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The Change of Catechin Antioxidant during Vacuum Roasting of Cocoa Powder

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Abstract

Concentration of catechin antioxidant in cocoa seed decreased greatly during processing into powder, especially in the conventional roasting process (without vacuum). Therefore, vacuum roasting process was developed. The objective of this research was to analyze the change of catechin in cocoa seed during vacuum-roasting of cocoa powder. The treatments consisted of roasting with vacuum (45.6 and 60.8 cmHg) and without vacuum at three roasting temperatures (100°C, 110°C, 120°C) for 25, 35, and 45 minutes. For catechin analysis, the standard catechin component of (+)-catechin was used, and LC-MS (Liquid Chromatography Mass Spectrometer) with formic acid solvent (0.1% in water, pH 2.5) and methanol solvent was used for identification. Result of research indicated that vacuum roasting at 60.8 cmHg in 100°C for 25 minutes resulted in the highest percentage of catechin in cocoa powder. The increase of catechin in cocoa powder during vacuum roasting was considered as strong due to the change or degradation of procyanidin (either in form of dimer, trimer or tetramer).

Keywords: Cocoa powder; Roasting; Vacuum; Catechin

Introduction

Cocoa seed is a main output of cocoa which contains protein, lipid, carbohydrate concentration and mineral. Cocoa seed also contains antioxidant, such as catechin, in high level of concentration. Catechin is a compound with some functions such as antioxidant, antibacterial, improving endothelial function, reducing blood pressure, increasing insulin sensitivity, and repairing platelet function [1-4].

Some researchers showed that the increase of cardiovascular function in human after consuming cocoa is related to the presence of metabolite, which is coming from catechin inside plasma [5,6]. However, catechin concentration of cocoa powder greatly decreases during processing in the conventional roasting stage.

Conventional roasting is usually conducted in open batch (without vacuum) such that free oxygen interferes into roasting space. Indeed, roasting can change catechin in cocoa seed [7,8]. Heating temperature in roasting process may trigger oxidation, degradation, and epimerization of catechin, thus reducing antioxidant activity. The reduction of catechin during processing is due to the combination of the effects of oxidation, epimerization, degradation and interaction with protein.

Heating (at 110-200°C) during conventional roasting system (without vacuum) allows oxygen in the roasting atmosphere to activate. During roasting, oxygen is heated which then can activate formation of singlet oxygen from the initially stable oxygen (triplet oxygen) this singlet oxygen is very reactive and easily reacted with organic molecule in the food material [9]. This reactive oxygen will then oxidize catechin in cocoa seed. Oxidation may change catechin structure into semiquinon compound, which it will change into quinone through further process [10]. Misnawi explained that quinone is one of the compounds which produce a distinctive taste of cocoa during roasting process in addition to reducer sugar, peptide and amino acid [11].

It is reasonable to say that oxygen in the roasting space can be minimized by vacuum roasting system because it seems possible to reduce oxidation. This system can reduce the possibility of catechin reduction but with the consequence of less maximum production of taste producer compounds. The final product may be then organoleptically different compared to conventional roasting system (without vacuum). Some effects in the vacuum roasting process must be further examined to ensure the reduction of the possibility of catechin reduction and loss.

Material and Methods

Sample preparation

Dried cocoa seeds are collected from public cocoa plantation at Lasusua subdistrict, North Kolaka District, Southeast Sulawesi. The seeds were processed into powder using a hydraulic press of 50 tones pressure strength, powder milling tool, 80-mesh screen, and some supporting tools (such as packager and others). The powder was then divided into two groups in which one group was not roasted and another group of cocoa powder was roasted either using vacuum frying device (equipped with water jet system) or without vacuum device to obtain dried cocoa powder. Following roasting process, the dried cocoa powder was treated sequentially to form dried extract of cocoa powder using the method of Ruzaidi and Othman [1,12] with few modifications. Briefly, dried cocoa powder is concentrated using ethanol 70% (1 gram dried cocoa powder is dissolved in 25 ml solvent (ethanol 70%) for 120 minutes at 50°C using orbital shaker. It was then cooled at room temperature and screened with No.1 Whatman Paper. Result of screening is re-extracted for twice with similar solvent, and then evapo-concentrated using rotary vacuum evaporator (HEIDOLPH

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Type Laborota 4000 Vacuum-Controller VC2) to obtain the dried extract of cocoa powder.

