UNIVERSITI PUTRA MALAYSIA

# EFFECT OF PROCESSING ON FLAVOUR PRECURSORS, PYRAZINES AND FLAVOUR QUALITY OF MALAYSIAN COCOA BEANS

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**By**

**PUZIAH HASHIM**

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**1997**

Dedicated to my beloved:

husband
Ahmad Fuaad Mohd Yatim

sons
Fayadh Al-wafi
Nabil Al-wafi
Zhafir Al-wafi

late parents
Hashim Mohamad
Halijah Abdul Hamid Merah

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| | |
|---|---|
| kg | kilogram |
| g | gram |
| mg | milligram |
| µg | microgram |
| ng | nanogram |
| m | metre |
| mm | millimetre |
| nm | nanometre |
| µm | micrometre |
| l | litre |
| µl | microlitre |
| ml | millilitre |
| cm | centimetre |
| sec | second |
| min | minute |
| hr | hour |
| mmole | millimole |
| M | molarity |
| N | normality |
| wt | weight |
| I.D. | internal diameter |
| bp | boiling point |
| std | standard |
| MW | molecular weight |
| sm | sentimeter |
| g | gravity (relative centrifugal force) |
| rpm | revolution per minute |

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Studies were conducted to determine the effect of processing (fermentation, drying and roasting) on flavour precursors and pyrazines concentration of cocoa beans and its flavour quality evaluation. Fermentation was carried out in a rotary drum reactor by subjecting the mixed hybrid of cocoa beans to 6-day fermentation. During fermentation, effect of mass and turning time on the concentrations of these compounds were determined. Drying of cocoa beans was carried out in a hot air oven at an airflow of 0.7$m^2$/sec. Similarly, during drying, effect of bean depth and temperature were determined. Thirteen treatments of fermentation and drying were carried out according to a central composite rotatable design configuration for two factors. The effect of roasting on the concentrations of flavour precursors and pyrazines was compared with air-blown and sun-dried of drum and pod-storage

fermentation and a tested representative Ghanaian sample. The resultant beans were made into cocoa liquor for flavour quality evaluation.

Fermentation significantly decreased the concentration of acidic free amino acids in cocoa beans by 15%, whereas total, hydrophobic and other amino acids increased significantly by 148, 280 and 127%, respectively; peptide-N and total reducing sugars increased by 55 and 208%, respectively. The study found six types of pyrazines, with trimethyl- and tetramethylpyrazine being the major compounds. During cocoa fermentation, an increase in cocoa mass and turning time significantly increased the concentrations of flavour precursors and pyrazines. Results from the Response Surface Methodology (RSM) plots of hydrophobic free amino acids, peptide-N and total reducing sugars recommended mass and turning time for optimum condition of cocoa fermentation were at 60 kg and 5 min turning per day after 48 hr of fermentation.

During drying, an increase in bean depth and temperature significantly decreased the concentrations of flavour precursors, but significantly increased the pyrazines concentration. In addition, total, acidic, hydrophobic and other amino acids decreased by 43, 41, 36 and 49%, respectively; peptide-N and total reducing sugars decreased by 56 and 71%, respectively; and trimethyl- and tetramethylpyrazine increased by 167 and 609%, respectively. Bean depth of 8.3 cm and temperature of 40°C were chosen as the optimum conditions for drying treatment. Under this condition, the concentrations of hydrophobic free amino acids,

peptide-N and total reducing sugars were highly significant, whereas those of trimethyl-, tetramethyl- and total pyrazines were significantly low.

Roasting the samples at 150°C for 30 min significantly decreased the concentrations of acidic, hydrophobic, total and other free amino acids, peptide-N and total reducing sugars but significantly increased the pyrazines concentration. There were no significant differences in the decrease of the concentration of hydrophobic free amino acids, peptide-N and total reducing sugars in the air-blown samples of different fermentation methods (drum and pod-storage); and in those of different drying treatments (air-blown and sun-dried). Air-blown drum fermentation samples had lower concentrations of 2,5-dimethyl-, trimethyl-, tetramethylpyrazine and total pyrazines than those of pod-stored (air-blown and sun-dried) and drum (sun-dried) samples.

