

ABSTRACT

Rania Ibrahim Mohammad Almoselhy. Application of Some Methods for Detecting Adulteration in Olive Oil. Unpublished M.Sc. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2010.

This study aimed to maintain the high quality of olive oil by investigating some analytical methods as a powerful tool to determine extra virgin olive oil adulteration with relatively cheap seed and vegetable oils such as sunflower oil, corn oil and refined olive oil.

The analytical methods ranged from the familiar manual physical and chemical tests such as refractive index (RI) which gives a good idea about the degree of unsaturation of the oil under investigation, as well as its correlation with iodine value (IV); acid value (AV) as indication of free fatty acid content of the oil; peroxide value (PV) which determines the amount of primary oxidation products and UV absorbencies at 232 and 270 nm, that measure the formation of conjugated dienes and trienes, respectively due to the formation of secondary oxidation products.

The analytical methods extended to the more sophisticated instrumental methods of analysis such as chromatographic separation and determination techniques which involved gas chromatography (GC) with flame ionization detector (FID) in order to investigate the composition of the fatty acids of the oils under investigations.

Fourier Transform Infra Red (FTIR) spectroscopic determination technique was employed and applied as a potent, nondestructive and effective analytical tool to study its potency to investigate the functional groups with their relative absorbencies or transmittances according to their concentrations in samples and their characteristic fingerprints. FTIR spectroscopy was used also to determine extra virgin olive oil adulteration with sunflower, corn and refined olive oils in their binary admixtures at different concentrations of 0, 5, 10, 20, 30, 40, 50, 100%; w/w.

FTIR spectral data collected in MIR range 4000-400 cm^{-1} showed major peaks representing triglyceride functional groups which could be observed around 2925 cm^{-1} [C–H stretching (asymmetry)], 2854 cm^{-1} [C–H stretching (symmetry)], 1747 cm^{-1} [C=O stretching], 1463 cm^{-1} [C–H bending (scissoring)], 1238, 1163, 1118 and 1097 cm^{-1} [C–O stretching] and 722 cm^{-1} [C–H bending (rocking)]. A peak around 1653 cm^{-1} is attributed to C=C stretching (*cis*). The spectral region (1300-1000 cm^{-1}) which contains FTIR fingerprints of the used oils was found to be very useful in detecting extra virgin olive oil adulteration.

A band shift at 3005 cm^{-1} ; assigned to C–H stretching vibration of *cis*- double bond (=C–H) characteristic to extra virgin olive oil, was observed at higher wavenumbers with increasing adulterant concentration which allowed the determination of adulteration of extra virgin olive oil.

The absorption intensity values of the spectral bands at 1163 cm^{-1} (assigned to C–O stretching vibration and CH₂ bending vibration) increased with increasing adulterant concentration.

There was a pronounced shift of the peak at 912 cm^{-1} (assigned to –HC=CH– of *cis*- double bond, bending out-of-plane) for extra virgin olive oil to higher wave numbers with increasing adulterant concentration.

Absorbance ratios (R1118/1097 and R1747/2925 cm^{-1}) decreased with increasing the concentrations of added adulterant oils (sunflower, corn and refined olive oils) with a fairly good linear relationship.

The spectral region selected between 1800-900 cm^{-1} mostly represented the combination of C–H bending, C=O stretching and C=C stretching and hence it was directly related to the unsaturated C=C bond. It played a very important role in the discriminant analysis.

In conclusion, FTIR spectroscopy proved its potency to detect extra virgin olive oil adulteration at 5% level of adulterant oils (sunflower, corn and refined olive oils) which is much lower than the limit at which there exists a threatening of adulteration of extra virgin olive oil.

KEY WORDS: Adulteration; extra virgin olive oil; FTIR spectroscopy; UV spectroscopy; GC analysis; sunflower oil; corn oil; refined olive oil.

