

**CHROMATOGRAPHIC SEPARATION OF PRECONCENTRATED VITAMIN E  
FROM PALM FATTY ACID DISTILLATE**

**CHU BOON SEANG**

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

**February 2004**

*To My Family*  
*Dad, Mom and Brothers*

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

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**By**

**CHU BOON SEANG**

**February 2004**

**Chairman : Associate Professor Badlishah Sham Baharin**

**Faculty : Food Science and Biotechnology**

The importance of vitamin E as a lipid-soluble antioxidant that protects unsaturated fatty acids against oxidative deterioration is widely known. An important source of vitamin E is the fatty acid distillate from deodorization during the refining of vegetable oils. The purpose of this study was to develop a method for separating vitamin E from palm fatty acid distillate (PFAD) obtained from palm oil refining. Vitamin E in PFAD was first concentrated by enzymatic hydrolysis. The free fatty acids liberated by the hydrolysis, together with those already present in PFAD, were neutralized by sodium hydroxide. A vitamin E-rich fraction was then extracted from the hydrolyzed and neutralized PFAD using hexane. The fraction obtained was finally subjected to normal phase adsorption chromatography with packed silica gel. Two elutions were done in the chromatography: the vitamin E in hexane was adsorbed on silica gel and the extraneous matter eluted out in the first elution, while the second elution released the vitamin E from the silica gel.

Reaction variables such as temperature, lipase concentration and water content in the reaction mixture affected the enzymatic hydrolysis of PFAD. Regression models generated from Response Surface Methodology adequately explained the data variation and significantly represented the actual relationships between the reaction parameters and responses. It was suggested from this study that for the maximum vitamin E concentration, the hydrolysis should be carried out with 2.5% w/w lipase and 45.2–47.3% v/w water for 5.5–5.7 h.

Screening tests concluded that silica gel was suitable for adsorption of vitamin E. Batch mode adsorption and desorption experiments were employed to study the equilibrium and kinetics of the adsorption and desorption processes. Vitamin E uptake by silica gel was rapid with adsorption equilibrium achieved in about 5 min. The adsorption isothermic data were in good agreement with the Langmuir model, suggesting that the vitamin E adsorbed on silica gel was monolayer. Kinetics study of batch adsorption revealed that the rate of vitamin E uptake by silica gel followed a pseudo-second order reaction and involved both external mass transfer and intraparticle diffusion. Intraparticle diffusion was the rate-limiting step during the adsorption as it needed a higher magnitude of activation energy ( $-25.45$  and  $-54.13$  kJ mol<sup>-1</sup> for external mass transfer and intraparticle diffusion, respectively). The adsorption of vitamin E on silica gel was exothermic, while the reverse was true for desorption. Desorption exhibited a bi-phasic characteristic with an initial fast release of the vitamin followed by a much slower phase. The two distinct rates were probably due to heterogeneities in the adsorbing surfaces. Since the silica gel surface is composed of both high-energy silanol groups and low-energy siloxane groups, it was postulated that the releases of vitamin E molecules from these two groups were responsible for the slow and rapid desorption processes, respectively. Entrapment of the

vitamin E molecules in the micropores of the silica gel may also have contributed to the slow desorption in the second phase. The desorption isotherm could be fitted in the Freundlich model.

Adsorption of vitamin E on silica gel was also tested in a fixed-bed column. The breakthrough curve of vitamin E adsorption in the column showed a typical S-shaped profile. The service time of the column increased with the column bed height, but decreased with increasing inlet vitamin E concentration, column temperature and flow rate. The column efficiency in terms of adsorbent usage rate could be improved by decreasing the inlet vitamin concentration and flow rate. Since adsorbing vitamin E on silica gel was exothermic, increasing the column temperature decreased the column capacity. The desorption of vitamin E in a column system reflected the “two-distinct-rate” desorption behavior found in batch desorption systems. This slow desorption was the rate-controlling step in the recovery of vitamin E. The desorption rate increased with column temperature but decreased with column bed height and flow rate. The recovery of vitamin E was high for all systems - 94.8 to 98.8% - with a vitamin E concentration in the extract of 18.5–21.5%.

