

ABSTRACT

This study focussed on the extraction and characterisation of carotenoid pigments and genes from the carotenoid biosynthetic pathway in *I. batatas* leaves. Six different local *I. batatas* varieties were studied namely, *I. batatas* var. Batu Kelantan (BK), *I. batatas* var. Batu Biasa (BB), *I. batatas* var. Oren (Oren), *I. batatas* var. Indon (Indon), *I. batatas* var. Vitato (Vitato) and *I. batatas* var. Biru Putih (BP).

The different *I. batatas* varieties were distinguished based on the morphological variations in their leaves and storage roots. General screening of the β -carotene and lutein levels conducted across the different developmental stages among the different varieties showed that 9 to 12 days old Oren variety leaves exhibited the highest levels of β -carotene and lutein pigments and thus was chosen as the suitable sample for subsequent experiments. Storage at 15°C was found to prolong storage of the leaf samples and the leaves showed a maximum storage of approximately 4 days before the pigments level deteriorated to below 80% of their total amount.

Acetone was found to be the suitable extraction solvent due to the higher intensity of yellow colour observed and the presence of higher levels of β -carotene and lutein pigments in the extract. Furthermore, 40% KOH and 2 hours of saponification time were selected as the optimum parameters for extraction. Stability experiments conducted on the yellow pigments extract kept in different storage conditions (temperature, illumination and matrix) for short term and long term durations showed that lutein and β -carotene exhibited lower stability in acetone and upon exposure to high temperature and light. Illumination was found to have had the greater effect on pigment stability compared to temperature.

LCMS/MS analysis showed that the yellow extract contained organic acids, lipids and traces of carotenoid, namely β - cryptoxanthin and 4-ketozeaxanthin, besides the two main pigments, β -carotene and lutein. Pesticide analysis conducted via GCMS showed no traces of herbicides, organochlorine insecticide and organophosphorus insecticide in the extract. Antioxidant assays performed on the extract showed that it contained 2.994 ± 0.078 g/ 100g gallic acid equivalents and 114.86 ± 4.35 μ g/g catechin equivalents respectively using the Folin-Ciocaltaeu and Vanillin HCl assays. The radical scavenging activity of the extract recorded an IC_{50} value of 491.86 μ g/ml, which was only slightly lower compared to vitamin C (IC_{50} = 471.6 μ g/ml).

Lycopene epsilon-cyclase (*LcyE*), lycopene beta-cyclase (*LcyB*) and phytoene synthase (*Psy*) genes were successfully isolated from the *I. batatas* leaves via RT-PCR. Pfam analysis showed that the *LcyB* and *LcyE* genes belong to the lycopene cyclase protein family while the *Psy* gene belongs to the squalene/phytoene synthase domain. BLASTp results on these genes further confirmed their identity and phylogenetic tree revealed their relationship with similar sequences from other plants. This study has shown that *I. batatas* leaves are suitable source for the extraction of carotenoids with high antioxidant properties.

ABSTRAK

Kajian ini memfokus kepada pengekstrakan dan karekterasi ekstrak pigmen karotenoid dan gen terlibat dalam sintesis karotenoid daripada daun *I. batatas*. Enam varieti ubi keledek tempatan dikaji termasuk varieti Batu Kelantan (BK), Batu Biasa (BB), Oren (Oren), Indon (Indon), Vitato (Vitato) dan Biru Putih (BP).

Ubi keledek dari varieti yang berlainan dikenalpasti melalui variasi morfologi pada daun dan ubi tumbuhan. Tahap beta karotin dan lutin dalam daun dari varieti dan tahap perkembangan yang berlainan dikenalpasti dan daun ubi keledek varieti Oren (9 hingga 12 hari) dipilih sebagai sampel mulaan kerana mempunyai tahap pigmen tertinggi jika dibandingkan dengan varieti-varieti lain. Suhu simpanan 15°C didapati boleh melanjutkan tempoh penyimpanan dedaun ubi keledek dan dedaun boleh disimpan pada jangka masa maksima selama 4 hari sebelum tahap pigmen jatuh dibawah tahap 80% daripada jumlah asal.

