DETERMINATION OF (+)-CATECHIN, (-)-EPICATECHIN, THEOBROMINE AND CAFFEINE IN COCOA BEANS FROM VARIOUS FERMENTARIES IN TENOM, SABAH

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ABSTRACT

(+)-Catechin, (-)-epicatechin, theobromine and caffeine had been identified and determined in cocoa beans from four fermentaries in Tenom, namely Nye Tai, Ying Hong, LNY, and Baru Jumpa using High Performance Liquid Chromatography. The average moisture content for cocoa beans at Nye Tai, Ying Hong, LNY, and Baru Jumpa are 7.80%, 7.77%, 6.53% and 6.67% respectively. In comparison, (+)-catechin content is within the range of 240 – 450mg/g, (-)-epicatechin content is within 40 – 145mg/g, theobromine content is within the range 90 – 102mg/g, and caffeine content is within the range of 4 – 10mg/g. In comparison, the highest (+)-catechin content is in Baru Jumpa beans and the lowest in Ying Hong. The highest (-)-epicatechin content is in LNY beans and the lowest is in Ying Hong. The highest theobromine content is in Baru Jumpa and the lowest is in LNY beans. Finally, the highest caffeine content is in LNY beans while the lowest is in Baru Jumpa beans.

ABSTRAK

(+)-Katekin, (-)-epikatekin, theobromin dan kafein telah dikenalpasti dan ditentukan dalam biji koko daripada empat fermentari di Tenom, Nye Tai, Ying Hong, LNY, dan Baru Jumpa menggunakan Kromatografi Cecair Prestasi Tinggi. Purata peratusan kelembapan bagi Nye Tai, Ying Hong, LNY, dan Baru Jumpa adalah 7.80%, 7.77%, 6.53%, dan 6.67% masing-masing. Secara perbezaan, kandungan (+)-katekin adalah dalam lingkungan 240 – 450mg/g, kandungan (-)-epikatekin dalam lingkungan 40 – 145mg/g, kandungan theobromin dalam lingkungan 90 – 102mg/g, dan kandungan kafein adalah dalam lingkungan 4 – 10mg/g. Secara perbandingan, kandungan (+)-katekin tertinggi adalah biji koko Baru Jumpa dan terendah ialah Ying Hong. Kandungan (-)-epikatekin tertinggi ialah LNY dan terendah ialah Ying Hong. Bagi theobromin, tertinggi adalah Baru Jumpa dan terendah adalah LNY. Dan akhir sekali, kandungan kafein yang tertinggi adalah LNY dan terendah adalah biji koko Baru Jumpa.

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LIST OF SYMBOLS AND ABBREVIATIONS

µm	micrometer
%	percentage
^{0}C	degree Celsius
g	gram
mL	milliliter
rpm	revolution per minute
AOAC	Association of Official Analytical Chemists
DAD	Diode Array Detector
HPLC	High Performance Liquid Chromatography
BJ	Baru Jumpa
LNY	LNY
NT	Nye Tai
YH	Ying Hong
R	Linear regression

CHAPTER 1

INTRODUCTION

1.1 Background

Chocolate is one of the most complicated natural flavours. Over 300 volatile compounds have been identified in roasted cocoa beans. Studies done shows that flavanoids (polyphenols) along with sugars and amino acids may be the flavour precursors in fermented cocoa beans (Kim *et al.*, 1983).

Cocoa is also rich in polyphenols content particularly in catechins (flavan-3-ols) and procyanidins. The polyphenols in cocoa beans are stored in the pigment cells of the cotyledons and cocoa leaves. Polyphenol and alkaloids, which comprise approximately 14 – 20% of the whole bean weight, are very important in affecting the quality of cocoa beans. Polyphenols in green and black tea, grape seeds, grapes and red wine have raised much attention but chocolate has not been investigated intensively up to now (Nazarudin *et al.*, 2006).

