Implementation of a physio-chemical approach coupled with a data fingerprinting methodology for the characterization of the Lebanese extra-virgin olive oils

Omar Dib

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Omar DIB

Composition du Jury

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*Allah is the Light of the heavens and the earth. The example of His light is that of a niche, in which there is a lamp; the lamp is in a glass – the glass looks like a brilliant star – it is lit by (the oil of) a blessed tree, the olive, which is neither eastern, nor western. Its oil is about to emit light even though the fire has not touched it – (it is) light upon light. Allah guides to His light whomsoever He wills; Allah describes examples for the people, and Allah knows everything well.
*Quran al Karim, An-Nur, Verse 35, Page 354
(Translated by: Mufti Taqi Usmani)

*اللَّهُ نُورُ السَّماواتِ والْأَرْضِ مَثَلُ نُورِهِ كَمِشْكاةٍ فِيها
مِصْباحٌ الْمِصْباحُ فِي زُجاجَةٍ الزُّجاجَةُ كَأَنَّها كَوْكَبٌ
دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبارَكَةٍ زَيْتُونَةٍ لا شَرْقِيَّةٍ ولا
غَرْبِيَّةٍ يَكادُ زَيْتُها يُضِيءُ ولَوْ لَمْ تَمْسَسْهُ نارٌ نُورٌ
عَلى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشاءُ ويَضْرِبُ اللَّهُ
الْأَمْثالَ لِلنَّاسِ واللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ
*القرآن الكريم، سورة النور، آية 35، صفحة 354

Table of Contents

List of Formation

Courses	***Categorie***
Scientific Writing Course	Outils et méthodes pour bien exercer son métier de doctorant
Synchronous Light	Course
Seminaire Olive Oil	Seminare
Seminaire La qualite de semences	Seminare
Poster at EDST (Doctoral Forum 2017-2018)	Définir son projet professionnel
Communiquer sur sa thèse : l'exposé oral et efficace - session 4	Communication et médiation scientifique
French Course	Langues étrangères et interculturalités
Committee meeting 2017	Séminaires scientifiques
Committee meeting 2018	Séminaires scientifiques
Doctoriales (Dina Darwish)	Supervising Master student
Journée de formation à la rédaction du manuscrit	Outils et méthodes pour bien exercer son métier de doctorant
Séminaires scientifiques (JDD ABIES)	Séminaires scientifiques
La Visite du Petit Palais : la culture par la connaissance de l'Art	Outils et méthodes pour bien exercer son métier de doctorant
Research integrity in scientific professions Université de Bordeaux (Course)	Outils et méthodes pour bien exercer son métier de doctorant
Become an expert or generalist consultant after your PhD (Course)	Parcours ' Conseil et expertise en innovation '
Forum professionnel des doctorants et docteurs - doc'avenir 2018	Définir son projet professionnel
Mon projet professionnel en 180 secondes	Définir son projet professionnel
Presentation skills in English - Getting your Message Across	Définir son projet professionnel

List of Publications

1. Tomé D, Cordella BY, Dib O, Péron C: Nitrogen and protein content measurement and nitrogen to protein conversion factors for dairy and soy protein-based foods: a systematic review and modelling analysis. In. Geneva: World Health Organization and Food and Agriculture Organization of the United Nations; 2019.

2. Omar H.Dib, Ali Bassal, Hussein Dib, Rita Yaacoub, Nathalie Locquet, Luc Eveleigh & Christophe B.Y. Cordella (2020). Impact of growing area and technological aspects on Lebanese olive oil: Characterization by unsupervised methods. Journal of Food Research. 9 (2): 48-57. DOI: 10.5539/jfr.v9n2p48

3. Omar H.Dib, Christophe B.Y. Cordella, Hussein Dib, Rita Yaacoub, Nathalie Locquet, Luc Eveleigh & Ali Bassal (2020). Conventional and Ultra-fast Analysis Exposing the Harvest Date Impact on Lebanese Olive Oil. Journal of Food Research.

4. Dib OH, Rizkalah J, Yaacoub R, Dib H, Locquet N, Eveleigh L, Cordella CBY, Bassal A. Does Variability Affect the Performance of Front-Face Fluorescence Spectroscopy? A Study Case on Commercial Lebanese Olive Oil. J Fluoresc (2020). doi: 10.1007/s10895-020-02634-8.

List of Communications

Place	Type	Title
International Research Conference, 2019 (Paris, France) *	Oral Presentation	Use of Front-Face Fluorescence Spectroscopy and Multiway Analysis for the Prediction of Olive Oil Quality Features
ABIES Doctoral Day 2018-2019 (Paris, France)	Poster	Utilization of Unsupervised Chemometric Applications to Geographically Characterize and Assess Lebanese Olive Oil
Doctoral Forum 2017-2018 (EDST, Beirut, Lebanon)	Poster	Geographical Characterization of Lebanese Olive Oil Based on Fatty Acid Profile and Detection of the Cause Disqualifying Olive Oil Virginity
Doctoral Forum 2016-2017 (EDST, Beirut, Lebanon)	Oral Presentation	Geographical Characterization of Lebanese Olive Oil Using Conventional and Miniaturized Fingerprint approach with Specific Emphasis on Sterols-Based Molecular Markers

* Received a “Best Presentation Award” from the ICSSA (International Conference for Spectroscopy and Spectral Analysis) on 24 April 2019.

List of Abbreviations

EU	European Union
IOC	International Olive Council
VOO	Virgin Olive Oil
EVOO	Extra Virgin Olive Oil
OVOO	Ordinary Virgin Olive Oil
LVOO	Lampante Virgin Olive Oil
IDAL	Investment Development Authority of Lebanon
GMP	Good Manufacturing Practices
ACTED	Agency For Technical Cooperation and Development
MOA	Ministry Of Lebanese Agriculture
GCC	Gulf Cooperation Council
MUFA	Monounsaturated Fatty Acid
SFA	Saturated Fatty Acid
ACP	Acyl Carrier Protein
PUFA	Polyunsaturated Fatty Acid
OLF	Olive Fruit Fly
UV	Ultra Violet
Vis	Visible
IR	Infra Red
NMR	Nuclear Magnetic Resonance
EEM	Exitation Emission Matrix
ROOs	Reactive Oxygen Speices
FGC	Flash Gas Chromatography
FID	Flame Ionization Detector
RT	Retention Time
PCA	Principle Component Analysis
PARAFAC	Parallel Factor Analysis
ICA	Independent Components Analysis
MLR	Multiple Linear Regression
PLS	Partial Linear Regression

PC	Principle Component
CANDECOMP	Canonical Decomposition
GC	Gas Chromatography
MS	Mass Spectroscopy
CORCONDIA	Core Consistency Diagnostic
MI	Maturity Index
PV	Peroxide Value
EC	European Council
FAME	Fatty Acid Methylation
PTFE	Ploytetrafluoroethylene
PMT	Photomultiplier
E-nose	Electric Nose
DTW	Dynamic Time Warping
COW	Correlation Optimized Warping

List of Symbols

t	Tons
mg/kg	Milligram per Kilogram
g	Gram
β	Beta
Δ	Delta
m^2	Meter square
L	Liter
kg	Kilogram
^oC	Degree celsius
meq/kg	Milliequivalent per kilogram
mg/L	Milligram per liter
S0	Ground singlet excited state
S1	Higher levelsinglet excited state
nm	Nanometer
K232	Specific extinction at a wavelength of 232 nm
K270	Specific extinction at a wavelength of 270 nm
mL	Milliliter
h	Hour
mm	millimeter
rpm	Rotation per minute
g/mol	Gram per mole
λ	Wavelength
ΔK	Variation of specific extinction
A	Absorbance
R^2	Correlation Coefficient
v/v	Volume per volume
µL	Microliter
min^{-1}	Per minute
N	Number of moles
M	Molar mass
cm	centimeter

V	volt
^{o}C /s	Degree Celsius per second

List of Figures

List of Tables

General Introduction

Olive trees cover 5.4 % of the Lebanese territory or 8 % of total agricultural lands in Lebanon, destined for both oil and table olives production (Chehade et al., 2016). The Lebanese olive germplasm is characterized by a high level of diversity. Four local varieties, namely, Abou-Chawki, Baladi, Del and Soury, are the most cultivated in commercial plantations. Oil groves are dispersed over the five provinces of Lebanon producing 90.307 thousand tons of olives per year where around 70 % of the total olive production is transformed into olive oil. In 2011, 11.3 thousand tons of olive oil were produced and only 30 % of total olive oil production consisted of extra virgin olive oil (IDAL, 2017b).

The inferior quality of olive oil in Lebanon is indicated by the low rate of exportable grade (about 30% of total olive oil production) (IDAL, 2017b). In the background of researchers and interested entities, heavy work has been done to improve the quality of olive and olive oil and subsequently enhance livelihood and economy of the Lebanese community. But little has been done regarding the improvement of commercial quality characteristics. The low percentage of Lebanese EVOO is an indicator of these limitations which are mainly due to the great variability shown in oil quality and chemical composition. Previous research work, though limited, attributed such inferior quality to non-compliance of micro-components such as sterols to IOC standards. Stigmastenol and some polyunsaturated fatty acids were reported to constraint international marketing of Lebanese EVOO (Youssef, 2002; Mireille, 2003; Claude, 2005; Rabih, 2005; Breidi, 2011).

This inconsistency is caused by several factors among them geographical location (soil composition, altitude, and latitude), climatic conditions, extraction process, cultivar, harvesting date etc... (Zamora et al., 2001; Temime et al., 2006; Rotondi et al., 2004; Kiritsakis et al., 1998; Franco et al., 2015; Dag et al., 2011; Baccouri et al., 2007; Abaza et al., 2005). Every one of the above-mentioned factors has its weight on the parameters that define the virginity of olive oil. These parameters include FFA, PV, UV, and other quality parameters such as fatty acid profile and total unsaponifiable matter.

Although Lebanon is considered a small-scale producer of olive oil as compared to other Mediterranean countries, it has the reputation of producing high-quality artisanal oils. This high-end product is increasingly being exported to countries like Saudi Arabia (2,635 tons), United States (1,315 tons), and others (IDAL, 2017a).

To keep up with such high-quality product, particularly in terms of olive oil nutritional and sensory properties, specialized institutions such as the International Olive Council (IOC) has set explicit olive oil quality controls where the complexity of some is reflected in the ample time and materials required to determine olive oil genuineness and quality (Guzman et al., 2015). Nowadays, the presence of new sample-holder accessories, software and numerous chemometrics applications gave way for faster and simpler analytical techniques, particularly front-face fluorescence spectroscopy and ultra-fast gas-chromatography (Flash-GC). Fluorescence spectroscopy has been successfully used as a rapid, non-invasive and highly sensitive technique for analysis of olive oil quality and showed to be more cost-efficient compared to other analytical procedures (Lleo et al., 2016). Never to mention the diversity of fluorescence applications especially in the field of olive oil analysis (Locquet et al., 2019). These include studies on detection of olive oil adulteration with vegetable oils or pomace oil (Dankowska & Małecka, 2009; Sayago et al., 2007), on monitoring thermal oxidation (Cheikhousman et al., 2005a; Poulli et al., 2009; Sikorska et al., 2008; Tena et al., 2012; Tena et al., 2009), on prediction of origin (Dupuy et al., 2005), and on quality parameters evaluation (Guimet et al., 2004a; Guzman et al., 2015). As for flash GC, it is a highly selective and sensitive technique used for analyzing volatile compound present in olive oil in matter of seconds. This type of chromatography has been used in discriminating geographical origins (Melucci et al., 2016), in detecting adulteration (Jabeur et al., 2014) and in determining quality grades of olive oil (Barbieri et al., 2020). These types of rapid analytical tools can be particularly useful as routine quality control especially with the aid of chemometric tools.

This work investigates the use of chemometrics like PCA, PARAFAC, ICA, MLR, and PLS to highlight the effect of growing area on the Lebanese olive oil based on the resulting physicochemical parameters, to detect the technological and agricultural factors leading to disqualification of Lebanese olive oil virginity and to test the usefulness of rapid analysis techniques in the grading of olive oil samples.

Objectives

The overall objectives may be summarized as:

- Elaboration of a control plan to evaluate the physico-chemical characteristics and quality grades of EVOO in comparison to an international set reference
- Improve the production, quality and eventually marketability and competitiveness of Lebanese produced EVOO in the international markets

The specific objectives include:

(1) Identifying sources and causes of non-conformities in the cultivation and/or processing of Lebanese olive oil while finding applicable solutions to fit with international requirements,

(2) Investigating the effect of growing area on the chemical composition of Lebanese olive oil.

(3) Development of a rapid assessment tool in order to replace the conventional analytical methods.

Study structure

The carried work is presented in the manuscript in six chapters. A general introduction is presented at the beginning of the manuscript.

Chapter 1 includes a bibliographic review to understand the situation of Lebanese olive oil, the impact of several environmental/ agricultural / technological factors on the main constituents of olive oil, and the alternatives for conventional analytical tests.

Chapter 2 presents the sampling locations, the protocols for collecting and preparing olive fruit and oil samples, as well as the different analysis techniques.

Chapter 3[1] deals with the influence of growing-area and technological aspects on the Lebanese Soury variety. The pedoclimatic conditions, quality parameters, and fatty acids of oil samples were examined using chemometric tools (PCA and ICA) to demonstrate the typicality of Lebanese olive oil.

Chapter 4[2] shows the impact of harvest date on the quality parameters, polyphenols, fatty acids, sterols, and volatile compounds of Lebanese olive oil from the Soury variety.

Chapter 5[3] presents the capability of front-face fluorescence spectroscopy coupled with chemometric techniques, namely multiple linear regression (MLR) applied on parallel factor (PARAFAC) scores and partial least squares (PLS), to predict Lebanese olive oil chemical parameters in spite of the variability present between the samples.

Chapter 6 provides a review of the main results and a general discussion around the main addressed scientific questions in addition to the drawn conclusions.

Chapters **3, 4, and 5** are presented under the article form. The first two articles have been published in international scientific journals and the last one has been accepted.

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[1] Omar H.Dib, Ali Bassal, Hussein Dib, Rita Yaacoub, Nathalie Locquet, Luc Eveleigh & Christophe B.Y. Cordella (2020). Impact of growing area and technological aspects on Lebanese olive oil: Characterization by unsupervised methods. Journal of Food Research. 9 (2): 48-57. DOI: 10.5539/jfr.v9n2p48

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Chapter 1: Literature Review

This chapter includes a bibliographic review to understand the situation of Lebanese olive oil, the impact of several environmental/ agricultural / technological factors on the main constituents of olive oil, and the alternatives for conventional analytical tests. In addition to an overview on the main preprocessing tools and chemometric methods that had been utilized in this work.

1. A general overview on olive oil

1.1. Olive oil: an introduction

Olive oil, a natural fatty liquid, is reported as one of the traditional crops in the world, in particular, in the Mediterranean region. Till now, the origin of the crop is still not known, but it can be traced to areas along the Mediterranean coast such as Syria, Lebanon, Turkey, Greece, and others as the olive tree can live for hundreds of years (Vossen, 2007). Olive cultivation (culture of olive trees, olive groves, and olive oil with oil mills) dates back to the period of the invention of agriculture and the cultivation of vines and wine, about 8,000 years ago, in the fertile crescent region of the Levant in the Near East and Mesopotamia (Recep et al., 2011).

Olive oil is solely produced from the fruit of the olive tree, *Olea europaea L.*, by mechanical means. Extraction, i.e., separating oil from the other fruit contents, is done merely through three main steps:

- Step one includes grinding the fruit into an olive paste.
- Step two is malaxation, where the resulting paste is slowly mixed to allow droplets of oil to aggregate.
- Step three is the collection of oil by centrifugation and decantation.

The end product of this process is primarily intended for human consumption and represents one of the emerging dietary trends worldwide (Souilem et al., 2017). Olive oil is well known for its nutritional and health protective characteristics. It is mainly composed of monounsaturated fatty acids, in particular, oleic acid, representing about 55-80 % of olive oil. Oleic acid is studied extensively due to its broad range of health benefits commencing from protecting against cardiovascular diseases (Kris-Etherton et al., 1999), high blood pressure (Gnoni et al., 2010), and to suppression of skin pigmentation (Ruiz et al., 2010). In addition to oleic acid, olive oil's minor chemical components like squalene, tocopherols, lipophilic and

hydrophilic phenols play an essential role in fighting cancerogenic cells due to their antioxidant capabilities (Cicerale et al., 2009). These functional bioactive compounds have rendered olive oil also to be used in the medical and pharmaceutical fields leading to a global increase in its production and consumption.

1.2. Geographic distribution of olive oil production and consumption

Consumers are motivated more and more by the nutritional and health assets of olive oil. The increase in demand has boosted its production, tripling over the last 60 years, reaching around 3,379,000 t in the 2017/2018 crop year. Countries responsible for olive oil production is mainly distributed across three groups (Figure 1):

1. Group one includes 28 EU countries, members of the international olive council (IOC), which dominates world production.
2. Group two is composed of 16 other current IOC member countries (Turkey, Tunisia, Syria, Morocco Albania, Algeria, Argentina, Croatia, Egypt, Iran, Iraq, Israel, Jordan, Lebanon, Libya, and Montenegro).
3. Group three is the non-IOC members (USA, China, Japan, Russia, etc.…).