The results from this work demonstrate the potential applicability of the current separation method for recovering vitamin E from PFAD. Detailed descriptions of the enzymatic hydrolysis and adsorption/desorption of vitamin E in adsorption chromatography have provided useful information for a better understanding of the current separation process. The method described offers an alternative to the existing vitamin E separation methods. It can be applied as one of a series of steps in producing a high-purity vitamin E concentrate from PFAD.

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

**PEMISAHAN KROMATOGRAFIK VITAMIN E  
YANG DIPEKATKAN DARIPADA  
SULINGAN PENYAHBAU MINYAK KELAPA SAWIT**

**Oleh**

**CHU BOON SEANG**

**Februari 2004**

**Pengerusi : Profesor Madya Badlishah Sham Baharin**

**Fakulti : Sains Makanan dan Bioteknologi**

Kepentingan vitamin E sebagai antioksidan larut lemak yang melindungi asid lemak tak tepu terhadap pengoksidaan memang diketahui umum. Satu sumber penting vitamin E ialah sulingan penyahbau minyak yang diperolehi semasa proses penyahbauan minyak sayuran di kilang pemrosesan. Tujuan utama kajian ini ialah untuk merekacipta satu kaedah pemisahan vitamin E daripada sulingan penyahbau minyak kelapa sawit (PFAD). Vitamin E di dalam PFAD dipekatkan terlebih dahulu dengan hidrolisis berenzim. Asid lemak bebas yang terhasil daripada hidrolisis itu, bersama-sama dengan yang tersedia ada di dalam PFAD, dineutralkan dengan sodium hidroksida. Fraksi yang kaya dengan vitamin E kemudiannya diekstrak dengan menggunakan heksana dan dialirkan melalui kromatografi penyerapan fasa normal yang disediakan dengan jel silika. Terdapat dua elusi yang terlibat dalam kromatografi penyerapan: semasa elusi pertama, molekul-molekul vitamin E dalam heksana terserap ke gel silika dan bahan-bahan lain

dikeluarkan; manakala pada elusi kedua molekul-molekul vitamin E dilepaskan daripada jel silika.

Suhu tindakbalas, kepekatan lipase dan kandungan air dalam campuran tindakbalas adalah faktor-faktor yang mempengaruhi hidrolisis berenzim PFAD. Model regresi yang dihasilkan daripada Metodologi Respon Permukaan dapat menerangkan variasi data dengan baik, di samping memberikan gambaran sebenar hubungan di antara parameter-parameter tindakbalas dengan respon. Adalah dicadangkan bahawa untuk mencapai kepekatan vitamin E yang maksimum, hidrolisis berenzim itu haruslah dijalankan dengan kepekatan lipase 2.5% w/w dan kandungan air 45.2–47.3% v/w selama 5.5–5.7 jam.

Ujian penyaringan memutuskan bahawa jel silika berkeupayaan untuk menyerap vitamin E. Eksperimen penyerapan dan nyahpenyerapan dalam mod sesekumpul telah dijalankan untuk mengaji keseimbangan dan kinetik proses penyerapan dan nyahpenyerapan. Penyerapan vitamin E oleh jel silika adalah cepat dan proses itu mencapai keseimbangan dalam masa lebih kurang 5 min. Data isoterma eksperimen penyerapan bersetuju dengan model Langmuir, dan ini menunjukkan vitamin E hanya membentuk satu lapisan molekul pada permukaan jel silika semasa penyerapan. Keputusan kajian kinetik penyerapan vitamin E menunjukkan kadar penyerapan vitamin E oleh jel silika adalah mengikut tertib tindakbalas pseudo-kedua dan proses ini melibatkan mekanisma pengangkutan jisim luaran dan difusi intrapartikal. Difusi intrapartikal adalah mekanisma yang menyekat kadar penyerapan kerana ia menunjukkan magnitud tenaga pengaktifan yang lebih tinggi ( $-25.45$  dan  $-54.13$  kJ mol<sup>-1</sup> untuk pengangkutan jisim luaran dan difusi intrapartikal masing-masing). Keputusan juga menunjukkan penyerapan vitamin E ke jel silika adalah bersifat eksotermik. Nyahpenyerapan pula bersifat endotermik. Nyahpenyerapan vitamin

