UNIVERSITI PUTRA MALAYSIA

CAROTENOIDS CONTENT AND ANTIOXIDANT CAPACITY OF UNDERUTILIZED TROPICAL FRUITS

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By

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July 2009

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This study was conducted to evaluate the total carotene content (TCC), *β*-carotene, antioxidant capacity and stability study of 12 Malaysian underutilized fruits. TCC and *β*-carotene content were determined using UV-Vis spectrophotometric and HPLC methods, respectively; while antioxidant capacity was determined using DPPH, FRAP and haemoglobin oxidation assays. TCC of the studied fruits was ranged from 1.4–19.8 mg/100 g edible portions. Jentik-Jentik had the highest *β*-carotene content followed by Cerapu 2, Durian Nyekak 2, Tampoi Kuning, Durian Nyekak 1 and Cerapu 1. TCC of the underutilized fruits was in the order of Jentik-Jentik > Durian Nyekak 2 > Durian Nyekak 1 > Cerapu 2 > Cerapu 1 > Tampoi Kuning > Bacang 1 > Kuini > Bacang 2 > Durian Daun > Bacang 3 > Tampoi Putih. Results of DPPH and FRAP assays showed that Cerapu had the highest antioxidant capacity, while Tampoi Kuning had the lowest capacity compared to other studied fruits. Cerapu had EC_{50} of 219.5 mg/L, while other fruits had no EC_{50}. For haemoglobin oxidation assay, the studied fruit extracts at higher concentration were able to significantly reduce the production of hydrogen peroxide-

induced malondialdehyde (MDA) compared to the control. A significant reduction in MDA level of red blood cell (RBC) was found after treated with Durian Nyekak 2 and commercial mango (control) extracts. In stability study, the degradation of TCC in Bacang 1, Bacang 3, Kuini and Tampoi Putih was more than 30% at day 12th of storage at −20°C. However, less than 5% degradation was observed in Cerapu 1 and Cerapu 2. For Cerapu 2, Durian Nyekak 2 and Jentik-Jentik, there were less than 15% of TCC of the studied samples degraded during storage at 0°C for 5 h. A rapid degradation of carotenoids occurred when the studied samples were stored at 0°C than −20°C. The study indicated that among the studied fruits, Jentik-Jentik and Cerapu had the highest carotene content and antioxidant capacity, respectively. Moreover, Cerapu extract had the slowest TCC degradation rate for the studied storage time and temperature.

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

KANDUNGAN KAROTENOID DAN KAPASITI ANTIOKSIDA BUAHAN TROPIKA YANG TERPINGGIR

Oleh

KHOO HOCK ENG

Julai 2009

Pengerusi: Profesor Madya Amin Ismail, PhD

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Kajian ini dijalankan untuk mengkaji jumlah kandungan karotena (JKK), *β*–karotena, kapasiti antioksida dan kajian kestabilan 12 buah-buahan terpinggir Malaysia. JKK dan kandungan *β*-karotena adalah ditentukan dengan menggunakan kaedah-kaedah "UV-Vis" spektrofotometrik and "HPLC" yang berkaitan; manakala kapasiti antioksida adalah ditentukan dengan menggunakan ujian-ujian "DPPH', "FRAP" dan pengoksidaan hemoglobin. JKK dalam buah-buahan yang dikaji adalah dalam lingkungan 1.4−19.8 mg/100 g bahagian yang boleh dimakan. Jentik-Jentik mengandungi kandungan *β*-karotena yang tertinggi diikuti dengan Cerapu 2, Durian Nyekak 2, Tampoi Kuning, Durian Nyekak 1 dan Cerapu 1. JKK buah-buahan terpinggir ini adalah dalam turutan Jentik-Jentik > Durian Nyekak 2 > Durian Nyekak 1 > Cerapu 2 > Cerapu 1 > Tampoi Kuning > Bacang 1 > Kuini > Bacang 2 > Durian Daun > Bacang 3 > Tampoi Putih. Keputusan ujian-ujian "DPPH" dan "FRAP" menunjukkan bahawa Cerapu mempunyai kapasiti antioksida yang tertinggi, manakala Tampoi Kuning mempunyai kapasiti yang terendah berbanding dengan buah-buahan