Among the EU countries and based on the latest data (5-year average, i.e., 2014/15 – 2018/2019) provided by the IOC, Spain stands out with 44.5 % of the world total production followed by Italy, Greece, and Portugal. Outside the EU, Tunisia has the highest production and constitutes around 7 % of the world total, followed by Turkey, Morocco, Algeria, and Egypt (IOC, 2018).

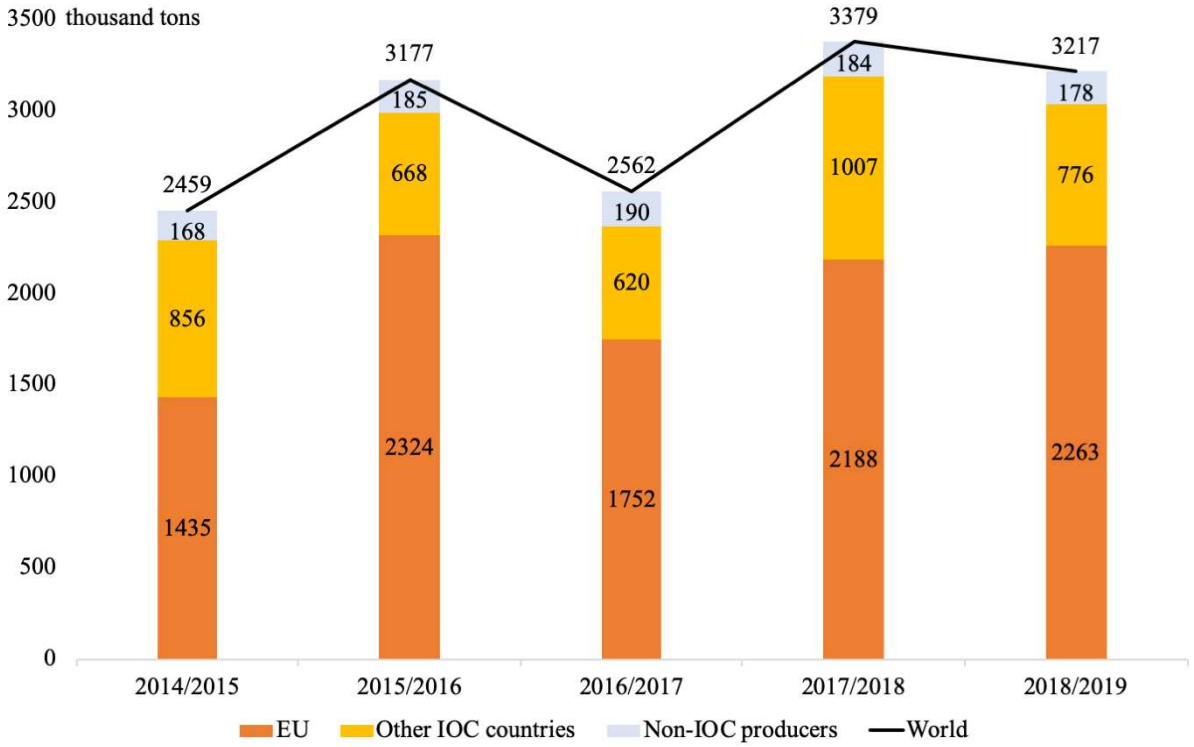

Figure 1. World olive oil production (Source: International Olive Council, 2018/2019)

This upward trend in production is followed by a similar one in consumption. A remarkable growth in olive oil consumption was observed since the 1990/91 crop year, where an increase of 82.4% is noted. The world consumption of olive oil reached about 2,909,000 t in the year 2018/2019. The EU countries hold the highest consumption per year in the world at about 1,433,000 t in 2018/2019. Spain and Italy alone, consume about 63 % (903,000 t) of the total EU olive oil consumption and about 36% of the world total. Concerning the other members of IOC, the consumption is 576,500 t, whereas, in the remaining countries, it is around 8999,599 t (IOC, 2018).

1.3. The rise of regulation

As olive oil production booms, regulations are a must to ensure product safety and standards uniformity. The objective of regulations is to provide the consumers with guarantees that the product is of high quality from farm to fork. Similarly, it offers the producers with assurances by leveling the market competition for imported products.

One of the most important bodies in laying down standards for olive oil and table olives is the international olive council (IOC). It was brought to light in Madrid, Spain, in 1959 and till now it is considered as the only international intergovernmental organization in the field of olive. The IOC currently has 17 countries plus the European union. The council main role is to develop the world olive economy by implementing measures to improve production, consumption and international trade. It does so by:

- Encouraging research and development projects to modernize olive growing and oil production technology, consequently improving fruit and oil quality, lowering costs, and sustaining the environment;
- Raising awareness through campaigns and expanding international trade to promote the consumptions of olive oil and table olives;
- Laying down trade standards and policies to ensure the fairness of the competition and improve the overall quality of the end product (IOC, 2020).

The application of these measures and principles is obligatory in international and domestic trade especially those related to olive oil trade. IOC has classified olive oil into several categories depending on the olive processing method and qualitative characteristics of the end product (Table 1).

Table 1. Olive oil categories.

Physical or mechanical extraction (virgin oils)	Mechanical and chemical extraction (refined oils)
Extra virgin olive oil	*Refined olive oil*
Virgin olive oil	*Olive oil (mix of virgin + refined oils)*
Ordinary virgin olive oil	*Crude olive-pomace oil*
Lampante virgin olive oil	*Refined olive-pomace oil*
	Olive-pomace oil

Most olive oil is produced with physical means without undergoing any treatment other than washing, grinding, malaxing, decantation, centrifugation and filtration. Although thermal treatment is applied (during malaxation), however, the application of the latter should not lead to alteration in the oil chemical composition. The finished product of such process is called virgin olive oil that is fit for human consumption and includes the following:

- *Extra virgin olive oil* is a VOO having an acidity (expressed as oleic acid) of not more than 0.8 g per 100 g;
- *Virgin olive oil* is an oil whose acidity is not more than 2 g per 100 g;
- *Ordinary virgin olive oil* is a VOO whose acidity is less than 3.3 per 100 g;
- *Lampante virgin olive oil* is an oil which has an acidity of more than 3.3 g per 100 g and it is not fit for human consumption. It is mostly used for refining purposes (IOC, 2011b);

Moreover, IOC lays down the standards detailing the minimum quality and purity criteria for each of the above olive oil grades. In addition, it also put standards pertaining to hygiene,

packaging, and labelling and it also sets analytical methods for determining various chemical parameters.

1.4. Chemical Composition

Olive oil can be separated into two portions: the saponifiable and the unsaponifiable (Figure 2). The saponifiable portion constitute about 98 to 99% of total weight and it is mainly composed of triacylglycerols. On the other hand, the unsaponifiable portion contains sterols, waxes, antioxidants, fat-soluble vitamins, aliphatic alcohols and aromatic compounds. These minor compounds are indicators of authenticity and stability of olive oil.

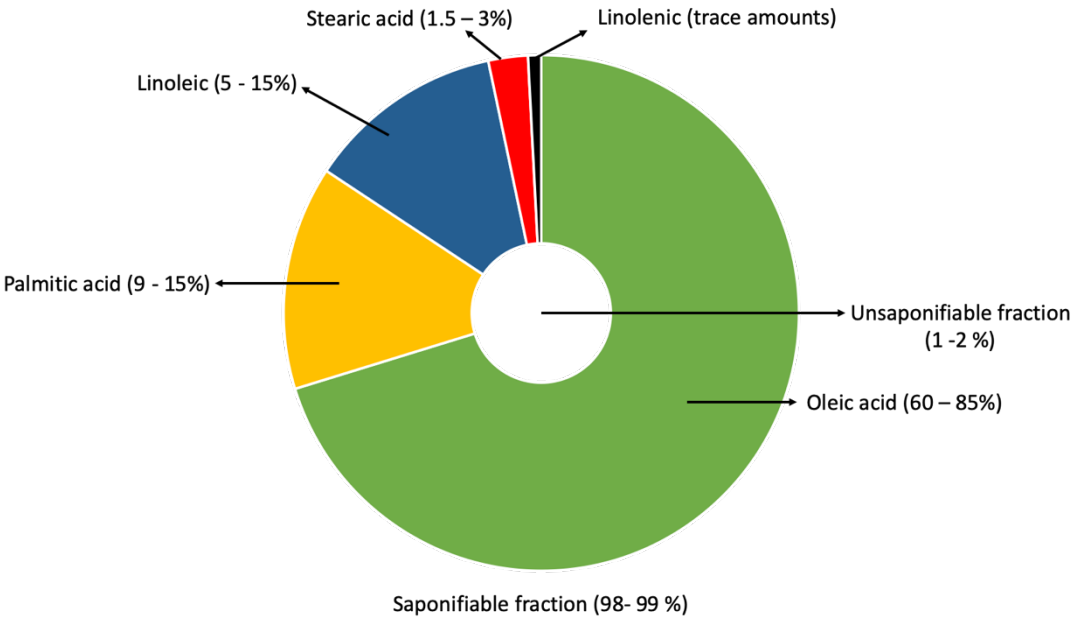

Figure 2. Chemical composition of olive oil

1.4.1. Fatty acids

Fatty acids can be either esterified to form triacylgycerides or be in free form which is the result of triglycerides hydrolysis. They can be saturated with no double bond (palmitic and stearic acids), monounsaturated with a single double bond (oleic acid) or polyunsaturated (linoleic and linolenic acids), if they have two or more double bonds (Gutiérrez et al., 1999). Several factors affect the fatty acids composition of olive oil including, variety, pedo-climatic conditions, and growing area.

For instance, growing area has an impact on fatty acid profile where countries close to the equator may have a higher palmitic, palmitoleic, linoleic and linolenic concentrations in contrary to those with a cold climate where a higher oleic level is observed (Aparicio et al., 1994). Furthermore, regions with higher altitudes have higher monounsaturated fatty acids content than those of lower altitudes where an increase in polyunsaturated and saturated fatty acids contents is noted (Di Bella et al., 2007).

1.4.2. Sterols

Plant sterols or particularly phytosterols, are structurally equivalent to cholesterols. Phytosterols are in the plant world what cholesterol is to the animal world. An essential element in the construction of cell walls and play a key role in the cell membrane function. In olive oil, they are one of the micro-components found in the unsaponifiable portion (Figure 2). The chief components are beta sitosterol, campesterol and stigmasterol. These sterol compounds are known for their stability even under prolonged storage of olive oil and are unique for each oily fruit (Thanh et al., 2006; Soupas et al., 2004). For this reason, their content in olive oil is regulated by the European Union legislations (EEC, 1991) and by the trade standards set by the IOC (IOC, 2011b) since these compounds are an important tool for the authentication of olive oil genuineness. As fatty acids, sterol compounds are also affected by the cultivar, climate, altitude, agronomic factors and other variables and can also be used for geographical discrimination (Giacalone et al., 2015).

1.4.3. Polyphenols

Phenolic compounds are of great organoleptic and nutritional interest. Several classes of phenolic compounds exist, mainly, phenolic acids, phenyl ethyl alcohols, flavonoids, hydroxy-iso-chromans and secoiridoids. That latter constitutes the main compounds of the phenolic fraction of *Oleaceae* plants. Polyphenols are generally linked to the bitter and pungency sensory attributes perceived when tasting olive oil. They have been also associated to color and to the stability of the oil due to their antioxidant properties (Nergiz & Ünal, 1991; Maga, 1978). Phenolic compounds such as gallic, vanillic, caffeic syringic, ferulic and cinnamic acids, inhibit the oxidation of triglycerides during storage (Kachouri & Hamdi, 2004). Thus, polyphenols influence long shelf-life compared to other vegetable oils. These compounds were also recognized as potential markers for geographical origin or olive fruit variety (Segura-Carretero et al., 2010).

1.4.4. Chlorophylls

In general, chlorophylls are responsible for the green color of olive fruits and their byproducts. The chlorophyll fraction present in the olive fruit decreases during the ripening of the fruit, as the color progresses from green, light green, red-spotted, purple to black, and during oil extraction process where 80 % of total chlorophylls are lost. These chemical and physical changes are the major influencers on the quantity/quality of the chlorophyll pigment profile present in olive oil. In both cases, most of the chlorophyll pigments (chlorophylls a and b) are lost either by converting the latter into pheophytins a and b due to prolonged ripening

throughout fruit milling or due to retention of the chlorophyll pigments in the pomace during extraction (Giuliani et al., 2011).

Chlorophyll pigments retained in olive oil can have either a negative or a positive effect on the stability of packaged olive oil. In presence of light and high temperatures, chlorophylls can act as prooxidant enhancing autooxidation by forming a singlet state oxygen. However, in suitable conditions, i.e. storage of olive oil in the dark and at low temperature, chlorophylls act as antioxidants preserving olive oil quality (Anniva et al., 2006).

1.4.5 Volatile compounds

Odor plays a significant role in virgin olive oil sensory quality and consumer acceptance. Volatile compounds are responsible for the odor/ fragrant and delicate flavor that virgin olive oil possess. The main precursors of the volatile formation are lipids, particularly linoleic and linolenic acids. Volatile compounds reach their maximum and mostly form during malaxation process through the so-called lipoxygenase pathway. The major volatile compounds formed by the oxidation of polyunsaturated fatty acids are aldehydes, alcohols and their corresponding esters. For instance, hexan-1-ol (Green), hexanal (Green – sweet), E-2-hexenal (Green, apple-like), and 3-methylbutan-1-ol (woody, sweet), are mostly present in extra virgin olive oils (EVOO) originating from the Mediterranean region (Salas et al., 1999; Morales et al., 1999; Aparicio et al., 1996).

A poorer quality of olive oil has a more complex volatile profile with higher number of volatiles particularly branched aldehydes and alcohols. These compounds which is a result of chemical oxidation (Morales et al., 1999) contribute to VOO organoleptic defects such as rancid, winey, vinegary, fusty and musty (Morales et al., 2005). Olive fruit maturity (Salas et al., 1999) and inadequate conditions in olive oil extraction especially during the malaxation step (Ranalli et al., 2001), are considered the most decisive parameters in the production of volatiles responsible for aromas appreciated by consumers.

Table 2. Standard quality parameters of olive oil based on IOC, 2011

Quality Parameters	**EVOO**	**VOO**	**OVOO**	**LVOO**
Acidity	≤0.8	≤2	≤3.3	>3.3
PV	≤20	≤20	≤20	No limit
K232	≤2.5	≤2.6		
Delta K	≤0.01	≤0.01	≤0.01	

Table 3. Micro and Macro-components of olive oil (Source: International Olive Council, 2011)

Micro-components	Level (mg/kg)
Aliphatic alcohols	60-200
β-carotene	0.33-3.69
Chlorophylls	0 - 10
α- tocopherol	150-300
Hydrophilic phenolics	40-1000
Lignans	41.53-100
Sterols	**Limits (%)**
Cholesterols	≤0.5
Brassicasterol	≤ 0.1
Campesterol	≤4
Stigmasterol	<campesterol
Δ7- stigmasterol	≤0.5
[b]Apparent β-sitosterol	≥93.0
Macro-components (Fatty acids)	**Limits (%)**
Palmitic acid	7.5 - 20
Palmitoleic acid	0.3-3.5
Stearic acid	0.5-5
Oleic acid	55-83
Linoleic acid	3.5-21
Linolenic acid	≤1
Arachidic acid	≤0.6

[b]Apparent β-sitosterol comprises β-sitosterol, Δ^5-avenasterol, $\Delta^{5,23}$-stigmastadienol, clerosterol, sitostanol, $\Delta^{5\text{-}24}$-stigmastadienol

2. Lebanese olive oil sector overview

2.1. Main Geographical features

Lebanon is a home to the oldest olive trees dating back centuries (at least 1,500 years). It has been renowned for its trade in crop products including olive and olive oil along the Mediterranean Basin (Mahfoud, 2007; Beayno et al., 2002; Thalman, 2000). The country's topography consists of a coast and two mountains running in parallel and separated by an agricultural plateau called Bekaa. Although landscape variations may give a rise to differentiated climatic conditions, Lebanon is characterized by a moderate Mediterranean climate where the average rainfall of 2.2 billion cubic meter per year allows the growth of olive trees.

Olive trees, mainly rain fed, cover approximately 5.4 % of the Lebanese territory or 8 % of total agricultural lands in Lebanon. Olive groves are dispersed over the main five territories of Lebanon: North, Nabatiyeh, South, Bekaa and Mount Lebanon covering a range of altitude from 45 to 900 m above sea level (Figure 3) (IDAL, 2017b). The Lebanese olive germplasm is characterized by a high level of diversity. Five local varieties, namely, Aayrouni, Abou-Chawki, Baladi, Del and Soury, are the most cultivated in commercial plantations used for both oil and table olives (Table 4). The Soury variety is the most cultivated variety where it occupies 85% of the cultivation areas. It originated from Tyr, and it is used for both table olive and oil production (Chehade et al., 2016).

