CHARACTERIZATION OF FLAVOUR IN DRIED COCOA BEANS FROM VARIOUS FERMENTARIES IN TAWAU DISTRICT

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

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ABSTRACT

Characterization of flavour profile of samples from 5 different fermentaries in Tawau district were carried out which include moisture content, titrable acidity and fermentation index. Moisture content and titrable acidity show quantity of water and acid in dried beans while fermentation index determine how well the beans had been fermented. Flavour compounds from dried cocoa beans were extracted using Likens-Nickerson apparatus with dichloromethane as solvent before being injected into gas chromatogram-mass spectrometer and analyzed. Sample from fermentary A with moisture content of 8.89 %, titrable acidity value of 0.318 mmoles and fermentation index of 1.019 had yielded 14.52 $x10^2$ ppm of tetramethylpyrazine. Sample from fermentary B with moisture content of 7.74 %, titrable acidity value of 0.264 mmoles and fermentation index of 1.04 had yielded 55.05 $x10^2$ ppm of tetramethylpyrazine. Sample from fermentary C with moisture content of 6.48 %, titrable acidity value of 0.164 mmoles and fermentation index of 0.816 had yielded 6.96 $x10^2$ ppm of tetramethylpyrazine. Meanwhile the sample from fermentary D with moisture content of 2.69 %, titrable value of 0.1 mmoles and fermentation index of 0.658 had no tetramethylpyrazine. Sample from fermentary E with moisture content of 8.17 %, titrable acidity value of 0.188 mmoles and fermentation index of 0.518 had yielded 0.987 $x10^2$ ppm of tetramethylpyrazine. The results showed that concentration of tetramethylpyrazine had varied with moisture content, titrable acidity and fermentation index. These parameters were greatly affected by different techniques used in the fermentary.

PENCIRIAN PROFIL SEBATIAN PERISA DARIPADA BEBERAPA FERMENTARI DI DAERAH TAWAU

ABSTRAK

Pencirian komponen perisa dalam biji koko kering daripada 5 fermentari di daerah Tawau dilakukan dengan menggunakan parameter kandungan kelembapan, kandungan asid boleh dititrat dan indeks fermentasi. Kandungan kelembapan ialah kandungan air dalam biji koko kering manakala kandungan asid boleh dititrat menunjukkan kandungan asid yang ada di dalam biji koko. Indeks fermentasi pula menunjukkan tahap proses fermentasi yang telah berlaku di dalam biji koko. Komponen perisa telah diekstrak menggunakan peralatan Likens – Nickerson dan kemudian dianalisa menggunakan instrumen gas kromotogram-spektrometer jisim. Sampel dari fermentari A menunjukkan kandungan kelembapan 8.89 %, asid boleh dititrat sebanyak 0.318 mmol dan indeks fermentasi 1.019 di mana ia memberikan kepekatan tetrametilpirazina sebanyak 14.52 x10^2 ppm. Sampel dari fermentari B mempunyai kandungan kelembapan 7.74 %, asid boleh dititrat sebanyak 0.264 mmol dan indeks fermentasi 1.019 di mana ia memberikan kepekatan tetrametilpirazina sebanyak 55.05 x10^2 ppm. Sampel dari fermentari C mempunyai kandungan kelembapan 6.48 %, asid boleh dititrat sebanyak 0.164 mmol dan indeks fermentasi 0.816 di mana ia memberikan kepekatan tetrametilpirazina sebanyak 6.96 x10^2 ppm. Manakala sampel dari fermentari D yang mempunyai kandungan kelembapan 2.69 %, asid boleh dititrat sebanyak 0.1 mmol dan indeks fermentasi 0.658 tidak memberikan puncak bagi tetrametilpirazina di dalam kromatogram. Sampel dari fermentari E mempunyai kandungan kelembapan 8.17 %, asid boleh dititrat sebanyak 0.188 mmol dan indeks fermentasi 0.518 di mana ia memberikan kepekatan tetrametilpirazina sebanyak 0.987 x10^2 ppm. Keputusan kajian ini menunjukkan kepekatan tetrametilpirazina bergantung kepada parameter-parameter di atas dan parameter ini pula bergantung kepada keadaan serta teknik yang telah digunakan oleh fermentari itu.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

DCM	dichloromethane
FA	fermentary A
FB	fermentary B
FC	fermentary C
FD	fermentary D
FE	fermentary E
FI	fermentation index
g	gram
GC-MS	gas chromatogram-mass spectrometer
M	molar concentration
mmoles	millimoles
mg/g	milligram over gram
mc	moisture content
NaOH	sodium hydroxide
nm	nanometer
ppm	parts per million
TA	titrable acidity
TRMP	tetramethylpyrazine
V	volume of fluid
w/w	weight over weight
° C	celsius
µL	microlitre
µm	micrometer
%	percentage