Although cocoa polyphenols received considerable attention in the past, no research or study have been carried out from Sabah area. With the availability of modern analytical techniques, it is appropriate to take a new look at this important class of compounds in cocoa. Most investigations of cocoa polyphenols utilized paper chromatography and spectrophotometric measurements. The disparity of such analytical methods requiring large samples and lengthy analysis time leads to difficulty in assessing the reliability of experimental results. However, high performance liquid chromatography (HPLC) suggests this approach as being promising in overcoming analytical difficulties (Kim *et al.*, 1983).

The cocoa beans flavour, taste and quality are the most important factor in cocoa beans production and usages. The current measurements and quality criteria do not objectively reflect the fine taste attributes. They increase the problems of manufacturers and dealers in classifying and standardizing their products. Because of this, it is necessary to evaluate physical and chemical parameters which allow us to determine the cocoa's quality. It is therefore important to analyze the polyphenols [(+)-catechin and (-)-epicatechin] and methylxanthines [theobromine and caffeine], because their level affect the flavour of the cocoa beans (Brunetto *et al.*, 2005).

Different analytical techniques have been developed for the determination of these catechins, theobromine and caffeine. High performance liquid chromatography is, however, the most frequently used method nowadays.

The polyphenols mentioned in this study are (+)-catechin and (-)-epicatechin while methylxanthines mentioned in these study are theobromine and caffeine.

1.2 Sampling Area

The sampling area is around Tenom, Sabah. The sample will be collected from 4 different fermentaries. These fermentaries are randomly chosen so that the results would not be biased upon the quality of beans produced by them. The cocoa beans that are taken from these fermentaries are fermented for 5 days according to a standard define by Malaysian Cocoa Board and dried.

Three replicates of sample will be taken from each fermentaries and there will be 12 samples altogether. Because of catechins, theobromine and caffeine are affected by the quality of the beans (Brunetto *et al.*, 2005), so the cocoa beans produced by the various fermentaries will have different level of (+)-catechin, (-)-epicatechin, theobromine and caffeine.

1.3 Objectives of Study

The objectives of this study are;

1 To extract and identify the (+)-catechin, (-)-epicatechin, theobromine and caffeine from cocoa beans.

2 To determine the composition of (+)-catechin, (-)-epicatechin, theobromine and caffeine in cocoa beans from various fermentaries using High Performance Liquid Chromatography.

1.4 Scope of Study

The scopes of this study are;

1 The determination of polyphenols comprising of (+)-catechin and (-)-epicatechin, and methylxanthines comprising of theobromine and caffeine respectively.

2 The samples are taken from 4 various cocoa bean fermentaries (labelled A, B, C, and D; and will be named after the sample taking has been done) around Tenom, Sabah. The fermentaries are chosen randomly regardless of the quality of the beans produced by them.

3 3 replicates will be taken from each fermentaries. The beans are fermented and dried in the respective fermentaries before making collections of samples.

CHAPTER 2

LITERATURE REVIEW

2.1 Cocoa Bean (*Theobroma cacao* L.)

A number of tropical plants belong to the *Theobroma* genus. Cuatrecasas (1964) has reported 22 species, one of which is the cacao bean (*Theobroma cacao*). The plant is native to America and is the only one at present with commercial significance. A very important cocoa industry has emerged with the development of cocoa, cocoa butter, syrups, pastes, and all kinds of chocolates. For this reason, and increasing demand for cacao beans exists, and the price is steadily rising, which encourages farmers to increase production. Therefore, there is a growing demand for research on cocoa beans and its contents (Sotelo *et al.*, 1991).

Cocoa beans are the seeds of the tropical cacao tree, *Theobroma cacao*, family *Sterculaceae*. Originating in the northern part of South America and currently grown within 20^0 latitude of the Equator, the tree flourishes in warm, moist climates with an average annual temperature of 24-28^0C and at elevations up to 600m. The tree, because of its sensitivity to sunshine and wind, is often planted and cultivated under shade trees ("cacao mothers"), such as forest trees, coconut palm and banana trees (Belitz *et al.*, 1999).

