



UNIVERSITI PUTRA MALAYSIA

**RAPID METHODS FOR ANALYSIS OF EDIBLE OILS AND FATS
BY FOURIER TRANSFORM INFRARED SPECTROSCOPY**

MOHAMED ELWATHIG SAEED MIRGHANI

FSMB 2002 14

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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

October 2002



ESPECIALLY DEDICATED TO MY BELOVED FAMILY



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

**RAPID METHODS FOR ANALYSIS OF EDIBLE OILS AND FATS BY
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October 2002

Chairman : Professor Dr. Yaakob B. Che Man

Faculty : Food Science and Biotechnology

Analysis using Fourier transform infrared (FTIR) spectroscopy techniques on edible fats and oils extracted from palm fruit, groundnut, sesame seed, cottonseed and animal body fats rendered from cow, chicken, lamb and lard were investigated. The studies included development and applications of rapid FTIR techniques to determine some quality parameters such as moisture content in crude palm oil (CPO), soap and hexane residues in refined palm oil and groundnut oil, malondialdehyde (MDA) as a secondary oxidation product in refined palm oil, minor components such as sesamol and gossypol in sesame and cottonseed oils, and aflatoxins in groundnut and groundnut cake. The detection of lard in different mixtures with other animals' body fats such as cow, chicken and lamb was also investigated.

Different sample handling techniques were used such as transmission cells of NaCl, BF₂, KBr and attenuated total reflectance (ATR) using internal reflectance element (IRE) of ZnSe. Partial Least Square (PLS) and Principle Component Analysis



(PCA) statistical methods were used to drive calibrations from FTIR versus actual or chemical values. In this study the frequency of 3700-3072 cm^{-1} was used to determine moisture content in CPO as it indicates the absorption of compounds containing hydroxyl groups (OH). The frequency at 1675-1500 cm^{-1} was used to determine soap residues in refined edible oils. For the determination of hexane residue in oils, the frequency used included all the data from 2935-2817 cm^{-1} , 1490-1333 cm^{-1} and 1200-1000 cm^{-1} for $-\text{CH}_3$ and $-\text{CH}_2$, and in-plane $-\text{CH}$ bending.

In the determination of MDA as a secondary oxidation product, the correlation and variance spectra were used to select the best regions (2900-2800 and 1800-1600 cm^{-1}) to derive calibration from FTIR versus values obtained by chemical methods with SEC of 1.49. The spectral regions included the data from 3650-3000, 1600-1450 and 1200-900 cm^{-1} that were used to determine sesamol in sesame seed oil. The study also included a qualitative and semiquantitative determination of palm and groundnut oils as adulterants in sesame seed oil using the spectral regions from 1504- 1503, 1400-1397 and 917-914 cm^{-1} . The gossypol was also determined as an important quality factor in cottonseed oil and cakes using the spectral regions from 3600-2520 and 1900-800 cm^{-1} . The study also covered the detection of lard in mixture of body fats of chicken, lamb and cow by using changes in frequency and absorbance of spectral regions 3009-3000, 1418-1417, 1385-1370, 1126-1085 and 966-967 cm^{-1} . The simple Beer-Lambert law was used to develop equations for the determination of mixtures.

Aflatoxins exhibit characteristic absorption bands at wavelengths of 3004-2969 cm^{-1} for CH_2 , aromatic $=\text{CH}$, $-\text{C}-\text{H}$, $\text{C}=\text{C}$ and phenyls, 1744-1720 cm^{-1} for $\text{C}=\text{O}$, 1364-369 cm^{-1} for methyl adjacent to epoxy ring, 1217-1220 cm^{-1} for in plane $-\text{C}-\text{H}$ bending.



CH bending of phenyl, 1035-1037 cm^{-1} for symmetric stretching of =C–O–C or symmetric bending of phenyl, and 900-902 cm^{-1} which may be for isolated H. In this calibration set the spectral regions that showed the highest correlation between concentration information and spectral response were set to include the data from 3000-2932, 1832-1693, 1400-1329 and 1250-1187 cm^{-1} for aflatoxins B₁, with standard errors of calibration (SEC) of 1.80 parts per million (ppm).