From the flavour precursors development of the beans and flavour quality evaluation by sensory of the cocoa liquor, fermentation at 60 kg mass and 5 min turning is recommended in typical cocoa fermentation. It produced good flavour as the pod-storage fermentation method currently recommended. Drying by air-blown produced beans with equally good flavour as the sun-drying method. Compared to Ghanaian beans, these samples had weak cocoa flavour and were less preferred by the taste panels.

Abstrak disertasi yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi syarat keperluan untuk Ijazah Doktor Falsafah

# KESAN PEMPROSESAN KE ATAS PELOPOR PERISA, PIRAZINA DAN KUALITI PERISA BIJI KOKO

Oleh

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FEBRUARI 1997

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Kajian telah dijalankan untuk mengesan proses fermentasi, pengeringan dan pemanggangan ke atas kepekatan pelopor perisa, pirazina dan kualiti perisa biji koko. Fermentasi dijalankan dalam reaktor drum berputar dengan menggunakan biji koko hibrid campuran selama enam hari. Semasa fermentasi, kesan jisim dan masa balikan ke atas sebatian tersebut ditentukan. Pengeringan koko dijalankan dalam oven panas pada kelajuan udara 0.7 $m^2$/saat. Semasa pengeringan, kesan kedalaman biji dan suhu ditentukan. Tiga belas kaedah fermentasi dan pengeringan dijalankan mengikut rekabentuk putaran campuran tengah susunan dua faktor. Kesan pemanggangan ke atas kepekatan pelopor perisa dan pirazina dibandingkan dengan pengeringan udara dan pengeringan melalui matahari dari kaedah fermentasi drum,

fermentasi penyimpanan buah dan fermentasi dari koko Ghana. Hasil biji koko tersebut dijadikan cecair koko untuk penilaian kualiti perisa.

Fermentasi menunjukkan penurunan yang nyata sebanyak 15% kepekatan asid amino asidik, sementara kenaikan yang nyata ditunjukkan oleh asid amino hidrofobik (280%), jumlah asid amino (148%), asid amino lain (127%), peptide-N (55%) dan jumlah gula penurun (208%). Kajian ini mendapati enam jenis pirazina semasa proses fermentasi, dengan trimetil- (69.20 μg/100 g) dan tetrametilpirazina (109.93 μg/100 g) merupakan pirazina yang terbanyak. Semasa fermentasi koko, kenaikan jisim dan masa balikan mempengaruhi dengan nyata kenaikan kepekatan pelopor perisa dan pirazina. Dari keputusan Metodologi Respons Permukaan untuk asid amino hidrofobik, peptide-N dan jumlah gula penurun, cadangan jisim dan masa balikan untuk proses fermentasi optimum ialah 60 kg dan 5 min balikan sehari selepas 48 jam fermentasi.

Semasa pengeringan, kenaikan kedalaman biji dan suhu menurunkan dengan nyata kepekatan pelopor perisa, tetapi kepekatan pyrazin menaik. Pengeringan menurunkan kepekatan asid amino sebanyak 41 (asidik), 36 (hidrofobik), 49 (asid amino lain) dan 43 (jumlah asid amino); menurunkan 56% peptide-N dan 71% jumlah gula penurun, sementara trimetil- dan tetrametilpirazina naik 167 dan 609%, masing-masing. Kedalaman biji 8.3 sm dan suhu 40°C dipilih sebagai pengeringan optimum koko. Ini berdasarkan kepada keputusan kepekatan asid amino hidrofobik,

peptide-N dan jumlah gula penurun yang tinggi dan kepekatan trimetil-, tetrametil- dan jumlah pirazina yang rendah semasa pengeringan koko.

