

Benha University
Faculty of Science
Chemistry Department



Some Chemical Studies on Detecting Olive Oil Adulteration

A Thesis

**Submitted to Benha University, Faculty of Science in Partial
Fulfillment of the Requirements for the Degree of**

**Ph.D. in Chemistry
(Organic Chemistry)**

By

Amany Nagah Ali Mostafa Farahat

B.Sc. Fac. Science., Al Azhar Univ., 2006

M.Sc. Fac. Science., Al Azhar Univ., 2014

Under Supervision of

Prof. Dr. Ashraf Abd Elhamid

Farouk Wasfy

Professor of Organic Chemistry

Dean of Faculty of Science Benha

University

Prof. Dr. Alaa Azouz Salama

Professor of Food Science and

Technology

Food Technology Research Institute

Agricultural Research Center

(2019)

CONTENTS

| Title | Page |
|---|-------------|
| List of tables | IV |
| List of figures | IX |
| List of abbreviations | XII |
| Introduction | 1 |
| Aim of the work | 6 |
| Review of Literature | 7 |
| -Authentication of olive oil | 9 |
| -Detection of EVOO Adulteration Using Chromatographic Analyses | 11 |
| -Detection of EVOO Adulteration by Gas Chromatography | 11 |
| -Detection of EVOO Adulteration by lipase hydrolysis | 19 |
| -Detection of EVOO Adulteration by HPLC | 21 |
| -Detection of EVOO Adulteration by Spectroscopic Analyses | 31 |
| -Detection of EVOO Adulteration by UV-VIS Spectroscopy | 31 |
| -Detection of EVOO Adulteration by Fourier Transform Infrared (FTIR) Spectroscopy | 35 |
| -Detection of EVOO Adulteration by Nuclear Magnetic Resonance (NMR) Spectroscopy | 46 |
| -Other Methods Used in Detection of EVOO Adulteration | 52 |
| -Detection of virgin olive oil adulteration using a voltammetric e-tongue | 52 |
| -Detection of virgin olive oil adulteration using chemiluminescence | 53 |
| -Detection of virgin olive oil adulteration using excitation–emission fluorescence spectroscopy | 54 |
| -Detection of virgin olive oil adulteration using non-thermal plasma | 55 |
| -Detection of virgin olive oil adulteration using Stimulated | 56 |

| | |
|--|----|
| Brillouin scattering in combination with visible absorption spectroscopy | |
| • Materials and Methods | 58 |
| • Materials | 58 |
| -Vegetable oils | 58 |
| -Pancreatin | 58 |
| • Methods of analysis: | 59 |
| - Chemical properties of oils: | 59 |
| -Acid value | 59 |
| -Peroxide value | 59 |
| -Saponification value | 59 |
| -Iodine Value | 59 |
| -Determination of K_{232} and K_{270} extinction coefficient | 60 |
| -Analysis of fatty acid composition | 61 |
| -Fatty acid methyl esters preparation. | 61 |
| -Determination of the glyceride structure | 61 |
| -Determination of the glyceride structure by lipase hydrolysis | 61 |
| -Pancreatic lipase hydrolysis | 62 |
| -Isolation of 2.monoglyceride | 63 |
| -Determination of the fatty acid composition of the 2. monoglycerides | 63 |
| -Determination of the glyceride structure using HPLC analysis | 63 |
| -Fourier Transform Infrared (FTIR) spectral analysis | 67 |
| -Nuclear Magnetic Resonance (NMR) Spectral Analysis | 68 |
| - ^1H -NMR Spectral Analysis | 68 |
| - ^{13}C Spectral Analysis | 69 |
| • Results and Discussion | 70 |
| -Physicochemical properties and fatty acids profile of pure | 70 |

| | |
|--|-----|
| oils | |
| -Detection of EVOO Adulteration by Gas Chromatography | 73 |
| -Detection of EVOO Adulteration by lipase hydrolysis | 81 |
| -Detection of EVOO Adulteration by HPLC | 126 |
| -Detection of EVOO Adulteration by Spectroscopic Analyses | 158 |
| -Detection of EVOO Adulteration by Fourier Transform Infrared (FTIR) Spectroscopy. | 160 |
| -Detection of EVOO Adulteration by Nuclear Magnetic Resonance (NMR) Spectroscopy. | 178 |
| 1. Detection of EVOO Adulteration by ^1H NMR spectroscopy | 179 |
| ^{13}C NMR spectroscopy of olive oil triacylglycerols | 199 |
| Summary | 217 |
| References | 223 |
| Arabic summary | v |