lain yang dikaji. Cerapu mempunyai EC_{50} sebanyak 219 mg/L, manakala buah-buahan lain tidak mempunyai EC_{50}. Untuk ujian pengoksidaan hemoglobin, ekstrak-ekstrak buah-buahan yang dikaji pada kepekatan yang tinggi dapat mengurangkan penghasilan "MDA" yang dirangsangkan oleh hidrogen peroksida secara ketara berbanding dengan kawalan itu. Satu pengurangan dalam tahap "MDA" secara ketara daridapa sel-sel darah merah didapati setelah dirawat dengan ekstrak-ekstrak Durian Nyekak 2 dan mempelan komersial (kawalan). Dalam kajian kestabilan, penguraian JKK dalam Bacang 1, Bacang 3, Kuini and Tampoi Putih ini adalah melebihi 30% pada hari simpanan yang ke-12 pada –20°C. Walau bagaimanapun, kurang daripada 5% penguraian adalah diperhatikan dalam Cerapu 1 dan Cerapu 2. Untuk Cerapu 2, Durian Nyekak 2 dan Jentik-Jentik, kurang daripada 15% JKK bagi sampel-sampel yang dikaji terurai semasa penyimpanan pada 0°C untuk 5 jam. Satu kehilangan karotenoid yang cepat berlaku semasa sampel-sampel yang dikaji ini adalah disimpam dalam 0°C daripada –20°C. Kajian ini menunjukkan bahawa diantara buah-buahan yang dikaji, Jentik-Jentik dan Cerapu mengandungi kandungan karotena dan kapasiti antioksida yang tertinggi yang berkaitan. Tambahan pula, ekstrak Cerapu mempunyai kadar penguraian JKK yang terlambat untuk masa dan suhu penyimpanan yang dikaji.

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I certify that a Thesis Examination Committee has met on 20 July 2009 to conduct the final examination of Khoo Hock Eng on his thesis entitled "**Carotenoid Content, Antioxidant Activity and Stability Properties of Underutilized Tropical Fruits**" 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.

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Date: 16 October 2009

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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KHOO HOCK ENG

Date: 30 November 2009

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List of Abbreviations

Abs	Absorbance
ABTS	2,2'-azinobis(3-ethylbenzothiazolline-6sulfonic acid)
AIBN	Azoisobutrylnitrile
AAPH	Amyloid protein homolog
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BC	Beta carotene
BEAC	Beta carotene equivalence antioxidant capacity
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
B-PE	β-phycoerthrin
BSA	Bovine serum albumin
DAD	Diode array detector
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
EHE	Ethanol-hexane extract
ESR	Electron spin resonance
FRAP	Ferric ion reducing antioxidant power
GAE	Gallic acid equivalence
GC	Gas chromatography

Hb	Hemoglobin
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
HRCM	House filters with a unique technology
ILP	Isolated limb perfusion
IOU	Inhibited Oxygen Uptake
LDL	Low-density lipoprotein
MARDI	Malaysian Agricultural Research and Development Institute
MDA	Malondialdehyde
MHE	Methanol-hexane extract
MS	Mass spectrometry
ORAC	Oxygen Radical Absorbance Capacity
PBS	Phosphate buffer saline
RBC	Red blood cell
ROS	Reactive oxygen species
RP	Reverse phase
RSD	Relative standard deviation
RSD(r)	Intra-batch repeatability relative standard deviation
SPSS	Statistic Package for Social Sciences
SD	Standard deviation
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TCC	Total carotene content

TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity
THF	Tetrahydrofuran
TOSC	Total Oxidant Scavenging Capacity
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine
TRAP	Total Radical Trapping Antioxidant Parameter
TRC	Total reducing capacity
UK	United Kingdom
USA	United State of America
UV	Ultraviolet
Vis	Visible

List of Annotations

α	Alpha
β	Beta
γ	Gamma
δ	Delta
ε	Epsilon
μ	Micro
μg	Micro gramme
λ_{max}	Lamda maximum
χ^2	Chi square
%	Percent
±	Plus and/or minus
<	Lesser than
>	More than
$A	Australian dollar
ºC	Celsius degree
$^1O_2$	Single oxygen
A	Absorbance value
A_0	Absorbance of the control
A_1	Absorbance of the extracts
B	Concentration of beta carotene (μg/ml)
C	*y* intercept of the curve
cm	Centimeter

Cu^{2+} / Cu (II)	Cupric ion
$CuSO_4$	Copper sulphate
Fe^{2+} / Fe (II)	Ferrous ion
Fe^{3+} / Fe (III)	Ferric ion
$FeCl_3$	Ferric chloride
$FeSO_4$	Ferrous sulphate
g	Gramme
h	Hour
H_2O	Water
$HO^{\bullet}$	Hydroxyl radical
L	Litre
M	Molar
m	Metre
M	Slope of the BC standard calibration curve
min	Minute
mm	Milimetre
mg	Micro-gramme
mL	Mililitre
N	Normality
Na_2CO_3	Sodium carbonate
NaK	Sodium potassium
NaOH	Sodium hydroxide
nm	Nanometer

pm	Pikometre
sec	Second
$0_2^{\bullet-}$	Superoxide anion
$ONOO^-$	Peroxynitrite
$ROO^{\bullet}$	Peroxyl radicals
V	Volt
V	Volume

CHAPTER 1

INTRODUCTION

Research Background

Underutilized fruit is defined as fruit that is less popular, with under-exploited potential and not ready for commercialization (Gruère et al., 2008). The underutilized fruits have been traditionally consumed as staple food and also for medicinal purposes. These fruits are growing wild, seasonal and not planted in commercial scale. However, most of the indigenous fruits in Malaysia are considered underutilized. These underutilized fruits are Bacang, Cerapu, Durian, Jentik-Jentik, Kuini and Tampoi. Among the underutilized fruits, some are known to have a great antioxidant capacity with potential health benefits. Certain underutilized fruits from the genera of *Baccaurea*, *Durio*, *Garcinia* and *Mangifera* are potential functional fruits.

