UNIVERSITI PUTRA MALAYSIA

APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR ANALYSIS, AUTHENTICATION AND MONITORING OF OXIDATIVE STABILITY OF EDIBLE OILS

ABDUL ROHMAN

IPPH 2010 1

APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR ANALYSIS, AUTHENTICATION AND MONITORING OF OXIDATIVE STABILITY OF EDIBLE OILS

ABDUL ROHMAN

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2010

APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR ANALYSIS, AUTHENTICATION AND MONITORING OF OXIDATIVE STABILITY OF EDIBLE OILS

BY

ABDUL ROHMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,

in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

October 2010

Dedicated To My beloved Parents, My wife and My Son
for Their Doa, Patience and Understanding

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

APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY FOR ANALYSIS, AUTHENTICATION AND MONITORING OF OXIDATIVE STABILITY OF EDIBLE OILS

By

ABDUL ROHMAN

October 2010

Chairman: Professor Dato' Yaakob bin Che Man, PhD

Institute : Halal Products Research Institute

Fourier transform infrared (FTIR) has become powerful analytical technique in the study of fats and oils. The objective of this study was to use FTIR spectroscopy combined with certain chemometrics techniques to analyze, authenticate, and to monitor the oxidative stability of selected edible fats and oils. FTIR spectra combined with principal component analysis (PCA) and cluster analysis using absorbances as variables at 16 frequencies have been successfully used for differentiation of lard (LD). Among sixteen edible fats and oils, extra virgin olive oil, rice bran oil, and chicken fat were close to LD compared with others.

The presence of LD in binary mixtures with beef (BF), chicken, (CF), and mutton fat (MF) was analyzed using FTIR spectroscopy combined with partial least square (PLS) at fingerprint region of 1,500 – 900 cm^{-1}. The coefficients of determination (R^2) for the relationship between actual value of LD (*x-axis*) and FTIR predicted

values (*y-axis*) in BF, CF, and MF were 0.999 0.998, and 0.995, respectively. Frequencies of 1,500 – 1000 cm^{-1} were selected for quantification of LD in the binary mixtures with selected vegetable oils of canola, corn, extra virgin olive, soybean, and sunflower oils. These frequency regions are also preferred for analysis of LD in quaternary systems using first derivative spectra with R^2 and root mean square error of calibration (RMSEC) values of 0.997 and 0.773 % (v/v), respectively. Furthermore, in food systems using meat ball formulation, LD has been successfully determined at 1,200 – 1,000 cm^{-1} with R^2 and RMSEC values of 0.999 and 0.442 % (v/v), respectively.

FTIR spectroscopy in combination with PLS and principle component regression (PCR) was also used for quantifying VCO. VCO in binary mixtures with palm oil (PO) is better determined using PLS at combined frequencies of 1,120 – 1105 and 965 – 960 cm^{-1} with R^2 and RMSEC values obtained were 0.999 and 0.758 % (v/v), respectively. These frequencies were also well suited for quantitative analysis of VCO in binary mixtures with olive oil (OO). Furthermore, frequencies at 1,200 - 1,000 cm^{-1} using PLS and second derivative spectra was applicable for quantitative analysis of VCO in ternary mixtures with PO and OO.

Authentication analysis of cod liver oil (CLO) from LD was performed using FTIR spectroscopy at frequency 1,035 – 1030 cm^{-1}. In addition, BF, CF, and MF were also successfully determined using PLS at certain optimized frequencies. Discriminant analysis (DA) can successfully classify CLO and CLO adulterated with animal fats at

certain frequency regions. FTIR spectroscopy was also used for authentication analysis of extra virgin olive oil (EVOO) and virgin coconut oil (VCO). FTIR normal spectra along with PLS was preferred over PCR for quantification of studied vegetable oils in EVOO and VCO, except for palm oil which was better determined with first derivative spectra. The R^2 values obtained were higher than 0.99 in calibration model. DA successfully classified EVOO and VCO and those adulterated with some vegetable oils. Analysis of fatty acid changes of the authentic oils (CLO, EVOO, and VCO) due to the adulteration practice can complement the FTIR spectral measurements.