LIST OF TABLES

| No. | Title | Page |
|------------|---|-------------|
| 1. | Chemical properties and fatty acids profile of pure oils | 76 |
| 2. | Physicochemical properties and fatty acids profile of Croatia olive oil and its binary admixtures. | 77-78 |
| 3. | Physicochemical properties and fatty acids profile of koroneiki olive oil and its binary admixtures | 79-80 |
| 4. | The total fatty acids and the fatty acid composition of the 2-monoglycerides of koroneiki olive oil and its binary admixtures | 84 |
| 5. | The total fatty acids and the fatty acid composition of the 2-monoglycerides of Croatia olive oil and its binary admixtures. | 85 |
| 6. | Component glycerides of Koroneiki olive oil. | 86-87 |
| 7 | Component glycerides of Koroneiki olive oil with 2% (w/w) corn oil. | 88-89 |
| 8 | Component glycerides of koroneiki olive oil with 5% (w/w) corn oil. | 90-91 |
| 9 | Component glycerides of koroneiki olive oil with 10% (w/w) corn oil. | 92-93 |
| 10 | Component glycerides of koroneiki olive oil with 2% (w/w) sunflower oil. | 94-95 |
| 11 | Component glycerides of Koroneiki olive oil with 5% (w/w) sunflower oil. | 96-97 |
| 12 | Component glycerides of Koroneiki olive oil with 10% (w/w) sunflower oil. | 98-99 |
| 13 | Component glycerides of Koroneiki olive oil with 2% (w/w) soybean oil. | 100-101 |
| 14 | Component glycerides of koroneiki olive oil with 5% (w/w) soybean oil. | 102-103 |

| | | |
|-----|--|---------|
| 15 | Component glycerides of koroneiki olive oil with 10% (w/w) soybean oil. | 104-105 |
| 16. | Component glycerides of Croatina olive oil. | 106-107 |
| 17. | Component glycerides of Croatina olive oil with 2% (w/w) corn oil. | 108-109 |
| 18. | Component glycerides of Croatina olive oil with 5% (w/w) corn oil. | 110-111 |
| 19 | Component glycerides of Croatina olive oil with 10% (w/w) corn oil. | 112-113 |
| 20 | Component glycerides of Croatina olive oil with 2% (w/w) Sunflower oil. | 114-115 |
| 21 | Component glycerides of Croatina olive oil with 5% (w/w) Sunflower oil. | 116-117 |
| 22 | Component glycerides of Croatina olive oil with 10% (w/w) Sunflower oil. | 118-119 |
| 23 | Component glycerides of Croatina olive oil with 2% (w/w) Soybean oil. | 120-121 |
| 24 | Component glycerides of Croatina olive oil with 5% (w/w) Soybean oil. | 122-123 |
| 25 | Component glycerides of Croatina olive oil with 10% (w/w) Soybean oil. | 124-125 |
| 26 | Triglycerides of koroneiki olive oil and its binary admixtures. | 132-133 |
| 27 | Triglycerides of Croatina olive oil and its binary admixtures. | 134-135 |
| 28 | Detection of extraneous oils in koroneiki olive oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 137 |
| 29 | Detection of extraneous oils in koroneiki olive oil with 2% sunflower oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 138 |
| 30 | Detection of extraneous oils in koroneiki olive oil | 139 |

| | | |
|----|---|-----|
| | with 5% sunflower oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | |
| 31 | Detection of extraneous oils in koroneiki olive oil with 10% sunflower oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 140 |
| 32 | Detection of extraneous oils in koroneiki olive oil with 2% soybean oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 141 |
| 33 | Detection of extraneous oils in koroneiki olive oil with 5% soybean oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 142 |
| 34 | Detection of extraneous oils in koroneiki olive oil with 10% soybean oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 143 |
| 35 | Detection of extraneous oils in koroneiki olive oil with 2% corn oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 144 |
| 36 | Detection of extraneous oils in koroneiki olive oil with 5% corn oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 145 |
| 37 | Detection of extraneous oils in koroneiki olive oil with 10% corn oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 146 |
| 38 | Detection of extraneous oils in Croatina olive oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 147 |
| 39 | Detection of extraneous oils in Croatina olive oil with 2% sunflower oil by a comparison of | 148 |