Carotenoids are classified into carotenes (carotenoid structure without oxygen atom) and xanthophylls (with one or more oxygen atom). Carotenoids are widely distributed in plant (O'Connell et al., 2007). Besides that, fruits and vegetables are also known to contain carotenoids (Holden et al., 1999). In plant, carotenoids are useful in protecting leaves from harmful UV irradiation (Niedzwiedzki et al., 2005). In human body, large quantity of carotenoid has been found, and it was believed to act as primary oxidation

defense system (Lako et al., 2007). Moreover, antioxidant activity of carotenoids has been widely studied (Palace et al., 1999; Choi et al., 2007; Lako et al., 2007).

Antioxidant has ability to scavenge the free radicals. It is able to inhibit lipid peroxidation induced by free radical that causes the deterioration of foods. The free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as $O_2^{\bullet -}$, H_2O_2, $ROO^{\bullet}$, $HO^{\bullet}$, $^1O_2$ and $ONOO^-$ (Huang et al., 2005). Free radicals produced in our body have contributed to various types of diseases. Besides, free radical scavenging are involve in hydrogen atom transfer and electron-transfer reactions (Huang et al., 2005), inhibition of Briggs-Rauscher oscillation reaction (Cervellati et al., 2002), chemiluminescence (Georgetti et al., 2003) and electro-chemiluminescence (Zhu et al., 2004).

The antioxidant activity of tropical fruits and fruit juices had been studied since antioxidant assays were developed. Mahattanatawee et al. (2006) reported that tropical fruits have a wide range of antioxidant capacity, which is due to the present of vitamins C and E, carotenoids, flavonoids, and thiol (SH) compounds. Supplementation of dehydrated juice concentrates from mixed fruit and vegetables was found to increase in the respondent plasma β-carotene, retinol, α-tocopherol, ascorbic acid, folic acid and homocysteine levels (Samman et al., 2003). The antioxidant capacity has been determined by Leong and Shui (2002) using ABTS assay shows that Ciku has higher antioxidant properties than other studied fruits.

Statements of Problem

Due to the popularity of fruit intake for maintaining good health and prevention of various diseases, there is a need to study the antioxidant properties of Malaysian underutilized fruits. These fruits are rich in antioxidants, such as carotenoids, flavonoids, isoflavones and proanthocyanidins (Kato et al., 1992; Monache et al., 1994). Besides, the fruits have antioxidant properties that can combat hidden hunger e.g. vitamin deficiency and promote better health (Bioversity International, 2004).

Fruits grown in Malaysia are claims to be rich in antioxidant compounds (Samman, 2002), but there is still a myth. Wilson and Shaw (1978) reported that guava (*Psidium guajava*) was rich in terpene. Besides, mango (*Mangifera indica*) is rich in antioxidants and medicinal properties (Adams, 1997; Samman, 2002). In traditional medicine, peoples had used mango to treat certain diseases, such as jaundice and pectoris stroke. Although mango is believes to be rich in medicinal values and dietary carotenoids, limited published data are available. Moreover, no study has been carried out to determine the antioxidant properties of particular fruits, such as *Baccaurea*, *Durio*, *Garcinia*, *Mangifera* and *Syzygium* fruits. In addition, limited research on antioxidant activities of underutilized fruits has been allocated in Malaysia.

Studies on carotenoid contents and antioxidant capacity of underutilized fruits are still lacking. A decade ago, numerous researches have been done on antioxidant capacities (Saija et al., 1995; Mitchell et al., 1998; Velioglu et al., 1998; Wei and Lau, 1998;

Soong and Barlow, 2004) and related potential health effects (Palace et al., 1999; Rui, 2007; van de Wiel et al., 2001), either *in*-vitro, animals or human subjects. Several studies have been carried out to determine the carotenoids (Yakushina and Taranova, 1995) and polyphenolic contents (Mulder et al., 2001; Wilkinson et al., 2002; Yáñez and Davies, 2005) that were presented in human biological fluids. However, no study has been performed to determine the carotenoid content of underutilized fruits, except for apple (Wach et al., 2007), berries (Marinova and Ribarovo, 2007), fruit juices (Belajová and Suhaj, 2004) and tea leaves (Zuo et al., 2002).

The stability of carotenoids in underutilized fruit extracts is questionable. Study has shown that some of the carotenoids found in fruits are not stabled to heat (Vásquez-Caicedo et al., 2007). Thermal processing was also found to affect the stability of carotenoids in fruits and vegetables (Mayer-Miebach and Behsnilian, 2006). Besides, carotenoid degradation occurred when expose to UV-irradiation (Cvetković and Marković, 2008). Moreover, carotenoid contents of some underutilized fruits were found to be degraded during low temperature storage, which will affect the carotenoid contents and antioxidant capacity of the studied fruits. Therefore, there is a need to obtain an optimal outcome of the study results.