DETERMINATION OF CATECHIN, EPICATECHIN, THEOBROMINE, AND CAFFIENE COMPOUNDS IN VARIOUS COCOA PLANTING MATERIALS IN SABAH

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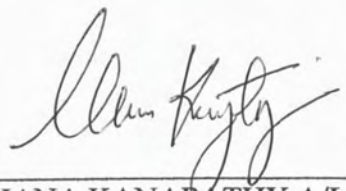
DECLARATION

I hereby declare that this dissertation is based on my original work, except for quotations and summaries each of which has been fully acknowledge.



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ABSTRACT

This study compares the quantity of the catechin, epicatechin, theobromine, and caffeine of cocoa beans from the various cocoa planting materials by using the reversed phase HPLC. Samples from different cocoa planting material were selected from the hybrid and clone varieties of cocoa pod. The cocoa fruits which contain the seeds were brought from the BAL cocoa plantation estate in Tawau. Only the ripe cocoa fruits were selected for the selected cocoa planting material. Cocoa beans were defatted before preparing the sample into two replicate. By using extraction method, catechin and epicatechin was extracted. Whereas theobromine and caffeine was extracted by using hot circulating bath. The result shows that theobromine compound for sample SCA9, BR25, PBC123, NA33, NA3 and mix hybrid are 2.43mg, 5.27mg, 2.90mg, 2.65mg, 5.34mg and 3.62mg, respectively. For caffeine, the amount of the compound in SCA9, BR25, PBC123, NA33, NA3 and mix hybrid are 0.520mg, 0.837mg, 0.385mg, 0.362mg, 0.610mg and 0.729mg respectively. Whereas for catechin, the compound in SCA9, BR25, PBC123, NA33, NA3 and mix hybrid are 133.26mg, 120.20mg, 13.41mg, 97.17mg, 7.302mg and 106.22mg, respectively. The result for the epicatechin cannot be identified because of systematic error occurred where there is no peak was identified in the graph obtained from the HPLC.

Determination of catechin, theobromine, and caffeine in various cocoa planting materials

ABSTRAK

Kajian ini bertujuan untuk menentukan komposisi katekin, epikatekin, theobromine dan kafein di dalam biji koko mentah daripada pelbagai jenis bahan tanaman koko di Sabah. Kajian ini membandingkan kuantiti katekin, epikatekin, theobromine dan kafein di dalam biji koko mentah daripada pelbagai bahan tanaman koko dengan menggunakan reverse phase HPLC, larutan piawai katekin, epikatekin, theobromine dan kafein. Sampel daripada pelbagai jenis bahan tanaman dipilih daripada koko kacukan dan jenis klon. Sampel koko didapati daripada estet penanaman koko BAL, Tawau. Selain itu hanya buah koko yang masak sahaja digunakan. Sampel koko dinyahlemah sebelum dijadikan kepada dua replika daripada setiap sampel. Katekin dan epikatekin diekstrak dan diuji dengan menggunakan HPLC. Theobromine dan kafein juga diekstrak dengan cara hot circulating bath. Hasil kajian menunjukkan kompaun theobromine bagi sampel SCA9, BR25, PBC123, NA33, NA3 dan mix hybrid adalah 2.43mg, 5.27mg, 2.90mg, 2.65mg, 5.34mg dan 3.62mg masing-masing. Bagi kandungan kafein bagi sampel SCA9, BR25, PBC123, NA33, NA3 dan mix hybrid adalah 0.520mg, 0.837mg, 0.385mg, 0.362mg, 0.610mg dan 0.729mg masing-masing. Kesemua kandungannya adalah didalam 0.01g sampel yang dikaji. Manakala bagi katekin pula, kandungannya adalah dengan susunan sampel diatas adalah 133. 26mg, 120.20mg, 13.41mg, 97.17mg, 7.302mg dan 106.22mg masing-masing. Kesemua kandungannya adalah didalam 0.5g sampel yang dikaji. Keputusan bagi epikatekin tidak dapat diperolehi disebabkan oleh berlakunya ralat sistematik yang menyebabkan puncak epikatekin tidak dapat diperolehi daripada graf HPLC.

