

APPLICATION OF SPECTROSCOPIC TECHNIQUES AS FAST DETECTION METHODS FOR THE QUALITY OF SOME FOODS

By

FATHY MOHAMED SABER MEHAYA

B.Sc. Agric. Sci. (Food Technology), Kafer EL-Sheikh University, 2001

M.Sc. Agric. Sci. (Food Technology), Cairo University, 2010

**A thesis submitted in partial fulfillment
of
the requirements for the degree of**

DOCTOR OF PHILOSOPHY

in

**Agricultural Science
(Food Science and Technology)**

**Department of Food Science
Faculty of Agriculture
Ain Shams University**

2016

Approval Sheet

APPLICATION OF SPECTROSCOPIC TECHNIQUES AS FAST DETECTION METHODS FOR THE QUALITY OF SOME FOODS

By

FATHY MOHAMED SABER MEHAYA

B.Sc. Agric. Sci. (Food Technology), Kafer EL-Sheikh University, 2001

M.Sc. Agric. Sci. (Food Technology), Cairo University, 2010

This thesis for Ph.D. degree has been approved by:

Dr. Adel Zaki Mohamed Badee

Prof. Emeritus of Food Technology, Faculty of Agriculture, Cairo
University

Dr. Mohamed Farag Khallaf

Prof. of Food Science and Technology, Faculty of Agriculture, Ain
Shams University

Dr. Hany Idrees Khalil

Prof. of Food Science and Technology, Faculty of Agriculture, Ain
Shams University

Date of Examination: / / 2016

APPLICATION OF SPECTROSCOPIC TECHNIQUES AS FAST DETECTION METHODS FOR THE QUALITY OF SOME FOODS

By

FATHY MOHAMED SABER MEHAYA

B.Sc. Agric. Sci. (Food Technology), Kafer EL-Sheikh University, 2001

M.Sc. Agric. Sci. (Food Technology), Cairo University, 2010

Under the supervision of

Dr. Hany Idrees Khalil

Prof. of Food Science and Technology, Faculty of Agriculture, Ain
Shams University, (Principal Supervisor)

Dr. Mohie Mostafa Kamil

Researcher Prof. Emeritus of Food Technology, Department of Food
Technology, National Research Center.

ABSTRACT

Fathy Mohamed Saber Mehaya. “Application of Spectroscopic Techniques as Fast Detection Methods for the Quality of Some Foods”. Unpublished Ph.D. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2016.

The present study aimed to evaluate the quality, including adulteration, of honey and meat by classical and advanced methods (HPLC, GC, SDS-PAGE and PCR) in parallel with FT-IR and Raman spectroscopic as fast and easy detection methods.

Physicochemical properties of honey and honey adulterated with glucose or sucrose were determined. Total soluble solids (TSS), pH and electrical conductivity of honey and its adulterated samples ranged between (84.10 - 84.50%), (3.80 - 4.63) and (11.73 – 232.32 μ S), respectively. The viscosity of adulterated honey with glucose syrup was the highest. Results of HPLC indicated that authentic honey had higher fructose/glucose ratio than adulterated honey samples. Also, honey adulterated with sucrose syrup had the highest HMF content.

The FT-IR and FT-Raman spectral peaks of sucrose, glucose and fructose were identified at different concentrations. Selected main specific peaks provide the best calibration curves with ($r^2 > 0.9$). Also, FT-IR and FT-Raman techniques were used to evaluate authentic honey and its adulterated with glucose and sucrose syrup.

Chemical composition of meat showed that moisture and fat content were higher in pork meat than in chicken and beef. On contrary, protein content of beef was the highest. Color parameters and pH demonstrated that beef meat had the highest redness parameter and pH value. While, the pure minced pork had low redness value (6.14), and minced beef meat adulterated with 25, 50 and 75 % of pork meat was 19.10, 16.22 and 11.87, respectively.

FT-IR spectral bands of beef, chicken and pork meats showed beef meat had a specific spectral bands at 900, 977, 1040, 1110, 1169 &

1303 cm^{-1} . Pork meat had unique peaks at 1075, 847 and 720 cm^{-1} , while chicken meat had specific peaks at 1152, 1093, 950 and 765 cm^{-1} .