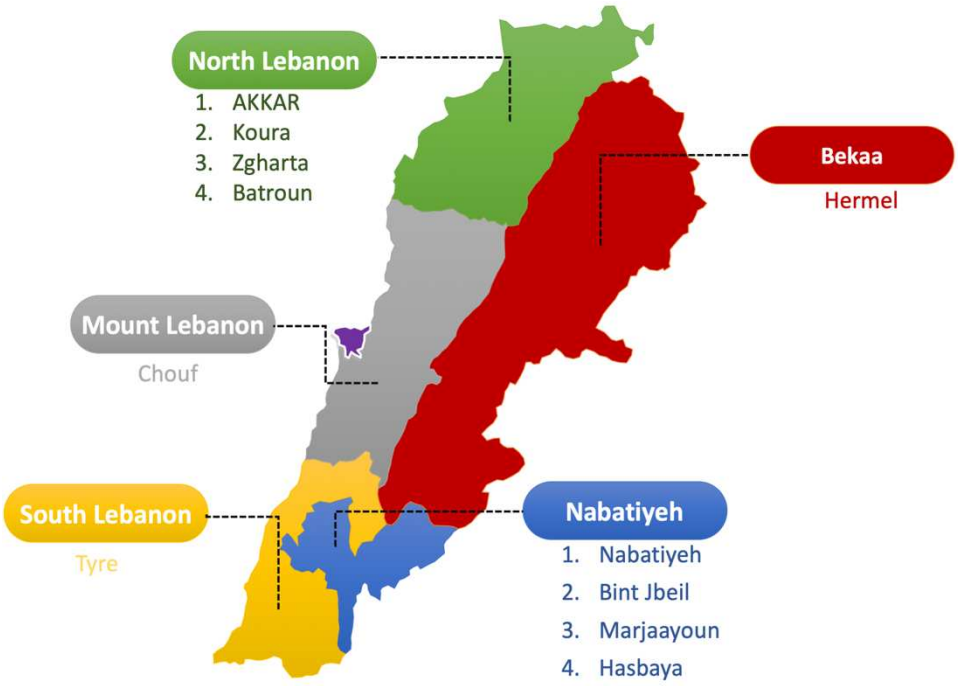

Figure 3. Main Olive groves distribution along the map of Lebanon

Table 4. Lebanese olive oil varieties and their distribution across Lebanon

Olive trees varieties	Main areas of cultivation	Purpose	Productivity	Tolerance to abiotic stress
Aayrouni	North and Mount Lebanon	Oil	Medium – High	High to drought
Abou chawkeh	North of bekaa	Dual Purpose	Medium – High	High to drought
Baladi	All Lebanon	Oil	High	High to drought
Smoukmoki	Mount Lebanon	Oil	High	High to drought
Del	West bekaa	Dual Purpose	High	High to drought
Soury	North and South	Dual Purpose	Medium to High	High to drought
Teliani	All Bekaa	Dual Purpose	High	High to drought

2.2. Olive oil production

41 % of Lebanese olive oil production takes place in North followed by Nabatiyeh (21%), 15 % in the South, 13% in Bekaa, and 10 % in Mount Lebanon. According to IOC latest data (2018/2019), Lebanon produces around 24,000 tons of olive oil. For the last six years, the production ranged between 16,500 t and 25,000t (Figure 4) (IOC, 2018).

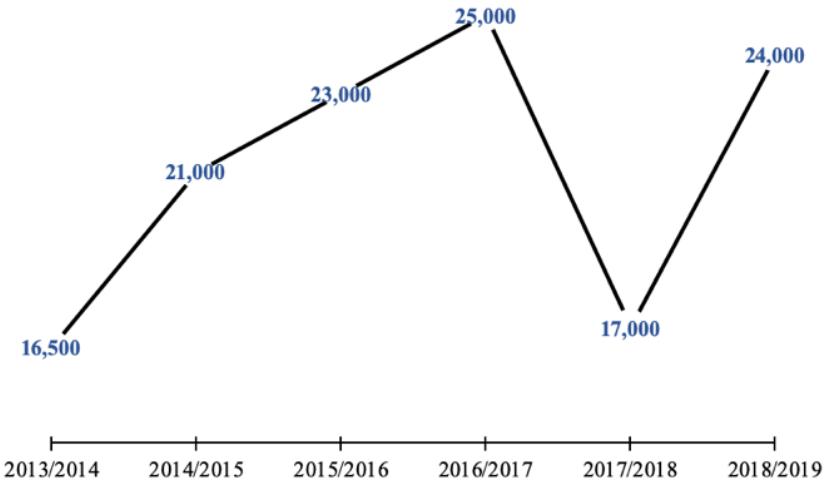

Figure 4. Yearly olive oil production in Lebanon (in tons) (Source: International Olive Council, 2018)

This fluctuation in production is due to several factors, among them are the following:

- The fact that olive trees are mostly rain fed. This would subject oil productivity to global warming affects and amount of rain fall per year.
- Farmers practices, especially those related to harvest time and to type of olive oil extraction method
- Most of the small growers (6,000 – 10,000 m^2), representing around 77% of olive producers in Lebanon, adopt traditional practices thus leading to a lower efficiency and productivity.
- Heavy alternate bearing phenomena, the tendency of olive trees to bear fruit in two-year cycles consisting of a large crop followed by a small crop, has a massive effect on Lebanese olive oil production.
- High cost of production
- Inadequate implementation of GMP and storage practices (ACTED, 2018).

This production is being managed through 485 registered mills scattered in Lebanon. About 85% of the mills still rely on traditional-extraction systems, particularly pressing method. The other 15 % rely on modern systems including 2-phase and 3-phase extractors (MOA, 2012).

As for olive oil consumption in Lebanon, it is about 4.3 L per capita. it is considered relatively low when compared to other countries in the region like Greece (10 L /capita) and Syria (20 L / capita). Lebanese consumers tend to buy olive oil in bulk from trusted family or neighbors, i.e. directly from the producers. Olive oil is also available in retail outlets; however, their demand is somehow low (ACTED, 2018).

2.3 Imports and exports

Lebanese olive oil holds the highest commercial prospects amongst all the other crops covering about 21% of the total cultivated area and 69% of the land cultivated with fruits. Although Lebanon is considered a small-scale producer of olive oil as compared to other Mediterranean countries, it has the reputation of producing high-quality artisanal oils. Exports of Lebanese olive has been increasing since 2006 at a rate of 15 % in value each year (Figure 5) especially to the gulf region and United States. For instance, Lebanon exported about 904 t of olive oil in 2005 to GCC market, whereas in 2016, 13,587 t was exported to GCC. Although a substantial reduction was noted in exports in 2017, a clear tendency of increase in Lebanese olive oil shows the ability of producers to infiltrate the export market (IDAL, 2017b).

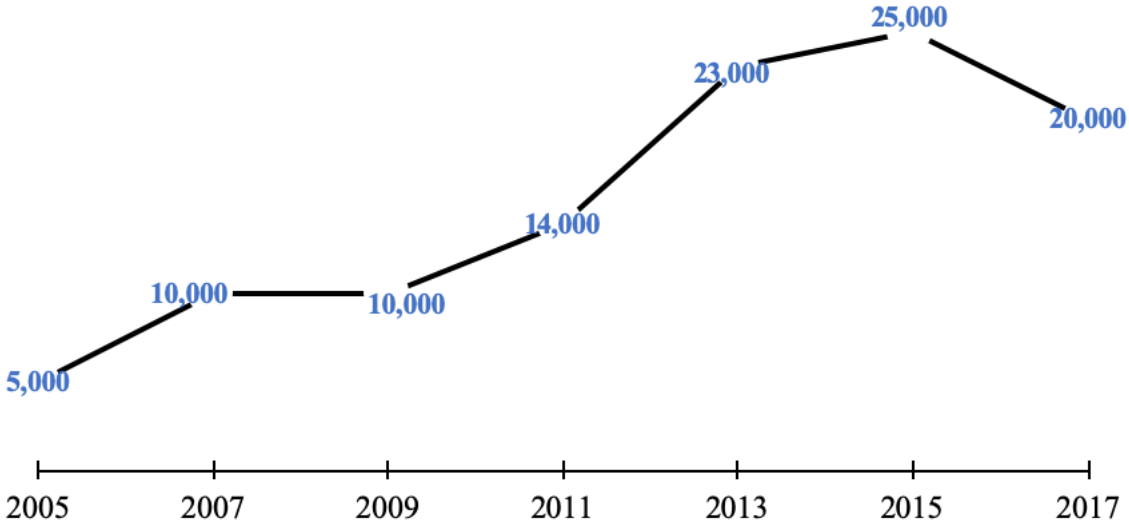

Figure 5. Export value of Lebanese olive oil (USD) (Source: Investment Development Authority of Lebanon, 2017)

Despite the export growth in Lebanese olive oil, Lebanon is still considered as an import dependent country. The average imports of olive oil are about 5,000 t per year and they are mostly imported from Syria and Tunisia (ACTED, 2018).

3. Fluctuation of EVOO/VOO components due to factors of different origins

A complex interaction exists between genetics, technological and agronomical factors (climate, soil, year, irrigation, growing area, fruit maturity) and the chemical composition of olive oil. Each of one of these factors and sub-factors affect oils' characteristics in its own manner.

3.1. Agronomical variables

3.1.1. Cultivar

The choice of which cultivar to grow is one of the most important decisions and the following factors should be taken into consideration before selection:

- Tree vigor, alternate bearing, maturity index, oil content and extractability.
- Orchard profitability (olive oil yield)
- Environmental factors (resistance to drought, cold hardiness…)
- Market demand
- Production costs
- Chemical composition

Each cultivar has its own chemical fingerprint. The triglyceride structure of olive oil is unique for each cultivar. For instance, 73 of the world's most common cultivars were compared and differences in the fatty acid content were found. For example, palmitic fatty acid percent ranged from 8.49 to 16.46, palmitoleic from 0.41 to 2.26, stearic from 1.46 to 3.79, oleic from 56.12 to 78.34, linoleic from 4.44 to 13.34 (Lombardo et al., 2008; Mannina et al., 2003; Zarrouk et al., 2009)

Minor components such as sterols and polyphenols are also highly influenced by the cultivar. The polyphenol content of olive oil varies from 182 to 1240 ppm depending on the cultivar as long as the olives and the extraction process are in excellent conditions and following good manufacturing processes guidelines (García-González et al., 2010; Tura et al., 2007). As for sterols, it constitutes the majority of the unsaponifiable fraction where an increased interest in the latter has been noticed due to its importance in the quality regulation of olive oil. Each cultivar has its own fingerprint represented by the sterol profile. A study by Kyçyk et al. (2016) showed that each of the 43 olive cultivars has their own distinct sterol profile. Because of this variability, sterols can be used as a discrimination tool among monovarietal VOOs. It can also be used in olive breeding projects to obtain new olive cultivars with improved sterol fraction.

3.1.2. Climate and elevation

Olive oil is mainly composed of fatty acids, especially monounsaturated fatty acids (oleic acids) in addition to polyunsaturated fatty acids like linoleic and linolenic (Salvador et al., 2003). The fatty acids profile of oils produced from the same cultivar is the same in oils produced in similar climates and growing conditions. However, a difference in the climate or altitude between regions has led to cases where large differences have been observed in the fatty acids composition of oil (Aparicio et al., 1994). Çetinkaya et al. (2017) have found that a difference in altitude has an effect on oleic acid, palmitic acid, linoleic acid and stearic acid. Other authors like Nergiz and Ergönül (2009) also found out that olives planted in high-altitude locations are rich in monounsaturated fatty acids (MUFA), while olives planted in low-altitude locations are rich in saturated fatty acids (SFA).

Furthermore, the change in altitude correspond to a decrease or increase in the environment temperature, relative humidity and thus the modification of the underlying physiological metabolic processes at the level of olive fruit, in particular, the process of fatty acid synthesis. Oleate desaturase, an enzyme responsible for desaturation of Oleoyl-ACP (precursor of longer-chain unsaturated fatty acids) into linoleate-ACP might be the reason behind the notable difference in the MUFA and PUFA concentrations between these two groups. This enzyme has been extensively discussed in seed oils. In sunflower seed, the low temperature lead to the

activation of the desaturase enzymes and the level of desaturated fatty acids such as linoleic acid increase as a mechanism to tolerate low temperatures during pod filling. However, at high temperatures (30 ^{o}C), desaturases enzymes are partially and reversibly inhibited reducing the linoleic acid content in the oil. This mechanism, known in sunflower, and the information regarding the desaturase enzymes responses to change in climatic conditions in olive is limited (Hernandez et al., 2011) Several studies reported that the gradual decrease in temperature from October to January, as olive ripening advances, may increase the linoleic acid content in the olive fruit (Inglese et al., 2011; Gutiérrez et al., 1999). In addition, García-Inza et al. (2014) studied the effect of high temperature especially at the beginning of oil accumulation, by placing fruiting branches in transparent plastic chambers with individualized temperature control. The results showed that high temperatures could decrease the oleic acid content and increase palmitic, palmitoleic, linoleic, and linoleic acid.

Micro-components also are affected by the climatic conditions. Several studies have shown that oil produced in warm coastal areas has fewer total polyphenols than those grown at higher altitudes and lower temperature regions (Osman, 1994). Sterols on the other hand are mostly related to fruit maturity and cultivar.

3.1.3 Fruit maturity

The effect of fruit maturity on olive oil chemical composition is significant. As olive fruit matures, the color of the fruit shifts from green at the beginning of the harvest period to small reddish-green spots to purple and lastly to black at the end of the harvest period (Motilva & Romero, 2010). Each stage imparts chemical variations on the level of metabolic processes of several compounds such as triglycerides, fatty acids, polyphenols, sterols and chlorophylls, which in turn have an impact on olive oil quality (Gargouri et al., 2016). These variations not only influence the quality but also the nutritional and organoleptic characteristics and the oxidative stability of olive oil (Maaitah et al., 2009). For instance, polyphenols and chlorophyll content decrease as fruit maturity increase especially when the olive fruit turns completely into black (last stage of fruit maturation).

As for fatty acids, Issaoui et al. (2010) related the increase in polyunsaturated fatty acids with fruit maturation. This is mainly due to continuing biosynthesis of triglycerides and to the actively desaturation of oleic acid into linoleic by oleate desaturase as the fruit ripens.

However, the variation based on the fruit maturity somehow depends on the cultivar. In some cases, the level of monosaturated fatty acids increases, whereas the level of saturated and polyunsaturated fatty acids increased as in the case of Barnea variety. Conversely, the delay of harvest has a detrimental effect on the quality of olive oil especially on the most widely spread

cultivar in Lebanon, the Souri variety. Lodolini et al. (2017) have reported that the latter variety should be harvested early. As it ripens, oleic content declines and linoleic increases, quality indices especially free fatty acids are negatively affected, while polyphenol levels and oxidative stability drop sharply (Noorali et al., 2014). Also, it has been reported that the sterol content decreases sharply from 2850 g/kg to 1644 g/kg. Moreover, fruit maturation has also been shown to affect olive oil quality parameters such as free fatty acids, peroxide value, specific UV absorbances and sensory attributes (Dag et al., 2011; Famiani et al., 2002b; Gomez et al., 2011; Lazzez et al., 2008; Mailer et al., 2010; Varzakas et al., 2010).

Several methods have been recommended to determine the exact or ideal harvest time with respect to the quantity and quality of olive oil. These methods have been developed using organic acids such as malic and citric ratio (Donaire et al., 1975), degree brix (Migliorini et al., 2011), sugars such as mannitol (Marsilio et al., 2001), fruit respiration (Ranalli et al., 1998) or dry matter (Mickelbart & James, 2003). Other methods have related a connection between fruit maturity and specific fatty acids and sterols present in the pulp. However, the most common used method to determine fruit maturity, recommended by IOC, is the measurement of the fruit external and internal colors allowing the calculation of maturity index.

3.1.4 Other agronomical factors

Other agronomical factors have an effect on olive oil's quality and quantity, and these include:

- Pest damages: oil obtained from olives damaged by olive fruit fly (*Bacrocera oleae*) show an increase in carbonyl and alcohol compounds. In addition, polyphenols contents decrease which leads to the lowering of the oxidative stability and in turn leads to the increase in free fatty acids and peroxide values. OLF attacks the fruit by consumption of the pulp which increases fruit drop and leads to the loss of the oil quality due to oxidation phenomena.
- Freeze injury prior to harvest can also reduce oil quality by decreasing the concentration of phenolic compounds and thus lower oxidative stability. Water within the fruit can crystallize and leads to the rupture of skin the olive fruit thus exposing it to oxidation phenomena (Trentacoste et al., 2020).
- Irrigation and rainfall: Both a deficit and a surplus of irrigation regimes have their own effect on the quality of olive oil. A deficit in water leads to early maturation and lower the accumulation of oil in the fruit. Whereas a surplus of water can lead to an oil with lower polyphenol content, bitterness and lower fruity, floral and herbaceous flavors. The latter flavors are enhanced in drought-stressed or deficit – irrigation trees (Gomez-Rico et al. 2007). In some cases, the fatty acid profile could be affected as a result of irrigation,

where the concentration of monounsaturated fatty acid witnessed an increase and those of polyunsaturated fatty acids and saturated fatty acids endorsed a decrease in concentration (Mailer et al., 2010; Mailer, 2005; Ben-Gal et al., 2011; Berenguer et al., 2006; Ceci & Carelli, 2007; Faci et al., 2002; Grattan et al., 2006; Pérez-López et al., 2007; Rapoport et al., 2004; Ripa et al., 2008; Servili et al., 2007; Stefanoudaki et al., 2009)

- Crop load and pruning mostly affect the quantity of olive oil obtained at harvest. The ratio of leaves to fruit and amount of fruits per tree (either heavy load or light load) can dramatically affect the fruit oil content. On the level of the olive oil components, heavy crop load can decrease the levels of palmitic and linoleic acids, and has no effect on free fatty acids, peroxide value or sensory characteristics.

