



UNIVERSITI PUTRA MALAYSIA

**BIOCHEMICAL DIFFERENTIATION OF COCOA COTYLEDON
PROTEINS FROM VARIOUS GENETIC ORIGINS**

AMIN BIN ISMAIL

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UNIVERSITI PUTRA MALAYSIA**

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By

AMIN BIN ISMAIL

**Thesis Submitted in Fulfilment of the Requirements for
the Degree of Doctor of Philosophy in
the Faculty of Food Science and Biotechnology
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August 2000



This dissertation is specially dedicated to.....

**My beloved wife Fatimah Omar, and sons
Amir Farhan and Amir Safuan**

**for their unconditional patience, love and
support.**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

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Chairman: Professor Jinap Selamat, Ph.D.

Faculty: Food Science and Biotechnology

Previous studies have reported that differences in the cocoa flavour of roasted beans from various genetic origins do exist; however, the findings were mainly based on sensory evaluation. In addition, the cocoa flavours differences were also claimed to be solely due to the maternal plants and not to other factors such as physiological factors. Cocoa cotyledon contains vicilin (7S)-class globulin (VCG), a major storage protein. It is a native source of oligopeptides and free amino acids, both of which have been identified as precursors of specific-cocoa aroma. These precursors are formed by proteolysis during fermentation. The VCG and the splitting specificity of proteases from cocoa cotyledons have been known to be responsible in the production of cocoa-specific aroma precursors during fermentation. The separation patterns of VCG which were separately isolated from cocoa cotyledons of various genotypes namely Forastero, Criollo, Trinitario, PBC 140, SCA 12 and UIT1 are shown. Comparative studies of VCG patterns and its multiple forms were conducted by means of SDS-PAGE and two-dimensional electrophoresis (2-D IEF/SDS-PAGE), whereas oligopeptide profiles were detected

using a high performance liquid chromatography. SDS-PAGE analysis of the molecular weights of the predominant polypeptides of VCG subunits (47 and 31 kDa) from various genotypes did not show qualitative differences. The 2-D IEF/SDS-PAGE analysis of the VCG subunits revealed at least 10 multiple forms with pIs in the range of 6.1 to 6.8. The additional polypeptides with their multiple forms which had pIs in the range of VCG subunits were also detected on 2-D IEF/SDS-PAGE. These polypeptides were most probably the resultant degradation proteolytic products from the action of aspartic endoprotease on VCG subunits. There were very strong similarities in the VCG subunits and their degradation products at the level of their polypeptide constituents from the standpoint of number, molecular weight and isoelectric point. The oligopeptide profiles of autolysis products of the acetone dry powder prepared from various genotypes did not revealed differences. It indicates that the action of aspartic endoprotease of the various cocoa genotypes on VCG during autolysis was similar. There was no analytical identity difference of VCG subunits and their degradation proteolytic products for all the genotypes. Thus, VCG seems not to be responsible for cocoa aroma differences in cotyledons of the various genotypes within methodical limits. The VCG was almost completely degraded as compared to the storage albumin at the end of fermentation. In conclusion, aroma differences in raw cocoa harvested from various genotypes are the result of other genotypes, physiological or curing related factors but are not due to genetic differences of aroma precursors derived from storage proteins.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

