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 µg/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 µg/ml, which was only slightly lower compared to vitamin C (IC_{50}= 471.6 µg/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, aceton, 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 menunjukan ketidakstabilan apabila disimpan dalam aceton, 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 antioksida mendapati

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

Gen lycopene epsilon-cyclase (*LcyE*), lycopene beta-cyclase (*LcyB*) dan phytoene synthase (*Psy*) daripada daun ubi keledek 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 mendapai bahawa dedaun ubi keledek sesuai dijadikan sebagai sumber pengekstrakan karotenoid (dengan aktiviti antioksida) untuk kegunaan industri.

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TABLE OF CONTENTS

List of Figures

List of Tables

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. batatas	*Ipomoea batatas*
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