UNIVERSITI PUTRA MALAYSIA

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By

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October 2009

Chairman : Jinap Selamat, PhD

Faculty : Food Science and Technology

Polyphenol is a naturally occurring compound in cocoa beans reported to reduce the concentration of flavour compounds, mainly pyrazines after cocoa roasting. This study was carried out to determine the effect of polyphenol concentrations and pH on cocoa flavour precursors (i.e. reducing sugars and amino acids) and flavour compounds (i.e. alkylpyrazines) during roasting. The study was conducted using a lipidic model system at roasting temperature 120^0C for 45 min. The concentration of amino acids and reducing sugars incorporated in the model systems was based on the results of the concentration found in fermented Malaysian cocoa beans.

To determine the effect of polyphenol concentration, crude polyphenol extracted from cocoa bean was added into the lipidic model system at 0, 58, 116 and 174 g kg^{-1}. Results showed that the higher concentrations of polyphenols (174 g kg^{-1}) significantly ($p<0.05$) decreased the concentration of reducing sugars and amino acids during roasting. As the concentration of polyphenol increased from 58, 116 to 174 g kg^{-1},

fructose was degraded at 33, 35, and 41% and glucose at 29, 30 and 33% respectively. Seventeen individual amino acids were detected, among all; alanine, tyrosine, valine and isoleucine were significantly ($p<0.05$) degraded higher than the others. These amino acids which are in the hydrophobic group were degraded at 41, 44, 45 and 43 % respectively at polyphenol concentration 174 g kg^{-1}. Changes in procyanidins monomeric and polymeric constituents were correlated with the reduction of the precursors. High polyphenol concentration (174 g kg^{-1}) significantly decreased the formation of alkylpyrazines in the model system.

The Response Surface Methodology (RSM) plots revealed that lower concentration of amino acids and reducing sugars was obtained at higher polyphenol concentration (120 g kg^{-1}) and lower pH value (4.5). Based on the constraints set on the obtained surface plots, the optimum region for polyphenol concentration of 43 – 58 g kg^{-1} and pH of 7.0 -7.5 was obtained for the optimum production of flavour precursors; while the optimum region polyphenol concentration of < 75g kg^{-1} and pH of > 6.0 was obtained for the production of alkylpyrazines. The optimum point was determined using response optimizer and it was found that the optimum polyphenol concentration of 49.5 g kg^{-1} and pH of 7.2 for flavor precursors and polyphenol concentration of 52.3 g kg^{-1} and pH of 7.0 for generated alkypyrazines.Thus, it is recommended that the acidity of the beans should be controlled during processing and extracting the excessive amount of polyphenol higher than 52.3 g kg^{-1} from the cocoa beans should be considered to lower its negative effect on reducing sugars and amino acids.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

KESAN POLIFENOL DAN pH KE ATAS PELOPOR RASA KOKO DAN SEBATIAN RASA KOKO MENGGUNAKAN MODEL SISTEM BERLIPID

Oleh

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Polifenol adalah salah satu sebatian yang terkandung secara semulajadi di dalam biji koko, dan dikatakan dapat merendahkan komponen perasa terutamanya pirazin selepas proses pemanggangan koko. Kajian ini dijalankan untuk mengenalpasti kesan kepekatan polifenol dan pH ke atas pelopor rasa koko (gula penurun dan asid amino) dan juga komponen rasa koko (alkilpirazin) selepas pemanggangan koko. Kajian ini dijalankan menggunakan model sistem berlipid pada suhu pemanggangan 120^0C selama 45 minit. Kepekatan asid amino dan gula penurun yang digunakan di dalam model sistem adalah berdasarkan data yang diperolehi daripada biji koko tertapai Malaysia.