Sampel koko yang dipanggang pada suhu 150°C selama 30 min menunjukkan penurunan yang nyata bagi kepekatan jumlah asid amino, asidik, hidrofobik, asid amino lain, peptide-N dan jumlah gula penurun, sementara kepekatan pirazina naik dengan nyata. Sampel-sampel yang dikering mengguna udara dari fermentasi drum dan penyimpanan buah dari kaedah pengeringan yang berlainan (udara dan matahari) tidak menunjukkan perbezaan yang nyata dalam penurunan kepekaatan asid amino hidrofobik, peptide-N dan jumlah gula penurun. Sampel dari pengeringan udara kaedah fermentasi drum mengandungi kepekatan 2,5-dimetil-, trimetil-, tetrametil- dan jumlah pirazina yang rendah berbanding dengan sampel dari fermentasi penyimpanan buah (pengeringan udara dan matahari) dan fermentasi drum (matahari).

Dari keputusan kualiti perisa biji koko dan penilaian kualiti perisa cecair koko, dicadangkan fermentasi dijalankan pada jisim 60 kg dan 5 min balikan kerana ia menghasilkan perisa yang baik sama seperti kaedah fermentasi penyimpanan buah yang diamalkan sekarang. Pengeringan dengan udara menghasilkan koko yang mempunyai perisa yang baik seperti kaedah pengeringan matahari. Berbanding dengan biji koko Ghana, sampel yang lain mempunyai kurang perisa koko dan kurang disukai oleh ahli panel.

# CHAPTER I

# GENERAL INTRODUCTION

Chocolate is one of the most widely used flavours. However, chocolate made from freshly harvested cocoa beans does not have any chocolate flavour. During fermentation, cocoa beans undergo a series of complex structural and chemical changes which produce flavour precursors, such as, amino acids, peptides and reducing sugars. Most of the reducing sugars are produced by the hydrolysis of sucrose to form glucose and fructose by invertase enzyme (Rohan and Stewart, 1967a). However, they can also be formed from the hydrolysis of anthocyanins to yield arabinose and galactose by glycosidase enzyme (Mamot, 1980). Proteolysis in cocoa beans during fermentation give rise to amino acids and peptides (Biehl and Passern, 1982; Macdonald et al., 1991; Mohr et al., 1971; Rohan and Stewart, 1967b). During this proteolysis, the aspartic proteinase and carboxypeptidase may split protein to hydrophobic free amino acids and hydrophilic peptides (Voigt et al., 1993; 1994a). All these flavour precursors interact through Maillard non-enzymatic browning reaction during the roasting process of the cocoa beans to produce chocolate flavour components such as alcohols, ethers, furans, thiazoles, pyrones, acids, esters, aldehydes, imines, amines, oxazoles, pyrazines, and pyrrroles (Hoskin and Dimick 1984a; 1995; Keeney, 1972; and Mottram, 1994).

Recently, focus has shifted to the pyrazines as being important compounds in cocoa flavour. Reviews on cocoa aroma have stressed the importance of pyrazines along with the effects of individual components, chocolate like flavours and flavour reinforcer (Maga, 1982; Zeigleder and Biehl, 1988). Although the concentration of pyrazines is very low, their contribution to the odour complex is very essential because many of them have low threshold (Takken et al., 1975).

Pyrazines, representing about 40% of the compounds identified in the aroma fraction of chocolate (Maga, 1992), are formed during the roasting of cocoa due to Maillard reaction (non-enzymatic browning reaction) between amino acids, peptides and reducing sugars (Barel et al., 1985; Mohr et al.,1971; Mohr et al., 1976; Rohan, 1972;). This fact was supported by the study using a model system in which the heating up of reducing sugar and amino acid led to the formation of pyrazines (Koehler et al., 1969; Koehler and Odell, 1970). Besides reducing sugars, Voigt et al. (1993; 1994a) concluded that hydrophilic peptides and hydrphobic free amino acids are the cocoa-specific flavour precursors. Although pyrazines are formed mostly during the roasting of well-fermented cocoa beans, Hashim and Chaveron (1994), Jinap et al. (1994a), Kosuge and Kamiya (1962), and Reineccius et al. (1972b) also found tetramethylpyrazine in unroasted and fermented cocoa beans. Thus, according to Zak et al. (1972), the levels of pyrazines, specifically the tetramethylpyrazine in unroasted beans might be of practical importance to the chocolate manufacturer as it could be used as an index of the degree of fermentation and the potential quality of beans prior to roasting. These results are

supported by Barel et al. (1985), who found the level of tetramethylpyrazines to be at a maximum on the seventh day of fermentation.