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Cocoa beans, the main raw material for cocoa production originated as seeds coated with white mucilage pulp from the fruit of cocoa plant (*Theobroma Cacao*). The seeds will have to undergo fermentation and drying processes before they are known as dried cocoa beans. The functions of fermentation are to get rid of the white pulp, bring bean death, avoid bean growth and most importantly to produce flavour precursors. Proper fermentation is crucial for flavour development which different methods that are use during this process will determine the quality of products. They methods may vary in the terms of pod storage time, bean spreading, size of fermentation box and duration of fermentation. These can influence parameters such as titrable acidity (TA), fermentation index (FI), moisture content and temperature which then will further affect flavour development (Biehl *et al.*,1990).

Cocoa beans in post-fermentation state are quite moist and need to be dried until the moisture content is around 6-7 % before they can be stored safely. The drying stage is

not only for moisture reduction, it also continues the fermentation process as the chemical changes in beans will only halt until the stated moisture content is reach. Oxidation of the beans occur during this stage and can be controlled by temperature and the rates of drying. Several techniques can be applied in the drying process and there are some characteristics that indicate the beans had good drying process (Hussein, 2006).

During both processes, the reactants for flavour formation which are the flavour precursors are develop. Specific flavour precursors for cocoa flavour formation are free amino acids, peptides and reducing sugars. Hydrophobic amino acids such as alanine, tyrosine, leucine and valine accumulate to far extent during fermentation and are reactive during roasting (Seiki, 1973). Fructose, glucose and sucrose are rich in the bean pulp with the two former being the main reducing sugars that will be utilized.

Roasting is the thermally process of flavour generation and Maillard reaction between amino acids and reducing sugars take place during this phase. Maillard reaction is a series of reactions, on which the outcome is dependent on precursors and reaction conditions (Weener *et al.,* 1997). Based on that, only fermented beans will develop cocoa flavour as unfermented beans lack the needed precursors. They are excessively astringent and bitter in taste (Biehl & Voight,1996; Puziah *et al.,* 1998).

Flavour compounds formed in cocoa beans through Mailard reaction are alkylpyrazines, alcohols, esters, ethers, furans, thiazoles, pyrones, acids, aldehydes, amines, imines, oxazoles and pyrroles (Hoskin & Dimick,1994; Kattenberg,2000; Jinap

et al.,1998). These compounds generate chocolate smell, flavour, sweet odour and they are influence by all the processes above. Different methods in fermentation and drying processes produce different results in titrable acids, moisture content, fermentation index and flavour compounds present after roasting.

Cocoa flavours can be extracted either by simultaneous distillation and extraction (SDE) or direct solid/liquid extraction. The compound then can be analyzed using gas chromatography-mass spectrometer (GC-MS) with the data being matched with the mass spectrometer library.

Studies had been made in comparing these parameters in several states in Malaysia including Sabah (Hii *et al.*, 2004). However, they did not extracted and analyzed flavour compounds of the samples and compare them with the parameters known earlier. This project specifically focused on the comparison of parameters and flavour compounds present in the cocoa samples due to different techniques used in various fermentaries in Tawau district where the best cocoa in the country is said to be produced (Wahab, 1994).

1.2 OBJECTIVES OF STUDY

The main objectives were:

1. To determine the titrable acidity (TA), fermentation index (FI), and moisture content values of dried cocoa beans samples.
2. To analyze the changes in the profile of flavour compounds of each fermentaries according to parameters using gas chromatography- mass spectrometer.

1.3.1 SCOPE OF STUDY

The focus of this project was to characterize flavour compounds in dried cocoa beans collected from several fermentaries in Tawau District. The beans were extracted using simultaneous distillation and extraction method and analyzed with gas chromatography-mass spectrometer. Titrable acidity, fermentation index and moisture content were also determined and compared among samples.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Theobroma cacao can be classified into Criollo and Forestero (Minifie, 1989). Forestero which is widely planted in Malaysia differs from Criollo in flavour profile and longer fermentation duration. Forastero characterizes strong cocoa flavour with bitter notes while Trinitario and Criollo produce fine grade flavour, mild and nutty notes (Eijk, 1994). Major chemical compositions in cocoa pulp are water, sugars, pentosans, organic acids namely citric acid meanwhile an additional of theobromine, fats, protein, caffeine and polyphenols exist in the bean (cotyledon). Cocoa plantations are dominated by Sabah (57.4 %) followed by West Malaysia (23.7 %) and Sarawak (18.9 %). Similar with the production of dried cocoa beans which is lead by Sabah (93.7 %) and the remaining is from Peninsular Malaysia. Therefore making Sabah as the main cocoa producer in this country and the best cocoa planted in this state is said to be from Tawau district (Malaysian Cocoa Board, 2004).