Laboratory analysis

Catechin analysis: Beside catechin content of roasted and unroasted cocoa powder, catechin content of dried cocoa seed, nib and unroasted cocoa powder were also analyzed and the results were used as control. The preparation of sample for the identification and analysis of catechin used the method of Othman [1] with modification. Briefly, sample solution (dried extract of cocoa powder made in the methanol (500 or 1000 times of dilution) was aspirated about 2 mL and 0.1 ml of 10% aluminum chloride and 0.1 ml of 1 M potassium acetate were added into the sample. It was further added by 2.8 ml deionized water prior to incubation for 30 minutes at room temperature and given a pressure at 415 nm. The absorbance concentration was compared to that in catechin standard curve (Sigma, Co. Chemical, St. Louis, USA) to determine the percentage of catechin concentration.

The identification of catechin with LC-MS: The sample was prepared with a modified method of Subagio and Calderon [13,14]. Briefly, one gram of dried extract of cocoa powder (extracted by rotary evaporator with ethanol 70%) was diluted into MeOH (50 ml) and put into ultrasonic (sonicator) for an hour. The sample was then resided for an hour before screened and dried using nitrogen gas. The LC-MS analysis was implemented using formic acid solvent (0.1% in air, pH 2.5) and methanol solvent. LC-MS type used was Shimadzu (Columbia, MD) LC-10A HPLC system and the Leap (Carrboro, NC) HTS PAL autosampler interfaced to an Applied Biosystems (Foster City, CA) API 4000 triple-quadruple mass spectrometer.

Design experiment and data analysis

The research consisted of three factors. Factor I was vacuum pressure (V) which consisted of 45.6, 60.8 (cmHg) and non-vacuum (Vo). Factor II was three levels of roasting temperature (T) (100°C, 110°C, 120°C). Factor III was roasting length (L) (25, 35, and 45 minutes). These three factors would produce 27 (3×3×3) combinations of treatment with 2 replications, and thus, 54 units of experiments were obtained.

Data were tabulated and analyzed using statistical procedure of the analysis of variance. The differences between treatments were compared by using Duncan Multiple Range Test at 5 levels.

Results and Discussion

The effect of temperature and length of vacuum roasting on catechin concentration of cocoa powder

Catechin concentration in dried cocoa seed, nib and unroasted-cocoa powder were analyzed and the results were used as a control of catechin change during roasting process based on treatments. The result of analysis of catechin concentration in the sample without treatment is shown in table 1.

Catechin concentration of unroasted-cocoa powder was higher than that of nib and dried cocoa seed. However, nib or cocoa seed without skin (unpeeled) had lower catechin than dried cocoa seed. It was indicated that preliminary roasting of (without vacuum) cocoa seed, could degrade catechin for 0.3%. During processing of the nib into powder, a synthesis of catechin about 0.47 % was occured. Result of analysis of catechin concentration in cocoa powder on various levels of vacuum pressure, temperature and length of roasting were shown in figures 1, 2, and 3, respectively.

Sample	Catechin Average (%)
Dried cocoa seed	3.20
Cocoa nib	2.90
Unroasted-Cocoa powder	3.37

Table 1: Catechin concentrations of seed, nib and unroasted-cocoa powder.

Figure 1 showed that at 100°C, degradation and synthesis of catechin appeared in all treatments. Vacuum roasting of 60.8 cmHg for 25 minutes resulted in highest catechin concentration (5.10%). This indicated that catechin concentration increased about 1.73% from early catechin concentration before roasting. Catechin concentration also increased when using vacuum roasting of 45.6 cmHg for 25 minutes, but the increase was only 0.52%. During non-vacuum roasting, catechin was degraded about 0.32%. When roasting the sample for 35 minutes

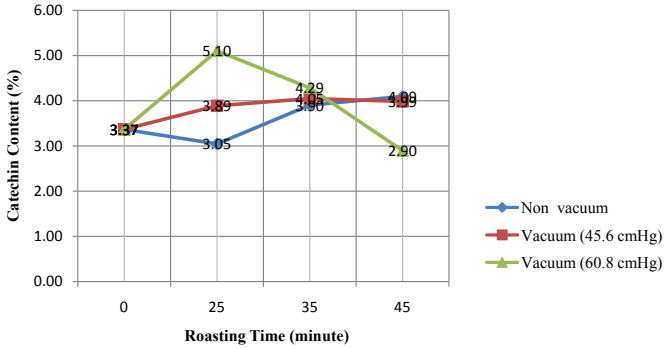

Figure 1: The effect of vacuum roasting of cocoa powder at 100°C on catechin concentration.