Polifenol kasar yang diekstrak daripada biji koko kemudiannya ditambah ke dalam model sistem berlipid pada kepekatan 0, 58, 116 and 174 g kg^{-1}. Keputusan mendapati kepekatan polifenol yang tinggi (174 g kg^{-1}) telah merendahkan kepekatan gula penurun dan asid amino secara signifikan ($p<0.05$). Apabila kepekatan polifenol meningkat dari 58, 116 ke 174 g kg^{-1}, fruktosa menurun sebanyak 33, 35, dan 41%; dan glukosa

sebanyak 29, 30 dan 33% masing-masing. Sebanyak tujuh belas asid amino dikesan dalam kajian ini, di mana alanina, tirosina, valina dan isoleusina didapati lebih menurun berbanding yang lain. Asid amino - asid amino ini, yang tergolong di dalam kumpulan hidrofobik telah menurun sebanyak 41, 44, 45 dan 43 % masing-masing pada kepekatan polifenol 174 g kg^{-1}. Perubahan pada komponen monomer dan polimer prosianidin juga dikaji dan dihubungkaitkan dengan penurunan pelopor rasa. Kepekatan polifenol yang tinggi (174 g kg^{-1}) juga didapati mempunyai kesan yang signifikan dalam merendahkan penghasilan alkilpirazina di dalam model sistem.

Plot yang dihasilkan menggunakan Kaedah Permukaan Respons menunjukkan bahawa kepekatan asid amino dan gula penurun yang rendah diperolehi pada kepekatan polifenol yang tinggi (120 g kg^{-1}) dan pH yang rendah (4.5). Bedasarkan kepada pencirian yang ditetapkan, kawasan kepekatan polifenol yang optimum ialah pada 43 – 58 g kg^{-1} dan pH pada 7.0 -7.5, bagi pelopor rasa koko. Manakala, kawasan kepekatan polifenol yang optimum pada <75 g kg^{-1} dan pH pada > 6.0 dapat mengoptimumkan penghasilan alkilpirazina. Titik optimum diperolehi menggunakan pengoptimum respons mendapati titik optimum polifenol pada 49.5 g kg^{-1} dan pH 7.2 bagi pelopor perisa koko, manakala titik optimum bagi penghasilan alkilpirazina adalah pada kepekatan polifenol pada 52.3 g kg^{-1} dan pH 7.0 . Oleh itu, adalah dicadangkan tahap keasidan biji koko dikawal semasa pemprosesan dan pengekstrakan polifenol yang tinggi dari 52.3 g kg^{-1} pula dapat menurunkan kepekatannya ke tahap yang diperlukan bagi menurunkan kesan negatifnya ke atas gula penurun dan asid amino.

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I certify that a Thesis Examination Committee has met on 23rd October 2009 to conduct the final examination of Noor Soffalina Binti Sofian Seng on her thesis entitled " Effect of polyphenol and pH on cocoa flavour precursors and alkylpyrazines in lipidic model system " in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science degree).

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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NOOR SOFFALINA BT SOFIAN SENG

Date: 25 February 2010

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LIST OF ABBREVIATIONS

%	: percentage
^{0}C	: degree Celsius
µg	: microgram
µl	: microlitre
bp	: boiling point
cm	: centimeter
Da	: Dalton
g	: gram
g	: gravity
GC	: Gas Chromatography
HPLC	: High Performance Liquid Chromatography
hr	: hour
i.d.	: internal diameter
l	: litre
M	: molarity
min	: minute
mg	: milligram
ml	: mililitre
mm	: milimetre
mM	: millimole
N	: normality
nm	: nanometer
PVP	: Polyvinylpyrolidone
rpm	: rotation per minute
RSM	: response surface methodology
s	: second
UV	: ultraviolet
v	: volume
w/w	: weight/weight
wt	: weight

