# RECOVERY OF PALM CAROTENE FROM PALM OIL AND HYDROLYSED PALM OIL USING ADSORPTION COLUMN CHROMATOGRAPHY

**By**

**YOU LI LING**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

**March 2006**

*Specially dedicated to:*

*My beloved*

*Daddy, Mummy, Brother,*

*Husband*

*and*

*Little Yan Yii*

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

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**March 2006**

**Chairman : Associate Professor Badlishah Sham Baharin**

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Crude palm oil (CPO) and crude palm olein (CPOlein) were hydrolysed with lipase from *Candida Rugosa* to produce free fatty acids (FFAs)- rich oil. The palm oil and hydrolysed palm oil were subsequently subjected to column chromatography process. Diaion HP-20 adsorbent was used for reverse phase column chromatography and the column temperature was kept at 50°C. Isopropanol (IPA) or ethanol (EtOH), and n-hexane were used as the first and second eluting solvents, respectively. The objective of hydrolysing the palm oil was to produce more polar FFA-rich oil in order to enhance the non-polar carotene to adsorb to the non-polar HP-20 adsorbent in the column chromatography. The results obtained showed that by hydrolysing CPO and CPOlein with lipase from *Candida rugosa*, gave 30- and 60-fold, respectively, of FFA production in the crude palm oil and crude palm olein in 8 h at 50°C. For column chromatographic process, using isopropanol or ethanol as the first eluting solvent, crude oil and hydrolysed oil showed the carotene recovery in fraction two (carotene-rich fraction) were about 36-37 and 90-96%, respectively. Over 90% of carotene recovery was obtained from hydrolysed palm oil

reflecting an increase of about 55% over CPO. Response Surface Methodology (RSM) for optimisation of carotene recovery from hydrolysed palm olein (HCPOlein) in adsorption chromatography was carried out. The level and interaction of three independent variables was investigated: column temperature (50 to 60°C), oil loading (25 to 200 g), and mobile phase flow rate (6 to 60 mL/min) was investigated. Based on the response as percentage of carotene recovery from 50 g of HP-20 adsorbent, the optimum conditions were achieved at 200 g of oil loading, column temperature at 55°C, and flow rate at 33 mL/min. Up to 98% of carotene recovery was able to obtain under this condition. Interaction of oil-oil and oil-flow rate could enhance percentage of carotene recovery. On the other hand, oil and flow rate as single factors could significantly reduce percentage of carotene recovery. Oil loading as a single factor could positively influence amount of carotene adsorbed. However, flow rate as a single factor and oil-oil interaction could negatively influence amount of carotene adsorbed. The predicted results according to the model for both responses were closed to the observed responses for experiments. The mean of difference (MD) of the experimental and predicted data for percentage of carotene recovery, and amount of carotene adsorbed were very small, -0.0067 and 0.0133, respectively. The probability (P) value showed no significant lack-of-fit for both equations of this model. Laboratory-scale batch studies were carried out to investigate the use of synthetic polymer adsorbent, HP-20, for carotene extraction from CPOlein and HCPOlein. The adsorption of carotene was determined by several adsorption isotherm models such as Langmuir, Freundlich and Scatchard plots. The effect of temperature, contact time, adsorbate concentration and the adsorbent mass were

examined. The equilibrium data fitted with both Langmuir and Freundlich models with correlation coefficients >0.9.

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

**PEMULIHAN KAROTIN SAWIT DARIPADA MINYAK SAWIT MENTAH DAN MINYAK TERHIDROLISIS DENGAN MENGGUNAKAN PROSES PENJERAPAN KROMATOGRAFI**