3.2. Technological variables

3.2.1. Olive transport and storage

The period between harvesting and milling are critical for oil quality. Improper conditions during handling and storage of olives may trigger a chain of degrading reactions such as lipolysis, lipid oxidation and the secretion of lipolytic and oxidative enzymes. For instance, the transport of olive fruits in plastic sacks that stay for several days in the mill before being pressed favor the enzymatic lipolysis of the fruit triacylglycerol and majorly affect the quality indicators of olive oil (Kiritsakis et al., 1998). Instead rigid plastic containers are used (15–20 kg crates or 200–300 kg bins) with olives in layers not thicker than 30cm. The crate should also have holes to ensure air circulation.

Therefore, two critical conditions should be taken into consideration during storage and transport of olive fruits:

- Avoid mechanical damage at any cost
- Control time and temperature

Olive fruits should be processed as soon as they arrive to the mill especially if they are ripened. In case of processing delay, limitation of the duration of the storage is a necessity taking into consideration the best practices during storage to conserve product quality (Famiani et al., 2002a).

3.2.2. Processing methods

The aim of olive processing is to acquire VOO as stated by the IOC. Until now, three mechanical systems are involved in olive oil extraction:

- Pressing method

- Percolation method
- Centrifugation method

All methods are supposedly to give olive oil with good quality (Figure 6). However, the distinctive features of each method have an effect on the micro components of olive oil unless contamination is a problem if necessary, precautions weren't taken into consideration, like in the pressing method. Pressing method uses granite millstones and mats (filtering diaphragms of nylon fiber) for olive fruit crushing and olive oil extraction. Olive paste is stacked in layers on pressing mats which are difficult to be kept clean and exempt from oxidized oil and fermentation defects possibly transferable to the oils. However, this method has an advantage over other methods by yielding the highest amount of extracted olive oil (Di Giovacchino et al., 1994; Di Giovacchino et al., 1996).

Percolation method is dated back to 1911, and it is based on the surface tension difference between oil and vegetation water. This method relies on a semi-cylindrical grating with small blades moving slowly through the slits, plunging into the olive paste, and oil drips off the blades when they are withdrawn. The percolation method doesn't give high oil yields, but it is considered as the most natural process as it takes place at ambient temperatures and without using water or employing mats (Mascolo, 1980).

Centrifugation method is the newest and most applied method. It is a continuous process using centrifugal force to separate VOO from vegetation water and pomace. Two types of this decanters are used in this method, the three-phase and the two-phase centrifugal systems. The latter reduce the amount of water added to the olive paste and improve the quality of olive oil.

Table 5 shows the values of some parameters obtained by oil produced the several extraction systems present above. As mentioned earlier, all systems give EVOOs when good quality olives are processed, and the mill is clean. However, the centrifugation system produces oil with lower content of phenols. In addition, the processing system can affect the content of some volatile compounds as present in Table 6 (Di Giovacchino et al., 1996).

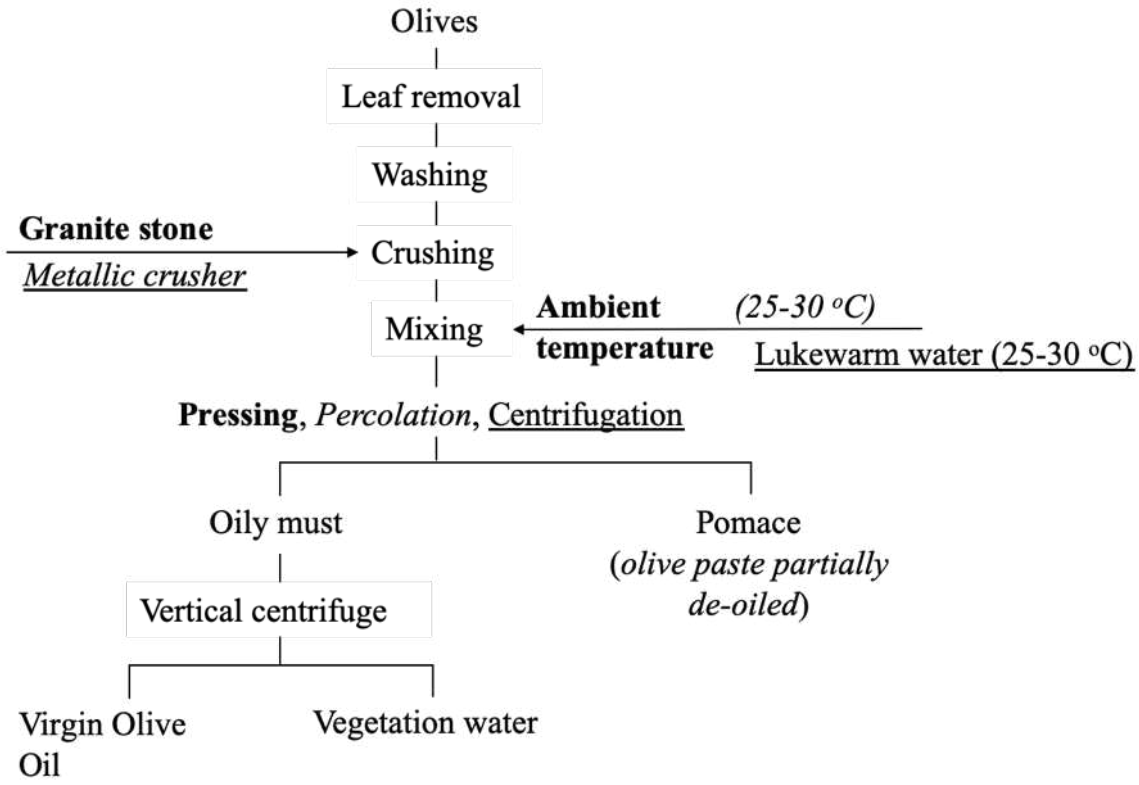

Figure 6. Diagram of olive oil extraction by different mechanical techniques (Giovacchino, 2000)

Table 5. Average value of key indicators on olive oil quality obtained by different types of mechanical systems (Di Giovacchino et al., 1996).

	Olive processing system		
	Pressing	Percolation	3-phase centrifugation
Free fatty acids (%)	0.23	0.23	0.22
Peroxide value (meq/kg)	4	4.6	4.9
K232	1.93	2.03	2.01
K270	0.12	0.12	0.13
Organoleptic assessment (score)	6.9	7	7
Total phenols (mg/L)	158	157	121
Chlorophyll pigments (mg/kg)	5	8.9	9.1

Table 6. Average values (%) of some the volatile contents of oils obtained by pressing and 3-phase centrifugation system (Di Giovacchino et al., 1996).

	Olive processing system	
	Pressing	3-phase centrifugation
n-Octane	24.3	7.1
Ethyl acetate	13.5	6.8
Hexanal	41.3	36.1
Isobutyl alcohol	20.1	4
Isoamyl alcohol	78.6	10.9
(E)-2-Hexenal	425.5	435.4
Hexanol	107.3	35.6
(E)-2-Hexenol	97.5	39.9
Acetic acid	6	1.7

Although IOC has made some restrictions on adding certain chemical compounds to enhance olive oil extraction, some researchers have been working on improving the VOO and EVOO production, for instance, by the utilization of solid carbon dioxide. Angela et al. (2015) introduced CO_2 to the olive fruits during the pre-milling phase, which induced intracellular water freezing and, consequently, the fruit cellular membrane's rapture. This process increased the extraction yield from 1 to 21 % without changing the olive oil's chemical composition.

3.2.3. Storage conditions

Olive oil storage is also one of the critical steps that have a big impact on the quality if not preserved well. The key points to consider during oil storage are:

- VOO should be stored as soon as it is extracted
- VOO should be stored in a container made of stainless steel or glass bottles
- VOO should be stored in a room at controlled temperatures (13-18 ºC) and away from light
- Storage containers should be filled to the tip and in case it is not filled with oil, air should be replaced with an inert gas like nitrogen.

In general, VOO contains a high level of oleic acid, a medium content of saturated fatty acids and a low concentration of polyunsaturated fatty acids. This explains why VOO has higher stability when compared to other vegetable oils, such as seed oils (higher percentage of polyunsaturated fatty acids). Basically, polyunsaturated fatty acids oxidize faster than monounsaturated ones. However, the above precautions need to be taken to avoid the chemical and biological reactions that leads to the deterioration of olive oil quality. Two major reactions are involved in the spoilage of olive oil:

1. Hydrolysis of triglycerides caused by enzymes and is caused by the presence of water during storage of olive oil
2. Chemical oxidations of fatty acids promoted by the presence of oxygen from either improper storage (presence of air) or due to light (Giovacchino, 2000).

The temperature at which the olive oil is stored has a direct impact on its chemical composition. Olive quality parameters, in general, remain intact or remain within the EVOO category when storing at freezing temperatures or temperatures above 4 ºC (not exceeding room temperature18 ºC). As for volatile compounds only, aldehydes, particularly hexanal (an indicator of oxidation), have increased when subjecting olive oil to -20 ºC temperatures. Others like esters and ketones show more stability, and the fresh oil volatile profile is preserved well at low temperatures (Brkić Bubola et al., 2014).

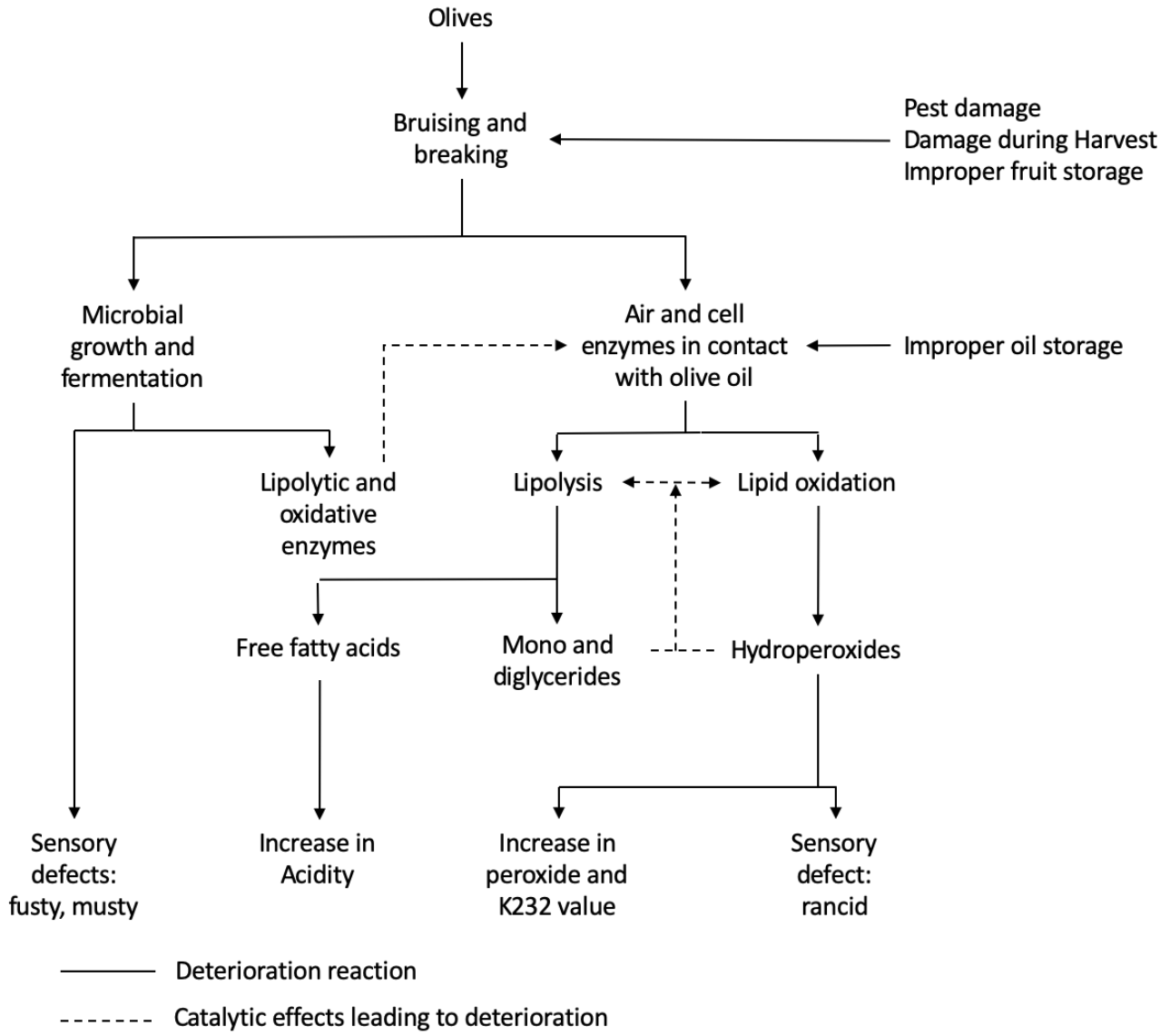

Figure 7. Mechanism of oil deterioration and oil's quality degradation

4. Rapid & non-destructive analysis techniques

4.1. Spectroscopy

Spectroscopy is the study of spectra originating from the interaction between electromagnetic radiation and matter. Several spectroscopic methods are available, each differ based on three important aspects:

1. Type of interaction between radiation and matter, i.e. diffraction, emission or absorption.
2. Type of species to be analyzed such as atomic or molecular spectroscopy.
3. Region of electromagnetic spectrum, which could be UV, Vis, IR or belonging to high radio frequency domain (NMR).

Spectroscopic methods are used by scientists from many disciplines, it is an informative technique and could be used for both qualitative and quantitative analyses. One of the spectroscopic techniques that is witnessing a flourishing, or a dramatic growth in the field of olive oil, is fluorescence spectroscopy (Penner, 2017).

Fluorescence is one of the categories of Luminescence. The phenomenon of luminescence has been known since ancient times. It is defined as the emission of light of any substance and it happens from electronically excited states. In case of fluorescence, it occurs as a result of a singlet excited states (Lakowicz, 2006).

4.1.1 Fluorescence spectroscopy

Fluorescence spectroscopy has been successfully used as a rapid, non-invasive and highly sensitive technique for analysis of olive oil quality and showed to be more cost-efficient compared to other analytical procedures (Lleo et al., 2016). This type of spectroscopy can be particularly useful as routine quality control since the analysis is carried out directly on the intact samples without any pre-treatment or usage of chemical reagents (Sikorska et al., 2004). These advantages have rendered front face fluorescence as an important tool in the evaluation of olive oils properties.

So, what is fluorescence? In simple words, it is a phenomenon resulting from the emission of light by the matter after absorption of light. Upon absorption of light in the UV or Vis range, the analyte absorbs a photon or is activated and reaches a higher energy level called the excited state. Jablonski's diagram (Figure 8), is often used to illustrate the processes occurring between absorption and emission of light (Szudy & Toruniu, 1998). Based on this diagram, a molecule resting at the ground state absorbs energy at an excitation wavelength (that is specific property to the molecule itself), and it gets excited. This will gradually transfer the molecule to a one or higher energy levels (S1). The next stage will be vibrational relaxation which permits the electron to return to its lowest energy level, from S1 to S0 (ground state).

The return to the ground state emits a photon, at a wavelength higher than that of the excitation state, thus producing the fluorescence phenomenon. This phenomenon which is called fluorescence takes about a few picoseconds or even a few nanoseconds. The only limitation to this phenomenon, is that it only works with fluorophore compounds mainly those consisting of aromatic molecules or into those having enough electronic density (high conjugated chemical structure).

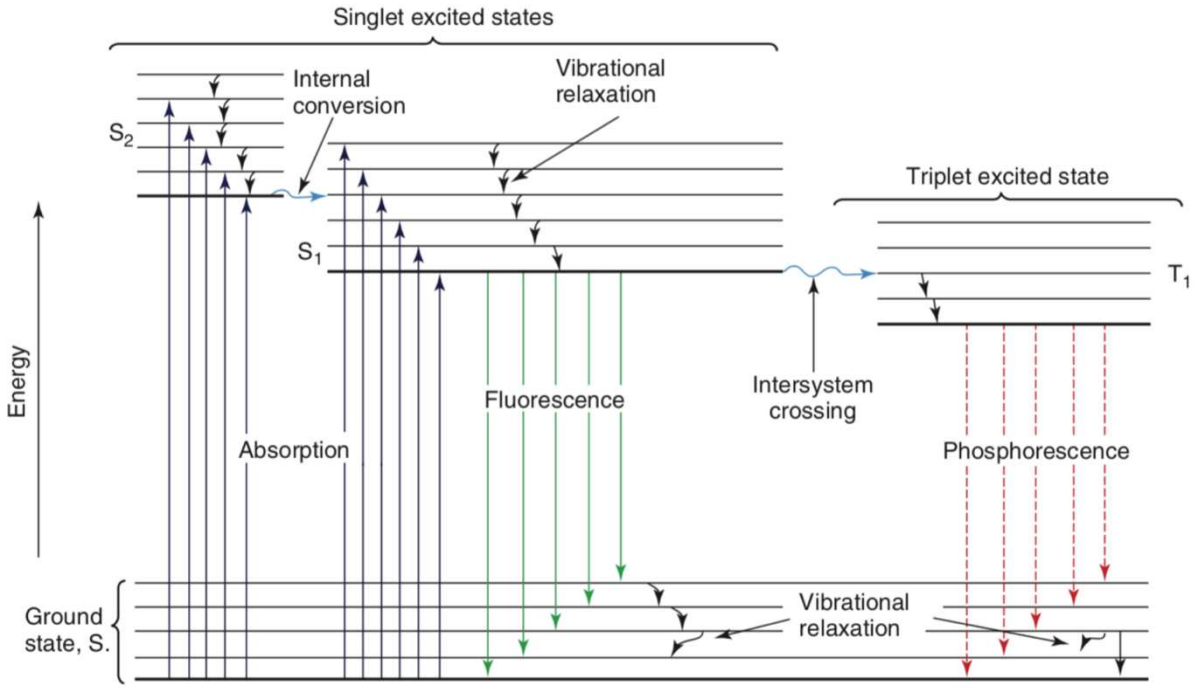

Figure 8. Jablonski diagram (Frackowiak, 1988)

Fluorescence spectroscopy instrument consists of:

- a light source, generally xenon or mercury lamp;
- two monochromators, used for selection of narrow band of excitation and emission wavelength
- a sample holder;
- a detector or a photodetector, used for converting the emitted light to an electric signal;
- a data acquisition and analysis unit (Figure 9).