Melalui proses pengoptimuman, acetone, 40% kalium hidroksida dan tempoh 2 jam saponifikasi dikenalpasti sebagai pelarut ekstrak, kepekatan kalium hidroksida dan tempoh saponifikasi optimum bagi proses pengekstrakan karotenoid daripada daun ubi keledek. Kestabilan ekstrak pigmen kuning apabila disimpan pada suhu, cahaya dan matriks yang berlainan dalam jangka masa pendek dan panjang turut dikenalpasti. Pigmen lutin dan beta karotin menunjukkan ketidakstabilan apabila disimpan dalam acetone, dan apabila terdedah kepada suhu tinggi dan cahaya lampu.

Analisa LCMS/MS mendapati bahawa pigmen karotenoid (β -carotene, lutein, β -cryptoxanthin dan 4-ketozeaxanthin), organik acid tumbuhan dan lilin tumbuhan hadir dalam sampel ekstrak. Analisa racun perosak menggunakan GCMS mendapati bahawa tiada sisa-sisa racun serangga didalam sampel ekstrak. Kajian antioksidan mendapati

sampel ekstrak mengandungi 2.99 ± 0.08 GAE g/100g dan 114.86 ± 4.35 $\mu\text{g/g}$ catechin apabila dikaji menggunakan kaedah Folin-Ciocalteu dan Vanillin HCl. Selain itu, kajian juga mendapati bahawa nilai IC_{50} untuk sampel ekstrak adalah 491.86 $\mu\text{g/ml}$ dan hanya sedikit kurang daripada vitamin C ($\text{IC}_{50} = 471.6$ $\mu\text{g/ml}$).

Gen lycopene epsilon-cyclase (*LcyE*), lycopene beta-cyclase (*LcyB*) dan phytoene synthase (*Psy*) daripada daun ubi keledak berjaya dikenalpasti melalui kaedah RT-PCR. Analisa Pfam menunjukkan bahawa gen *LcyB* dan *LcyE* termasuk dalam famili protein lycopene cyclase manakala gen *Psy* adalah dalam domain squalene/phytoene synthase. Secara keseluruhan, kajian ini mendapati bahawa dedaun ubi keledak sesuai dijadikan sebagai sumber pengekstrakan karotenoid (dengan aktiviti antioksidan) untuk kegunaan industri.

ACKNOWLEDGEMENT

I would like to express my greatest gratitude to god for giving me strength and opportunity to pursue my dream of obtaining a doctorate degree in my field of interest. It has been a great journey throughout my candidature with lots of learning opportunities and self-developments. I would also like to acknowledge the guidance and support from my supervisors Dr. Chandran Somasundram and Prof. Datuk Dr. Amru Nasrulhaq Boyce throughout my research and thesis writing period. This thesis would not be possible without the help from both of them.

Besides that, I am also truly indebted and thankful to both of my parents who supported and believed in me, encouraging me during my ups and downs and my younger brother who consistently cheered me up with his jokes during my stressful moments, and not to forget my supporting and encouraging friends. I would also like to thank the Post Harvest Biotechnology laboratory mates and friends who willingly taught and shared their experiences with me, which helped me greatly throughout my research.

Sincere thanks also go to En. Abdul Karim for his help and willingness in supplying the *I. batatas* leaves used for this study. Without his help, my field trips and sample collections would not be as smooth. Besides, I would also like to thank the University of Malaya for providing grant (PS263/2010B) and scholarship for me to conduct and complete this research project. Last but not least, I would like to thank everyone for their help in every other ways in making this thesis a reality.