There have been several studies regarding the changes of amino acid composition and concentration in unfermented, fermented and roasted cocoa beans (Kirchhoff et al., 1989a; Mohr et al., 1971; Rohan, 1964; Rohan and Stewart, 1966a; 1967a; and Zeigleder and Sandmeier, 1982). The changes in peptide-N concentration during fermentation were observed by Biehl et al. (1985) and Biehl and Passern (1982), however very limited study was available on the peptide-N concentration upon roasting. Quantification and measurement of sugars in cocoa beans during fermentation have been reported by Rohan and Stewart (1966b; 1967b), whereas Reineccius et al. (1972a; 1972b) reported the changes of sugar composition and concentration during the third and seventh day of fermentation, in unroasted and roasted cocoa beans samples. During cocoa fermentation, the effect of mass and turning time on the formation of flavour precursors concentration are very important (Mamot and Samarkhody, 1984), however very limited data is available.

During drying, the flavour precursors may undergo some chemical changes associated with thermal reactions, such as Maillard non-enzymatic browning reaction. Several researchers have reported on the decreased of free amino acids concentration during drying process (Eichner and CinerDoruk, 1981; Mottram, 1994; Riggin and Kissinger, 1976; and Ziegleder, 1982); whereas Rizzi, (1988; 1989) and Ho et al. (1992) observed the decrease of peptide concentration. No data was reported on the concentration of sugars during drying treatment. Besides rate

of airflow, temperature and bean depth are the key factors affecting the drying process; however, no study was reported on the effect of temperature and bean depth on the concentrations of amino acids, peptide-N, sugars, and pyrazines. Reineccius et al. (1972b) studied the changes in the concentration of amino acids, sugars, and pyrazines during roasting of cocoa beans, whereas Rohan and Stewart (1966b) reported that the concentration of reducing sugars was decreased by 80 to 90%.

## Statement of Problem

Cocoa beans are largely used in the manufacture of cocoa and chocolate products. A good quality cocoa is one with the inherent flavour of the type of beans concerned. Besides agriculture variations, such as, climatic conditions, cocoa disease, type of beans and soils, the major stages that also contribute to good chocolate flavour are the harvesting of the ripe pods, fermentation, drying of the beans, as well as, roasting process. If any of these stages is mishandled, the proper mixture of the components in the beans may not be present to develop a good chocolate flavour.

Considerable improvements have been achieved by the estates and some researchers in reducing the acidity of the cocoa beans through the manipulation of the fermentation and drying techniques (Mamot, 1982). However, the flavour of Malaysian cocoa is described by the market and manufacturers as being weak as compared to Ghanaian cocoa. The beans are normally used in blends but this will

be an additional cost to the manufacturers. Consequently, the beans are sold at a discounted price in the world market.

Thus, it is important to study the chemical changes of flavour precursors and the formation of pyrazines in cocoa beans during fermentation, drying and roasting. The results of this study will aid in determining the optimum conditions for the fermentation and drying treatments and in improving the weak flavour of Malaysian cocoa beans.

The objectives of this study are as follows:

1. To determine the changes in flavour precursors and pyrazines concentration during cocoa fermentation and the effect of mass and turning time on these compounds.
2. To determine the changes in flavour precursors and pyrazines concentration during cocoa drying and the effect of bean depth and temperature on these compounds.
3. To determine the effect of roasting on flavour precursors and pyrazines concentration of cocoa beans and its flavour quality.