To obtain a fluorescence spectrum, light is emitted from an excitation source and passes through a monochromator. Then the narrow band of excitation wavelength selected by the latter strikes the sample. The incident light is absorbed by the sample, where some of the molecules in the sample fluoresce. It should be noted that light only penetrates some um inside the matter as it can do in an ATY mode in infrared spectroscopy. It is more like a surface analysis than a comprehensive and deep analysis. The emitted fluorescent light passes through the second monochromator, reaches the detector and gets recorded by the data acquisition unit (Locquet et al., 2019).

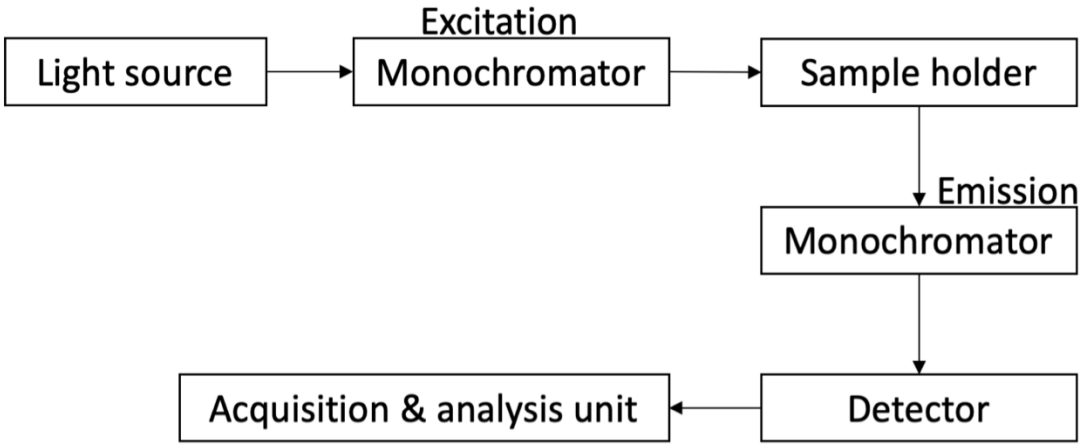

Figure 9: Basic elements required for obtaining a fluorescence spectrum

4.1.2. Fluorescence spectra

Different types of fluorescence spectra exist, and these include:

- Absorption spectrum is the result from the partial absorption of excitation beam. It reflects the absorption capacities of a molecule and its behaviour with respect to exciting light.
- Excitation spectrum is obtained by fixing the emission wavelength while advancing the excitation wavelength over a smaller or longer wavelength range. For a pure product the absorption and the excitation spectrum should be the same. In case of difference, this means that fluorescence is occurring from one component.
- Emission spectrum represents the characteristics of a given analyte. Its obtained by fixing the excitation wavelength as opposed to the light emission intensity as it moves from the excited state towards the ground state.
- Synchronous fluorescence spectrum involves the simultaneous scanning of excitation and emission wavelength at a fixed wavelength difference step (delta λ).
- EEM or a 3D fluorescence spectrum is one of the leading approaches since it provides massive amount of data and multidimensional information. The EEM spectrum is composed of the excitation wavelength and the emission wavelength (Figure 10). It is obtained by measuring emission spectra for different excitation wavelengths with a constant step. This is possible by using the excitation and emission monochromators successively (Albani, 2001; Valeur, 2004).

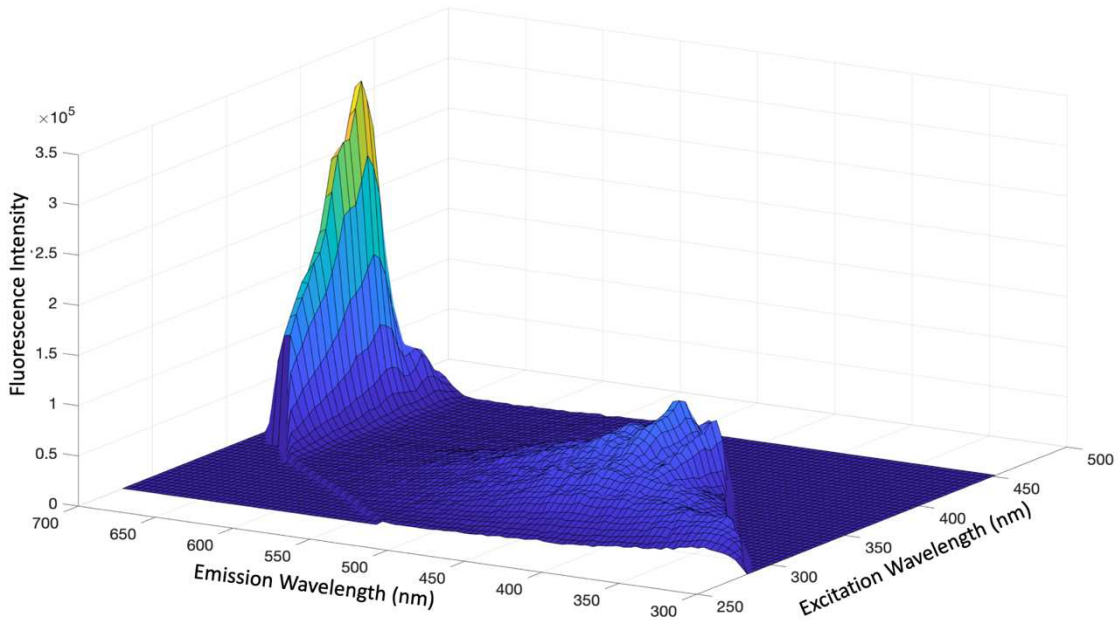

Figure 10. Three-dimensional fluorescence (3D-EEM)

4.1.3. Application of fluorescence in olive oil analysis

Frehse was the first to use fluorescence to analyse olive oil in 1925 (ref). He studied the possibility of finding reactive oxygen species (ROOs) in virgin olive oil (VOO) by placing olive oil under a quartz lamp equipped with a wood filter (Täufel, 1964). This was followed up by Englesen (Engelsen, 1997) whose study showed that fluorescence coupled with multivariate analysis could be considered as a powerful tool for monitoring of deteriorating frying oil. Moreover, Kyriakidis and Skarkalis (Kyriakidis & Skarkalis, 2000) showed that fluorescence spectra can be directly obtained on VOO and other vegetable oils without the need of dilution. Olive oil contain several natural fluorescent components including tocopherols, phenolic compounds, pheophytins and their oxidation products. The typical fluorescence spectrum of olive oils measured with the use of front face geometry exhibits three intense bands:

- One with excitation at about 270–330 nm and emission at about 295–360 nm
- The second with excitation at about 330–440 nm and emission at about 660–700 nm
- The third band appears in spectra of deteriorated olive oil, located in the intermediate range, with excitation at 280-330 nm and emission at 372-480 nm products (Sikorska et al., 2011).

There are several published studies showing the diversity of fluorescence applications especially in the field of olive oil analysis (Locquet et al., 2019). These include studies on detection of olive oil adulteration with vegetable oils or pomace oil (Dankowska et al., 2009; Sayago et al., 2007), on monitoring thermal oxidation (Ammari et al., 2012; Cheikhousman et

al., 2005a; Cheikhousman et al., 2005b; Poulli et al., 2009; Sikorska et al., 2008; Tena et al., 2012; Tena et al., 2009; Valderrama et al., 2011), and on quality parameters evaluation (Guimet et al., 2004a; Guzman et al., 2015).

4.1.3.1 Quality parameters evaluation

Olive oil is considered as an economically important product and its quality control is of great interest. The potential of fluorescence as being a rapid, non-invasive and highly sensitive technique and the data obtained from the analysis of its excitation-emission spectra, reveals notable information on olive oil's quality parameters such as acidity, peroxide value, UV absorbance at 232 nm (K232) and 270 nm (K270).

Acidity, based on IOC recommendations, is one of the crucial quality parameters for classification of olive oil into different categories. The increase in acidity is due to free fatty acids (palmitic, oleic and linoleic acids) resulting from triglyceride decomposition. Studies on this parameter have focused on the fluorescence band at 429-545 nm which is due to oleic acid (Poulli et al., 2005). Kyriakidis and Skarkalis (Kyriakidis et al., 2000) also suggested that the low fluorescence intensities at 445 and 475 nm are attributable to the high content of monounsaturated fatty acids (oleic acid). The study by Poulli, Mousdis, and Georgiou (Poulli et al., 2005) also showed the link between the fluorescent bands at 273 and 325 nm and palmitic and linoleic acids respectively. Other important quality parameters are peroxide value and K270. These two latter which are the precursors of primary and secondary oxidation products and have also been linked to high fluorescent intensities at 450-460 nm and 470 nm respectively (Guimet et al., 2005; Guzman et al., 2015). The increase in the fluorescence intensity in these regions is due to the oxidation of fatty acids into conjugated hydrogen peroxides and conjugated triene and diene systems. The fluorescence emission spectra also contain information on fluorescent molecules such as chlorophyll (600-700 nm), Vit E (525 nm), polyphenols and α-tocopherol (300–390 nm) (Kyriakidis et al., 2000).

4.2. Flash GC

Flash gas chromatography (FGC), or Ultra Fast-GC, is a rapid technique used for analyzing volatiles present in olive oil and other materials. In general, FGC process depends on the volatilization of chemical compounds and then their separation through chromatographic analysis.

4.2.1. Sampling

Samples introduced into FGC can be either diluted which require larger volumes samples, or concentrated, like in the case of olive oil, where smaller sample size is required and

consequently shorter sample times. Then the samples are injected, and then concentrated in a colder trap zone.

Before entering into the trap and at the end of the inject time, a carrier gas flows through the trap and out to vent to remove excess air, moisture, or solvent.

Samples are then injected and concentrated in the trap, where the latter starts heating to the appropriate desorption temperature under no-flow conditions. The advantages of the trap zone are:

- Avoid the mismatch between the internal gas flow with the flows through the capillary columns
- Provide flexibility in the type of the samples accepted for analysis as well as sample concentration.

When pre-heating time elapses, the sample is injected into the capillary column for chromatographic analysis.

4.2.2. Chromatographic separation

After the trap reaches its desorption temperature, the desorbed sample passes back out of the trap into two separation columns (one is polar DB5 and the second is semi-polar DB1701) and the carrier gas is established again.

The amount of sample that enters the columns is determined by the split flow rate. High split flow results in narrower injection and sharper peaks whereas a lower split flow results in wider injection plugs, which allows larger quantities of the sample to be injected.

Split flow depends on the partition coefficients of the compounds to be eluted. For instance, if a low split flow is used and if the compounds have a low partition coefficient, which in other words, have significant column migration at the column's initial temperature, their peak shapes will be broader, resulting in possible lower resolution. However, if the compounds have larger partition coefficient, a lower split flow results in greater loading of the column and lower detection limits. Usually, splitless conditions are used to analyse aroma/odor compounds from food samples in order to recover the maximum quantity of volatile compounds inside the detection chamber.

After establishing the correct split flow rate, the compounds are eluted at a different time (known as retention time) and detected using two flame ionization detectors (FID).

4.2.3 Sensory and chemical characterization

After recording the retention time and calculating the reference indices (Kovats indices) of the eluted compounds, a list of possible matching compounds is created using AroChemBase library (Figure 11).

The candidate molecules are recognized based on the measured parameters (retention time, peak area on 1 or 2 columns) and theoretical ones found in literature (Table 7).

Table 7. An example on a list of some of the possible olive oil volatile compounds based on Kovats indices calculated on RT for two columns

RT on DB5 (sec)	RT on DB1701 (sec)	Kovats Index DB5	Kovats Index DB1701	Possible chemical candidates
3.1	3.8	401	531	acetaldehyde
5	6	607	671	Butanal / methyl ethyl Ketone / methyl butenol
7.4	10.5	690	765	1-penten-3-one / pentenol
7.5	12.2	688	797	1-penten-3-ol / 2,3 pentanedione
25	27	994	1078	2-octanol/ octanone / octadien-3-ol

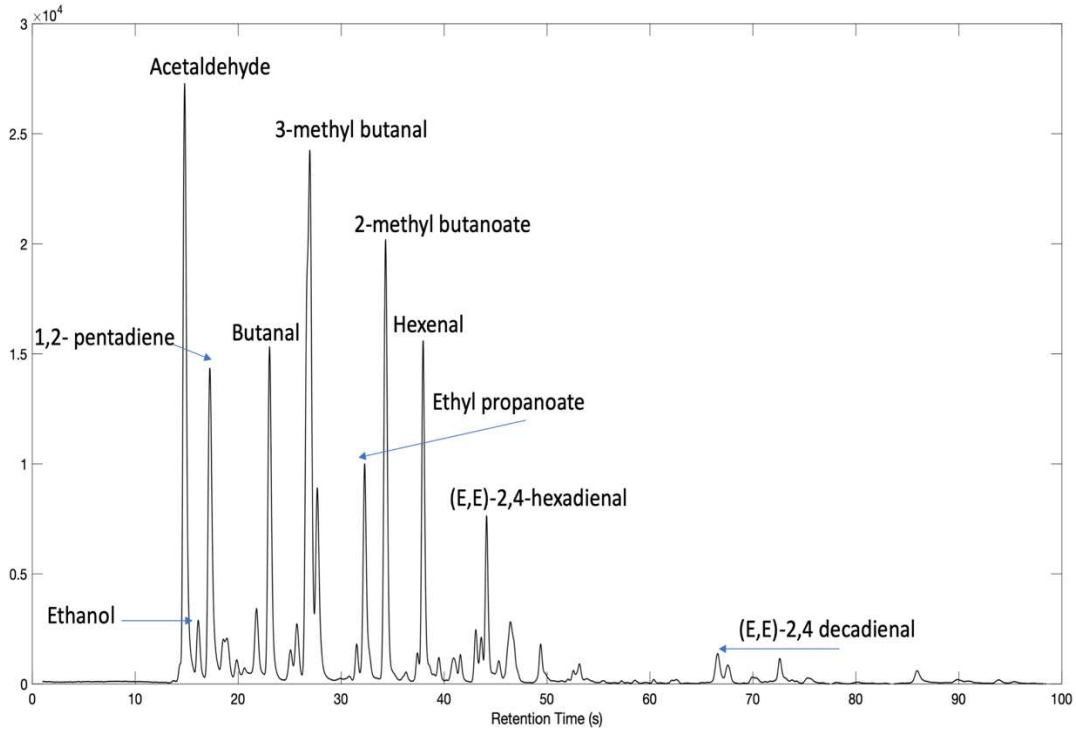

Figure 11. Flash GC chromatogram of virgin olive oil volatile compounds

5. Chemometrics

In this section, an overview on the chemometric methods used in this thesis is provided. These methods were divided in two groups. The first group contains methods belonging to exploratory analysis or "factorial displaying methods" (principle component analysis (PCA), parallel factor analysis (PARAFAC), and independent component analysis (ICA)). The second group contains methods belonging to quantitative analysis (multiple linear regression (MLR), partial least square (PLS)).

5.1 Principal component analysis

Principal component analysis (PCA) is one of the most extensively used methods in social and natural sciences (Abdi & Williams, 2010). It was developed by Hotelling (1933). PCA is considered as a starting point when dealing with big data and its main goal lies in minimizing the dimensionality of the data (Leardi, 2002; Siebert, 2001). PCA uses orthogonal transformation to decompose a big set of data X (original matrix) into two matrices (T and P), the scores matrix and the loading matrix, while maintaining the maximum variance (Figure 12).

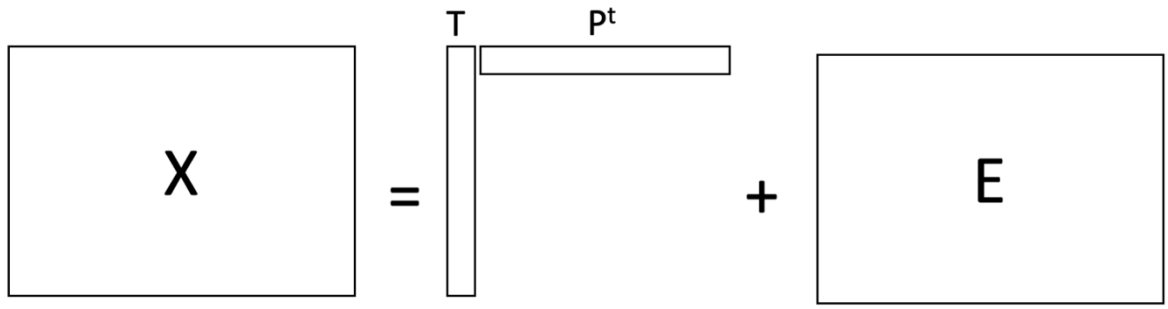

Figure 12. PCA decomposition principle

PCA generates new variables by linear combination of the original variables which results in a change of axes, referred to as principal components (PCs). The first PC is calculated representing the max amount of information, the second components representing smaller amount of information and so on. Scores are represented by the coordinates of the observations on two PC components (score plots) and loadings are represented by original variables in the space of principal components (loading plot) (Cordella, 2012).