FTIR spectroscopy was used to evaluate the oxidative stability of vegetable oils with high mono- and polyunsaturated fatty acids, namely corn, rice bran, soybean, and sunflower oils. The oils were subjected to thermal oxidation at 160 ^{o}C for 120 h. Each 12 h interval, the oils were analyzed for its specific absorptivity of conjugated dienes (CDs) and trienes (CTs), *p*-anisidin values (*p*-AV) and FTIR spectra changes at frequencies of 3470, 3008, 1743, 1655, 967, and 721 cm^{-1}. FTIR spectral changes at 3008 cm^{-1} can be used to predict the level of oxidation, especially for determination of CDs, CTs, and *p*-AV values.

In conclusion, this study showed that FTIR spectroscopy combined with suitable chemometrics technique can provide reliable analytical tool to analyze and to authenticate edible fats and oils. FTIR spectroscopy could also potentially be used for routine monitoring the oxidative stability of fats and oils.

Abstrak Tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

APLIKASI SPEKTROSKOPI TRANSFORMASI FOURIER INFRA MERAH UNTUK ANALISIS, PENGESANAN DAN PEMANTAUAN KESTABILAN OKSIDASI MINYAK MAKAN

Oleh

ABDUL ROHMAN

Oktober 2010

Pengerusi : Professor Dato' Yaakob bin Che Man, PhD

Institut : Institut Penyelidikan Produk Halal

Kajian ini bertujuan untuk menggunakan spektoskopi Fourier transform inframerah (FTIR) untuk menganalisis, mengesahkan dan memantau kestabilan oksidasi beberapa minyak dan lemak terpilih telah dilakukan.

Kombinasi spektra FTIR dengan analisis komponen utama dan analisis cluster menggunakan absorban sebagai variabel di 16 frekuensi telah berjaya untuk membezakan lemak babi dari minyak dan lemak lainnya. Diantara 16 minyak dan lemak makan, minyak zaitun, minyak dedak padi, dan lemak ayam sangat rapat dengan lemak babi dibandingkan dengan minyak dan lemak lainnya.

Adanya lemak babi dalam campuran dengan lemak haiwan seperti lemak lembu (BF), lemak ayam (CF), dan lemak kambing (MF) telah dianalisis dengan kombinasi spektroskopi FTIR dan kalibrasi "partial least square" (PLS) dalam

lingkungan spectra cap jari iaitu antara 1500 - 900 cm^{-1}. Frekuensi diantara 1500-100 cm^{-1} digunakan untuk mengesan kehadiran lemak babi dalam minyak sayuran terpilih. Lemak babi dalam campuran empat lemak dapat dikesan pada frekuensi diantara 1500-1000 cm^{-1} dengan nilai R^2 dan RMSEC yang diperolehi adalah 0.997 dan 0.773 % (v/v). Kehadiran lemak babi dalam bebola dapat dikesan pada frekuensi diantara 1200-100 cm^{-1} dengan korelasi antara nilai sebenar (*paksi x*) dan nilai anggaran FTIR (*paksi y*) ialah y = 0.999x + 0.004, dimana nilai R^2 dan RMSEC adalah 0.999 dan 0.442 % (v/v).

Spektroskopi FTIR juga digunakan untuk mengesan campuran minyak kelapa dara (VCO). VCO dalam dengan PO lebih baik dikesan dengan PLS pada frekuensi 1120 - 1105 dan 965 - 960 cm^{-1}, dengan nilai R^2 dan RMSEC ialah 0.999 dan 0.758 % (v/v). Kombinasi antara frekuensi in dan PLS juga sesuai untuk menentukan campuran VCO dan minyak zaitun (OO). Gabungan frekuensi 1200 - 1000 cm^{-1} dan PLS dapat digunakan untuk analisis tiga campuran minyak (VCO, PO dan OO).