CONTENTS

Title	Page
LIST OF TABLES	iv
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	vii
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	7
2.1 -Physicochemical Quality Parameters of Virgin Olive Oil	7
2.2 -Fatty Acid Composition of Virgin Olive Oil	8
2.3 -Unsaponifiable Matter Composition of Virgin Olive Oil	10
2.4 -Detection of Adulteration of Virgin Olive Oil by DSC	11
2.5 -Detection of Adulteration of Virgin Olive Oil by Hyphenated Chromatographic Separation and Determination Techniques	14
2.6 -Detection of Adulteration of Virgin Olive Oil by UV Spectroscopy	28
2.7 -Detection of Adulteration of Virgin Olive Oil by IR Spectroscopy	28
2.8 -Detection of Adulteration of Virgin Olive Oil by NMR Spectroscopy	42
2.9 -Detection of Adulteration of Virgin Olive Oil by Mass Spectroscopy	46
2.10-Detection of Adulteration of Virgin Olive Oil by CL Spectroscopy	48
2.11-Detection of Adulteration of Virgin Olive Oil by Fluorescence Spectroscopy	50
2.12-Detection of Adulteration of Virgin Olive Oil by XRF Spectroscopy	52
2.13-Detection of Adulteration of Virgin Olive Oil by Electronic Nose	54

3. MATERIALS AND METHODS	56
3.1- MATERIALS	56
3.1.1- Oils	56
3.1.1.1- Extra Virgin Olive Oil (EVOO)	56
3.1.1.2- Refined Olive Oil (ROO)	56
3.1.1.3- Sunflower Oil (SO)	56
3.1.1.4- Corn Oil (CO)	56
3.1.2- Solvents	56
3.1.3- Standards	56
3.2- METHODS	57
3.2.1- Preparation of Binary Admixtures Containing Different Adulterant Oils; (w/w %)	57
3.2.1.1- Binary admixtures of SO in EVOO	57
3.2.1.2- Binary admixtures of CO in EVOO	57
3.2.1.3- Binary admixtures of ROO in EVOO	57
3.2.2- ANALYTICAL METHODS	58
3.2.2.1- Refractive Index (RI)	58
3.2.2.2- Acid Value (AV) and Free Acidity	58
3.2.2.3- Peroxide Value (PV)	58
3.2.2.4- Iodine Value (IV)	58
3.2.3- Ultraviolet (UV) Spectroscopic Characteristics	58
3.2.4- Gas Chromatography (GC) Analysis	59
3.2.4.1- Gas Chromatography Analysis of Fatty Acids	59
3.2.4.2- Useful Relationships Obtained from Fatty Acid Composition	60
3.2.4.3- Determination of Total Unsaponifiable Matter	60
3.2.5- FTIR Spectroscopic Characteristics	61
3.2.5.1- FTIR Spectral Data Analysis	61
3.2.5.2- Useful Relationships Obtained from FTIR Spectral Data Analysis	62

4. RESULTS AND DISCUSSION	63
4.1- Physical and Chemical Properties	63
4.1.1- Refractive Index	63
4.1.2- Acid Value and Free Acidity	67
4.1.3- Peroxide Value	72
4.1.4- Iodine Value	75
4.2- Ultraviolet (UV) Spectroscopic Characteristics	80
4.3- Gas Chromatography (GC) Analysis	89
4.3.1- GC Analysis of Fatty Acids	89
4.3.1.1- Detection of adulteration of EVOO with adulterant SO using fatty acids composition	91
4.3.1.2- Detection of adulteration of EVOO with adulterant CO using fatty acids composition	92
4.3.1.3- Detection of adulteration of EVOO with adulterant ROO using fatty acids composition	95
4.3.2- Total Unsaponifiable Matter	97
4.4- Fourier Transform Infra Red Spectroscopic Characteristics	99
4.4.1- FTIR Spectral Data Analysis	99
4.4.2- Detection of adulteration of virgin olive oil by FTIR Spectroscopy	100
4.4.3- Detection of adulteration of EVOO with adulterant SO using FTIR spectral data analysis	115
4.4.4- Detection of adulteration of EVOO with adulterant CO using FTIR spectral data analysis	126
4.4.5- Detection of adulteration of EVOO with adulterant ROO using FTIR spectral data analysis	142
4.4.6- Detection of adulteration of EVOO with adulterant oils (SO, CO and ROO) using FTIR spectral data analysis and useful relationships	144
5-SUMMARY	156
6-REFERENCES	160
ARABIC SUMMARY	----