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°C	degree Celsius
g	gram
rpm	rotation per minute
%	percentage
ml	milliliter
min	minute
HPLC	High Performance Liquid Chromatography
m	meter
mm	millimeter
nm	nanometer
$CaSO_4$	Calsium sulphate
Na_2SO_4	Natrium sulphate

CHAPTER 1

INTRODUCTION

1.1 Research Background

The cocoa tree(*Theobroma cacao. L*) is a native of the dense and one of the oldest forests of the Amazon. The forest accommodates a very good environment for the cocoa tree to grow in the wild such as semi shade, warmth, and high humidity (Minifie, 1999). Ancient civilizations such as Mayans and the Aztecs of the Mexico have been planting and cultivating the cocoa production long before it was brought to Europe and Asia continent. The Mayans use cocoa bean from its tree as active ingredient in their favorite drink "chocolatl" which they made by mashing the roasted and grinded cocoa nibs which then mixed with water, maize, and spice (Minifie, 1999). There are quit many species of the tropical plant which is belonged to the *Theobroma* genus. As reported by Cuatrecasas (1964) *Th.cacao* which is one of the 22 species which have been found which presents commercial value (Sotelo and Alwarez, 1991).

In Malaysia, the cocoa plantation was first started commercially in 1950 in Jerangau, Terengganu (Thompson, 1950). In 1955, the Borneo Abaca Limited BAL Plantation Sdn. Bhd.) Sabah, started the cocoa plantation in Sabah by allocating

as much as 76 acres to be planted with cocoa (Yusuf, 1981). In the ranking of the largest production for the cocoa beans in the world, Malaysia is holding the fifth place. Malaysia is also the main producer of the cocoa based products and the biggest in Asia. But when compared to the West African beans, Malaysian cocoa beans are sold at lower price, due to some weakness to some quality (Azizah *et al.*, 2006). The raw cocoa beans which produced in Malaysia have an acidic taste, high astringency and low cocoa flavour (Meyer *et al.*, 1989). One of the reasons which could result this is high content of phenolic substance (Azizah *et al.*, 2006).

Cocoa bean is rich with polyphenol content. Cocoa bean polyphenols, comprising 12-18% of whole dry bean weight, have long been associated with the flavor and colour of chocolate. Studies show that astringency is reduced through complex chemical reaction and the burnt feather character of roasted protein was depressed (Kim and Keeney, 1984). The complex reaction here is meant by the polyphenol's epicatechin and catechin will be oxidized to quinones and later on the polyphenols and proteins will undergoes a condensation reaction where it will decrease the astringency effect and the bitter flavour as been stated before (Niemenak *et al.*, 2006).

Recently, polyphenols have been receiving a very good attention from the public and the health concerned people. It is because of its potentials in human's health such as its physiological functions, which includes the antioxidant potentials, antimutagenic and antitumour activities. The antioxidative potential in cocoa beans is due to its redox reaction of the phenolic compound which will act as reducing agents, hydrogen donators and singlet oxygen quenchers (Azizah *et al.*, 2006).

Based on the studies and findings, cocoa beans are developed to produce the downstream products such as chocolate and other commercial confectionary products. Cacao industries have been emerged as a very important industry with development of cocoa, cocoa butter, syrup, pastes, and all kinds of chocolates. Farmers have been encouraged to increase production of the cocoa beans because of the increasing demand and the steady price of the cocoa beans (Sotelo and Alvarez, 1991).

1.2 Objective of the Study

The objectives of the study are:

- To determine the composition of catechin, theobromine, caffeine and epicatechin content from the cocoa beans from various cocoa planting materials in Sabah.

1.3 Scope of the Study

The scope of the this study is to compare the quantity of the catechin, epicatechin, theobromine, and caffeine of cocoa beans from the various cocoa planting materials by the reversed phase HPLC, using standart solution of the catechin, (-)-epicatechin, theobromine, and caffeine.

CHAPTER 2

LITERATURE REVIEW

2.1 History of Cocoa

Cocoa tree is originated from the South America. It is planted and grows well around the Amazon River which well known for its dense forest and well equipped with its tropical climate which supports the growth of this tree. Initially, *Theobroma cacao.L*, or well know as cocoa tree, was believed founded firstly in the Amazon Valley and Orinoco. It is belonged to the *Theobroma* genus and from the sterculiacea family. The sterculiacea family is indeed a very big family and only *Theobroma cacao* is receiving a wide range of attention from all around the globe (Minifie, 1999).