CHAPTER 1

GENERAL INTRODUCTION

1. 1 Introduction

The cocoa tree, *Theobroma cacao*, *L*., is usually found in warm and moist climates in areas about 20^0 latitude north and south of the equator (Wollgast and Anklam, 2000a; 2000b). The Maya and Aztec Indians of central America cultivated the cocoa tree before Columbus discovered America. When Hernando Cortes and his men conquered the Aztecs, they found the Indians, who consider cocoa to be divine and delivered to them directly by God, using the beans to create a drink name chocolatl, an Aztec word meaning acrid or sour (Minifie, 1980, Ensminger *et al*.,1994, Dillinger *et al*., 2000). The Swedish botanist, Carolus Linnaeus, must have been familiar with the pleasant flavour of cocoa or chocolate when he classified it in the first edition of his 'Species Plantarum' published in 1753, since the Latin term *theobroma* means 'food of the gods'. This pleasant chocolate flavour builds ups during fermentation and roasting of cocoa beans (Woods and Lass, 1985, Ensminger *et al*., 1994). In Malaysia, there is report stating that cocoa plant was found planted in Sabah in the 1700 after having been brought in by the Spaniards from Latin America to the Philippines and then to Indonesia (Wood and Lass, 1985).

Freshly harvested cocoa beans do not have any chocolate flavour. The unique chocolate flavour derived from cocoa beans will only be produced after series of processing involving fermentation and roasting. A correct fermentation process is crucial in order to produce suitable cocoa flavour precursors. The flavour precursors produced by fermentation are resulted from the breakdown of protein and carbohydrate into reducing sugars, amino acids and peptides (Foster, 1978).

Reducing sugars, free amino acids and oligopeptides have been identified as important (non volatile) precursors of hundreds of volatile compounds assembling the characteristic of cocoa aroma (Ziegleder and Biehl, 1988; Voigt and Biehl, 1995). Incorrect fermentation process affect the flavour quality badly, this is because unfermented or partly fermented cocoa beans that is further roasted does not develop any chocolate flavour and the resulting flavour was found to be extremely astringent and bitter (Lopez, 1986; Biehl and Voigt, 1996; Puziah *et al*., 1998a, 1998b).

Sucrose was almost completely hydrolysed to fructose and glucose by invertase present in the beans during the fermentation (Puziah *et al*., 1998a, 1998b). However, the reducing sugars can also be formed from the hydrolysis of anthocyanins to arabinose and galactose by glycosidase enzyme (Mamot, 1989; Hoskin and Dimick, 1994).

Free amino acids, namely hydrophobic amino acids, are aroma precursors of cocoa beans produces during cocoa fermentation, which will develop into cocoa-specific aroma upon roasting (Barel *et al*., 1985; Voigt *et al*., 1994a; 1994b). During cocoa fermentation, proteolysis, catalyzed by aspartic proteinase and carboxypeptidase, increased the content of free amino acids and oligopeptides (Biehl *et al*., 1982b; Ziegleder and Biehl, 1988; Puziah *et al*., 1998a; 1998b). The aspartic proteinase is an endopeptidase that breakdowns proteins at hydrophobic sites to produce hydrophobic amino acids and oligopeptides. The carboxypeptidase is an exopeptidase that breakdowns proteins at the carboxy terminals producing hydrophilic amino acids and oligopeptides (Biehl *et al*., 1993; Voigt *et al*., 1993; 1994a).

Roasting of the whole cocoa bean or nib is an essential step to further develop the chocolate flavour, which should already present in the precursors form arising from correct fermentation. Cocoa roasting is a process that depend on time-temperature relationship, where the time can ranges from 5 to 120 min and the temperature ranges from 120 to 150 ^{0}C (Wollgast and Anklam, 2000a). All the aroma precursors which include free amino acids, peptides and reducing sugars will involved in heat-induced Maillard non-enzymatic browning reaction to produce subsequent cocoa-specific flavour compounds (Barel *et al*., 1985; Hoskin and Dimick, 1984a). Maillard non-enzymatic browning is a reaction between the carbonyl group of reducing sugars and a nitrogen source from free, uncharged amine group of amino acids or protein with the loss of one mole of water (Davies *et al*., 1997; Hofmann and Schieberle, 2000; Jinap *et al*., 1998; Labuza and Braisier, 1992).