All of the results were in good correlation and of comparable accuracy to the classical wet chemical methods such as the American Oil Chemists Society (AOCS), Association of Official Analytical Chemists (AOAC) and International Union of Pure and Applied Chemistry (IUPAC) methods. This study represents the use of FTIR spectroscopy as a new rapid analytical technique developed for determination of some quality parameters of fats and oils, together with the detection of adulterants and contaminants. The FTIR spectroscopic technique has the potential to replace the time- and effort-consuming chemical methods for fast analysis of fats and oils. This can also eliminate the use of toxic chemicals that are hazardous to the analysts as well as to the environment in the analysis.



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

**KAEDAH PANTAS UNTUK MENGANALISA MINYAK DAN LEMAK
MASAK DENGAN PENGGUNAAN
SPEKTROSKOPI FOURIER TRANSFORM INFRARED**

Oleh

MOHAMED ELWATHIG SAEED MIRGHANI

Oktober 2002

Pengerusi: Profesor Yaakob Bin Che Man, Ph.D.

Fakulti: Sains Makanan dan Bioteknologi

Kajian telah dijalankan untuk menganalisa minyak dan lemak yang diekstrak daripada buah kelapa sawit, kacang tanah, biji bijan, biji kapas dan lemak haiwan dari lembu, ayam, kambing dan khinzir dengan menggunakan Spektroskopi Fourier Transform Infrared (FTIR). Kajian ini merangkumi pembangunan dan penggunaan teknik pantas FTIR untuk menentukan beberapa parameter kualiti seperti kandungan lembapan dalam minyak sawit mentah, sabun, sisa heksana dalam minyak yang ditulin; kandungan aflatoksin dalam kacang tanah dan hampas kacang tanah; malondialdehid (MDA) dari 2900 – 2800 dan 1800 – 1600 cm^{-1} sebagai produk oksidasi sekunder; dan komponen minor seperti sesamol dan gosipol dalam minyak bijan dan biji kapas. Pengesanan lemak khinzir dalam campuran lemak binatang lain seperti lembu, ayam dan kambing juga telah dijalankan.



Pelbagai teknik pengendalian sampel yang berlainan telah digunakan, contohnya sel-sel telusan NaCl, BF₂, KBr dan pantulan keseluruhan « attenuated » (ATR) dengan menggunakan elemen pantulan dalaman (IRE) ZnSe. Partial Least Square (PLS) dan kaedah statistik Prinsip Analisis Komponen (PCA) telah digunakan untuk mengukur kalibrasi FTIR berbanding dengan nilai kimia atau nilai sebenar. Dalam kajian ini, frekuensi pada 3700–3072 cm⁻¹ telah digunakan untuk menentukan kandungan lembapan dalam minyak sawit mentah (CPO) kerana ia mewakili penyerapan gelombang bagi bahan yang mengandungi kumpulan hidroksil (OH) seperti air (H-OH). Frekuensi pada 1675–1500 cm⁻¹ telah digunakan untuk menentukan sisa sabun dalam minyak makan tulin. Manakala bagi penentuan sisa heksana dalam minyak, frekuensi yang digunakan termasuk semua data dari 2935–2817 cm⁻¹, 1490–1333 cm⁻¹ dan 1200–1000 cm⁻¹ bagi CH₃ dan –CH₂ dan pembengkakan –CH dalam planar.

Bagi penentuan MDA sebagai produk oksidasi sekunder, korelasi dan variasi spektra telah digunakan untuk memilih kawasan spektra terbaik (2900–2800 dan 1800–1600 cm⁻¹) untuk menghasilkan kalibrasi FTIR berlawanan nilai-nilai yang diperoleh dari kaedah kimia dengan SEC 1.49. Kawasan spektra merangkumi data dari 3650–3000, 1600–1450 dan 1200–900 cm⁻¹ telah digunakan untuk menentukan sesamol dalam minyak bijan. Kajian ini juga termasuk penentuan secara kualitatif dan semi-kuantitatif bagi minyak sawit dan minyak kacang tanah yang telah bercampur dengan minyak bijan (1504 - 1503, 1400 - 1397 dan 917 - 914 cm⁻¹). Gosipol dalam minyak biji kapas juga telah ditentukan sebagai satu faktor kualiti penting dalam perdagangan minyak biji kapas dan hampas kapas (3600 - 2520 dan 1900 - 800 cm⁻¹). Kajian ini juga meliputi pengesanan lemak khinzir dalam campuran lemak binatang

seperti ayam, kambing dan lembu dengan menggunakan perubahan frekuensi dan penyerapan kawasan spektra 3009-3000, 1418-1417, 1385-1370, 1126-1085 dan 966-967 cm^{-1} . Kaedah Beer-Lambert telah digunakan untuk menghasilkan formula penentuan campuran.