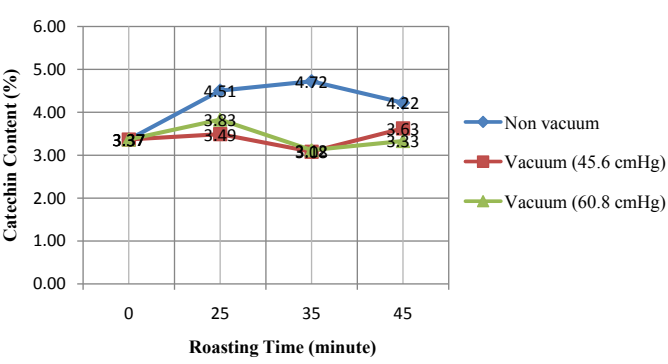

Figure 2: The effect of vacuum roasting of cocoa powder at 110°C on catechin concentration.

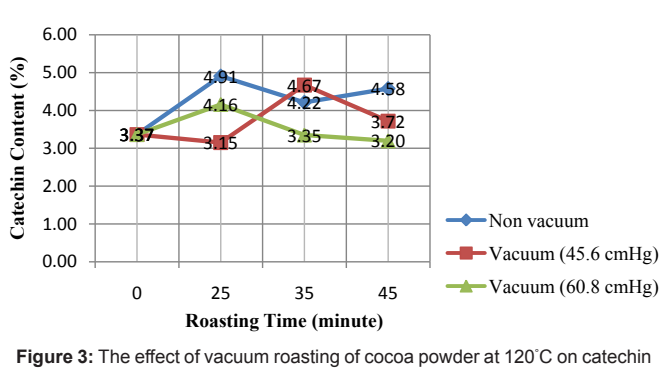

Figure 3: The effect of vacuum roasting of cocoa powder at 120°C on catechin concentration.

using both vacuum roasting at 45.6 cmHg and non-vacuum roasting, catechin concentration increased to about 4%, and this concentration was stable until 45 minutes. Vacuum roasting at 60.8 cm Hg forced catechin to decrease at minute 35-45. These search results showed that vacuum roasting of 60.8 cmHg at 100˚C might increase catechin concentration of the sample but the length of roasting is not more than 25 minutes.

Based on the temperature treatments, roasting at 100˚C and 110˚C were different. Roasting temperature of 110˚C might increase catechin concentration higher enough in the non-vacuum roasting. The detail is given in figure 2.

Figure 2 indicated that catechin concentration increased during non-vacuum roasting to about 4.51 and 4.72% when samples were roasted for 25 and 35 minutes, respectively. However, catechin concentration decreased slightly when roasting prolonged up to 45 minutes. Both levels of vacuum roasting (45.6 cmHg and 60.8 cmHg) gave almost similar effect on catechin concentration changes. During vacuum roasting at 110˚C, there were few syntheses and degradations of catechin in almost similar percentage, but catechin concentration was relatively stable from the beginning of roasting till the end (i.e., 45 minutes). It illustrated that the increased temperature beyond 100˚C during vacuum roasting would not establish a significant increase on catechin concentration. This fact was supported by the result of catechin concentration analysis during vacuum roasting at 120˚C, as shown in figure 3.

Figure 3 showed that vacuum roasting of 45.6 cmHg at 120˚C increased catechin concentration about 4.67 % in 25 minutes of roasting but it decreased in 45 minutes of roasting. This catechin concentration was still higher than that produced from vacuum roasting of 60.8 cmHg at 100˚C for 25 minutes. In non-vacuum roasting at 120˚C, catechin concentration increased to about 4.91% I 25 minutes of roasting. Although catechin was degraded in 35 minutes of roasting, there was a synthesis of catechin in 45 minutes of roasting; hence catechin concentration did not change much.

Vacuum roasting at 60 cmHg in figures 1, 2, and 3 showed a similar graphic pattern, in which a catechin synthesis only occurred in the roasting length of 25 minutes. However, catechin was degraded in the next minute. This condition might explain why the role of oxygen is lower in catechin compound and its precursor (such as dimmer or oligomer) oxidation during roasting.