Keaslian minyak ikan kod (CLO) dari minyak babi dapat dikesan pada frekuensi 1035-1030 cm^{-1} dengan R^2 dan RMSEC adalah 0.990 dan 1.04 % (v/v). Lemak lembu (BF), ayam (CF) dan kambing (MF) dapat dikesan juga dengan menggunakan PLS pada frekuensi tertentu. Analisis diskriminasi (DA) pula mampu mengklasifikasikan diantara CLO dan campuran CLO dengan lemak haiwan pada frekuensi tertentu. FTIR turut digunakan untuk mengesan EVOO dan VCO. Spektra FTIR normal dan digabungkan dengan kalibrasi PLS lebih terpilih

dibandingkan dengan PCR untuk mengesan minyak sayuran dalam EVOO. Minyak sawit dapat dikesan dengan lebih baik menggunakan spectra turunan pertama, dengan nilai R^2 lebih tinggi daripada 0.99 pada model kalibrasi. Analisis diskriminasi telah berjaya memisahkan EVOO dan VCO dengan EVOO dan VCO bercampur oleh minyak sayuran ini. Analisis perubahan acid lemak boleh melengkapi data spectra FTIR untuk mengkasi praktik pemalsuan minyak.

Spektroskopi FTIR berpotensi untuk pengukuran kestabilan oksidasi minyak sayuran terpilih. Minyak dengan asid lemak mono tak tepu dan asid lemak poli tak tepu iaitu minyak jagung, minyak dedak padi, minyak soya, dan minyak bunga matahari dikenai panas tinggi (160 ^{o}C) dalam oven selama 120 jam. Nilai absorptiviti spesifik diena terkonjugasi (CDs) dan triena terkonjugasi (CTs), nilai anisidin (p-AV), serta spektra FTIR pada frekuensi 3470, 3008, 1743, 1655, 967, dan 721 cm^{-1} pada minyak tersebut ditentukan setiap 12 jam. Perubahan absorban pada frekuensi 3008 cm^{-1} dapat digunakan untuk mengetahui tahap oksidasi minyak-minyak sayuran ini.

Sebagai kesimpulan, kajian ini memberi petunjuk tentang kemampuan spektroskopi FTIR untuk melakukan analisis dan autentikasi minyak dan lemak. Tambahan pula, spektroskopi FTIR dapat dicadangkan untuk pemantauan kestabilan oksidasi minyak dan lemak.

ACKNOWLEDGEMENTS

In the name of Allah, The Most Merciful and The Most compassionate. All praise is to Allah. With the blessings and Allah's guidance, I have completed my research and preparation of this PhD thesis.

I would like to deeply express my gratitude and utmost appreciation to my supervisory committee chairman, Professor Dr. Yaakob bin Che Man who consistently motivated and supported me with his insight, experience, knowledge, and financial as well as for his invaluable guidance and advice during the course of my study. I am also indebted to my supervisory committee members, namely Assoc. Prof. Dr. Amin bin Ismail and Dr. Puziah Hashim for their assistance and encouragement.

My appreciation was also directed to Directorate of Human Resources (Direktorat Ketenagaan), Directorate General for Higher Education (Dikti), The National Education Ministry, Republic of Indonesia for financial supports during my study. I also would like to express my deepest thanks to Halal Products Research Institute (HPRI) UPM for providing research facilities and conducive environment for conducting this research. The appreciation is also due to the Faculty of Pharmacy, Gadjah Mada University for its approval to pursue my PhD program at HPRI, Universiti Putra Malaysia.

Acknowledgement is also due to all HPRI staffs (academic and supporting staffs) for their kindness as well as to all my friends in HPRI, namely Dias Indrasti, Mohd. Hazim, Haniff, Nurrulhidayah, Nina, Naim, Fiqah, Tengku Salwani, Yanty, Norakasha,

Juliana, Wan Siti Faizan, Siti Aishah, Fariha, Norhayati, Farah, Norazihan, who had given me the moral encouragement and kind discussions during my study. I can not imagine conducting this project without the kindness and friendship from all of you.

I also tank to Indonesian student association, especially to my housemates namely Pak Bambang, Pak Suliadi, Pak Malik, Pak Sofyan, Aris and Dr. Asep Edi Kusnaedi for the conducive house environment, kind discussion, and togetherness during the study. I hope all of you can success in your journey.

Finally, my deepest gratitude goes to my parents (Kamsinah, Sukamdi, H. Budiredjo, MA and Hj. Sri Asriati) for their constant doa, supports and guidance. To my wife, Rizqi Amalia Rohmah, STP and My son Ahza Nabil Muhammad as well as my late sons Syafi' and Naim (who died during my study), all of you are my sources of motivation and inspiration for the better future.

