# DETERMINATION OF NATURAL AND SYNTHETIC ANTIOXIDANTS IN PALM OIL USING FOURIER TRANSFORM INFRARED SPECTROSCOPY

**By**

**WANNA AMMAWATH**

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

**March 2006**

**Dedicated To My Parents, My Husband and My Son**

**for Their Love Patience and Understanding**

Abstract of 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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**Chairman: Professor Yaakob bin Che Man, PhD**

**Faculty: Food Science and Technology**

Lipid oxidation is one of the major deteriorative reactions in cooking oils and often results in a significant loss of quality. Various natural and synthetic antioxidants are used in the prevention or retardation of lipid oxidation. Routine quality control of antioxidants in oil turns out to be more and more important due to the increased environmental concern on the use of large volumes of solvents for analysis. Simple, accurate and rapid methods for determination of antioxidants in oils and fats industry are necessary to be developed. Fourier transform infrared (FTIR) spectroscopy has come of age in terms of price, performance and ease of use on determination of some natural and synthetic antioxidants.

FTIR spectroscopy was developed in conjunction with partial least squares (PLS) technique for determining of α-tocopherol in refined bleached and deodorized (RBD) palm olein. The calibration and validation samples were prepared by spiking known amounts of α-tocopherol to produce a wide range of α-tocopherol up to 2000 ppm. The accuracy of the method was comparable to that of the International Union of Pure and Applied Chemistry (IUPAC, 1992) method, with coefficients of determination ($R^2$) from calibration samples of 0.9922 and standard error of calibration (SEC) of 53.54 at the FTIR spectral region 3100-2750 $cm^{-1}$. For determination of β-carotene in RBD palm olein using PLS calibration models coupled with FTIR spectral region at 980-915 $cm^{-1}$, fifty RBD palm olein samples spiked with a known amount of standard (95%) β-carotene to produce a wide range of concentrations up to 2000 ppm. The accuracy of the method was comparable to that of the PORIM method with $R^2$ of 0.9950 and SEC of 19.47.

FTIR spectra of RBD palm olein samples between 3600 and 2800 $cm^{-1}$ were used for quantitative determination of tert-butylhydroquinone (TBHQ). Fifty stripped oil samples spiked with known amounts of TBHQ up to 300 mg/kg (ppm), were separated into two sets that of the calibration and validation models based on PLS analyses. The accuracy of the method was comparable to that of IUPAC method with $R^2$ of 0.9961 and SEC 5.06. In the determination of butylated hydroxytoluene (BHT) content in RBD palm olein and RBD palm oil using FTIR spectroscopy, the accuracy of the method in both oils were comparable to that of the IUPAC method

with an $R^2$ of 0.9907 and SEC 8.47 for RBD palm olein, while an $R^2$ of 0.9848 and SEC 10.73 was obtained for RBD palm oil. For determining butylated hydroxyanisole (BHA) of RBD palm oil and RBD palm olein, PLS coupled with the 'leave-one-out" cross-validation procedure was used to verify the calibration model. FTIR spectral regions 3486-3170 and 1960-719 $cm^{-1}$ gave an $R^2$ of 0.9939 in RBD palm olein and an $R^2$ of 0.9884 in RBD palm oil samples. Also, FTIR spectroscopy coupled with the PLS and PCR techniques was employed to construct the calibration models for determining propyl gallate (PG) in RBD palm olein. The results indicated that FTIR was a useful analytical tool for simple and rapid quantitative determination of PG in RBD palm olein in the spectral region 3707-3262 $cm^{-1}$.

New FTIR methods developed for determining some synthetic and natural antioxidant used in palm oil product were found to be useful analytical tools, which were shown to significantly improved analysis time and avoided solvent-disposal problems. The results were found to be in good correlation and of comparable accuracy to PORIM and IUPAC methods. FTIR spectroscopy is advantageous as it is simple, rapid, accurate and requires minimum solvent as only acetone was used for cleaning NaCl windows. The method is suitable for routine quality control analysis with results obtainable in about 2 min.

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

**PENENTUAN ANTIOKSIDAN SEMULAJADI DAN SINTETIK DI DALAM MINYAK KELAPA SAWIT MENGGUNAKAN SPEKTROSKOPI FOURIER TRANSFORM INFRARED (FTIR)**

Oleh

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**Mac 2006**

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Pengoksidaan lipid adalah salah satu daripada tindakbalas kerosakan utama bagi minyak masak dan selalu mengakibatkan kemerosotan kualiti. Pelbagai antioksida sintetik dan semulajadi digunakan untuk menghalang atau menghentikan pengoksidaan lipid. Kawalan kualiti rutin bagi antioksida di dalam minyak menjadi isu yang semakin penting berikutan daripada pengggunaan kuantiti pelarut yang tinggi di dalam analisis. Kaedah yang mudah, tepat dan pantas bagi penentuan antioksida di dalam industri minyak dan lemak adalah amat perlu untuk dibentuk. Spektoskopi ‘ Fourier Transform Infrared’ adalah alat yang sesuai, dari segi harga, prestasi dan mudah digunakan untuk penentuan sesetengah antioksidan semulajadi dan sintetik.

