



UNIVERSITI PUTRA MALAYSIA

**LYCOPENE CONTENT AND ANTIOXIDANT PROPERTIES OF PINK
GUAVA INDUSTRY BY-PRODUCTS**

KONG KIN WENG

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**LYCOPENE CONTENT AND ANTIOXIDANT PROPERTIES OF PINK GUAVA
INDUSTRY BY-PRODUCTS**

By

KONG KIN WENG

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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**LYCOPENE CONTENT AND ANTIOXIDANT PROPERTIES OF PINK
GUAVA INDUSTRY BY-PRODUCTS**

By

KONG KIN WENG

February 2010

Chair: Associate Professor Amin Ismail, PhD

Faculty: Medicine and Health Sciences

Malaysia is one of the largest pink guava (*Psidium guajava*) puree exporters in the world. Daily production of the industry produces huge amount of the by-products. Thus, exploitation of these by-products as functional sources could reduce the disposal cost and problem. This study was aimed to investigate the lycopene content and antioxidant properties of these by-products and the lycopene-rich fractions produced from decanted by-product. The content of lycopene and lipophilic antioxidant capacity were compared among pink guava fruit by-products named accordingly to the processing points as refined by-product, sieved by-product and decanted by-product. Lycopene content was estimated using UV-VIS spectrophotometer and high performance liquid chromatography (HPLC) method. Atmospheric pressure chemical ionization liquid chromatography-mass spectrometry (APCI-LC-MS) was used to confirm the presence of lycopene. Lipophilic antioxidant capacity was assessed using lycopene equivalent antioxidant capacity (LEAC) and β -carotene bleaching assays. Lycopene content and antioxidant capacity were in the order of fruits > decanted by-product > sieved by-

product > refined by-product. Decanted by-product was found to be the highest lycopene content and antioxidant capacity among the by-products. Heat treatment through steam blanching was applied to find the best conditions for lycopene content in the studied by-products. Steam blanching at 60°C for 20-60 min significantly ($p < 0.05$) increased lycopene content in decanted by-product to about 13% from the freeze-dried control. To further optimise drying conditions for lycopene and lipophilic antioxidant capacity in the studied by-products, decanted by-product was dried with different temperatures (50-80°C) and drying times (4-6 h) using an oven dryer based on the response surface methodology (RSM). Response surface plots showed increase in temperature and time significantly ($p < 0.05$) reduced the response variables. The optimum oven conditions for drying of decanted by-product with minimum lycopene degradation were at 43.8°C for 6.4 h. Besides, the effects of solvent and supercritical carbon dioxide (SC-CO₂) extractions on antioxidant capacity and cytotoxicity in lycopene-rich fractions (hydrophobic fractions) were also determined using LEAC, β -carotene bleaching and MTT (3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide) assays. Extraction with SC-CO₂ exhibited higher lycopene content than the solvent extraction. No cytotoxicity was found on human normal liver cell lines supplemented with both extracts at concentrations ranged from 6.25-200 μ g/ml. Protective effects of lycopene-rich fraction against H₂O₂-induced cells damage were observed at concentrations of 2.32 μ M of lycopene (25 μ g/ml) and 5.09 μ M of lycopene (200 μ g/ml) for solvent and SC-CO₂ extracts, respectively. However, treatments with 200 μ g solvent extract/ml containing 18.65 μ M lycopene and 10 μ M lycopene standard (Sigma Chemical Co.,USA) were found to provide the protective effect towards

oxidative DNA damage evaluated by Alkaline Comet assay. Lycopene-rich fraction from pink guava by-products have the potential to be used as functional food ingredients in preventing the promotion of oxidative stress.



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

**KANDUNGAN LIKOPEN DAN CIRI-CIRI ANTIOKSIDAN DALAM HAMPAS
DARIPADA INDUSTRI JAMBU BATU MERAH**

Oleh

KONG KIN WENG

Februari 2010

Pengerusi: Profesor Madya Amin Ismail, PhD

Fakulti: Perubatan dan Sains Kesihatan

Malaysia merupakan antara negara pengeksport puri jambu batu merah (*Psidium guajava*) yang terbesar di dunia. Pengeluaran harian industri ini telah menghasilkan hampas daripada buah dengan jumlah yang banyak. Maka, pengeksploitan terhadap hampas ini sebagai sumber makanan berfungsi dapat membantu mengurangkan kos dan masalah penglupusan. Matlamat kajian ini adalah untuk mengkaji kandungan likopen dan ciri-ciri antioksidan hampas dan fraksi kaya-likopene daripada “decanted by-product”. Perbandingan kandungan likopen dan kapasiti antioksidan lipofilik telah dijalankan antara buah jambu batu merah dan hampasnya yang dikenali mengikut tempat prosesannya iaitu “refined by-product”, “sieved by-product” dan “decanted by-product”. Kandungan likopen ditentukan dengan menggunakan alat “UV-VIS” spektrofotometer dan kromatografi cecair berprestasi tinggi (HPLC). Kromatografi cecair-spektrofotometri jisim pengionan kimia tekanan atmosfera (APCI-LC-MS) telah digunakan dalam pengenalpastian likopen. Kapasiti antioksida lipofilik telah diukur dengan menggunakan kapasiti antioksida bersamaan likopen (LEAC) dan ujian