Hundreds of years later, Columbus first brought cocoa beans to the Europe but Don Cortes who is his fellow countryman, realized that the beans has a commercial value and send it to Spain with the recipe to make the chocolate. Later on, the Spaniards added sugar to the recipe and make it a popular drink to be consumed. Eventually, the trees were grown in the other part of the West Indian Islands and in the Philippines, where as the Dutch probably introduced cocoa in the Indonesia and Ceylon. Its richness has made the people to believe that the cocoa tree was of divine

origin, and later led the Swedish botanist Linnaeus initiate the name "Theobroma"- Food of the Gods-to the genus, including the cacao species (Minifie, 1999).

Cocoa was planted in Peninsular Malaysia in Melaka since 1778, but only in the 1960's the cocoa plantation's hectarage was increasing rapidly. There were some plantation which was successful even thought the plantation was situated on the deep basaltic soils of the Tawau district of Eastern Sabah (Mainstone, 1966).

In 1950, the Department of Agricultural imported a number of varieties of cocoa beans from Ghana, Peninsular Malaysia and Celebes to the Agricultural Research Centre (Central Agricultural Station) at Tuaran for preliminary observation. In 1952, the first batch of seedling of 750 seedlings were produced and supplied to the Borneo Abaca Ltd., in Tawau for planting starting with 2.02 hectares. At the same time, there are other estates started planting cocoa around the Quoin Hill area Tawau on the volcanic basaltic soils and by 1958, there were ten estates operating around the area. From 30.8 hectares in 1955, it expands to 254 hectares in 1958. In 1958 also, Sabah has recorded the first export of 15 tons of dried cocoa beans which values at RM 2,454 (Tze, 1982).

2.2 Cocoa

The cocoa tree can only be cultivated within narrow limits of altitude, latitude and humidity. According to Woods (1985), 75 % of worlds cocoa cultivation comes from region which is within eight degrees of either side of the equator, with exception in some areas to about 18 degree north or south. Temperature which is within 18°C to

32°C is the optimum for the cocoa tree grows healthy in cocoa plantation. Lower temperature or changes in the climate will then yields lower cocoa beans and also damages its quality (Minifie, 1999).

Rain is very for the growth of the cocoa tree. Around 1500 and 2000mm per year is required and preferable for the plantation. This rainfall requirement suits the tropical climate. Relative humidity under these conditions will be 70 to 80 percent during the day, increasing to the saturation point at night. The tree will grow to a height of 20 to 30ft and requires some shade from larger forest tree its flower are about half an inch in diameter and are formed in small group on the trunk and the lower main branches of the trees (Minifie, 1999).

The chemical composition of fresh cocoa bean which is stated in Table 2.1 determines the flavour of the cocoa bean which will be produced. The genotype of the seeds is the main factor which is influencing the quality and the taste of the cocoa beans (Lopez and Dimick, 1995).

Table 2.1 Chemical composition of fresh cocoa beans.

Cotyledon		Pulp	
Water	32-39%	Water	82-87%
Cellulose	2-3%	Sugar	10-13%
Starch	4-6%	Pentosane	2-3%
Pentosane	4-6%	Citric acid	1-2%
Fat	30-32%	Salt	8-10%
Protein	8-10%	pH	3.5
Theobromine	2-3%		
Caffeine	1%		
Acid	1%		
Polyphenol	5-6%		

Sucrose	2-3%		

(Source: Lopez and Dimick, 1995)

Special attention is given in choosing the best planting material. It is because influence the quality of the cocoa beans and its quality. There were many studies that have been done in producing the best cocoa planting material which is giving good quality and good yield of cocoa beans (Clapperton *et al.*, 1994).

2.3 Cocoa Planting Material

Cocoa beans which are having a very good quality are produced by a very good planting material. The planting material also should yield good flavoring compound and should resists to diseases (Masaruddin Mohd. Yusuf, 2006). Earlier studies have been conducted and show that the estimation of a cocoa plantation which shows that 25 percent of the cocoa plants in a population will be high-yielders and another 25 percent are from the poor yielders. The remaining 50 percent are from the average yielders which gives average quality of the cocoa beans (Lamin & Fong, 1994)

Vegetative methods have been used to propagate the cocoa planting material on a commercial scale using the budding or cuttings. There are many investigations have been done locally in Sabah and overseas on the production techniques management and planting of the vegetatively propagated materials. The advantage of this method is that it preserves the characters of the selected trees true to type (Kien, 1980).