TABLE OF CONTENTS

CONTENT	PAGE
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENT	vi
TABLE OF CONTENTS	vii
LIST OF FIGURES	xii
LIST OF TABLES	xvii
LIST OF ABBREVIATIONS	xviii
CHAPTER 1	1
Introduction	
CHAPTER 2	7
Literature Review	
2.1 Introduction to <i>Ipomoea batatas</i> (Sweetpotato)	
2.1.1 Distribution and Growth Habitat of <i>Ipomoea batatas</i>	
2.1.2 Classification and Taxonomy of <i>Ipomoea batatas</i>	
2.1.3 Growth and Usages of <i>Ipomoea batatas</i>	
2.1.4 <i>Ipomoea batatas</i> (Sweetpotato) Leaves	
2.1.5 The Future of <i>Ipomoea batatas</i> (Sweetpotato) Plant	
2.2 Plant Secondary Metabolites	
2.2.1 Types of Plant Secondary Metabolites	
2.2.2 Roles of Plant Secondary Metabolites	
2.2.3 Qualitative and Quantitative Analyses of Plant Secondary Metabolites	
2.2.4 Future of Plant Secondary Metabolites	
2.3 Carotenoids	
2.3.1 Nomenclature and Structures	
2.3.2 Roles and Functions of Carotenoids	
2.3.3 Carotenes	
2.3.4 Xanthophylls	
2.3.5 Carotenoids Biosynthesis Pathway	
2.3.6 Sources of Carotenoids	
2.3.7 Extraction and Stability of Carotenoids from Biological Sources	
2.3.8 Past and Current Biotechnological Approaches in Studying Carotenoids Biosynthesis	

- 2.4 Antioxidants
 - 2.4.1 Benefits and Roles of Antioxidants
 - 2.4.2 Antioxidant Assays
 - 2.4.3 Previous Studies on Antioxidants from Plants
- 2.5 Introduction to Colouring Dyes
 - 2.5.1 Synthetic Dye Versus Natural Dye
 - 2.5.2 Safety Issues Concerning Natural Dye from Plants

CHAPTER 3

65

Material Selection for Pigments Extraction: General Screening of Pigments

- 3.1 Introduction
- 3.2 Materials and Methods
 - 3.2.1 Plant Materials
 - 3.2.2 Morphological Variation between the Different Varieties of *Ipomoea batatas* Leaves
 - 3.2.3 Morphological Variation between the Different Varieties of *I. batatas* Storage Roots
 - 3.2.4 General Screening of Chlorophyll *a*, Chlorophyll *b*, Lutein and β -Carotene in *Ipomoea batatas* Leaves Using Spectrophotometric Method
 - 3.2.5 Statistical Analysis of Data
 - 3.2.6 Sample Handling and Storage
- 3.3 Results
 - 3.3.1 Morphological Variation between the Different Varieties of *Ipomoea batatas* Leaves
 - 3.3.2 General Screening of Chlorophyll *a*, Chlorophyll *b*, Lutein and β -Carotene in *Ipomoea batatas* Leaves using Spectrophotometric Method
 - 3.3.3 Sample Handling and Storage
- 3.4 Discussion

CHAPTER 4

99

Optimisation of Extraction Method and Stability of the Yellow Pigments Extracted from *Ipomoea batatas* Leaves

- 4.1 Introduction
- 4.2 Materials and Methods
 - 4.2.1 Optimisation of Extraction Solvent
 - 4.2.2 Optimisation of Potassium Hydroxide (KOH) Concentration Used in Saponification
 - 4.2.3 Optimisation of Saponification Duration during Extraction
 - 4.2.4 Stability of Yellow Pigments from *Ipomoea batatas* Leaves
 - 4.2.4.1 Sample Preparation

4.2.4.2 Stability of Extracted Yellow Pigments Stored in Different Conditions

4.3 Results

- 4.3.1 Optimisation of Extraction Solvent
- 4.3.2 Optimisation of Potassium Hydroxide (KOH) Concentration Used in Saponification
- 4.3.3 Optimisation of Saponification Duration during Extraction
- 4.3.4 Stability of the Extracted Yellow Pigments from *Ipomoea batatas* Leaves
 - 4.3.4.1 Short Term Analysis on the Stability of Extracted Yellow Pigments from *Ipomoea batatas* Leaves
 - 4.3.4.2 Long Term Analysis on the Stability of Extracted Yellow Pigments from *Ipomoea batatas* Leaves

4.4 Discussion

CHAPTER 5

148

Qualitative Analysis and Antioxidant Activity of Yellow Extract from *Ipomoea batatas* leaves