Aflatoksin menunjukkan ciri rantai serapan pada jarak gelombang 3004–2969 cm^{-1} bagi $\text{CH}_2 = \text{CH}$ aromatik, $-\text{C}-\text{H}$, $\text{C}=\text{C}$ dan kumpulan fenil, 1477–1720 cm^{-1} bagi $\text{C}=\text{O}$, 1364–11369 cm^{-1} bagi kumpulan metil yang bercantum dengan gelang epoksi, 1217–1220 cm^{-1} bagi fenil akibat pembengkokan $-\text{CH}$ dalam planer, 1035 1037 cm^{-1} bagi getaran simetri $=\text{C}-\text{O}-\text{C}$ atau pembengkokan simetri fenil, dan 900–902 cm^{-1} bagi $-\text{H}$ yang telah diasingkan. Set kalibrasi bagi kawasan spektra maklumat kepakatan. Reaksi spektra telah ditetapkan dan merangkumi semua data dari 3000–2932 cm^{-1} , 1832–1693 cm^{-1} , 1400–1329 cm^{-1} dan 1250–1187 cm^{-1} bagi aflatoxin B₁, dengan ralat standard kalibrasi (SEC) 1.80 ppm.

Semua keputusan kajian ini telah menunjukkan korelasi yang baik dan kejutuan yang setanding dengan kaedah kimia klasik seperti dalam kaedah American Oil Chemists Society (AOCS), Association of Official Analytical Chemists (AOAC) dan International Union of Pure and Applied Chemistry (IUPAC). Kajian ini membuktikan bahawa spektroskopi FTIR adalah satu kaedah analitikal baru yang pantas bagi penentuan sesetengah parameter kualiti di dalam lemak dan minyak juga pengesanan bahan pencemaran. Teknik spektroskopi FTIR mempunyai potensi untuk menggantikan kaedah kimia yang memakan masa dan tenaga. Ini juga boleh mengelakkan penggunaan bahan kimia bertoksik yang membahayakan juru analisis dan alam sekitar.

AKNOWLEDGEMENTS

I pray to Almighty ALLAH Subhanahu wa Ta'ala who give me the thoughts, the will, and guided me to complete this work. I pray that ALLAH will bless this work and make it useful for mankind, and that He will forgive us.

My sincere and deepest gratitude to Professor Dr. Yaakob B. Che Man, the chairman of my supervisory committee for his guidance, encouragement, patience and continuous follow up during the course of this study. My appreciation and gratitude is also extended to members of my supervisory committee, Prof. Dr. Jinap Selamat, Assoc. Prof. Dr. Jamilah Bakar and Assoc. Prof. Badlishah Sham Baharin for their advice, punctuate comments and support.

My gratitude is also due to all the staff of the Department of Food Technology, and the Faculty of Food Science and Biotechnology, UPM for their co-operation. My special appreciation is extended to my colleagues Dr. Irwandi Jaswir, Dr. Tan C. Ping, Ms. Gabby Setiowaty, Ms. Wanna Ammawath, Mr. Kambis Shams and Ms. Mariam Abdulatif for their kind help and friendly attitude.

I would like to acknowledge the financial support provided by IRPA fund for this study awarded to Prof. Dr. Yaakob B. Che Man. Acknowledgement is also due to the National Oilseed Processing Research Institute (NOPRI), University of Gezira, Sudan, especially Prof. Dr. Ismail H. Hussein, the Director of the Institute for granting me the opportunity to pursue my PhD studies.



I am gratefully indebted to the one who have lighted the way for me, my mother Haja Waqeya (Um-Aboha), my sisters, brothers and rest of our extended family for their support, encouragement and invaluable assistance.

Finally but first in my thoughts, I owe my sincere gratitude thanks to my beloved wife, Sabah and daughters Sarrah, Faizah and Doaa for their understanding, patience, care and love.



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