E berlaku dalam dua peringkat di mana molekul-molekul vitamin E dilepaskan daripada jel silika dengan cepat pada permulaan dan diikuti dengan tahap pelepasan yang lebih perlahan. Ini mungkin disebabkan sifat permukaan penyerapan jel silika yang tidak seragam. Oleh kerana permukaan jel silika mengandungi kumpulan silanol yang bertenaga tinggi dan kumpulan siloxane yang bertenaga rendah, adalah dicadangkan bahawa perlepasan molekul-molekul vitamin E dari kedua-dua kumpulan ini masing-masing menyebabkan proses nyahpenyerapan peringkat perlahan and cepat. Sekatan molekul vitamin E di ruang-ruang jel silika yang bersaiz mikron mungkin juga menyumbang nyahpenyerapan vitamin E peringkat perlahan. Didapati isoterma nyahpenyerapan adalah bersetuju dengan model Freundlich.

Penyerapan vitamin E ke atas jel silika juga dikaji dalam sistem turus. Keputusan menunjukkan kelok *breakthrough* berbentuk “S” yang tipikal. Masa servis turus meningkat apabila turus dipanjangkan, tetapi menurun apabila kepekatan vitamin E, suhu turus dan kadar aliran ditingkatkan. Keputusan juga menunjukkan kecekapan turus dalam konteks kadar penggunaan adsorben dapat ditingkatkan dengan mengurangkan kepekatan vitamin E pada inlet turus dan kadar aliran larutan. Oleh kerana penyerapan vitamin E ke jel silika adalah bersifat eksotermik, peningkatan suhu menyebabkan penurunan kapasiti turus. Kelok nyahpenyerapan vitamin E dalam turus menggambarkan sifat “dwi-kadar-nyata” seperti yang ditunjukkan dalam nyahpenyerapan mod sesekumpul. Sekali lagi, fasa nyahpenyerapan perlahan adalah tahap yang menyekat kadar nyahpenyerapan vitamin E. Didapati kadar nyahpenyerapan meningkat dengan pengurangan tinggi turus dan kadar aliran larutan, tetapi menurun dengan pengurangan suhu turus. Peratusan pungutan hasil vitamin E selepas selesainya nyahpenyerapan adalah



tinggi bagi semua sistem, yaitu di antara 94.8–98.8%, dengan kepekatan vitamin E dalam ekstrak 18.5–21.5%.

Keputusan kajian ini menunjukkan potensi teknik pemisahan ini untuk mendapatkan vitamin E daripada PFAD. Perbincangan terperinci tentang hidrolisis berenzim dan penyerapan/nyahpenyerapan vitamin E dalam turus kromatografi telah menyediakan informasi yang berguna untuk pemahaman yang lebih mendalam tentang teknik pemisahan ini. Teknik ini juga memberikan satu alternatif bagi pemisahan vitamin E. Ia boleh digunakan sebagai salah satu langkah di antara satu siri langkah bagi menghasilkan vitamin E yang berkepekatan tinggi.

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I certify that an Examination Committee met on 19<sup>th</sup> February 2004 to conduct the final examination of Chu Boon Seang on his Doctor of Philosophy thesis entitled “Chromatographic Separation of Preconcentrated Vitamin E from Palm Fatty Acid Distillate” 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:

**MOHD. YAZID ABDUL MANAP, Ph.D.**

Associate Professor  
Faculty of Food Science and Biotechnology  
Universiti Putra Malaysia  
(Chairman)

**BADLISHAH SHAM BAHARIN**

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

**QUEK SIEW YOUNG, Ph.D.**

Lecturer  
Faculty of Food Science and Biotechnology  
Universiti Putra Malaysia  
(Member)

**YAAKOB CHE MAN, Ph.D.**

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

**ANDREW PROCTOR, Ph.D.**

Professor  
Department of Food Science  
University of Arkansas  
Fayetteville, Arkansas, USA.  
(External Examiner)

---

**GULAM RASUL RAHMAT ALI, Ph.D.**

Professor/Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy.

**BADLISHAH SHAM BAHARIN**

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

**QUEK SIEW YOUNG, Ph.D.**

Lecturer  
Faculty of Food Science and Biotechnology  
Universiti Putra Malaysia  
(Member)

**YAAKOB CHE MAN, Ph.D.**

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

---

**AINI IDERIS, Ph.D.**

Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## **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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**CHU BOON SEANG**

Date:

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