5.1 Introduction

5.2 Materials and Methods

- 5.2.1 Qualitative analysis
 - 5.2.1.1 Lutein and β -carotene Standards
 - 5.2.1.2 Sample Preparation
 - 5.2.1.3 Determination of Carotenoids using Liquid Chromatography Mass Spectrometry/ Mass Spectrometry (LCMS/MS)
 - 5.2.1.4 General Screening for Active Carotenoids
- 5.2.2 Pesticide Analysis on Yellow Pigments Extract
- 5.2.3 Antioxidant Analysis on the Yellow Pigments Extract from *Ipomoea batatas*
 - 5.2.3.1 Sample Preparation
 - 5.2.3.2 Total Polyphenols (Folin-Ciocalteu) Assay
 - 5.2.3.3 Vanillin-HCl Assay
 - 5.2.3.4 DPPH Radical Scavenging Assay
 - 5.2.3.5 Reducing Power Assay

5.3 Results

- 5.3.1 Qualitative Analysis of Yellow Pigments Extract
- 5.3.2 Pesticide Analysis on Yellow Pigments Extract
- 5.3.3 Total Polyphenols (Folin-Ciocalteu) Assay
- 5.3.4 Vanillin-HCl Assay
- 5.3.5 DPPH Radical Scavenging Assay
- 5.3.6 Reducing Power Assay

5.4 Discussion

Isolation and Characterisation of Lycopene Epsilon-Cyclase (LcyE), Lycopene Beta-Cyclase (LcyB) and Phytoene Synthase (Psy) Genes from *Ipomoea batatas* Leaves

6.1 Introduction

6.2 Materials and Methods

- 6.2.1 Sample Preparation
- 6.2.2 RNA extraction from *Ipomoea batatas* leaves
- 6.2.3 Quantitative Analysis of RNA
- 6.2.4 Primer Design for RT-PCR
- 6.2.5 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)
- 6.2.6 Purification of RT-PCR Product and Sequencing
- 6.2.7 Sequence Analyses using Bioinformatics Tools
 - 6.2.7.1 NCBI
 - 6.2.7.2 ExPASy
 - 6.2.7.3 Pfam
 - 6.2.7.4 ClustalW

6.3 Results

- 6.3.1 Quantitative Analysis of RNA
- 6.3.2 LcyE gene
 - 6.3.2.1 Agarose Gel Electrophoresis
 - 6.3.2.2 Complementary DNA (cDNA) Sequence of LcyE
 - 6.3.2.3 Translated Amino Acid Sequence
 - 6.3.2.4 Sequence Homology Using BLASTp
 - 6.3.2.5 Signature Pattern
 - 6.3.2.6 Analysis of Amino Acid Sequence using ProtParam
 - 6.3.2.7 Multiple Sequence Alignment for LcyE Using ClustalW
 - 6.3.2.8 Phylogenetic Tree for LcyE
 - 6.3.2.9 Protein Structure Prediction
- 6.3.3 LcyB Gene
 - 6.3.3.1 Agarose Gel Electrophoresis
 - 6.3.3.2 Complementary DNA (cDNA) Sequence of LcyB
 - 6.3.3.3 Translated Amino Acid Sequence
 - 6.3.3.4 Sequence Homology Using BLASTp
 - 6.3.3.5 Signature Pattern
 - 6.3.3.6 Analysis of Amino Acid Sequence using ProtParam
 - 6.3.3.7 Multiple Sequence Alignment for LcyB Using ClustalW
 - 6.3.3.8 Phylogenetic Tree for LcyB
 - 6.3.3.9 Protein Structure Prediction
- 6.3.4 Psy Gene
 - 6.3.4.1 Agarose Gel Electrophoresis
 - 6.3.4.2 Complementary DNA (cDNA) Sequence of Psy

6.3.4.3	Translated Amino Acid Sequence
6.3.4.4	Sequence Homology Using BLASTp
6.3.4.5	Signature Pattern
6.3.4.6	Analysis of Amino Acid Sequence Using ProtParam
6.3.4.7	Multiple Sequence Alignment for Psy Using ClustalW
6.3.4.8	Phylogenetic Tree for Psy
6.3.4.9	Protein Structure Prediction