PCA is widely used for the geographical origin discrimination of olive oils. Various studies relied on PCA to discriminate several olive varieties or cultivars (Aparicio et al., 1994; Stefanoudaki et al., 1999; López-Feria et al., 2008), to study the influence of growing region on certain components of olive oil mainly fatty acids and sterol compounds, to reveal the effect of pedo-climatic conditions like temperature, altitude, humidity, precipitation on a particular variety of olive oil (Aparicio et al., 1994; López-Feria et al., 2008; Piravi-Vanak et al., 2012;

Hlima et al., 2017). Other studies used PCA to analyze how several agronomic factors such as fruit ripening, harvest time, oil content affects the micro and macro components of olive oil (Gomez et al., 2011; Lukić et al., 2013)

All studies show that PCA is an important starting tool and is of great usefulness for the geographical characterization of olive oil. It gives an overview on the relationship of quantitative/qualitative olive oil's chemical profiles and geographical origin of olives.

5.2 Parallel factor analysis

Parallel factor analysis (PARAFAC) was developed by Harshman (1970) and by Carroll and Chang (1970) who referred to it as CANDECOMP (CANonical DECOMPosition). It is a multi-way method used in the decomposition of 3-way data arrays. The purpose of this decomposition tool is to facilitate the identification and quantification of the underlying signals or in another word the "components or factors". PARAFAC is used in diverse research fields (medical, environmental, social, pharmaceutical and food) and applied to a wide range of data (spectral, GC-MS, sensory, NMR, etc.…). However, its main practice lies in analyzing fluorescence excitation emission matrices (EEMs) (Bro, 1997).

The PARAFAC model is written as:

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}$$

Equation (1)

where:

- x_{ijk} is the intensity of the ith sample (mode 1) at the jth (mode 2) and kth variables (mode 3).
- a_{if} is the relative concentration of compound *f* in sample *i* (on the first mode of X),
- b_{jf} is the fth loading of variable j (estimated emission spectrum),
- c_{kf} is the fth loading of variable k (estimated excitation spectrum),
- e_{ijk} contain the variation not accounted by the model.
- F is the number of extracted signals or components,

F is an important parameter, as determining the number of factors or PARAFAC components is crucial to perfectly reconstruct the data. There are several ways to determine this parameter among which the scree plot, residual investigation, cross validation, etc. Core Consistency Diagnostic (CORCONDIA) is another interesting method, where a core array is estimated from the PARAFAC loadings and from the data, and then compared to the ideal PARAFAC core array (composed of 0's and 1's). If the computed core array is appropriate, another PARAFAC

model, with an additional component, is calculated (Bro, 1997). The CORCONDIA is usually expressed in percentage and the closer it is to 100 % the better the model.

Another method for determining the number of PARAFAC components is split half analysis. This method relies on dividing the data set into two halves and then applying PARAFAC on both halves. Due to the uniqueness of the latter chemometric method, the same results are obtained on both data sets which means that the correct number of factors are chosen (DeRose & Resch-Genger, 2010).

After choosing the right number of components, PARAFAC can be constrained to help in interpreting or stabilizing the components. In spectral data, non-negativity constraint is mostly selected based on the fact that concentrations and spectral values are always positive.

PARAFAC as mentioned earlier, is widely used decomposition tool especially in the field of fluorescence spectroscopy. The use of PARAFAC to interpret and analyze EEMs has increased in recent years. The advantages of using PARAFAC especially in analyzing olive oil fluorescence spectra are that PARAFAC's components can be interpreted to represent independent fluorophores present in the olive oil mixture. Several studies have used PARAFAC to identify olive oil fluorophores like chlorophyll, polyphenols, VIT E and oxidation products (Table 8) (Zandomeneghi et al., 2005). Guimet et al. (2004) applied PARAFAC in the evaluation of complete fluorescence landscapes of olive oils where its decomposition revealed four different fluorophores of which the far dominating one was assigned to chlorophyll and two of the derived fluorophores were assumed to be oxidation products with an excitation around 350 nm.

Table 8. Excitation and emission maxima (nm) of the 7 PARAFAC factors

Factor	Excitation max (nm)	Emission max (nm)	Expected fluorophores
F1	300	325	Tocopherols and tocotrienols (Ammari et al., 2012)
F2	280	315	Polyphenols (Zandomeneghi et al., 2005)
F3	400	675	Chlorophyll (Sikorska et al., 2008)
F4	320	370	Oxidation products (Guimet et al., 2004b)
F5	360	430	Hydrolysis/Oxidation products (Kyriakidis et al., 2000)
F6	360	675	Chlorophylls and pheophytins (Sikorska et al., 2005)
F7	340	515	Oxidation products (Ammari et al., 2012) Riboflavin (Cheikhousman et al., 2005a)

5.3 Independent components analysis

ICA is an unsupervised method developed by Common in 1990s. It is known for recovering underlying source signals mixed together based on an assumption that the latter are statistically

independent In recent years, the number of applications of the ICA technique has increased significantly (Comon, 1994). It has been applied to medical signal processing (Makeig et al., 2002), batch process monitoring (Yoo et al., 2004), speech recognition (Brown et al., 2001; Kim et al., 2003), fault detection (Kano et al., 2003), statistical process monitoring (Lee et al., 2003), and spectroscopic data (Chen & Wang, 2001; Shao et al., 2004; Visser & Lee, 2004). Its application in analytical signals has also expanded especially when it comes in analyzing fluorescence spectroscopy (Bouveresse et al., 2007; Valderrama et al., 2011), NIR (Chen et al., 2001; Garcia et al., 2016; Mishra et al., 2016), MIR (Visser et al., 2004), and GC/MS data (Shao et al., 2004).

So far PCA shares the same applications as ICA. However, several differences lie between these two factorials displaying methods:

- The interpretations of PCA loadings vector doesn't describe physical reality and is often more complicated than those of ICA. On the other hand, ICA loading vectors describes statistically independent phenomenon. ICs describe a more "chemically pure" vectorial space.
- PCA calculates principal components to describe the maximum sample dispersion (maximum variance direction) where there not all PCA loading vectors (eigenvectors) corresponds to the underlying signals. ICA calculates independent components (ICs) where the variation in one pure signal is as much as possible not related to the variations in another signal.
- PCA uses the Variance-Covariance matrix to calculate vectors, whereas ICA uses higher order statistics (4^{th} cumulants for JADE algorithm).

The general model of ICA is:

$$X = AS + E$$

Equation (2)

where

X: the matrix of the recorded signals,

S: the matrix of "pure" source signals,

A: the unknown mixing matrix corresponding to the "proportions" ("scores") of source signals in each mixture.

E: error (all remaining information not taken into account in A.S)

5.3 Regression

The main purpose of regression analysis is to study the relation between a dependent variable and an independent one. The result of this regression is a linear relation equation explaining the correlation between the involved variables and making predictions for the topic in hand.

5.3.1 Multiple linear regression

Regression models having more than one independent variable and one dependent variable are called multiple linear regression. The most important assumptions of MLR are:

- Normal distribution,
- Linearity,
- Collinearity or no ties between the independent variables (Tabachnick et al., 2018).

The MLR model is same as the univariate analysis, however with addition of more than one independent variables

$$\boldsymbol{y} = b_1\boldsymbol{a_1} + b_2\boldsymbol{a_2} + \cdots + b_7\boldsymbol{a_7} + b_0 + \varepsilon$$

Equation (3)

where

y = dependent variable

a_i = independent variable

b_0 = intercept

b_i = regression coefficient

ε = error

5.3.2 Partial least squares

Partial least squares (PLS) have the same principle as MLR, however more than one dependent variable can be included in the PLS model. It is a generalization of the above method (MLR) (Wold et al., 2001). The algorithms for PLS was put by Herman Wold in 1982 (Wold, 1982).It is designed to deal with multiple regression when the data has more variables than observations or has missing values or if multicollinearity problem is present.

The goal of PLS is to predict Y from X by identification of the underlying factors that are a linear combination of the independent variables or X (which may also be known as latent variables) which best model the dependent variables or Y (Abdi, 2004).

Several studies have conducted MLR and PLS prediction models to show the suitability of fluorescence in detecting the quality parameters of olive oil. For instance, Guimet et al. (2005) studied the relation between fluorescence EEM and some quality parameters (peroxide value, K232, and K270) of different oil qualities (13 extra virgin, 2 virgin, 16 pure and 2 pomace olive oils). Sikorska et al. (2008) demonstrated the use of fluorescence spectroscopy for monitoring

the effect of light on the chlorophyll and total polyphenols content of extra virgin olive oil. Also, Tena et al. (2012) showed the potential of EEM to study virgin olive oils thermo-oxidized at a fixed pattern (190°C for 94 h, where a sample of 40 mL was collected every 2 h).

Chapter 2: Materials and Methods

Planning of the sampling strategy as well as preparation of oil samples, essential in the development of the work plan, are presented in this chapter. In order to understand the behavior of the macro-and micro-components found in olive oil, the physico-chemical parameters are measured. The techniques for analyzing the various elements are also presented in this chapter.

1. Sampling

1.1. Olive fruit and oil sampling

About 100 samples of proposed extra-virgin olive oil and olive fruits were collected (harvesting seasons 2016 and 2017) from the following locations:

- Mount Lebanon (Chouf)
- South Lebanon (Tyre, Hasbaya, Marjaayoun, Bint Jbeil)
- North Lebanon (Zgharta, Koura, Batroun, Akkar) (Figure 13)

in order to screen oil for their forensic quality characterization as an introductory step toward geographical indication and molecular fingerprint. These included major and minor chemical components.

During the first harvesting season, which extended from 1st of November 2016 till January 2017, farmers were interviewed, questionnaires were filled, fields and mills were assessed and about 54 samples of olive oil were collected. In addition, interviews were conducted with the Ministry of Agriculture regional officers of each province to have a wider prospective on the condition of this culture.

In 2017, field visits were conducted to each of the designated regions twice. During the visit, which extended from 2nd of October till October 29th, 2017, olive fruits were picked from two fields within the same area. Then the fruits were transferred to LARI in Riyaak for oil extraction using an Abencor analyzer.

Afterward, olive oil samples were collected during the second round of visits which extended from November 12th, 2017 until January 2018. During both phases, the farmers were interviewed, questionnaires were filled, and fields and mills were assessed.

Figure 13. Sampling locations across Lebanon (more than 40 locations)

1.2. Olive fruit sampling technique

Olive fruits, mainly of "Soury variety", were sampled based on IOC recommendations (IOC, 2011b) to obtain a representative sample of the selected field:

- Olive trees with comparable load of homogenous color (maturity) were selected. In large plots, 10 trees were selected and in small plots 20 trees were selected.
- The number of collected olives per trees were selected based on size of tree. In Lebanon, olive fields are not homogenous (traditional orchards), so a different number of olive fruits were collected from each tree. About 5 kg of olive fruits were collected from each field.
- As per collection per one tree, fruits were collected from different branches of the tree avoiding taking olives from the same place twice.

Samples maturity index was then calculated based on IOC directives (IOC, 2011a). 100 olive fruits were selected randomly from each batch. The color of fruit's skin flesh was tested visually and categorized based on the following scoring system:

- Category 0: skin color deep green
- Category 1: skin color yellow-green
- Category 2: skin color green with reddish spots on < half the fruit surface. Start of color change
- Category 3: skin color with > half the fruit surface turning reddish or purple. End of color change
- Category 4: skin color black with white flesh
- Category 5: skin color black with < half the flesh turning purple
- Category 6: skin color black with not all the flesh purple to the stone
- Category 7: skin color black with all the flesh purple to the stone

The maturity index was obtained by applying the following formula where a, b, c, d, e, f, g, and h are the number of fruits in each of the color categories 0, 1, 2, 3, 4, 5, 6, and 7 respectively:

$$M.I = \frac{A0 + B1 + C2 + D3 + E4 + F5 + G6 + H7}{100}$$

Equation (4)

All samples were then stored in crates until further extraction (less than 24 hours).

1.3. Olive oil extraction

The oil was obtained at laboratory scale by cold extraction within 24 h from harvesting using an Abencor analyzer (Mc2 Ingenieria y Sistemas, Seville, Spain), simulating commercial oil-extraction systems (Figure 14). This system consists of three essential elements: hammer mill, thermomixer, and a centrifuge. Before milling, olive fruits were sorted and cleaned, removing infected or damaged fruit, leaves, and other debris. Olives were crushed using a hammer mill equipped with 5.5 mm sieve. The resulting olive paste was distributed into stainless steel containers and kneaded in a mixer at 50 rpm for 30 mins at 28ºC. The obtained paste was then centrifuged at 3500 rpm for 2 mins. All oil samples were decanted and stored in glass bottles at 4ºC without headspace until analysis.

Figure 14. Olive oil extraction process

2. Conventional chemical analysis of olive oil

Olive oil samples were subjected to several chemical tests. These tests include the following: free fatty acids, peroxide values, fatty acid determination, total chlorophyll and Beta-Carotene content, total polyphenols, total un-saponifiable content (sterol extraction), and spectrophotometric investigation in the ultraviolet light. The analyses that were carried out according to the EC Regulation No 2568/91 (EEC, 1991), International Olive Council (IOC) standards and subsequent amendments and supplements (IOC, 2011a).

2.1. Quality Indices

2.1.1. Acidity

A common parameter in specifications of fats and oils, expressed as a percentage of oleic acid. These molecules are produced by triglycerides hydrolysis and their content increases if olives preservation from day of harvest to oil storage was not appropriately managed (Figure 15).

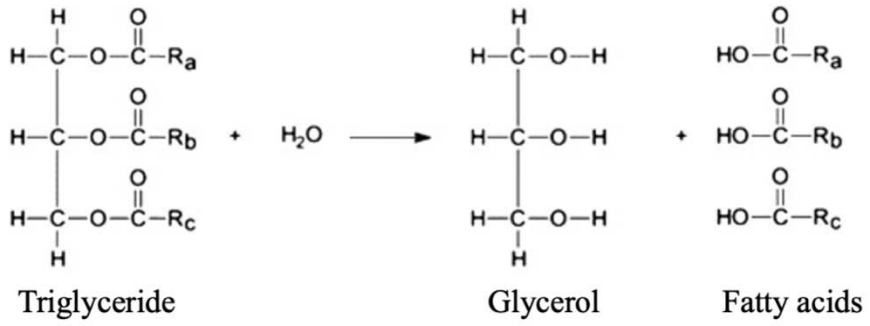

Figure 15: Triglyceride Hydrolysis. Source: (Díaz et al., 2014)

The determination of free fatty acids in olive oil was carried out based on EC Regulation No 2568/91. 5 g of olive oil was dissolved in 50-150 mL of neutralized ethanol (95%). Phenolphthalein was added as an indicator. The mixture was then titrated while stirring with a 0.1 ml/l potassium hydroxide solution and the endpoint was determined when the pink color of the added indicator persisted for 10 seconds.

Acidity as percentage by weight was then calculated based on the following equation:

$$V \times c \times \frac{M}{100} \times \frac{100}{m} = \frac{V \times c \times M}{10 \times m}$$

Equation (5)

where:

V = the volume of potassium hydroxide used, in milliliters.

c = the concentration of the titrated solution (potassium hydroxide), in moles per liter

M = the molar weight of the acid used (M = 282), g / mol

m = the weight of olive oil sample

2.1.2. Peroxide value

PV test for fats and oils, expressed as milliequivalents of active oxygen per kilogram, include a measure of the decomposed glycerides by the lipase action. This action is induced by the exposure of olive oil to light, air and moisture especially during oil extraction and storage.

Generation of hydroperoxides

$$R - H + O_2 \rightarrow ROOH$$

Equation (6)

The determination of amount of oxygen (as peroxide) in olive oil was carried out based on EC Regulation No 2568/91. 5 grams of olive oil were dissolved in 10 mL chloroform. Then 15 mL of acetic acid and 1ml of potassium iodide were added to the mixture. The stopper was inserted

as quick as possible to avoid entrance of air, and the solution was shaken for one minute and left for five minutes away from light at a temperature between 15 to 25 °C.

After 5 mins, 75 mL of distilled water is added, and the liberated iodine was titrated with 0.002 N sodium thiosulfate solution using starch as an indicator. Titration was done in artificial light avoiding direct sunlight.

Generation of iodine

$$KI + CH_3COOH \rightarrow HI + CH_3COO^-K^+$$

$$ROOH + 2\,HI \rightarrow ROH + I^2 + starch\ indicator$$

Equation (7)

Titration

$$I_2\,(purple) + 2Na_2S_2O_3 + 2\,Nal\,(colourless)$$

Equation (8)

PV was calculated using the following equation:

$$PV = \frac{V \times T \times 1000}{m}$$

Equation (9)

where:

V is the volume of sodium thiosulphate solution in mL

T = the normality of sodium thiosulphate solution

m = the weight of olive oil sample.