Oleh

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Minyak yang kaya dengan asid lemak bebas telah dihasilkan daripada hidrolisis minyak sawit mentah dan olein mentah. Proses hidrolisis ini mengunakan enzyme lipase daripada *Candida rugosa* sebagai mangkin. Hasil minyak sawit dan olein mentah yang telah dihidrolisiskan kemudiannya dituju kepada proses kromatografi. Diaion HP-20 digunakan sebagai penjerap dalam

proses kromatografi ini. Suhu ditetapkan pada 50°C semasa proses pengasingan karotin daripada minyak sawit melalui teknik kromatografi. Isopropanol atau etanol dan *n*-heksana telah digunakan sebagai sebagai pelarut. Tujuan menghidrolisiskan minyak sawit mentah dan olein mentah adalah untuk menghasilkan molekul minyak berkutub yang boleh meninggikan penjerapan karotin pada permukaan HP-20. Keputusan kajian hidrolisis dengan enzim lipase daripada *Candida rugosa* menunjukkan bahawa minyak sawit mentah dan olein mentah yang dihidrolisis selama 8 jam pada suhu 50°C dapat meninggikan kuantiti penjerapan karotin sebanyak 30 dan 60 kali ganda, masing-masing. Proses kromatografi yang menggunakan isopropanol atau etanol sebagai pelarut elutan pertama, telah menunjukkan bahawa peratus pemulihan karotin daripada minyak mentah dan minyak yang dihidrolisis, pada pecahan kedua, iaitu pecahan yang kaya dengan karaotin adalah 36-37% dan 90-96% masing-masing. Peratus pemulihan karotin yang melebihi 90% pada minyak sawit yang dihidrolisis telah menunjukkan peningkatan peratusan pemulihan melebihi 55% berbanding minyak sawit mentah. Satu program yang bernama "Response Surface Methodology (RSM)",untuk mengoptimisasi pemulihan karotin daripada minyak olein yang dihidrolisis dengan kaedah penjerapan kromatografi juga dilaksanakan. Kajian interaksi antara tiga pengubah yang bebas iaitu, suhu turus (50-60°C), kuantiti minyak yang digunakan (25-200 g), dan kadar aliran fasa bergerak(6-60 mL/min) juga dikaji. Berdasarkan kepada peratus pemulihan dengan menggunakan 50g penjerap HP-20, keputusan RSM menunjukkan keadaan optimum kromatografi penjerapan adalah seperti berikut: suhu turus 55°C; beban minyak yang masukkan dalam turus, 200 g; kadar aliran 33 mL/min. Dalam keadaan optimum yang telah ditentukan, peratus pemulihan karotin sebanyak 98% boleh didapati. Interaksi antara minyak-minyak, minyak-kadar pengaliran, didapati dapat meningkatkan peratus pemulihan karotin. Kuantiti minyak dan kadar pengaliran sebagai factor tunggal boleh menyebabkan

pengurangan peratus pemulihan karotin dengan ketara. Kuantiti minyak jika diambil sebagai factor tunggal akan mempengaruhi kuantiti penjerapan karotin secara positif, manakala pengambilan kadar pengaliran sebagai factor tunggal dan interaksi antara minyak-minyak akan memberi kuantiti penjerapan karotin secara negatif. Keputusan eksperimen yang diperolehi adalah menepati ramalan yang didapati daripada model RSM dengan pembezaan peratusan pemulihan karotin dan kuantiti penjerapan karotin yang amat kecil, iaitu, -0.0069 dan 0.0133, masing-masing. Nilai kebarangkalian (P) menunjukkan kedua-dua persamaan ini adalah padan dengan model RSM ini. Kajian kelompok pada peringkat makmal dengan penggunaan penjerap HP-20 sintetik untuk penyarian karotin daripada minyak olein mentah dan olein yang telah di hidrolisis juga telah dijalankan. Mekanisma yang diperolehi dalam proses penjerapan karotin ini dapat digambarkan dengan pelbagai garis sesuhu penjerapan seperti plot Langmuir, Freundlich, dan Scatchard , Kajian mendalam tentang pengaruh suhu turus, jangkamasa sentuhan, kepekatan pelarut, dan kuantiti bahan penjerap telah dibuat. Data pada keadaan kesimbangan yang dipadan kepada model Langmuir dan Freundlich memberi nilai pekali sekaitan > 0.9.

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I certify that an Examination Committee has met on 16th March 2006 to conduct the final examination of You Li Ling on her Doctor of Philosophy thesis entitled " Recovery of Palm Carotene from Palm Oil and Hydrolysed Palm Oil Using Adsorption Column Chromatography" 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 the Examination Committee are as follows:

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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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**YOU LI LING**

Date:

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