6.4 Discussion

CHAPTER 7	238
General Discussion	
BIBLIOGRAPHY	243
PUBLICATIONS	267
APPENDIX 1	292

List of Figures

	Page	
Fig 2.1	The <i>Ipomoea batatas</i> plant	8
Fig 2.2	The route of <i>Ipomoea batatas</i> dispersion	9
Fig 2.3	Taxonomy of the <i>Ipomoea batatas</i>	11
Fig 2.4	<i>Ipomoea batatas</i> leaves	13
Fig 2.5	Structures of phenolics compounds	20
Fig 2.6	Structure of terpenoids compounds	22
Fig 2.7	Structure of alkaloids compounds	23
Fig 2.8	Isoprene structure in carotenoids	30
Fig 2.9	Structures of α -carotene, β -carotene, γ -carotene and ϵ -carotene	35
Fig 2.10	Structures of lutein and zeaxanthin	37
Fig 2.11	Carotenoids biosynthesis pathway	39
Fig 2.12	Examples of reactive oxygen species (ROS)	51
Fig 3.1	Method of measuring the maximum width and length for the leaves	68
Fig 3.2	Photographs of different varieties of <i>Ipomoea batatas</i> leaves	73
Fig 3.3	Photographs of storage roots for the different varieties of <i>I. batatas</i>	79
Fig 3.4	Total chlorophyll levels in the different growth stage of <i>I. batatas</i> leaves	81
Fig 3.5	Lutein concentration in the different growth stage of <i>I. batatas</i> leaves	83
Fig 3.6	β -carotene concentration in different growth stage of <i>I. batatas</i> leaves	83
Fig 3.7	Correlation between total chlorophyll and lutein and total chlorophyll and β -carotene	84
Fig 3.8	Percentage of remaining lutein in the <i>I. batatas</i> leaves kept at 4°C	86
Fig 3.9	Percentage of remaining β -carotene in <i>I. batatas</i> leaves kept at 4°C	86
Fig 3.10	Percentage of remaining lutein in <i>I. batatas</i> leaves kept at 15°C	88
Fig 3.11	Percentage of remaining β -carotene in <i>I. batatas</i> leaves kept at 15°C	88
Fig 3.12	Percentage of remaining lutein in <i>I. batatas</i> leaves kept at room temperature (25°C)	90

Fig 3.13	Percentage of remaining β -carotene in <i>I. batatas</i> leaves kept at room temperature (25°C)	90
Fig 4.1	Yellow pigments extracted from <i>I. batatas</i> leaves using different extraction solvents	107
Fig 4.2	Concentrations of β -carotene and lutein in the yellow pigments extract using different extraction solvents	108
Fig 4.3	Yellow pigments extracted from <i>Ipomoea batatas</i> leaves using different concentrations of potassium hydroxide (KOH) during saponification	109
Fig 4.4	Concentrations of β -carotene and lutein in the yellow pigments extract using different concentrations of potassium hydroxide (KOH) for saponification	110
Fig 4.5	Yellow pigments extract from <i>I. batatas</i> leaves using different saponification durations	111
Fig 4.6	Concentrations of β -carotene and lutein in the yellow pigments extract using different saponification durations	112
Fig 4.7	Graph of yellow pigments extract in acetone analysed using scanning spectrophotometer	113
Fig 4.8	Graph of yellow pigments extract in soybean oil analysed using scanning spectrophotometer	114
Fig 4.9	Picture of the yellow pigments extract in acetone and soybean oil	115
Fig 4.10	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in -20°C under dark condition	116
Fig 4.11	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 4°C under dark condition	117
Fig 4.12	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 4°C in the presence of light	118
Fig 4.13	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 25°C under dark condition	119
Fig 4.14	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 25°C in the presence of light	120
Fig 4.15	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 40°C under dark condition	121
Fig 4.16	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 40°C in the presence of light	122
Fig 4.17	Percentage of β -carotene pigments in acetone when kept at different storage conditions	123
Fig 4.18	Percentage of lutein pigments in acetone when kept at different storage conditions	124