The FT-IR spectra afford information on the functional groups of lard. Calibration curve of pork fat (lard) was prepared by concentration levels at 10, 20, 40, 50 and 70%. The obtained standard curves of selected important peaks related to lipids had higher ($R^2 > 0.9$). The identification of beef burger adulterated with pork meat using FTIR and Raman techniques showed that the intensity of wave numbers that referred to amide I and amide II was decreased when the adulteration percentage of pork meat increased, while the major lipid peaks were relatively more intense when the adulteration ratio of pork meat was increased. Moreover, the FT-IR and FT-Raman spectra of extracted fat from beef burger that adulterated by pork fat at (10 & 20%), indicated that there are good correlations ($R^2 > 0.9$) between control burger and adulterated burger by pork fat in some specific spectral band corresponding to pork fat structure.

Protein profile of chicken, beef and pork by SDS-PAGE showed that pork meat had specific bands at 142, 97 and 17 kDa, while beef meat had unique bands at 139, 65 and 8 kDa, and chicken meat had specific bands at 129, 110 and 88 kDa.

Simplex and Multiplex PCR with species specific primers were successively applied for discriminating between beef, chicken and pork in minced meat and its mixtures without interfering.

Gas chromatography-flame ionization detector was used to detect the adulteration of minced beef and chicken meat with pork meat based on fatty acid profiles. The results indicated that the proportion of oleic C18:1/ linoleic C18:2 ratio was decreased when the adulteration ratio with pork was increased. In addition, the proportion of C18:1/ C18:2 ratio was 1.23 and 4.07 for chicken and pork fat, respectively. This ratio increased when the adulteration ratio with pork was increased.

Keywords: Spectroscopic techniques, FTIR, Raman, PCR, SDS-PAGE, GC, honey, meat, quality, adulteration, lard

ACKNOWLEDGMENT

First of all, grateful thanks to **ALLAH** who enabled me to overcome all the problems, which faced me through this work and blessed me with his unlimited graces.

I wish to express my thanks, special gratitude to **Prof. Dr. Hany Idrees Khalil**, Prof. of Food Science, Faculty of Agriculture, Ain Shams University, for his supervision, kind assistance and guidance through revision of the manuscript of this thesis and his effort provided to achieve this work.

I wish to express my thanks, deepest gratitude to **Prof. Dr. Mohie Mustafa Kamil**, Prof. Emeritus of Food Technology, National Research Center for his moral support, encouragement throughout study guidance through revision of the manuscript of this thesis and his effort provided to achieve this work.

I gratefully acknowledge the Science and Technology Development Fund (STDF) Egypt, Grant No 1062. for its financial support to this work.

Also, my sincere thanks go to **Prof. Dr. Nefisa Abbas Hegazy**, Prof. Emeritus of Food Technology, National Research; **Dr. Ihab Salah Ishoush** Assistant Prof. of Food Sci., Food Sci. Dept., Fac. of Agric., Ain Shams Univ. and **Prof. Dr. Hisham Moharram** Prof. of Food Technology, National Research, who gave me the confidence and guidance to explore my research.

My sincere thanks also go to **Prof. Dr. Abdel Hfeez Shouk** Prof. of Food Sci. and Technol., Food Technol. Dept., National Research Center; **Dr. Mohamed Gad Allah** Assistant Prof. of Food Sci. and **Dr. Abdel Fattah Abdel Kareem** Lecture of Food Sci., Food Sci. Dept., Fac. of Agric., Ain Shams Univ., who gave me the confidence and guidance to explore my research.

I gratefully acknowledge the research and technical staff of the Central Lab. of Food and Nutrition Division, and **all staff members of Food Technology lab**, National Research Centre for their assistance and support for using the various laboratory equipments.

Words fail me to express my appreciation to **my family** for their support and help me through my life and study.

CONTENTS

LIST OF TABLES.....	vi
LIST OF FIGURES.....	ix
LIST OF ABBREVIATIONS.....	xiii
1. INTRODUCTION.....	1
2. REVIEW OF LITERATURE.....	6
2.1. Honey	6
2.1.1. Honey quality.....	6
2.1.2. Physicochemical properties of authentic and adulterated honey.....	7
2.1.3. Spectroscopic techniques.....	11
2.1.3.1. Fourier-transform Infrared (FT-IR) technique	11
2.1.3.2. Raman spectroscopy	16
2.2. Meat	18
2.2.1. Spectroscopic methods	18
2.2.1.1. Fourier-transform Infrared (FT-IR) technique	19
2.2.1.2. Raman spectroscopy.....	23
2.2.2. protein- based analysis.....	25
2.2.2.1. Sodium dodecyl sulfate- PAGE (SDS-PAGE)	26
2.2.3. DNA-based methods.....	28
2.2.3.1 PCR-based techniques.....	28
2.2.3.2. PCR using species-specific primers.....	29
2.2.3.3 Species-specific multiplex PCR.....	33
2. 2.4. Identification of beef, chicken and pork by GLC.....	36
3. MATERIALS AND METHODS.....	39
3.1. MATERIALS.....	39
3.1.1. Raw materials	39
3.1.2. Chemicals and reagents.....	39
3.2. METHODS.....	40
3.2.1 Honey.....	40