This thesis was 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 were as follows:

Dato' Yaakob Bin Che Man, PhD
Professor
Halal Products Research Institute
Universiti Putra Malaysia
(Chairman)

Amin Ismail, PhD
Associate Professor
Halal Products Research Institute
Universiti Putra Malaysia
(Member)

Puziah Hashim, PhD
Research Fellow
Halal Products Research Institute
Universiti Putra Malaysia
(Member)

_____
HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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 other institutions.

_____

ABDUL ROHMAN
Date: 22 October 2010

TABLE OF CONTENTS

DEDICATION ii
ABSTRACT iii
ABSTRAK vi
ACKNOWLEDGEMENTS ix
APPROVAL xi
DECLARATION xiii
LIST OF TABLES xix
LIST OF FIGURES xxiii
LIST ABBREVIATIONS xxviii

CHAPTER

1 **GENERAL INTRODUCTION** 1

2 **LITERATURE REVIEW** 8
2.1 Fourier Transform Infrared (FTIR) Spectroscopy 8
2.1.1 Introduction 8
2.1.2 Infrared Absorption process 9
2.1.3 Instrumentation 11
2.1.4 FTIR advantage 13
2.1.5 IR spectra of fats and oils 15
2.1.6 Sampling techniques 17
2.1.7 Quantitative Analysis 20
2.2 FTIR spectroscopy for analysis of fats and oils quality 22
2.3 Edible fats and oils 24
2.3.1 Virgin coconut oil 25
2.3.2 Olive oil 26
2.3.3 Lard 29
2.3.4 Cod liver oil 30
2.4 Fats and oils authentication 34
2.5 Analytical methods for authentication of fats and oils 36
2.5.1 FTIR spectroscopy 36
2.5.2 Chromatography-based techniques 38
2.5.3 Differential scanning calorimetry (DSC) 39
2.5.4 Electronic nose (EN) 40

2.6 Meat ball 41
2.7 Stability of oil 42
2.7.1 Measurement of oxidative stability 44
2.7.2 FTIR spectroscopy for monitoring oxidative stability of oils 45
2.8 Antioxidant 48

3 **CHARACTERIZATION OF LARD AND SELECTED ANIMAL FATS AND VEGETABLE OILS USING FTIR SPECTRA** 50
3.1 Introduction 50
3.2 Materials and Methods 52
3.2.1 Materials 52
3.2.2 Sample preparation 52
3.2.3 Fatty acid (FA) analysis 53
3.2.4 Measurement of FTIR spectra 54
3.2.5 Statistical Analysis 55
3.3 Results and Discussion 56
3.3.1 Fatty acid composition of lard and others 56
3.3.2 Principal component analysis (PCA) of lard and other studied fats and oils using FTIR spectra 63
3.3.3 Cluster Analysis 71
3.4 Conclusions 73

4 **QUANTITATIVE ANALYSIS OF LARD IN OTHER ANIMAL FATS, VEGETABE OILS AND IN MEATBALL USING FTIR SPECTROSCOPY** 74
4.1 Introduction 74
4.2 Materials and Methods 77
4.2.1 Materials 77
4.2.2 Sample preparation 78
4.2.3 Quantitative analysis of lard (LD) in binary mixtures with animal fats and vegetable oils 78
4.2.4 Analysis of lard in quaternary mixture with other animal fats 78
4.2.5 Analysis of pork fat in meatballs formulation 80
4.2.6 Determination of precision and sensitivity of analytical method 81
4.2.7 FTIR Instrumental Analysis 81
4.2.8 Statistical Analysis 81

4.3 Results and Discussion 82
4.3.1 Spectral analysis 82
4.3.2 Analysis of lard in the binary mixtures with other animal fats and vegetable oils 84
4.3.3 Analysis of lard in quaternary mixtures with other animal fats 93
4.3.4 Quantification of pork fat (lard) in meat ball formulation 97
4.4 Conclusions 101