Fig 4.19	Percentage of β -carotene pigments in soybean oil when kept at different storage conditions	125
Fig 4.20	Percentage of lutein pigments in soybean oil when kept at different storage conditions	126
Fig 4.21	Picture of the yellow pigments extract in acetone and soybean oil after six months of storage in -20°C	127
Fig 4.22	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in -20°C in the dark	128
Fig 4.23	Picture of the yellow pigments extract in acetone and soybean oil after six months of storage at 4°C	129
Fig 4.24	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 4°C in the dark	130
Fig 4.25	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 4°C in the presence of light	131
Fig 4.26	Picture of the yellow pigments extract in acetone and soybean oil after six months storage in 25°C (Room Temperature)	132
Fig 4.27	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 25°C in the dark	133
Fig 4.28	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 25°C in the presence of light	134
Fig 4.29	Picture of the yellow pigments extract in acetone and soybean oil after 6 months storage in 40°C	135
Fig 4.30	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 40°C in the dark	136
Fig 4.31	Stability of β -carotene and lutein pigments in acetone and soybean oil stored in 40°C in the presence of light	137
Fig 5.1	Detection of β -carotene standard peak using LCMS/MS	164
Fig 5.2	Detection of β -carotene peak in the yellow extract from <i>Ipomoea batatas</i> leaves using LCMS/MS	165
Fig 5.3	Detection of lutein standard peak using LCMS/MS	166
Fig 5.4	Detection of lutein peak in the yellow extract from <i>Ipomoea batatas</i> leaves using LCMS/MS	167
Fig 5.5	Chromatogram of the yellow extract from <i>Ipomoea batatas</i> leaves detected using LCMS/MS	169
Fig 5.6	Mass spectrum of lutein in the yellow extract from <i>Ipomoea batatas</i> leaves	170
Fig 5.7	Mass spectrum of β -carotene in the yellow extract from <i>Ipomoea batatas</i> leaves	171

Fig 5.8	Mass spectrum of beta cryptoxanthin in the yellow extract from <i>Ipomoea batatas</i> leaves	172
Fig 5.9	Mass spectrum of fumaric acid in the yellow extract from <i>Ipomoea batatas</i> leaves	173
Fig 5.10	Mass spectrum of phytofluene in the yellow extract from <i>Ipomoea batatas</i> leaves	174
Fig 5.11	Mass spectrum of 4-ketozeaxanthin in the yellow extract from <i>Ipomoea batatas</i> leaves	175
Fig 5.12	Mass Spectrum of 1-monolinoleoylglycerol trimethylsilyl ether in the yellow extract from <i>Ipomoea batatas</i> leaves	176
Fig 5.13	Mass Spectrum of malonic acid in the yellow extract from <i>Ipomoea batatas</i> leaves	177
Fig 5.14	Mass Spectrum of decanoic acid in the yellow extract from <i>Ipomoea batatas</i> leaves	178
Fig 5.15	Standard curve for gallic acid used in the folin-ciocalteau assay	180
Fig 5.16	Standard curve for catechin used in vanillin-HCl assay	181
Fig 5.17	Radical scavenging activity of the vitamin C, leaf extract and yellow extract from <i>Ipomoea batatas</i> leaves depicted by the percentage of inhibition	182
Fig 5.18	Reducing power of the leaf and yellow extract from <i>Ipomoea batatas</i> leaves	183
Fig 6.1	Simplified carotenoid biosynthesis pathway in plants	195
Fig 6.2	Young leaves of <i>I. batatas</i> var. Oren used in RNA extraction	197
Fig 6.3	Gel Image of RT-PCR Product of LcyE Gene Amplification	205
Fig 6.4	cDNA sequence for LcyE	206
Fig 6.5	Translated amino acid sequence for LcyE	206
Fig 6.6	Multiple sequence alignment for <i>I. batatas</i> LcyE amino acid sequence with <i>C. canephora</i> , <i>I. kenyan</i> , <i>C. sinensis</i> and <i>R. communis</i>	209
Fig 6.7	Phylogeny tree showing the evolutionary relationship and distance between the <i>I. batatas</i> LcyE amino acids sequence and other sequences	210
Fig 6.8	Predicted protein structure of LcyE from <i>I. batatas</i>	211
Fig 6.9	Gel Image of RT-PCR Product of LcyB Gene Amplification	212
Fig 6.10	cDNA sequence for LcyB	213
Fig 6.11	Translated amino acid sequence for LcyB	213