2.2 FERMENTATION

Fermentation is the first stage of cocoa processing after the fruits were harvested. It can be summarized as a microbiatic biochemical process that produces metabolic-end products from energy source in an anaerobic condition. These substances diffuse into bean and initiate bean death which triggers arrays of biochemical reactions that generate precursors for chocolate flavour, colour and aroma (Lehrian and Patterson, 1983; Lopez and Dimmick, 1995). It's a sequence of anaerobic ethanolic, acetic acid and lactic acid fermentation, proteolysis, hydrolysis and oxidation (Appendix A).

Pulp sugars are utilized during fermentation giving rise in the concentration of ethanol, acetic acid, lactic acid and heat. Intense metabolic activities causes temperature to increased while citric acid decreased by about 55% and sucrose decreased to non-detectable level. Multiple reactions in cocoa bean do not occur uniformly as the reactants are stored in different cells and they are dependable on variable events at histological level during fermentation. For example, on the acetic acid gradient formed due to microbial pulp degradation which penetrates the seed through fermentation (Quesnel, 1965).

Microbial degradation of the pulp around the seed affects the reactions in the seed due to the production of heat, ethanol and mainly acetic acid. Furthermore, this process controls temperature and pH-value within the cell, temperature of bean-pulp increased meanwhile pH of the pulp increase while that of the bean decrease. Table 2.1 displays the

changes in the chemical compositions of the pulp and bean fractions during cocoa beans fermentation (Ardhana and Fleet, 2003).

Temperature of beans are around 45 – 50 °C during fermentation and will remain around this range until the process is complete. Thus it is necessary to mix the beans occasionally to ensure that those initially on the outside are expose to the interior temperature (Kim and Keeney, 1984). It is impossible to reconstitute during manufacturing process the properties of cocoa beans which are lost during fermentation. Composition of amino acids and oligopeptides in the seeds which are important flavour precursors therefore are also strongly affected by the variable course of pulp fermentation (Biehl, 1989).

A number of studies had been carried out to improve cocoa beans flavour quality in Malaysia where they had introduced the method of pulp preconditioning. Pulp preconditioning keep pH at ≥ 5.0 in the nibs throughout fermentation, especially during proteolysis and to prevent the nib's pH from increasing at the end of fermentation. With this, flavour potential was found to be improved. Pulp preconditioning can be done either by post harvest pod storage which is the time the pods were stored after being harvested but before splitting and by bean spreading. These treatments cause a significant decrease in pulp volume per seed, pulp water per seed and pulp sugar per seed without changing the original percentage. Pulp volume reduction allows microaeration which increase the respiration of sugar and decreases alcoholic fermentation whom responsible for the formation of acetic acid (Biehl *et al.*, 1989 & 1990).

Table 2.1 Changes in the chemical composition of the pulp and bean fractions during cocoa bean fermentation (Ardhana & Fleet, 2003).

Components	Concentration							
	Before fermentation				After fermentation			
	A		B		A		B	
	Pulp	Bean	Pulp	Bean	Pulp	Bean	Pulp	Bean
Fructose (mgg^{-1})	62	1.0	42	0.8	11	0.4	9	0.3
Glucose (mgg^{-1})	41	0.7	24	0.6	7	0.1	5	0.1
Sucrose (mgg^{-1})	32	19	21	18	0	0	0	0
Ethanol (%w/w)	0.5	0.2	0.3	0.2	0.1	0.4	1.6	1.6
Citric acid (mgg^{-1})	24	9.0	21	7.4	11	4.0	9	3.5
Lactic acid(mgg^{-1})	0.3	0.1	0.3	0.1	6	2.0	5	1.8
Acetic acid (mgg^{-1})	0.4	1.0	0.4	0.7	12	25	10	15
pH	3.7	6.3	4.8	6.5	3.9	5.1	4.9	5.0

2.3 DRYING PROCESS

The drying of cocoa beans is the next stage in primary processing phase. Both of these events are known to give distinct effect on the flavour afterwards. The essential physical and chemical occurrences that happen are water and acid evaporation and oxidation of beans. It is crucial to determine the starting time of the mentioned process as drying on

wet beans after consumption of pulp substrates will cause black, over-fermented and hammy beans with brittle shells (Ostovar and Keeney, 1973). Bacterias then attack seed constituents which destroy amino acids and peptides and give rise to off-flavours (Biehl, 1994).

Moisture content of the beans which is around 55 % after fermentation need to be reduced to approximately 6 % - 7 %. This is to provide good condition for storage where it is important in order to stop any microbial activities. Oxidation of the beans occurs in the drying process where the seeds are accessible to air. The browning reaction due to oxidation of polyphenols in the seed is responsible in giving flavour and colour as well as reducing bitterness and astringency.