The vegetative methods that have been recognized and implemented for large-scale multiplication are:

1) Budding- 12 month's growth is restored by budding on the root.
2) Green Budding- selected bud wood which is greenish or greenish brown stages.
3) Rooting of Cuttings- selective branches were used for cuttings for rooting though chupons (Kien, 1980).

Other than the vegetatively propagated cocoa planting material, the different type of genotype of cocoa seeds also yields different type of quality in raw cocoa beans. From this method, hybrid and clonal cocoa beans are produced. Cocoa plantation which uses the hybrid and clonal cocoa seeds as planting material shows a high degree of variability in all the characters including yield (Lamin & Fong, 1994). The cocoa bean which is recommended for plantation according to the location in Sabah is shown in Table 2.2 and 2.3.

Table 2.2 Some of the hybrid which is recommended for plantation.

No.	Hybrid	Bean size (g)	Cocoa fruit index	Cocoa bean's shell composition (%)	Yield (Kg/ha/year)
1.	UIT X NA 33	1.13	22.34	16.40	2692
2.	PA 156 X 1MC 67	0.97	23.88	18.94	2646
3.	PA 138 X SCA 9	1.00	25.10	17.14	2574
4.	PA 173 X SCA9	1.00	27.35	16.42	3215

(Source: Aloysius *et al.*, 2006)

Table 2.3 Clone suitability according to location in Sabah.

Location	Clones used for plantation
Tawau	BR 25; PBC 123, KKM 1, 4, 5, 22; QH 22, 37, 326, 986, 1003, 1176, 1287.
Lahad Datu	PBC 123; KKM 25, 1, 4, 5, 22, BR 25; QH 22, 37, 326, 968, 1003, 1176, 1287
Sandakan	PBC 123; KKM 25; QH 22, 37, 1003, 1176
Tenom	PBC 123, 140, 159, 326, 968, 1003, 1176, 1287; QH 22, 37; KKM 1, 2, 4, 5, 22, 25

(Source: Aloysius *et al.*, 2006)

The common types of cocoa beans are Criollo and the Forastero beans. Criollo type of bean produces the fine grade of cocoa beans (Clapperton *et al.*, 1994). It is originated from Central and South American region and makes up only 5% to 10% of the world harvest. It is considered as a "fine flavour" beans because of its subtle aroma and complex flavour. Criollo is also in much demand for producing high quality chocolate bars (Zonis, 2005). This bean also requires shorter fermentation period but yields a high grade of quality cocoa beans (Hidayahtullah Hussien, 2006).

The Amazonian Forastero cocoa beans are originated from Ghana. It is also called as "bulk beans" because of its usage for blending, milk chocolate, and for the most of the cocoa powder (Clapperton *et al.*, 1994). This type of beans also has thick-walled cocoa pods and also gives strong aroma and more bitter taste (Zonis, 2005). Forastero beans also requires longer fermentation period than the Criollo beans and it can be found more in Malaysia (Hidayahtullah Hussien, 2006).

Other than the types that have stated before, there are Amelonado types of cocoa beans. It is known as West African Amelonado which is from the lower Amazonian Forestero type (Clapperton *et al.*, 1994).

In Sabah, the Criollo cocoa beans were brought from Sri Lanka in the year 1895 and were planted in the agricultural station in Sandakan and Silam. Later on, the Trinitario and Forastero types were introduced from Philipines, Celebes and Malaya. Then, in 1950, about 5000 Amelonado seedlings were imported from the Ghana (Mainstone, 1978)

Most of the successful clones are from the crosses between a parent derived from Pound's (1938) collection and either an unrelated Upper Amazon or, more commonly, a lower Amazon Forastero or a Criollo/Trinitario (Clapperton *et al.*, 1994). Some examples of the cocoa planting material which is used in Sabah are shown in Photo 2.1, 2.2, 2.3. Until 1958, there were 40 imported clones in total which were established and these comprised the Amelonado, ICS, I, WA, NGK, and UA types (Mainstone, 1978).

Choosing a clonal planting material based on its performance usually varies with location depending on the local climatic and soil condition. However, these following criteria used in consideration of choosing of clonal planting material (Lamin and Fong, 1994):-

1) High yielding, exceeding 2000 kg/ha
2) Good pod value, < 25
3) Acceptable bean sizes, > 1.0 g
4) Adaptable to a wider range of environmental condition
5) Tolerant to the prevailing diseases of the locality

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