Fig 6.12	Multiple sequence alignment for <i>I. batatas</i> LcyB amino acids sequence with <i>I. kenyan</i> , <i>C. sinensis</i> , <i>S. lycopersicum</i> and <i>C. papaya</i>	217
Fig 6.13	Phylogeny tree showing the evolutionary relationship and distance between the <i>I. batatas</i> LcyB amino acid sequence and other sequences	218
Fig 6.14	Predicted protein structure of LcyB from <i>I. batatas</i>	219
Fig 6.15	Gel Image of RT-PCR Product of Psy Gene Amplification	220
Fig 6.16	cDNA sequence for Psy	221
Fig 6.17	Translated amino acids sequence for Psy	222
Fig 6.18	Multiple sequence alignment for <i>I. batatas</i> Psy amino acid sequence with <i>P. mume</i> , <i>A. deliciosa</i> , <i>C. papaya</i> and <i>I. kenyan</i>	225
Fig 6.19	Phylogeny tree showing the evolutionary relationship and distance between the <i>I. batatas</i> Psy amino acids sequence and other sequences	226
Fig 6.20	Predicted protein structure for Psy from <i>I. batatas</i>	227

List of Tables

		Page
Table 2.1	Common Pesticides in Agricultural Products	62
Table 3.1	Leaf Morphology for Six Varieties of <i>Ipomoea batatas</i>	75
Table 3.2	Mean Width of Different Varieties of <i>Ipomoea batatas</i> Leaves at Different Development Stages	76
Table 3.3	Mean Length of Different Varieties of <i>Ipomoea batatas</i> Leaves at Different Development Stages	77
Table 3.4	Storage Root Morphology for the Different <i>I. batatas</i> Varieties	80
Table 4.1	Parameters used in the optimisation of the extraction of natural yellow pigments from <i>Ipomoea batatas</i> leaves	104
Table 5.1	Gallic Acid Standard Preparation	155
Table 5.2	Catechin Standard Preparation	157
Table 5.3	Vitamin C Standard Preparation	159
Table 5.4	Butylhydroxyanisole (BHA) Standard Preparation	162
Table 5.5	Compounds Detected Using LCMS/MS	168
Table 5.6	Pesticide Analyses on Yellow Pigments Extract	179
Table 6.1	Primers Used in RT-PCR	199
Table 6.2	Reagents Used in RT-PCR	200
Table 6.3	Signature Patterns for LcyE Amino Acids Sequence	208
Table 6.4	Physico-Chemical Properties of LcyE Amino Acids Sequence	208
Table 6.5	Signature Patterns of LcyB Amino Acids Sequence	215
Table 6.6	Physico-Chemical Properties of LcyB Amino Acids Sequence	216
Table 6.7	Signature Patterns for Psy Amino Acids Sequence	223
Table 6.8	Physico-Chemical Properties of Psy Amino Acids Sequence	224

List of Abbreviations

A	Absorbance
BHA	Butylhydroxyanisole
BHT	Butylatedhydroxytoulene
BLASTp	Basic Local Alignment Search Tool Protein
cDNA	Complementary deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
ExPASy	Expert Protein Analysis System
FW	Fresh weight
ESI	Electrospray ionisation
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
<i>I. batatas</i>	<i>Ipomoea batatas</i>
KOH	Potassium hydroxide
LCMS/MS	Liquid chromatography mass spectrometry/ mass spectrometry
LcyB	Lycopene beta cyclase
LcyE	Lycopene epsilon cyclase
NCBI	National Centre for Biotechnology Information
nm	nanometer
Psy	Phytoene synthase
RNA	Ribonucleic acid
rpm	Revolutions per minute
sec	second
UV	Ultraviolet