Spektroskopi 'Fourier Tramform Infrared' digandingkan dengan teknik 'Partial Least Squares' telah di bentuk untuk menentukan kandungan α-tocoferol di dalam minyak sawit olein tertapis, terluntur dan ternyahbau (RBD). Sampel kalibrasi dan validasi telah disediakan dengan memasukkan kandungan tertentu α-tocoferol untuk menghasilkan julat α-tocoferol sehingga 2000 bahagian per sejuta (ppm). Ketepatan kaedah ini adalah setara dengan kaedah 'International Union of Pure and Applied Chemistry (IUPAC)'. dengan coefficient of determination ($R^2$) ialah 0.9922 dan sisihan piawai kalibrasi (SEC) 53.54 daripada set kalibrasi pada julat spektrum FTIR 3100-2750 $cm^{-1}$. Bagi penentuan β-karotene di dalam minyak sawit olein RBD menggunakan model kalibrasi Partial Least Square (PLS) digandingkan dengan julat spektra FTIR 950-915 $cm^{-1}$, dua set yang terdiri daripada 50 sampel minyak sawit olein RBD yang dimasukkan kandungan paiwai yang diketahui (95%) β-karotene untuk menghasilkan julat kepekatan yang besar sehingga 2000 ppm digunakan. Ketepatan kaedah adalah setara dengan kaedah PORIM dengan $R^2$ 0.9950 dan SEC 19.47.

Spektra FTIR bagi sampel minyak sawit olein RBD antara 3600 dan 2800 $cm^{-1}$ digunakan untuk penentuan kuantitatif kandungan tert-butylhydroquinone (TBHQ). Lima puluh sampel yang dimasukkan kandungan TBHQ dengan kepekatan sehingga 300mg/ kg (ppm) yang dibahagikan kepada dua set untuk model calibrasi dan validasi berasaskan analisa PLS. Ketepatan kaedah adalah setara dengan kaedah IUPAC dengan $R^2$ ialah 0.9961 dan SEC 5.06. Bagi penentuan kandungan butylated

hydroxytoluene (BHT) di dalam minyak sawit olein RBD dan minyak sawit RBD menggunakan spektroskopi FTIR, ketepatan kaedah ini bagi kedua-dua jenis minyak adalah standing dengan kaedah IUPAC dengan $R^2$ ialah 0.9907 dan SEC 8.47 untuk minyak sawit olein RBD, manakala $R^2$ ialah 0.9848 dan SEC 10.73 untuk minyak sawit olein RBD. Untuk menentukan kandungan butylated hydroxyanisole (BHA) di dalam minyak sawit RBD dan minyak sawit olein RBD, PLS digabungkan dengan prosedur validasi silang 'leave-one-out' telah digunakan untuk mengesahkan model kalibrasi. Julat spektra FTIR daripada 3486-3170 dan 1960-719 $cm^{-1}$ memberikan $R^2$ = 0.9939 di dalam minyak sawit olein RBD dan $R^2$ = 0.9884 di dalam minyak sawit RBD. Manakala, spektroskopi FTIR digabungkan dengan teknik PLS dan Principal Component Regression (PCR) digunakan untuk membina model kalibrasi untuk menentukan kandungan propyl gallate di dalam minyak sawit olein RBD. Keputusan menunjukkan bahawa FTIR sebagai alatan analitikal yang berguna untuk penentuan kuantitatif pantas untuk menentukan propyl gallate di dalam minyak sawit olein pada julat spektum 3707-3262 $cm^{-1}$.

Kaedah FTIR yang baru dibentuk untuk menentukan beberapa antioksida sintetik dan semulajadi yang digunakan di dalam produk minyak sawit telah menunjukkan peningkatan yang bermakna dari segi tempoh analisis dan mengelakkan masalah pelupusan sisa pelarut. Keputusan yang diperolehi menunjukkan korelasi yang baik dan setara dengan kaedah PORIM dan IUPAC. Spektroskopi FTIR adalah berguna sebagai kaedah yang mudah, pantas, kuantitatif, tepat dan memerlukan jumlah

pelarut yang minimum, dimana hanya acetone digunakan untuk membersihkan sel telusan NaCl. Kaedah ini adalah sesuai untuk analisa kawalan mutu rutin dengan keputusan boleh dicapai dalam masa dua minit.

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I certify that an Examination Committee has met on 20th March 2006 to conduct the final examination of Wanna Ammawath on her Doctor of Philosophy thesis entitled "Determination of Natural and Synthetic Antioxidants in Palm Oil using Fourier Transform Infrared Spectroscopy" 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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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory 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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**WANNA AMMAWATH**

Date:

## TABLE OF CONTENTS