3.2.1.1. Preparation of sugar standard solutions	40
3.2.1.2. Adulterated honey samples.....	40
3.2.1.3. Determination of electrical conductivity, pH and TSS.	40
3.2.1.4. The apparent viscosity	40
3.2.1.5. Color measurement	41
3.2.1.6. Fourier transform infrared spectroscopy (FT-IR).....	41
3.2.1.7. FT-Raman spectroscopy	41
3.2.1.8. Determination of sugars by high performance liquid chromatography (HPLC)	41
3.2.1.9. Determination of hydroxymethylfurfural by HPLC....	42
3.2.1.10. Sensory evaluation	42
3.2.2 Meat	43
3.2.2.1. Preparation of meat samples for PCR.....	43
3.2.2.2. Preparation of meat samples for FTIR and SDS-PAGE electrophoresis.....	43
3.2.2.3. Preparation of adulterated beef meat with pork meat or lard for FTIR and Raman spectroscopy.....	43
3.2.2.4. Preparation of meat samples for GLC.....	44
3.2.2.5. Determination of chemical composition	44
3.2.2.6. Color measurement	44
3.2.2.7. Determination of pH	44
3.2.2.8. Extraction of proteins for SDS-PAGE	45
3.2.2.9 Preparation of electrophoresis buffers, solutions and stains.....	45
3.2.2.10. DNA extraction for PCR technique.....	48
3.2.2.11. Oligonucleotide primers design	49
3.2.2.12. Simplex PCR	49
3.2.2.13. Multiplex PCR	50
3.2.2.14. Amplified product detection.....	50
3.2.2.15. Fat extraction	50

3.2.2.16. Determination of fatty acid profile of meat samples by GLC.....	50
3.2.2.17. Statistical analysis	51
4. RESULTS AND DISCUSSION.....	52
4.1. Fast and easy detection methods of honey quality.....	52
4.1.1. Physicochemical properties of honey.....	52
4.1.2. Identification and quantification of sugar profiles in honey by HPLC	59
4.1.3. Determination of HMF in honey by HPLC.....	62
4.1.4.Evaluation of honey quality by spectroscopic techniques	64
4.1.4.1. FT-IR for the identification and quantification of sucrose, glucose and fructose.....	64
4.1.4.2. FT-IR spectral of authentic and adulterated honey....	75
4.1.5. Evaluation of some commercial honey by FT-IR and HPLC.....	81
4.1.5.1. FTIR.....	81
4.1.5.2. High performance liquid chromatography.....	84
4.1.6. Raman spectroscopy.....	85
4.1.6.1. Identification and quantification of sucrose, glucose and fructose	85
4.1.6.2. Detection of honey adulteration by Raman spectroscopy.....	95
4.2. Easy and fast detection methods of local meat.....	99
4.2.1. Physicochemical properties of raw meats	99
4.2.1.1. Chemical composition of meat.....	99
4.2.1.2. Color parameters and pH of beef meat and its adulterated with pork meat.....	100
4.2.2. Spectroscopic techniques.....	102
4.2.2.1 FT-IR spectral bands of beef, chicken and pork meats.....	102

4.2.2.2. Identification and detection of pork meat and lard using FT-IR.....	106
4.2.2.2.1. Lard standard curve of FT-IR spectroscopy.....	106
4.2.2.2.2. FT-IR spectra of adulterated beef burger by pork meat.....	110
4.2.2.2.3. FT-IR spectra of extracted fat from adulterated burger by pork meat.....	111
4.2.2.2.4. FT-Raman spectra of adulterated beef burger by pork meat	116
4.2.2.3. FT-IR spectra of extracted fat from adulterated burgers by lard.....	122
4.2.2.4. FT-Raman spectra of adulterated beef burger by lard.....	125
4.2.2.5. Raman assignments of extracted fat from adulterated beef burger by lard.....	129
4.2.3. Electrophoreses SDS technique to identify protein pattern of chicken, beef and pork meat.....	132
4.2.4. PCR technique to detect beef meat adulteration with pork meat.....	136
4.2.4.1. DNA extraction.....	136
4.2.4.2. Simplex PCR specificity.....	136
4.2.4.3. Multiplex PCR specificity.....	137
4.2.4.4. Multiplex PCR for adulterated meat samples.....	138
4.2.5. Identification of beef, chicken and pork by GLC.....	141
4.2.5.1. Fatty acids profile of beef, pork and their mixtures...	141
4.2.5.2. Fatty acids profile of chicken, pork and their mixtures.....	145
5. SUMMARY AND CONCLUSION.....	149
6. REFERENCES.....	159
ARABIC SUMMARY.....	---