| | | |
|----|--|-----|
| | mathematical algorithms with a data base built from genuine olive oils. | |
| 40 | Detection of extraneous oils in Croatia olive oil with 5% sunflower oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 149 |
| 41 | Detection of extraneous oils in Croatia olive oil with 10% sunflower oil a comparison of mathematical algorithms with a data base built from genuine olive oils. | 150 |
| 42 | Detection of extraneous oils in Croatia olive oil with 2% soybean oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 151 |
| 43 | Detection of extraneous oils in Croatia olive oil with 5% soybean oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 152 |
| 44 | Detection of extraneous oils in Croatia olive oil with 10% soybean oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 153 |
| 45 | Detection of extraneous oils in Croatia olive oil with 2% corn oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 154 |
| 46 | Detection of extraneous oils Croatia olive oil with 5% corn oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 155 |
| 47 | Detection of extraneous oils in Croatia olive oil with 10% corn oil by a comparison of mathematical algorithms with a data base built from genuine olive oils. | 156 |
| 48 | Comparison of triglycerides composition from lipase hydrolysis and HPLC analysis. | 157 |

| | | |
|----|---|---------|
| 49 | Comparison of the chemical assignment of bands in the FTIR spectra for pure oils (Croatina and koroneiki olive oils, sunflower, soybean and corn oils). | 168-169 |
| 50 | Assignment of the main resonances in the ^1H NMR spectrum of Koroneiki olive oil and its binary admixtures with 10% (w/w) (corn, sunflower and soybean oils). | 184 |
| 51 | Assignment of the main resonances in the ^1H NMR spectra of Croatia olive oil and its binary admixtures with 10% (w/w) (corn, sunflower and soybean oils). | 185 |
| 52 | Integrals of the ^1H NMR main resonance groups for Koroneiki olive oil and its binary admixtures with 10% (w/w) corn, sunflower and soybean oils. | 194 |
| 53 | Integrals of the ^1H NMR main resonance groups for Croatia olive oil and its binary admixtures with 10% (w/w) corn, soybean and sunflower oils. | 195 |
| 54 | Assignment of the main resonances in the ^{13}C NMR spectrum of Koroneiki olive oil and its binary admixtures with 10% (w/w) (corn, sunflower and soybean oils). | 205-206 |
| 55 | Assignment of the main resonances in the ^{13}C NMR spectrum of Croatia olive oil and its binary admixtures with 10% (w/w) (corn, sunflower and soybean oils). | 207-208 |

LIST OF FIGURES

| No. | Title | Page |
|------------|---|-------------|
| 1 | Chemical structure of triacylglycerol species | 126 |
| 2 | Flow diagram of the sequential procedure | 136 |
| 3 | Comparison of the chemical assignment of bands in the FT-IR spectra for Koroneiki olive oil, sunflower,soybean and corn oils. | 170 |
| 4 | Comparison of the chemical assignment of bands in the FT-IR spectra for Croatia olive oil, sunflower,soybean and corn oils. | 171 |
| 5 | Comparison of the chemical assignment of bands in the FT-IR spectra for Koroneiki olive oil and 2%, 5% and 10% corn oil. | 172 |
| 6 | Comparison of the chemical assignment of bands in the FT-IR spectra for Koroneiki olive oil and 2%, 5% and 10% sunflower oil. | 173 |
| 7 | Comparison of the chemical assignment of bands in the FT-IR spectra for Koroneiki olive oil and 2%, 5% and 10% soybean oil. | 174 |
| 8 | Comparison of the chemical assignment of bands in the FT-IR spectra for Croatia olive oil and 2%, 5% and 10% corn oil. | 175 |
| 9 | Comparison of the chemical assignment of bands in the FT-IR spectra for Croatia olive oil and 2%, 5% and 10% sunflower oil. | 176 |
| 10 | Comparison of the chemical assignment of bands in the FT-IR spectra for Croatia olive oil and 2%, 5% and 10% soybean oil. | 177 |
| 11 | Typical ^1H NMR spectra of koroneiki olive oil | 186 |