5 **ANALYSIS OF VIRGIN COCONUT OIL IN THE MIXTURES WITH OTHER OILS USING FTIR SPECTROSCOPY AND MULTIVARIATE CALIBRATION** 102
5.1 Introduction 102
5.2 Materials and Methods 104
5.2.1 Materials 104
5.2.2 Sample preparation 105
5.2.3 Analysis of VCO in binary mixtures with olive oil (OO) and palm oil (PO) 105
5.2.4 Analysis of VCO in ternary mixtures with olive oil (OO) and palm oil (PO) 106
5.2.5 Analysis of lard in VCO 107
5.2.6 FTIR spectroscopic analysis 107
5.2.7 Statistical analysis and validation procedure 108
5.3 Results and Discussion 108
5.3.1 Spectral analysis 108
5.3.2 Analysis of VCO in binary mixtures with olive oil (OO) and palm oil (PO) 112
a. VCO in the mixture with olive oil 112
b. VCO the in mixture with palm oil 116
5.3.3 Analysis of VCO in ternary mixtures with olive oil (OO) and palm oil (PO) 120
5.3.4 Analysis of lard blended with VCO 123
5.4 Conclusions 125

6 **APPLICATION OF FTIR SPECTROSCOPY FOR ANALYSIS OF COD-LIVER OIL ADULTERATION** 127
6.1 Introduction 127
6.2 Materials and Methods 129
6.2.1 Sample preparation 129

6.2.2 Preparation of oil mixtures for gas chromatography (GC) analysis 129
6.2.3 Calibration and validation in FTIR analysis 130
6.2.4 Discriminant analysis 130
6.2.5 FTIR instrumental analysis 131
6.2.6 Statistical analysis 131
6.3 Results and Discussion 132
6.3.1 Fatty acid composition of cod liver oil (CLO) and animal fats 132
6.3.2 FTIR spectra analysis 134
6.3.3 Quantification of animal fats as adulterants in cod liver oil 136
6.3.4 Discriminant analysis 154
6.4 Conclusions 157

7 APPLICATION OF FTIR SPECTROSCOPY FOR AUTHENTICATION OF EXTRA VIRGIN OLIVE OIL AND VIRGIN COCONUT OIL 158
7.1 Introduction 158
7.2 Materials and Methods 161
7.2.1 Sample preparation 161
7.2.2 Quantitative analysis 161
7.2.3 Discriminant analysis 162
7.2.4 Instrumentation 162
7.2.5 Fatty acid analysis 162
7.2.6 Chemometrics and statistical analyses 163
7.3 Results and Discussion 163
7.3.1 Fatty acid (FA) analysis of selected oils 163
7.3.2 Authentication of extra virgin olive oil (EVOO) from oil adulterants using FTIR spectroscopy 166
a. Quantification of oil adulterants in EVOO 168
b. Classification of EVOO and adulterants 175
c. Analysis of fatty acid composition 179
7.3.3 Authentication of virgin coconut oil (VCO) 181
7.4 Conclusions 187

8 POTENTIAL USE OF FTIR SPECTROSCOPY FOR MONITORING THE OXIDATIVE STABILITY OF SELECTED VEGETABLE OILS 188

8.1 Introduction 188
8.2 Materials and Methods 190
8.2.1 Sample preparation 191
8.2.2 Determination of iodine value and fatty acid analyses 191
8.2.3 Measuremet of conjugated dienes (CDs) and conjugated trienes (CTs) 191
8.2.4 Determination of *p*-anisidin value (*p*-AnV) 192
8.2.5 Measurement of FTIR spectra 193
8.2.6 Statistical analyses 193
8.3 Results and Discussion 194
8.3.1 Fatty acid (FA) compositions 194
8.3.2 FTIR studies of non-oxidized and oxidized vegetable oils 195
8.3.3 Specific absorptivity values of CDs and CTs of vegetable oils 208
8.3.4 Analysis of *p*-anisidine value (*p*-AV) 215
8.4 Conclusions 218

9 **GENERAL CONCLUSIONS AND RECOMMENDATIONS** 219
9.1 General conclusions 219
9.2 Recommendations 222

REFERENCES 224
LIST OF PUBLICATIONS 248
BIODATA OF STUDENT 251
APPENDICES 252