LIST OF TABLES

No.	TITLE	Page
1	Effect of honey adulteration with glucose or sucrose syrup on total soluble solids (TSS), pH and electrical conductivity (EC) (mean $\pm$ SD).....	53
2	Color parameter of adulterated honey with glucose or sucrose syrup	54
3	Apparent viscosity properties of honey and its adulterated samples.....	56
4	Sensory evaluation of natural and adulterated honey.	58
5	Sugars profiles of adulterated honey as determined by high performance liquid chromatography (HPLC).....	61
6	FT-IR spectral bands of sucrose solution at wave numbers 1048, 1138 and $990 \pm 5 \text{ cm}^{-1}$	65
7	FT-IR spectral bands of glucose solution at 1152 (A), 1105 (B) 1077 (C), 1030 (D) and $985 (E) \pm 5 \text{ cm}^{-1}$	68
8	FT-IR spectral bands of fructose solution standard curve at 1632 (A), 1084 (B) and $1057 (C) \pm 5 \text{ cm}^{-1}$	72
9	FT-IR ATR spectral bands of authentic and adulterated honey with glucose syrup (GS) and sucrose syrup (SS).....	78
10	FT-IR ATR spectral bands of commercial honey samples from local market.....	82
11	Sugars contents of some commercial honey products that determined by high performance liquid chromatography (HPLC)...	84
12	Assignments of FT-Raman spectral bands of fructose at different concentration levels.....	88

No.	TITLE	Page
13	Assignments of FT-Raman spectral bands of glucose.....	90
14	Assignments of FT-Raman spectral bands of sucrose.....	94
15	FT-Raman spectral bands of authentic and adulterated honey.....	97
16	Physicochemical properties of meat (mean $\pm$ SD)	99
17	Color parameters and pH of fresh beef, chicken and pork meats.....	100
18	Color parameters of adulterated minced beef with pork meat.	101
19	Assignments of FT-IR ATR spectral bands of beef chicken and pork meat samples.....	105
20	FT-IR assignments of selected spectral bands of lard	108
21	FT-IR spectral peaks of adulterated beef burger by different pork meat ratios.....	110
22	FT-IR assignments of extracted fat from beef burger contains pork meat.....	114
23	FT-Raman assignments of minced beef with different ratio of pork meat.....	120
24	FT-IR functional groups of extracted fat from beef burger adulterated by lard.....	122
25	FT-Raman assignments of adulterated beef by different ratio of lard.....	128
26	FT-Raman assignments of beef burger adulterated by 10 and 20% lard.....	130
27	Electrophoresis SDS separation patterns of from pork, chicken and beef proteins	134

No.	TITLE	Page
28	Detection of beef meat adulteration with pork based on fatty acid profiles.....	143
29	PLS regression of predictions and actual pork meat percentage in beef meat.....	144
30	Detection of chicken meat adulteration with pork depending on fatty acid profile (%).	147
31	PLS regression of predictions and actual pork meat percentage in chicken meat.....	148

LIST OF FIGURES

No.	TITLE	Page
1	HPLC standard curves of fructose (A), glucose (B) and sucrose (C).....	60
2	Typical HPLC chromatograms of HMF contents in honey: A- Authentic and adulterated honey with 25, 50 and 100 % sucrose syrup. B- Authentic and adulterated honey with 25, 50 and 100 % glucose syrup.....	62
3	HMF content (mg/100g) of natural and adulterated honey with glucose or sucrose syrup.....	63
4	FT-IR spectra of sucrose solution at 5, 10, 15, 20 and 40% concentration levels.....	66
5	FT-IR of sucrose standard curves at wave numbers 1048, 1138 and $990 \pm 5\text{cm}^{-1}$	67
6	FT-IR spectra of glucose solution at concentration levels 10, 15, 20 and 40%.....	69
7	Standard curves of glucose at several wave numbers (A-E).....	71
8	FT-IR spectra of fructose at 10, 15, 20 and 40% concentration levels.....	73
9	Standard curves of fructose at 1632 (A), 1084 (B) and 1057 (C) $\pm 5\text{ cm}^{-1}$	74
10	FT-IR spectral bands of authentic and adulterated honey with different concentration levels of glucose.....	75
11	FT-IR spectral bands of authentic and adulterated honey with different sucrose concentration levels.....	77