| | | |
|----|--|-----|
| 12 | Typical ^1H NMR spectra of koroneiki olive oil with 10% Corn oil. | 187 |
| 13 | Typical ^1H NMR spectra of koroneiki olive oil with 10% sunflower oil. | 188 |
| 14 | Typical ^1H NMR spectra of koroneiki olive oil with 10% soybean oil. | 189 |
| 15 | Typical ^1H NMR spectra of Croatina olive oil. | 190 |
| 16 | Typical ^1H NMR spectra of Croatina olive oil with 10% Corn oil. | 191 |
| 17 | Typical ^1H NMR spectra of Croatina olive oil with 10% sunflower oil. | 192 |
| 18 | Typical ^1H NMR spectra of Croatina olive oil with 10% soybean oil. | 193 |
| 19 | Assignments of the resonances around 2.04 ppm in the ^1H NMR spectra of olive oils and their binary admixtures. | 196 |
| 20 | Relationship between the total sum of linoleic and linolenic acids and their corresponding integrals at 2.7 ppm for Koroneiki olive oil and its binary admixtures with 10% (w/w) corn, sunflower and soybean oils. | 197 |
| 21 | Relationship between the total sum of linoleic and linolenic acids and their corresponding integrals at 2.7 ppm for Croatina olive oil and its binary admixtures with 10% (w/w) corn, sunflower and soybean oils. | 198 |
| 22 | ^{13}C NMR spectrum of an extra virgin olive oil sample in CDCl_3 solution. | 200 |
| 23 | Typical ^{13}C NMR spectra of Koroneiki olive oil. | 209 |
| 24 | Typical ^{13}C NMR spectra of Koroneiki olive oil with 10% corn oil. | 210 |
| 25 | Typical ^{13}C NMR spectra of Koroneiki olive oil with 10% sunflower oil. | 211 |

| | | |
|----|---|-----|
| 26 | Typical ^{13}C NMR spectra of Koroneiki olive oil with 10% soybean oil. | 212 |
| 27 | Typical ^{13}C NMR spectra of Croatina olive oil. | 213 |
| 28 | Typical ^{13}C NMR spectra of Croatina olive oil with 10% corn oil. | 214 |
| 29 | Typical ^{13}C NMR spectra of Croatina olive oil with 10% sunflower oil. | 215 |
| 30 | Typical ^{13}C NMR spectra of Croatina olive oil with 10% soybean oil. | 216 |

LIST OF ABBREVIATIONS

| | |
|----------|--|
| APCI | Atmospheric Pressure Chemical Ionization |
| APCI-MS | Atmospheric Pressure Chemical Ionization - Mass Spectrometry |
| APPI | Atmospheric Pressure Photoionization |
| ATR | Attenuated Total Reflectance |
| ATR-FTIR | Attenuated Total Reflection - Fourier Transform Infrared |
| CALB | <i>Candida antarctica lipase B</i> |
| CDA | Canonical Discriminant Analysis |
| CVA | Canonical Variate Analysis |
| CGC | Capillary Gas Chromatography |
| CGC-FID | Capillary Gas Chromatography -Flame Ionization Detector |
| CN | Carbon Number |
| CAD | Charged Aerosol Detector |
| CBTs | Classification Binary Trees |
| DSC | Differential scanning calorimeter |
| DA | Discriminant Analysis |
| DEPT | Distortionless Enhancement By Polarization Transfer |
| DHA | Docosahexaenoic Acid |
| ECN42 | Equivalent Carbon Number 42 |
| ELSD | Evaporative Light Scattering Detection |
| EEFS | Excitation-Emission Fluorescence Spectroscopy |
| EVOO | Extra Virgin Olive Oil |
| FA | Fatty Acid |
| FAME | Fatty Acid Methyl Ester |
| FTIR | Fourier Transform Infrared |
| FT-NIR | Fourier Transform Near-Infrared |
| FT-Raman | Fourier Transform Raman |

| | |
|--------------|--|
| FT | Fourier-Transform |
| GC | Gas Chromatography |
| GLC | Gas Liquid Chromatography |
| GA-PLS | Genetic Algorithms - Partial Least Square |
| GA-PLS | Genetic algorithms- Partial Least Square |
| HDL | High Density Lipoprotein |
| HPLC | High Performance Liquid Chromatography |
| HPLC-UV | High Performance Liquid Chromatography Method With Ultra-Violet Detection |
| HT-GC/EI-MS | High Temperature-Gas Chromatographic Method Coupled To Electron Ionization-Mass Spectrometry |
| IR | Infrared Spectrophotometry |
| IOC | International olive council |
| iPLS | interval Partial Least Square |
| KNN | K-Nearest Neighbours |
| LODs | Limit Of Detections |
| LDA | Linear Discriminant Analysis |
| OOL | Linoleo-Diolein |
| LC | Liquid Chromatography |
| LC-APPI-MS | Liquid Chromatography-Atmospheric Pressure Photoionization-Mass Spectrometry |
| MS | Mass Spectrometry |
| MALDI-TOF/MS | Matrix-Assisted Laser Desorption Ionization - Time Of Flight-Mass Spectrometry |
| MAEV | Mean Absolute Error of Validation |
| MdAEV | Median Absolute Error of Validation |
| MUFA | Mono-Unsaturated Fatty Acid |

