

Data table of determination of Catechin content showed that the fermented cocoa beans contains catechins at 2329.639 ± 14.618 g / ml while the non fermented cocoa beans contains catechins at 2258.781 ± 31.623 g / ml. The data results showed that the fermented cocoa beans contained catechins more greater than the non fermented cocoa beans. This shows that the fermentation process can improve the content of catechins in cocoa beans. Increasing of catechin content in fermented cocoa bean was due in the fermented cocoa beans occuring hydrolysis process which change the catechin glycosides become cathechin and sugar. So that, the cathechin and sugar content in fermented cocoa increased. therefor, it was normal if the fermented cocoa more sweeter than the non fermented cocoa.

Increased content of catechins was also an impact on the antioxidant activity of fermented cocoa beans, wherein the fermented cocoa extract provide data IC50 lower than the non fermented cocoa extract. This condition showed that just required little concentration for fermented cocoa beans to produce IC 50 compared with non fermented cocoa beans

CONCLUSION

The IC_{50} for fermented cocoa extract was 0.201 time from the power of vitamin C and the IC_{50} for non fermented cocoa extract was 0.141 time from the power of vitamin C. The catechin content in fermented cocoa fraction was 2329.63 $\pm$ 14.618 µg/ml, whereas catechin content in the fraction of non fermented cocoa was 2258.781 $\pm$ 31.625 µg/ml

DISCLOSURE : -

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