2.3.1 Techniques of drying and its control

Drying rate is not only control by the temperature but also by the fluctuations of temperature during the process and the depth of beans in the drying platform. During this process, dissipation of remaining acetic acid will decrease the acidic content in the beans but if the drying temperature is too high it will lessen the evaporation rate causing high acidic residue (Duncan *et al.*, 1989). Furthermore beans will shrivel and inhibit air from going into the bean thus reducing browning effect. Poor or slow aeration plus high temperature will dry the surface moisture and shrink beans. However, if the rate is too slow hammy will occur due to butiric acid and ammonia produced (Said, 1988). From research conducted before, the temperature of the beans must not exceed 65 ° C.

There are different techniques of drying, categorized as natural or artificial with the former having better flavour quality due to gentle and slow drying process which allows browning to progress from bean surface to its centre. Obstacles in this technique is that it is very weather dependent with rain and dampness could cause moldy bean and the duration needed is longer (Hii *et al.,* 2004). This off-flavour is very resistant and can not be removed during manufacturing process. Artificial drying has large probability of producing smoky beans if open-fire method is used. Several physical indicators for good drying process are known such as good storage-life with crisp, plump and satisfactory shell characteristic. Complete browning reaction that had occurred in beans can be examined through cut-test procedure and low pH of nibs without smoky or hammy flavour.

2.4 PHYSICAL AND CHEMICAL PROPERTIES OF FERMENTED BEANS

2.4.1 Acidity of beans

Acidity in dried cocoa beans is influence by the former processes, fermentation oxidizes ethanol to acetic acid and lactic acid which then diffuse into bean while drying evaporates remaining acids. High pH of beans initially is due to citric acid originally present it will decrease after fermentation. The main acids in cocoa beans are lactic acid and acetic acid with the latter being volatile and higher in concentration. High acidity predicts bad flavour, possibly due to over degradation of storage protein which associates with pH of less than 5.2. Jinap *et al.,* (1995) reported that chocolate made from medium pH beans

received a higher response in strong chocolate flavour than those of low and high pH beans. Low quality in Malaysian cocoa beans are due to high acid contains.

However, it is the uptake of acids during fermentation that is more important than the final acidity at the end of fermentation. Slow acidification leads to slow proteolysis ensuring the formation of certain oligopeptides with hydrophobic residues. This will release more hydrophobic amino acids which are the flavour precursors (Biehl *et al.*, 1985). Titrable acidity however is a better indicator of acidity than pH (Nazaruddin, 2006).

2.4.2 Moisture content

Low moisture content, 6 % - 7 % terminate beans re-growth and any microorganism activities which may promote low quality of beans. Beans must show moisture content around the above percentage in order to give optimum flavour production. Moisture content below the minimal standard results in crack beans while over 8% makes growth of mould possible and even faster in tropical climate (Zijderveld, 1994). It leads to development of off-flavour, increases in free fatty acids and produces of mycotoxins. However natural mycotoxins inhibitor quinine, in cocoa bean makes it difficult to produce such hazardous product. Water content should be distributed equally between shell and nib. Moisture content also plays crucial role during roasting as it prevents uncontrolled loss of desirable volatile flavour substances.

2.4.3 Fermentation Index

Fermentation index refers to the formation of brown-yellow in the beans as a result of the polyphenols oxidation. Through this parameter the degree of fermentation can be monitored. Polyphenols in cocoa beans can be classified into catechins, anthocyanins and proanthocyanins. Fully fermented beans are important for full flavour development during roasting and they always display fermentation index higher than 1 while below indicate under-fermented beans (Gourieva and Tserrevitinov, 1979). This is due to the condensation of polyphenols compounds namely anthocyanins which will increase absorbance at 460 nm but decrease absorbance at 530 nm (Kim and Keeney, 1983).

Dried under fermented and partly fermented cocoa beans are rich in polyphenols, comprising of 12 – 18 % of their dry weight (Kim and Keeney, 1984). Polyphenols are the compounds that are responsible for the astringency and contribute to bitter and green flavours. However, the most important characteristic of polyphenols are their tendency to form complexes with proteins, polysaccharides and alkaloids. Misnawi *et al.*, 2004 found that there is a linear relationship between concentration of polyphenols in beans and concentration of flavour compound pyrazine. It can significantly affects pyrazines formation mainly on 2,5- dimethylpyrazine, 2,3-dimethylpyrazine and tetramethylpyrazine.

Phenolic hydroxyl groups are excellent hydrogen bond donors and form strong hydrogen bonds with amide carbonyls of the peptide backbone therefore decreasing the

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