Botanically, the term "cacao" refers to the tree and its fruits (pods and seeds). Cocoa describes the bulk commercial dried fermented beans, as well as the powder produced from the beans (Minifie, 1999).

Cacao seeds are the source of commercial cocoa, chocolate, and cocoa butter. Fermented seeds are roasted, cracked, and ground to give a powdery mass from which fat is expressed. Per 100g, the seed is reported to contain as below;

Table 2.1 Compounds contained per 100g of cocoa seed

Calories	456
H_2O	3.6g
Protein	12.0g
Total Carbohydrate	34.7g
Fat	46.3g
Fibre	8.6g
Ash	3.4g
Calcium	106mg
Phosphorus	537mg
Ferum	3.6mg
Beta-carotene Equivalent	30μg
Thiamine	0.17mg
Riboflavin	0.14mg
Niacin	1.7mg
Ascorbic Acid	3mg

Cocoa also contains about 18% proteins, fats, amines and alkaloids, including theobromine (0.5-2.7%), caffeine (0.25% in cocoa and 0.7-1.70% in fat-free beans) (Duke, 1983).

The correct fermentation and drying of cacao are of vital importance as no subsequent processing of the bean will correct bad practice at this stage. A good flavour in the final cocoa or chocolate is related closely to good fermentation, but if the drying after fermentation is retarded, moulds develop and these also impart very unpleasant flavours even if fermentation has been carried out correctly (Minifie, 1999).

Fermentation and drying are two important processes in cocoa processing activity after harvesting. Both of these processes play a major role in producing cocoa beans high in quality where chocolate flavour is formed through chemical reactions of compounds in the cotyledons of the beans. A correct cocoa processing method is important to produce high quality cocoa bean that is less acidic, high in chocolate flavour, enough fermentation, dry, and low count of damaged beans. Moisture content 6.0 – 7.5% is the correct condition for beans storing. Drying also permits the oxidation process to continue where it gives less bitterness and astringency and also the brown colour of the cotyledon. Environmental factors such as temperature, air humidity, pests, and fungal infection must be watched from time to time so as to guarantee that dry cocoa beans can be stored safely (Hidayatullah, 2006).

2.2 Polyphenols

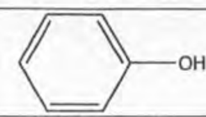
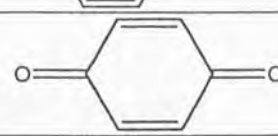
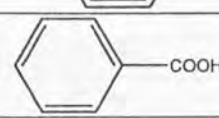
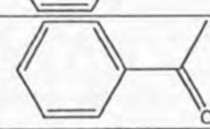
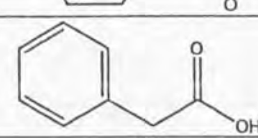
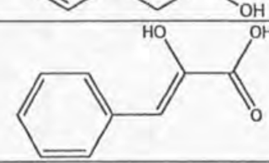
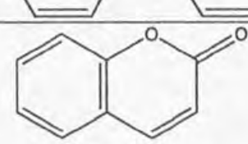
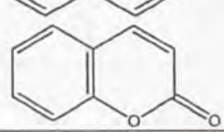
Cocoa (*Theobroma cacao* L.) is an important crop in the economics of several countries such as Ghana, Ivory Coast, Nigeria, Indonesia and Malaysia. Malaysia is the fifth largest producer of cocoa beans in the world. It is one of the main producers of cocoa-based products in the world and the biggest in Asia. However, Malaysian beans are sold at a lower price compared to the West African beans, due to some weaknesses in its quality (low cocoa aroma, astringent and bitter taste). One of the factors which could cause this could be a high amount of phenolic substances (Othman *et al.*, 2006).