The low oxygen in the roasting space caused low oxidation of catechin which made degradation of catechin slow. However, there was also a possibility of the degradation of procyanidin or proanthocyanidin (precursors), as both were the constructors of catechin compound which made the synthesis rate always higher than the degradation rate. As the roasting space was vacuum, the roasting process and many of its components occurred faster. On the other hand, in non-vacuum roasting, high concentration of oxygen could possibly make catechin oxidation fast.

Catechin degradation occurred at vacuum roasting of 60.8 cmHg for a period of more than 25 minutes. It showed that in addition to oxygen, there were other factors in the vacuum roasting space which affected the process. Artnasewa explained that the drying is fast with the reduction of vacuum rate. Based on this condition, it was estimated that some reactions were fast during vacuum roasting because there was a convergence between vacuum drying and vacuum roasting principles.

It was estimated that catechin oxidation at the 25th minute could produce a quinon compound. Janeiro and Oleveira [10] asserted that a catechin oxidation mechanism initially constructs a semiquinon, which then converts into a quinon. A quinon compound can react with amino acid, protein or other polymers to produce proanthocyanidin [15,16]. Proanthocyanidin can degrade again into catechin because proanthocyanidin represents oligomer or polymer from flavan-3-ol (catechin/epicatechin) [17-19].

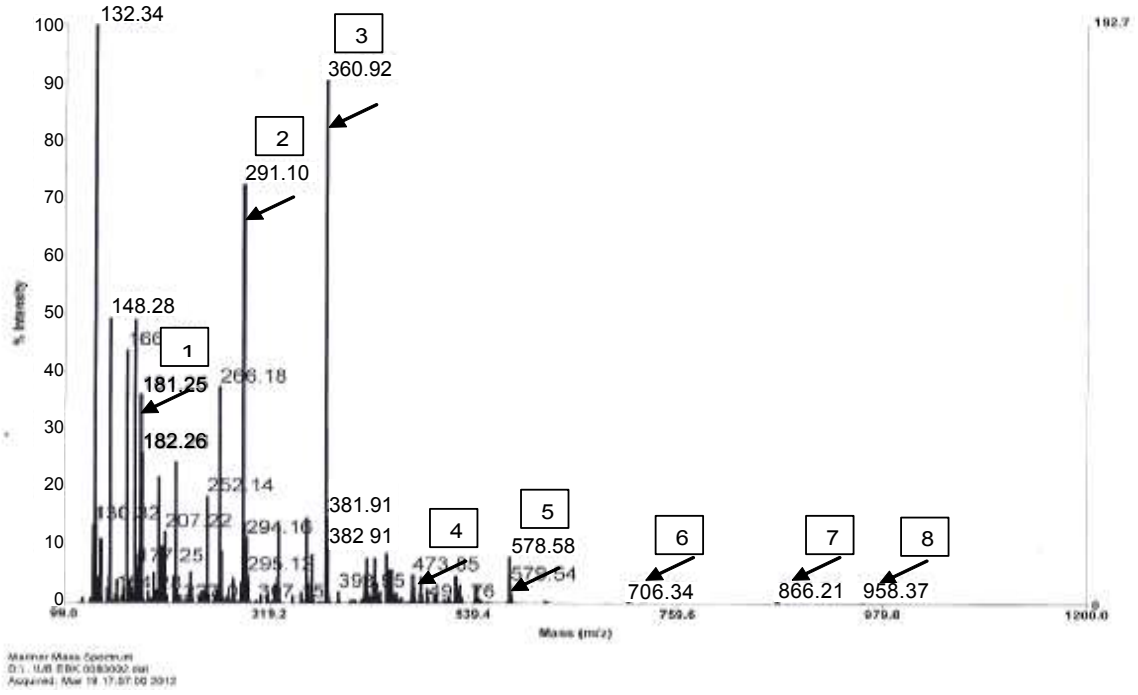

Figure 4: Cocoa powder extract in the vacuum roasting of 60.8 cmHg at 100˚C for 25 minutes using LC-MS.

Proanthocyanidin (58%), along with catechin (37%) and anthocyanine (4%), are three main polyphenols found in cocoa seed or its processed product [16,20]. Proanthocyanidin in cocoa may take a form of monomer, dimer and tetramer [21]. A dimer structure can be degraded into a catechin molecule and an anthocyanidin molecule (and other secondary results) during heating with acid. The treatment with hot acid and oxygen will produce an oxidative reaction, which then results in cyanidin, epicatechin and other products [22].