LIST OF ABBREVIATIONS

APCI	Atmospheric Pressure Chemical Ionization
APPI	Atmospheric Pressure Photospray Ionization
ATR	Attenuated Total Reflectance
AV	Acid Value
CEC	Capillary Electrochromatography
CL	Chemiluminescence
CLA	Conjugated Linoleic Acids
CO	Corn Oil
CVA	Canonical Variate Analysis
DEPT	Distortionless Enhancement by Polarization Transfer
DSC	Differential Scanning Calorimetry
DTGS	Deuterated Tri-Glycine Sulfate
ECN	Equivalent Carbon Number
ED	Energy-Dispersive
EEFM	Excitation–Emission Fluorescence Matrices
EEFS	Excitation–Emission Fluorescence Spectroscopy
ESI	Electrospray Ionization
EVOO	Extra Virgin Olive Oil
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
FID	Flame Ionization Detector
FIR	Far-IR
FTIR	Fourier Transform Infra Red
GC	Gas Chromatography
GC–FID	Gas Chromatography – Flame Ionization Detector
GC-MS	Gas Chromatography–Mass Spectrometry
GILS	Genetic Inverse Least Squares
GLC	Gas Liquid Chromatography
HPLC	High-Performance Liquid Chromatography

HPLC- APCI- MS	High-Performance Liquid Chromatography combined with Atmospheric Pressure Chemical Ionization coupled with Mass Spectrometry
HT-GC	High Temperature – Gas Chromatography
ID	Internal Diameter
IOOC	International Olive Oil Council
IV	Iodine Value
LC-MS	Liquid Chromatography with Mass Spectrometry
LDA	Linear Discriminant Analysis
MIR	Mid-infrared
MRI	Magnetic Resonance Imaging
MS	Mass Spectroscopy
MSC	Multiplicative Signal Correction
MUFA	Mono-unsaturated Fatty Acid
NIR	Near-infrared
NMR	Nuclear Magnetic Resonance
OPO	Olive–Pomace Oil
OSC	Orthogonal Signal Correction
PCA	Principal Component Analysis
PCR	Principal Component Regression
PDO	Protected Denomination of Origin
PLS	Partial Least Square
PLS-DA	Partial Least Square-Discriminant Analysis
PR	Pattern Recognition
PRESS	Prediction Residual Error Sum of Squares
PUFA	Poly-unsaturated Fatty Acid
PV	Peroxide Value
QC	Quality Control
QqTOF	Quadrupole Time-Of-Flight
R	Absorbance Ratio
ROO	Refined Olive Oil

RP- HPLC	Reversed Phase – High Performance Liquid Chromatography
SD	Standard Deviation
SEP	Standard Error of Prediction
SFA	Saturated Fatty Acid
SFC	Supercritical Fluid Chromatography
SFE	Super-critical Fluid Extraction
SIMCA	Soft Independent Modeling of Class Analogy
SO	Sunflower Oil
SPE	Solid Phase Extraction
SPME– MDGC	Solid Phase Microextraction coupled with Multidimensional Gas Chromatography
TAGs	Triacylglycerols
TG	Triglyceride
TH	Total Hydrocarbons
TLC	Thin-Layer Chromatography
ToFMS	Time of Flight Mass Spectrometry
TS	Total Sterols
TSFA	Total Saturated Fatty Acids
TSyF	Total Synchronous Fluorescence
TUFA	Total Unsaturated Fatty Acids
U-PCA	Unfold Principal Component Analysis
USFA	Unsaturated Fatty Acids
UV	Ultra Violet
VOO	Virgin Olive Oil
WD	Wavelength-Dispersive
XRF	X-Ray Fluorescence