**PEMBEZAAN BIODOKIMIA PROTIN KOTILEDON KOKO DARI PELBAGAI
GENETIK ASAL**

Oleh

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Ogos 2000

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Kajian-kajian terdahulu dilaporkan bahawa wujud perbezaan citarasa koko yang dipanggang dari pelbagai genetik asal. Walau bagaimanapun, penemuan-penemuan ini pada keseluruhannya berasaskan penilaian deria. Di samping itu, perbezaan-perbezaan citarasa koko ini hanya disebabkan oleh pokok induk tanpa mengambilkira faktor-faktor lain iaitu faktor-faktor fisiologi. Protein kotiledon koko mengandungi “vicilin (7S)-class globulin” (VCG) merupakan protein simpanan utama. Ia merupakan sumber oligopeptida dan asid amino bebas yang dikenalpasti sebagai pelupor koko aroma yang spesifik. Pelupor-pelupor ini terbentuk disebabkan oleh proteolisis semasa fermentasi. VCG dan spesifik penguraian oleh protease dari kotiledon koko telah diketahui bertanggungjawab dalam penghasilan pelupor aroma koko yang khusus sewaktu fermentasi. Corak pemisahan VCG yang telah disepara tulen daripada koko kotiledon yang berlainan genotaip iaitu Forastero, Criollo, Trinitario, PBC 140, SCA 12 dan UIT1 telah ditunjukkan. Kajian-kajian perbandingan corak-corak VCG dan komposisinya telah dikendalikan dengan menggunakan SDS-PAGE dan dua dimensi elektroforesis (2-D IEF/SDS-PAGE)

manakala profil-profil oligopeptida dikesan dengan kromatografi cecair tekanan tinggi. Analisa SDS-PAGE untuk berat molekul polipeptida-polipeptida utama bagi VCG (47 dan 31 kDa) dari pelbagai genotaip tidak menunjukkan perbezaan-perbezaan kualitatif. VCG yang dianalisa dengan 2-D IEF/SDS-PAGE menunjukkan sekurang-kurangnya 10 bentuk-bentuk berganda dan mempunyai titik isoelektrik di antara julat 6.1 hingga 6.8. 2-D IEF/SDS-PAGE juga dapat mengesan polipeptida-polipeptide tambahan yang mana bentuk-bentuk bergandanya mempunyai titik isoelektrik di antara julat subunit-subunit VCG. Polipeptida-polipeptida ini berkemungkinan besar hasil produk-produk penguraian proteolitik oleh tindakan aspartik endoprotease terhadap subunit-subunit VCG. Terdapat persamaan-persamaan yang jelas pada subunit-subunit VCG dan produk-produk penguraiannya pada peringkat juzuk-juzuk polipeptidanya dari segi bilangan, berat molekul dan titik isoelektrik. Produk-produk autolisis “Acetone dry powder” yang disediakan dari pelbagai genotaip menghasilkan oligopeptida yang sama profilnya. Ini menandakan semasa autolisis tindakan aspartik endoprotease dari pelbagai genotaip koko terhadap VCG adalah sama. Tiada perbezaan identiti analitikal di antara subunit-subunit VCG dan produk-produk penguraian proteolitiknya bagi semua genotaip. Dengan ini VCG nampaknya tidak bertanggungjawab untuk menghasilkan perbezaan aroma koko dari pelbagai genotaip. Penguraian VCG hampir keseluruhannya dibandingkan dengan albumin simpanan di akhir fermentasi. Kesimpulannya, perbezaan-perbezaan aroma koko yang dituai dari pelbagai genotaip disebabkan faktor genotaip lain, faktor fisiologi atau faktor berkaitan fermentasi dan pengeringan, tetapi bukan disebabkan perbezaan-perbezaan genetik pelopor citarasa hasil daripada protein simpanan.

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I certify that an Examination Committee met on 09 August, 2000 to conduct the final examination of Amin bin Ismail on his Doctor of Philosophy thesis entitled “Biochemical Differentiation of Cocoa Cotyledon Proteins from Various Genetic Origins” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

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LIST OF ABBREVIATION

A	amphere
AcDP	acetone dry powder
DTT	DL-dithiothreitol
HPLC	high performance liquid chromatography
hr	hour
ICRG	Institute of Cocoa Research Ghana
IEF	isoelectric focusing
IPG	immobilised pH gradient
kDa	kilodalton
L	litre
M	molar
MARDI	Malaysian Agriculture Research Development Institute
mM	millimolar
min	minute
ml	millilitre
Mr	molecular weight
PBC	Prang Besar clone
pI	isoelectric point
SCA	Scavina clone
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
TFA	trifluoroacetic acid

2-D IEF/SDS-PAGE	two dimensional electrophoresis
UIT1	Unidentified Trinitario clone
V	volt
VCG	vicilin (7S)-class globulin
v/v	volume/volume
w/v	weight/volume
μ	micro