2.1.3. UV spectrophotometric investigation

This kind of examination in the UV wavelength range provide information on the quality of fat present in olive oil especially those related to the presence of diene and triene conjugated fatty acids. These kinds of fatty acids if present in virgin olive oil are the results of oxidation or refining processes and can be detected by light absorption at specified wavelengths. These absorptions are expressed by K also referred to as extinction coefficients.

Spectrophotometric determinations were carried out based on IOC Regulation COI/T.20/Doc. No 19/Rev. 3 and were made using a UV spectrophotometer (Hitachi U-2900, Spectrophotometer, Japan). 0.25 g of olive oil was dissolved in 25 mL cyclohexane in a graduated cylinder for absorbance measurements at 268 nm and 270 nm whereas 0.05 g of olive oil diluted in 25 mL cyclohexane for absorbance measurements at 232 nm. In both measurements, the solution was homogenized properly. Before introducing the sample into the UV spectrophotometer, the baseline (220-290 nm) was corrected with solvent (cyclohexane) in

quartz cells then the test solution was introduced and the extinctions at 270 nm, and 232 were measured. After measuring the absorbance at 270 nm, the absorbance at $\lambda_{max,}$ λ_{max+4} and λ_{max-4} were measured.

The recorded extinctions at various wavelength were then introduced into the below equation below:

$$K\lambda = \frac{E\lambda}{c \times s}$$

Equation (10)

where:

K = specific extinction at wavelength λ

E = extinction measured at wavelength λ

c = concentration of the solution in g/100 mL

s = path length of the quartz cell in cm

As for ΔK, it was calculated:

$$\Delta K = \left| Km - \left(\frac{K\lambda m - 4 + K\lambda m + 4}{2} \right) \right|$$

Equation (11)

where K_m is the specific extinction at the wavelength for maximum absorption at 270 nm and 268nm

2.2. Pigments in olive oil

2.2.1. Total chlorophylls and β-carotene

Chlorophyll concentrations of the samples were determined according to Isabel Minguez-Mosquera et al. (1991) with a slight modification in the procedure. 5 g of olive oil was dissolved in 25 mL of acetone, and the absorbance of the samples at 670 nm and 642,5 nm were measured with a UV spectrophotometer (Hitachi U-2900, Spectrophotometer, Japan).

Total chlorophyll content was measured by the following equation

:

$$\textit{Total chlorophyll (mg/ml)} = 7.12\ A\ (670) + 16.8\ A\ (642.5)$$

Equation (12)

As for β carotene, the absorbance was measured at 436 nm and the concentration was determined based on a standard curve (R^2 = 0.9997) (Figure 16).

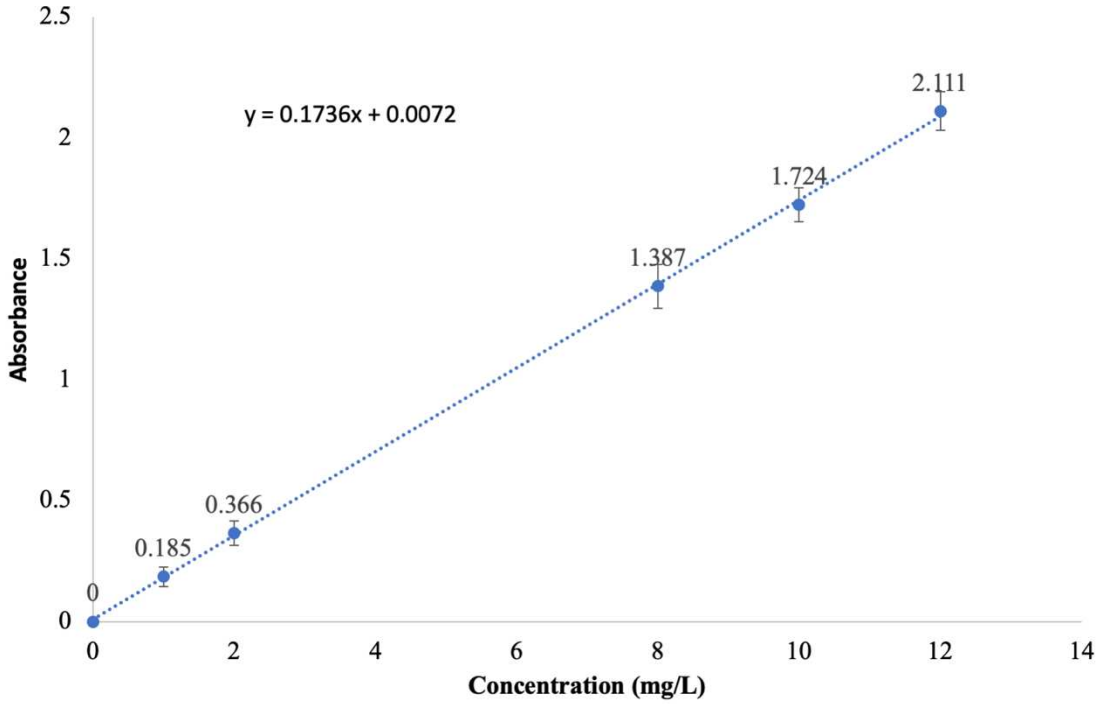

Figure 16. B-carotene calibration curve

2.2.2. Total polyphenols

The total polyphenol content of olive oil was determined according to Montedoro et al. (Montedoro et al., 1992) at 765 nm by a UV spectrophotometer with a slight modification (Hitachi U-2900, Spectrophotometer, Japan).

Extraction was carried out on a 5g of oil by adding 10 mL hexane and 15 mL methanol/water (60:40 v/v). 0.2 mL of the aqueous phase was collected after vortexing for 3 minutes and centrifuging at 1900 rpm for 12 minutes at 5°C. 0.2 mL of the aqueous phase was mixed with 5 mL of Folin–Ciocalteu (1:10 v/v in distilled water) reagent and after 3 mins, with 1 mL of saturated Na_2CO_3. The final solution was filled with distilled water to the mark and was maintained in a dark place at room temperature for 60 minutes. Caffeic acid standard solutions were used to calibrate the method ($R^2 = 0.9995$) (Figure 17).

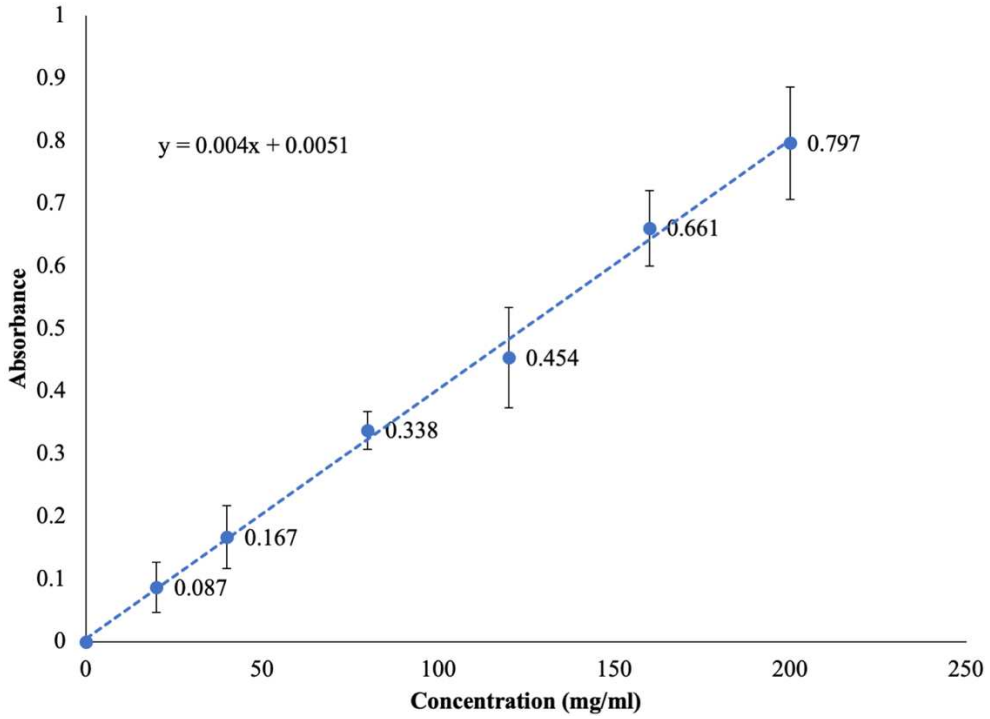

Figure 17. Total polyphenol calibration curve

2.3. Fatty acids analysis

The fatty acid methyl esters (FAME) of olive oil were extracted according to the International Olive Council (IOC) method COI/T.20/Doc. No 33 (IOC, 2017) and EC Regulation No 2568/91. The FAME was prepared according to Part I and Part II of this method.

2.3.1. Preparation of the fatty acid methyl esters from olive oil (acid value ≤ 2.0 %)

0.1 g of olive oil was added to a 5 mL screw-top test tube. 2 mL of heptane were then added to the test tube and the latter was shaken using a vortex.0.2 mL of the methanolic potassium hydroxide solution was then added, the cap fitted with a PTFE joint was put on, tightened, and shaken vigorously for 30 seconds. The solution was left to stratify until the upper solution becomes clear. The upper layer containing the methyl esters was decanted and the heptane solution was ready for injection into the gas chromatograph.

The solution was kept in the refrigerator until gas chromatographic analysis not exceeding the 12 hours limit.

2.3.2. Preparation of the fatty acid methyl esters from olive oil (acid value > 2.0 %)

2g of olive oil was placed in a 100 mL flask. 35 mL of methanol was added, and the condenser was placed in a water bath for few minutes.

The condenser was then removed, and 3.5 mL of sodium methylate solution were added, and the condenser was refitted and boiling under reflux was initiated for 3 hours. The methylation

process was stopped when all fatty substances were liquified and the mixture looked perfectly clear at room temperature.

The mixture was then transferred to 250 mL separating funnel. 30 mL of diethyl ether, 100 mL of water and 5 mL of 10 % sodium chloride solution were added to the mixture. The separating funnel was shaken and left for few minutes for layers formation. The resulting aqueous solution was transferred to a second separating funnel and 25 mL of diethyl ether was added for a second extraction.

Water present in the aqueous solution was then removed by adding 50 mL of 40 to 60 ^{o}C petroleum ether. The resulting ether phase was then washed three times with 15 mL of distilled water, then dried on sodium sulphate and filtered using a filter paper. The filtrate was collected in 200 mL flask. The solvent was then evaporated to 20 mL under current of nitrogen.

2.3.3. Analysis of FAME by GC-FID

Gas chromatography (GC) is a commonly used in analytical chemistry for separating and analyzing compounds that can be vaporized without changing or decomposing the analyte chemical's composition.

The mobile and the stationary phases constitute the core of gas chromatography. The carrier gas (mobile phase), usually is an inert gas such as helium or hydrogen. The stationary phase is composed of a minute layer of liquid or polymer on a solid support, presented inside a piece of glass or metal tubing called a column.

The gaseous compounds to be analyzed interact with the stationary phase. This causes each compound to elute at a different time, known as the retention time. The comparison of retention times of the different compounds is what gives GC its analytical importance.

GC is mostly used in testing the purity or separating the different components of a mixture and in some situations, it may help in identifying a compound.

A gas chromatograph Shimadzu GC-2025 (Kyoto, Japan) equipped with a capillary column SP-2380 (30 m × 0.32 mm i.d. x 0.20 μm film thickness; Supelco, Bellefonte, PA, USA) and a flame ionization detector was used for the determination of fatty acid profile. Helium was employed as the carrier gas at a flow rate of 1 mL/min. Injector and detector temperatures were held at 230°C and 240°C, respectively. The injection volume into a split GC port was 1 μL, and a split injection mode (1/100) was used. The following oven temperature program was used: initial temperature 165°C held for 10 min; ramped at 1.5°C min^{-1} up to 200°C. Fatty acids were identified by comparing the retention time of experimental peaks with those obtained by the external standard mixture.

2.3.4. Method of calculation

The mass percentage of the FAME was calculated using the following formula:

$$w_i = \frac{A_i}{\sum A} \, x \, 100$$

Equation (13)

where:

A_i is the area under the peak of the individual fatty acid methyl ester i; ΣA is the sum of the areas under all the peaks of all the individual fatty acid methyl esters.

The results were expressed by two decimal places.

2.4. Sterol analysis

The sterols of olive oil were extracted according to the International Olive Council (IOC) method COI/ T.20/ Doc. No 30/Rev. 1.

2.4.1. Preparation of the unsaponifiable matter

500 μL of the α-cholestanol solution (internal standard solution) was introduced into the 250 mL flask using a 500 μL micro-syringe. The internal standard was then evaporated until dryness with a gentle current of nitrogen. After drying, 5g of olive oil was introduced to the same flask. 50 mL of 2 N ethanolic 2M potassium hydroxide solution was added and the flask was fitted to the reflux condenser and heat to gentle boiling was applied until saponification takes place i.e. the solution becomes clear (Figure 18). Heating was continued for a further 20 minutes, then 50 mL of distilled water was added from the top of the condenser, the condenser was detached, and the flask was cooled to approximately 30°C.

Figure 18. Olive oil saponification process

The contents of the flask were transferred into a 500 mL separating funnel using several portions of distilled water (50 ml). 80 mL of diethyl ether was added, and the funnel was shaken vigorously for approximately 60 seconds. The funnel was allowed to stand still until there is complete separation of the two phases.

The soap solution was then drawn off into a second separating funnel. Two further extractions were then done using 70 mL of diethyl ether.

The three ether extracts were combined in one separating funnel containing 50 mL of water. The extracts were then washed with water (50 mL) until the wash water no longer gives a pink color upon the addition of a drop of phenolphthalein solution.

The extracts were filtered on anhydrous sodium sulphate into a previously weighed 250 mL flask. The funnel was washed with small amounts of diethyl ether to ensure no residues are left. The filtered solvent was evaporated by distillation in a rotary evaporator at 30 ^{o}C under vacuum. 5mL of acetone was added to the remaining solvent (5 mL left) and then completely remove the solvent using a gentle current of nitrogen. The residues were dried in the oven at 103 ± 2 ^{o}C for 15 min. The flasks were then cooled in the desiccator and then weighed to the nearest 0.1 mg.

2.4.2. Preparation of the basic thin layer chromatography plates

The silica gel plates were immersed about 4 cm in the 0.2 N M ethanolic potassium hydroxide solution for 10 seconds, then allowed to dry in a fume cupboard for two hours and finally placed in an oven at 100 ^{o}C for one hour. Then they were removed from the oven and kept in a calcium chloride desiccator (3.13) until use (not more than 15 days).

A mixture of hexane/diethyl ether mixture (50:50 V/V) was placed into the development chamber, to a depth of approximately 1 cm. The chamber was closed and left for at least half an hour, in a cool place until the liquid-vapor equilibrium was established. The developing mixture was replaced for every test, in order to achieve perfectly reproducible elution conditions (Figure 19).

Figure 19. Developing chamber for TLC plates

5% solution of the unsaponifiable was prepared in chlorofom and, using the 100 µL micro-syringe, 0.3 mL of the solution was disposed on a narrow and uniform streak on the lower end (2 cm) of the chromatographic plate. In line with the streak, 2 to 3 µl of the material reference solution was placed so that the sterol and triterpene dialcohols band can be identified after developing.

The plate was then placed in the prepared developing chamber and allowed eluting until the solvent reaches approximately 1 cm from the upper edge of the plate. The plate was then removed from the developing chamber and the solvent was evaporated by leaving the plate for a short while, under a hood.

The plate was then sprayed by 0.2% 2,7-dichlorofluorescence to identify the sterol area. Then the sterol band was identified by using UV light and the silica gel on the marked area was scraped off by using a metal spatula (Figure 20). The finely comminuted material was transferred into a filter funnel. 10 mL of hot chloroform was added and then mixed carefully with the metal spatula and filtered, collecting the filtrate in the conical flask attached to the filter funnel.

The residue in the flask was washed three times with diethyl ether (10 mL each time), collecting the filtrate in the same flask attached to the funnel. Then the filtrate was evaporated to a volume of 4 to 5 mL using a rotary evaporator, and the residual solution was transferred to a previously weighed 10 mL test tube. The solution was then evaporated to dryness by mild heating, in a gentle flow of nitrogen, acetone was then added which in turn was evaporated again to dryness (Figure 21).

Total mass of sterol (mg) for each sample was calculated by subtracting the test tube holding the sterols from the empty test tube.