pelunturan β -karotena. Kandungan likopen dan kapasiti antioksida adalah dalam turutan buah > “decanted by-product” > “sieved by-product” > “refined by-product”. “Decanted by-product” didapati tertinggi dalam kandungan likopen dan kapasiti antioksida berbanding hampas yang dikaji. Pengolahan haba melalui kaedah penceluran dengan wap telah digunakan untuk memperolehi keadaan yang terbaik untuk kandungan likopen dalam hampas-hampas tersebut. Penceluran dengan wap pada suhu 60°C selama 20-60 min meningkatkan kandungan likopen di “decanted by-product” secara signifikan ($p < 0.05$) sebanyak 13% daripada kawalan yang dikering sejuk beku. Seterusnya, pengoptimuman keadaan pengeringan bagi kandungan likopen dan kapasiti antioksidan lipofilik dalam hampas yang dikaji iaitu “decanted by-product” telah dikeringkan dalam suhu (50-80°C) dan masa pengeringan (4-6 jam) yang berlainan dengan ketuhar berdasarkan kaedah permukaan gerak balas (response surface methodology) (RSM). Plot permukaan gerak balas menunjukkan kenaikan suhu dan masa telah menurunkan pemboleh ubah gerak balas secara signifikan ($p < 0.05$). Keadaan ketuhar yang optima untuk pengeringan “decanted by-product” dengan degradasi likopen yang minima adalah 43.8°C selama 6.4 jam. Selain itu, kesan pengekstrakan pelarut dan karbon dioksida superkritikal (SC-CO₂) terhadap kapasiti antioksidan dan sitotoksikiti dalam fraksi kaya-likopen (fraksi hidrofobik) telah ditentukan melalui asai LEAC, ujian pelunturan β -karotena dan asai MTT (3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide). Pengekstrakan SC-CO₂ menunjukkan kandungan likopen yang lebih tinggi berbanding pengekstrakan pelarut. Tiada kesan sitotoksikiti dipamerkan oleh kedua-dua ekstrak pada kepekatan antara (6.25-200 $\mu\text{g/ml}$) terhadap sel hepar manusia normal yang dirawat. Kesan perlindungan

fraksi kaya-likopen terhadap kerosakan sel yang terawat H_2O_2 dapat dilihat bagi ekstrak pelarut dan SC-CO_2 masing-masing pada kepekatan likopen $2.32 \mu\text{M}$ ($25 \mu\text{g/ml}$) dan $5.09 \mu\text{M}$ ($200 \mu\text{g/ml}$). Manakala, rawatan dengan $200 \mu\text{g}$ ekstrak pelarut/ml mengandungi $18.65 \mu\text{M}$ likopen dan $10 \mu\text{M}$ piawai likopen (Sigma Chemical Co., USA) telah memberikan kesan perlindungan terhadap kerosakan oksidatif DNA yang ditentukan melalui asai Komet Beralkali. Fraksi kaya-likopen daripada hampas jambu batu merah berpotensi digunakan sebagai ramuan makanan berfungsi mengelakkan tekanan oksidatif.

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I certify that a Thesis Examination Committee has met on **22 Februari 2010** to conduct the final examination of Kong Kin Weng on his thesis entitled “**Lycopene Content and Antioxidant Properties of Pink Guava Industry By-products**” 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.

Members of the Thesis Examination Committee were as follows:

Zaitun Yassin, PhD

Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Asmah Rahmat, PhD

Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Muhajir Hamid, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Wan Aida Wan Mustafa, PhD

Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was 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:

Amin Ismail, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Tan Chin Ping, PhD

Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Nor Fadilah Rajab, PhD

Associate Professor
Faculty of Allied Health Sciences
Universiti Kebangsaan Malaysia
(Member)

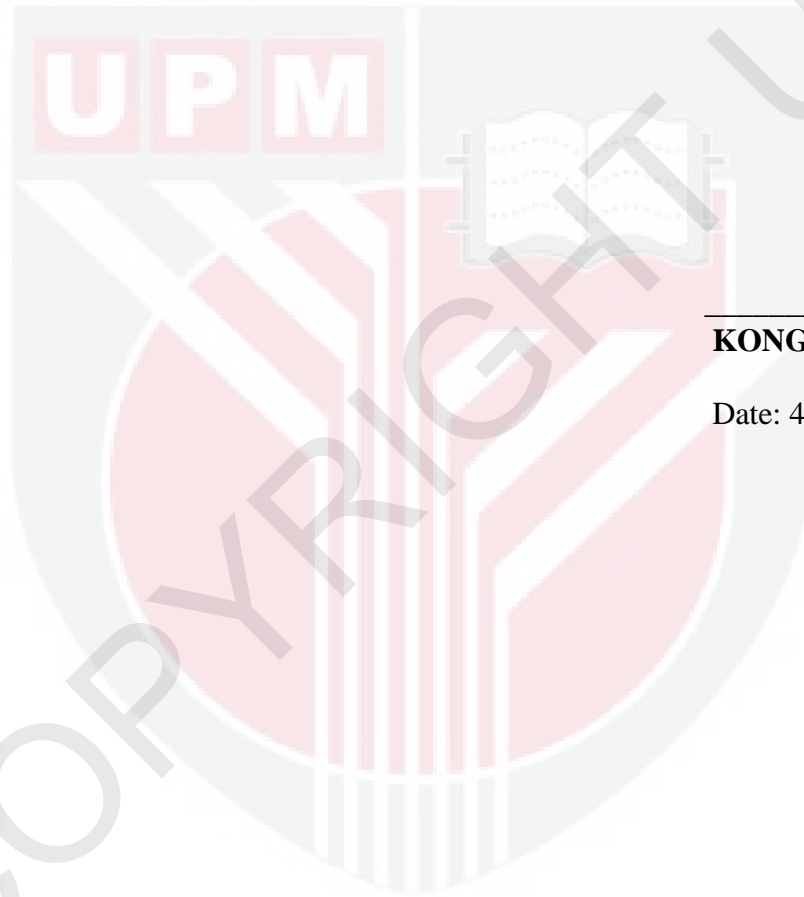
HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 8 April 2010

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.



KONG KIN WENG

Date: 4 March 2010

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