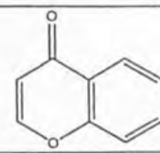
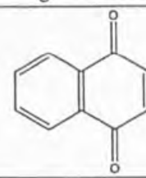
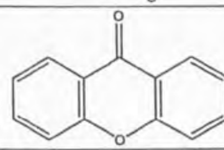
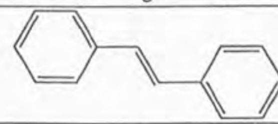
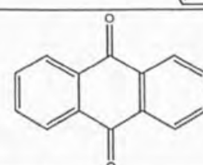
Polyphenols are a large group of natural and synthetic small molecules that are composed of one or more aromatic phenolic rings. Natural polyphenols are a class of phytochemicals found in high concentrations in wine, tea, nuts, berries, cocoa and a wide variety of other plants. More than 8000 polyphenolic compounds have already been identified, and their natural function is correlated to protection of plants from diseases and ultraviolet light and prevention of damage to seeds until they germinate. Natural polyphenols are grouped into several categories: vitamins (e.g., β-carotene and α-tocopherol), phenolic acids (e.g., benzoic acid and phenylacetic acid), flavonoids (e.g., flavanone and isoflavanone), and other miscellaneous polyphenols (ellagic acid, sesamol, eugenol, thymol, etc.) (Porat *et al.*, 2006).

The nib cotyledons consist of two types of parenchyma cells. More than 90% of the cells are small and contain protoplasm, starch granules, aleurone grains and fat globules. The larger cells are scattered among them and contain all the phenolic compounds and purines. These polyphenol storage cells (pigment cells) make up 11-13% of the tissue and contain anthocyanins and, depending on their composition, are white to dark purple. Three groups of polyphenols are present: catechins 37%, anthocyanins 4% and leucoanthocyanins 58%. The main catechin is (-)-epicatechin, (+)-catechin, (+)-gallocatechin and (-)-epigallocatechin (Belitz *et al.*, 1999).

Polyphenols have become an intense focus of research interest because of their perceived health-beneficial effects. Polyphenols are products of the secondary metabolism of plants. Polyphenols can be divided into at least 10 different classes depending on their basic structure. Table 2.1 shows the basic chemical structure of the main polyphenolic compounds. Flavonoids, one the polyphenol group, which constitute the most important single group, can be further divided into 13 classes, with more than 5000 compounds described by (Table 2.2) (Wollgast *et al.*, 2000).

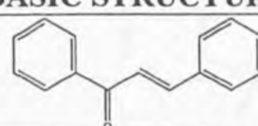
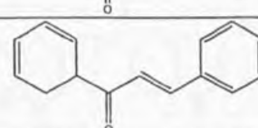
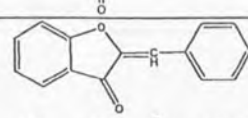
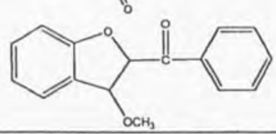
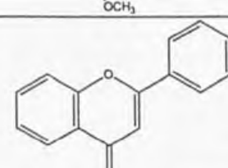
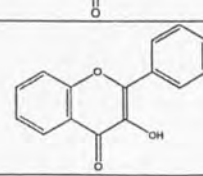
Table 2.2 Main classes of polyphenolic compounds

CLASS	BASIC STRUCTURE	BASIC STRUCTURE
Simple phenols	C_6	
Benzoquinones	C_6	
Phenolic acids	C_6-C_1	
Acetophenones	C_6-C_2	
Phenylacetic acids	C_6-C_2	
Hydroxycinnamic acids	C_6-C_3	
Phenylpropenes	C_6-C_3	
Coumarines, isocoumarines	C_6-C_3	 

Chromones	C_6-C_3	
Naphthoquinones	C_6-C_4	
Xanthones	C_6-C_1-C_6	
Stilbenes	C_6-C_2-C_6	
Anthraquinones	C_6-C_2-C_6	
Flavonoids	C_6-C_3-C_6	*(continued in next table)

Source: Wollgast *et al.*, 2000

Table 2.3 Classification of Food Flavanoids

FLAVONOID	BASIC STRUCTURE
Chalcones	
Dihydrochalcones	
Aurones	 
Flavones	
Flavonols	

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