| | |
|-------------|---|
| MRM | Multiple reaction monitoring |
| N-PLS | Multi-way Partial Least Squares |
| NIR | Near Infrared |
| NARP-LC-RID | Non-Aqueous Reverse-Phase - Liquid Chromatography- Refractive Index Detector |
| NTP | Non-Thermal Plasma |
| NP | Normal-Phase |
| NMR | Nuclear Magnetic Resonance |
| NOE | Nuclear Overhauser Enhancement |
| OLL | Oleo- Di-Linolein |
| OPO | Olive-Pomace Oil |
| OCPLS | One-Class Partial Least Squares |
| OSC | Orthogonal Signal Correction |
| PLL | Palmito-Di-Linolein |
| POO | Palmito-Di-Olein |
| POL | Palmito-Oleo-Linolein |
| PARAFAC | Parallel Factor Analysis |
| PLS | Partial Least Square |
| PLS-R | Partial Least Square-Regression |
| PLS-DA | Partial Least Squares Discriminant Analysis |
| PCI | Photo-Induced Chemical Ionization |
| PUFAs | Polyunsaturated Fatty Acids |
| PCA | Principal Component Analysis |
| PCR | Principle Component Regression |
| RI | Refractive Index |
| RID | Refractive Index Detector |
| REP | Relative Error of Prediction |

ABSTRACT

Amany Nagah Ali Mostafa, Some Chemical Studies on Detecting Olive Oil Adulteration, Ph.D., Department of chemistry, Faculty of Science, Benha University, 2019.

This study focused on the application of three different chromatographic and three different spectroscopic methods for identification of adulteration of two olive oil varieties (Koroneiki and Croatia) with cheaper vegetable oils (sunflower, soybean and corn oils). It was found from fatty acids profile that, adulteration of extra virgin olive oils with high linoleic acid oils could be detected with 10% (w/w) addition of both soybean and corn oils while, sunflower oil addition could not be found out at 10% (w/w). 1,3 specific lipase hydrolysis followed by gas chromatography and HPLC were used for detection of adulteration by studying the change in triglycerides composition. It was found that a decrease in OOO and POO and an increase in OOL, POL, OLL and PLL occurred with 2, 5 and 10% additions of sunflower, soybean and corn oil for Koroneiki and Croatia olive oils respectively. According to global method procedures, only pure Koroneiki and Croatia olive oils were genuine oils while olive oils admixtures with sunflower, soybean and corn oils were not genuine olive oils.

¹H NMR spectra of olive oils and their binary admixtures with 10% (w/w) (sunflower, soybean and corn oils) were discussed. The most obvious difference between the spectra of olive oils and their binary admixtures spectra was the appearance of the signal around 2.04 ppm due to allylic protons of linoleic acid in the binary admixtures while being absent in pure olive oils. This peak could be functioned as indicator of

adulteration of olive oil with high linoleic acid oils. Furthermore, the peak integrals at 2.7 ppm showed a good correlation with the total sum of linoleic and linolenic acids.



جامعة بنها
كلية العلوم
قسم الكيمياء

بعض الدراسات الكيميائية على كشف الغش فى زيت الزيتون

رسالة مقدمة من

أمانى نجاح على مصطفى فرحات

لجنة التحكيم

| م | الاسم | الوظيفة | التوقيع |
|---|----------------------------------|--|---------|
| ١ | أ.د/ صلاح الدين حسين سيد أبوريه | أستاذ علوم وتكنولوجيا الأغذية كلية الزراعة – جامعة القاهرة | |
| ٢ | أ.د/ محمد جمعه محمد عاصي | أستاذ الكيمياء العضوية كلية العلوم- جامعة الزقازيق | |
| ٣ | أ.د. أشرف عبد الحميد فاروق وصفى | أستاذ الكيمياء العضوية التطبيقية ووكيل كلية العلوم جامعة بنها | |
| ٤ | أ.د. علاء عزوز سلامه عبد المقصود | أستاذ علوم وتكنولوجيا الأغذية معهد بحوث تكنولوجيا الاغذية مركز البحوث الزراعية | |