Results of the analysis against cocoa powder extract sample in the vacuum roasting of 60 cmHg at 100°C for 25 minutes using LC-MS (Liquid Cromatography Mass Spectrometry) described a possibility of degradation which occurred during vacuum roasting. The details were shown figure 4.

Peak 1 in figure 4 had molecule weight of m/z 181.25. Some ionic distributions were estimated as a product of degradation of catechin/epicathecin monomer component. Peak 2 was identified as a catechin/epicathecin monomer. Wollgast [16] insisted that monomer (+)-catechin/epicathecin has moleculer weight of 290, with a molecule ion distribution ($[M-H]^-$) at m/z 289. It was supported by Calderon [14] who analyze the antioxidant of cocoa powder extract using LC-MS/MS, which was found that the ionic distribution of m/z catechin and epicathecin is m/z 289→245. The molecule weight m/z 290 was also proposed by Callemien and Collin [23] as catechin/epicathecin.

Catechin/epicatechin has been recognized as the composer units of procyanidin polymer in cocoa [16] or also called as dehydrocatechin. Procyanidin of cocoa has Type A and Type B, which are differed by the bond between individual monomer subunits. Type A is established at cross-link 4-8 and 2-7, while Type B is constructed at cross-link 4-8. Type B is consisting of procyanidin B1 which is detected on ionic distribution m/z 577→407 and procyanidin B2 which is detected on m/z 577→259 [23]. Peak 3 (m/z 380.92) and Peak 4 (m/z 473) remained in the ionic distribution of procyanidin B2, while Peak 5 (m/z 578.56 and m/z 579.54) was located at ionic distribution of procyanidin B1.

According to Wollgast [16], procyanidin dimer, trimer, tetramer and others have molecule weights of 578 (290+288), 866 (578+288), 1154 (866+288) and others, which are giving a disguise of molecule ion at m/z 577, 865, 1153, 1441 and 1729 from dimer throughout hexamer.

Peak	m/z	Identification	Ionic Distribution
1	181.25	As a compound resulted from the degradation of monomer component (catechin/epicathecin)	m/z 207→181
2	291.10	Catechin/epicathecin	m/z 289→245
	266.18		
	252.14		
		Procyanidin Dimer / Dehydrocatechin	m/z 739→577
3	380.92	Procyanidin B2	m/z 577→259
	381.91		
	382.91		
	398.55		
4	473.85	Procyanidin B2	
	491.76		
5	578.56	Procyanidin B1	m/z 577→407
	579.54		
6	706.34	Procyanidin Trimer	m/z 867→579
7	866.21	Procyanidin Trimer	
8	958.37	Procyanidin Tetramer / Poranthocyanidin	

Table 2: Compounds in cocoa powder extract analyzed by LC-MS.

Calderon et al. added that the ionic distribution of m/z 739→577 is procyanidin dimer, while that of m/z 867→579 is procyanidin trimer. Peak 6 (m/z 706.34) and Peak 7 (m/z 866.21) remained at the ionic distribution of procyanidin trimer, while Peak 8 (m/z 958.37) was located at ionic distribution of procyanidin tetramer. Tomas-Barbaren [24] explained that all tetramers ([M-H])" at m/z 1153 have almost identical ionic fragments (m/z 1136, 1027, 1001, 983, 908, 866, 865, 813, 739, 695, 575, and 425).

Concerning this overview, the detail result of the identification of compound in the cocoa powder extract using LC-MS was summarized in table 2.

Cathecin compound (either in monomer, dimer, trimer and tetramer) shown in table 2 produced a very strong estimation that the catechin was increased due to the degradation of procyanidin. The degradation of dimer, trimer and tetramer occurred simultaneously during roasting. However, the order of degradation was from tetramer to produce trimer, dimer and monomer. Monomer catechin was also degraded into smaller component, but the detailed explanation of this component was lacking.

Conclusion

Vacuum roasting of 60.8 cmHg at 100°C for 25 minutes produced the highest percentage of catechin concentration of cocoa powder. The increased temperature to beyond 100°C for the length of more than 25 minutes would reduce catechin concentration of cocoa powder. The increased catechin in cocoa powder during vacuum roasting occurred due to the degradation of procyanidin (either in form of dimer, trimer or tetramer).

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