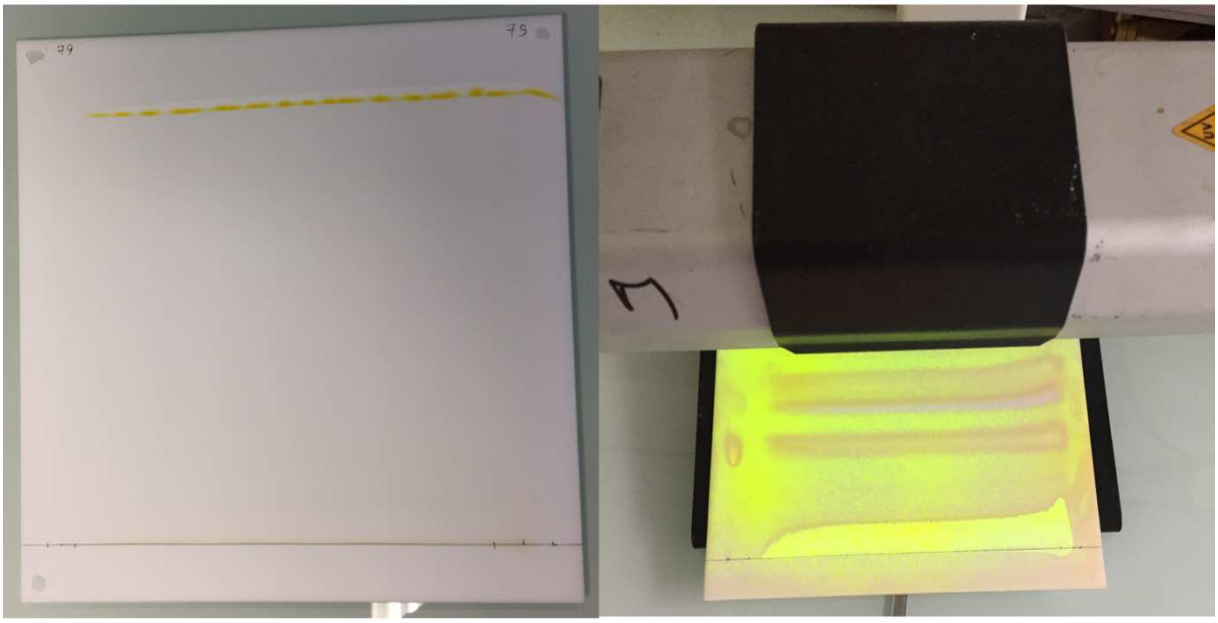

Figure 20. Sterol band identification under UV Light

2.4.3. Preparation of the trimethylsilyl ethers

The silylation reagent consisting of:

- Pyridin
- hexamethyldisilazane
- trimethylchlorosilane

was added in the ratio of 50 µl for every milligram of sterols and triterpene dialcohols in the test tube while avoiding any uptake of moisture.

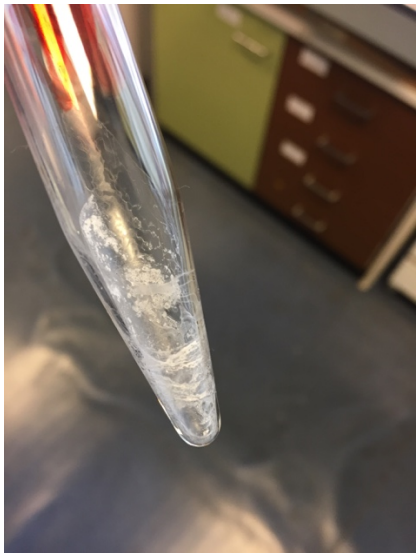

Figure 21. Sterol powder before adding the silylation agents

The test tube was closed and shaken carefully (without overturning) until the compounds are completely dissolved. Then it was left to stand for at least 15 minutes at ambient temperature and then centrifuged for few minutes. Then the clear solution was injected in the GC-MS.

2.4.4. Sterol analysis by GC-MS

Sterols analysis was conducted using ITQ 900 GC-MS (Trace 1310 GC, Thermo Scientific, USA) system with quadrupole ion trap mass analyzer supplied with split/splitless injection and autosampler (Thermo Scientific AI/AS 1310).

A DB-5 MS fused-silica capillary column (5% phenyl–95% methyl polysiloxane), (30 m x 0.25 mm x 0.25 um, Agilent Technologies, USA), was chosen for testing of the different sterol samples.

After column condition, several chromatographic and mass spectrometer conditions trials were conducted to find the best peak resolution and method sensitivity. The best separation was attained under the following GC-MS conditions. Helium carrier gas was used at a flowrate of 1.2 ml/min. The oven temperature was initially set at 100 ºC for 2 mins, then gradually raised to 267 ºC at 40 ºC min1 rate and held for 40 mins. Injection was spitless at 280 ºC. The ion source temperature was set at 230 ºC and the transfer line was at 290 ºC. No calibration ranges were needed as all samples have an internal standard (α-cholestanol). Sterols were identified by comparing the retention time of the obtained peaks with the retention time of IOC reference peaks (similar experimental conditions were executed).

2.4.5. Method of calculation

The areas of the α-cholestanol and the sterol and triterpene dialcohols peaks were calculated using Xcalibur software (ThermoFisher, USA).

The concentration of each individual sterol (mg/kg) was calculated as follows:

$$x = \frac{A_x . m_s . 1000}{A_s . m}$$

Equation (14)

where:

A_x = peak area for sterol x

A_s = area of the α-cholestanol peak.

m_s = mass of added α-cholestanol, in milligrams;

m = mass of the sample used for determination, in grams.

The percentage of each individual sterol (%) was calculated as follows:

$$sterol\ x = \frac{A_x}{\sum A}$$

Equation (15)

where:

A_x = peak area for x;

ΣA = total peak area for sterols

As for the percentage of erythrodiol and uvaol, it was calculated as follows:

$$Erythrodiol + Uvaol = \frac{Er. + Uv.}{Er. + Uv. + \sum A}\ x\ 100$$

Equation (16)

where:

ΣA = sum area for sterol

Er = area of Erythrodiol

Uv = area of Uvaol

The results for individual sterols and that of erythrodiol and uvaol were expressed by one decimal point. As for total sterol concentration it was expressed without any decimal point.

3. Rapid techniques for olive oil analysis

3.1. 3D front-face fluorescence spectroscopy

3D front-face spectra were measured directly on the olive oil samples without prior preparation using a fluorescence spectrophotometer (Hitachi, F-7000 FL, 2240-001) equipped with a xenon lamp, excitation and emission monochromators and a front-face cell holder. Measurements were carried out on the samples filled in quartz cuvettes. As for the front-face angle, it was set at 56 ºC. This angle maximize signal to noise ratio and overcome the drawbacks of right-angle fluorescence spectroscopy especially when using highly viscous samples such as olive oil. The excitation wavelengths ranged from 280 to 540 nm and emission wavelengths from 280 to 700 nm, with 20 and 5 nm intervals in excitation and emission acquisitions respectively.

Excitation and emission monochromator slit widths were set at 4 nm and the scan rate was 240 $nm.min^{-1}$. The photomultiplier (PMT) detector voltage of 350 V was used. All samples were analyzed in duplicates on two different sides of the cuvette. The excitation-emission matrices (EEMs) were placed in a in a 3-way cubic array with the sample in the first mode [96 x 14 x 85] x 2 duplicates.

3.2. Flash-GC

FGC E-nose Heracles II (AlphaMos, Toulouse, France) was used for all samples (Figure 22). The Heracles II is equipped with two columns: a non-polar column (MXT5: 5% diphenyl, 95% methylpolysiloxane, 10 m length and 180 mm diameter) and a slightly polar column (MXT1701: 14% cyanopropylphenyl, 86% methylpolysiloxane, 10 m length and 180 mm diameter).

An aliquot of each sample (500 µL) was placed in a 20 mL vial and sealed with a cap. The vial was placed in the Heracles' auto-sampler, which placed it in a shaker oven where it remained for 15 min at 80 °C, shaken at 500 rpm. Next, a syringe pierced the silicone septum of the cap and sampled 5 mL of the head space. Then the 5-mL headspace aliquot was adsorbed on a CARBOWAX trap maintained at 30 °C for 36 s while the carrier gas (H_2) flowed through it in order to concentrate the analytes and to remove excess air and moisture. Then desorption of the analytes was obtained by increasing the temperature of the trap up to 240 °C in 90 s and the sample was injected. The thermal program started at 40 °C (held for 2 s) and increased up to 280 C at 4 °C /s. The final temperature was held for 21 s. The total separation time was 100 s. At the end of each column, an FID detector was placed, and the acquired signal was digitalized every 0.01 s.

For calibration, an alkane solution (from n-hexane to n-hexadecane) was used to convert retention time in Kovats indices and identify the volatile compounds using specific software (AroChemBase, Alpha MOS, France). Samples were analyzed duplicates.

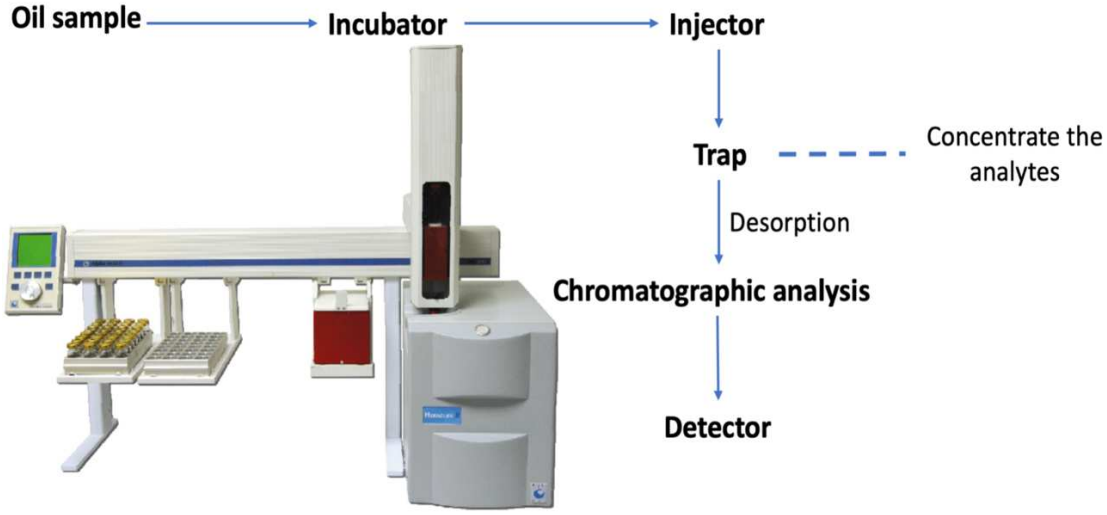

Figure 22. FGC step by step for identifying olive oil volatile compounds

4. Chemometrics prerequisites

4.1 Notation

The mathematical notation used in this thesis and commonly accepted by the scientific community (Kiers, 2000) are:

- *x:* indicates scalars (zero-order data))
- **x:** indicates vectors (i.e. first-order data)
- **X:** indicate matrices (i.e. second-order data)
- ***X:*** indicate three-way arrays.
- $\mathbf{X}^T$ or $\mathbf{x}^T$: Transposition of a matrix or a vector
- $\mathbf{X}^{-1}$: indicates the inverse matrix.

4.2 Three-way arrays

The data was arranged in three-way array (I x J x K) when three dimensions are used. Each dimension of this array is referred to as mode and the number of levels in this mode is called the dimension of the mode (Bro, 2001). For instance, the excitation emission matrices obtained from fluorescence spectroscopy were arranged in a three-way array or a cube where, the first mode is the samples, the second mode is the emission matrix and the third mode is the excitation matrix (Figure 23).

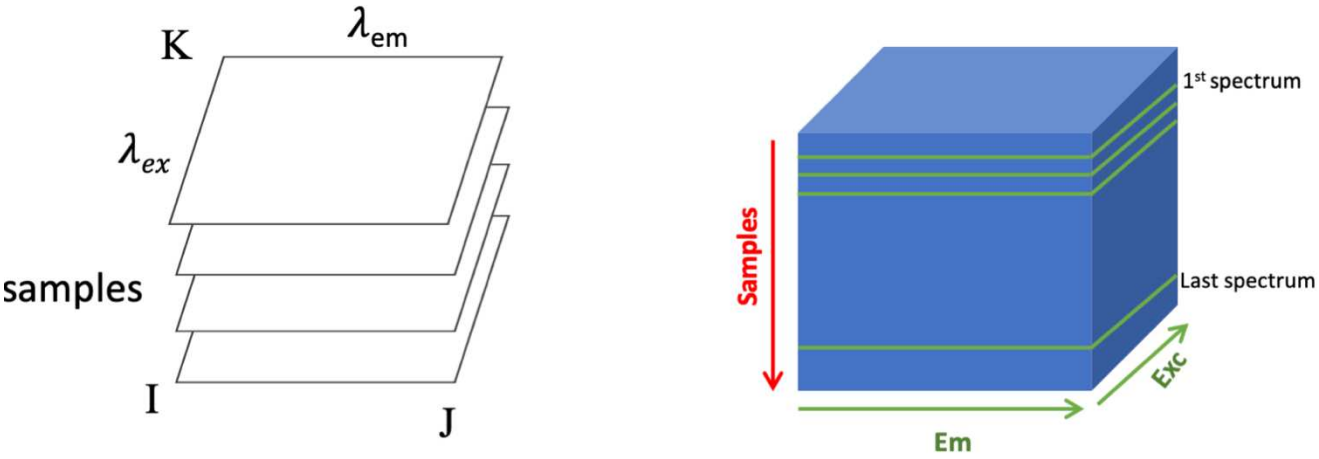

Figure 23. Three-way arrangement

Where, i=1,...,I, j=1,...,J, k=1,...,K, ...

4.3 Preprocessing

4.3.1. Unfolding

Unfolding is the rearranging of a three-way array into a matrix (Bro, 2001; Kiers, 2000). This was accomplished by fixing one of the modes while combining the other two modes. For

example, the matricization of a three-way structure, I x J x K, can be done in three ways, depending on which modes are concatenated:

1. The first and the second, i.e., K x IJ
2. The second and the third, i.e. I x JK
3. The third and the first, i.e. J x KI

The unfolding process executed in this thesis refers to the second type of concatenation. This allows to keep the sample mode (mode 1) and to combine the other two spectral modes (mode 2 and 3) (Figure 24).

Although unfolding can be helpful, however several disadvantages exits for such a process. For instance, unfolding a three way to a two-way array leads to the loss of the three-way structure and increases the number of variables as a result of combining the variables of the two modes (Bro, 2001).

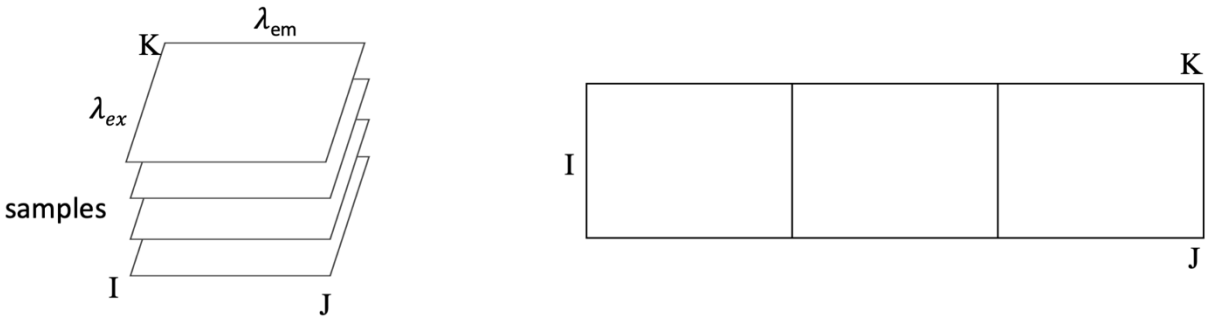

Figure 24. Graphical representation of unfolding a three-way array

4.3.2. Scaling

Scaling aims at eliminating the difference of magnitude between samples or variables. Various scaling methods exists. Standardization and normalization were the two scaling methods used in this thesis.

Standardization is one of the common scaling methods, which involves dividing each element of a matrix by its column standard deviation:

$$x_{ij(st)} = \frac{x_{ij}}{S_j}$$

Equation (17)

where

$$S_j = \sqrt{\frac{\sum_{i=1}^{n} [\![\left(x_{ij} - \bar{x}_i\right)]\!]^2}{n-1}}$$

Equation (18)

Another form of scaling is normalization. It is performed to remove systematic variation associated with the total amount of sample (Beebe et al., 1998). It is done by dividing the vector by the Euclidean norm.

$$x_{ij\,(norm)} = \frac{x_{ij}}{\|\mathbf{X}_j\|}$$

Equation (19)

where

$$\|\mathbf{X}_j\| = \sqrt{x_{1j}^2 + x_{2j}^2 + \cdots + x_{nj}^2}$$

Equation (20)

In general, when dealing with a three-way data, scaling should be done only on the rows of a matrix. EEMs were unfolded according to the excitation mode (transformed into a matrix of the concatenated emissions); then for each sample, the standard deviation of all the emissions were used to scale the data (dividing each emission by the standard deviation). The scaled emissions of all the samples were finally refolded back to reform the three-way array.

4.3.3. Rayleigh scatter

Scattering is formed as a result of light diverging from its original path and spreading in different directions. This occurs due to the presence of small particles in the sample. The information obtained by scattering provides no information on the sample's properties.

Rayleigh scatter is a type of scattering that is produced when dealing with matter-light interaction, and hence fluorescence EEMs. Two Rayleigh scatterings were produced as a diagonal line across the obtained spectra as shown in Figure 25 (Townshend, 1995). These lines appeared when:

- Emission wavelengths was equal to excitation wavelengths (first-order Rayleigh)
- Emission wavelengths were twice the excitation wavelengths (second-order Rayleigh)

In the obtained EEMs, Rayleigh signals were removed by inserting zero values at $\lambda emission \leq \lambda excitation$ (since no emission is present at wavelengths below the excitation wavelength) and by inserting missing values at $\lambda emission = \lambda excitation + 5$ to $\lambda emission = \lambda excitation + 25$ (Elcoroaristizabal et al., 2015; Andersen & Bro, 2003).

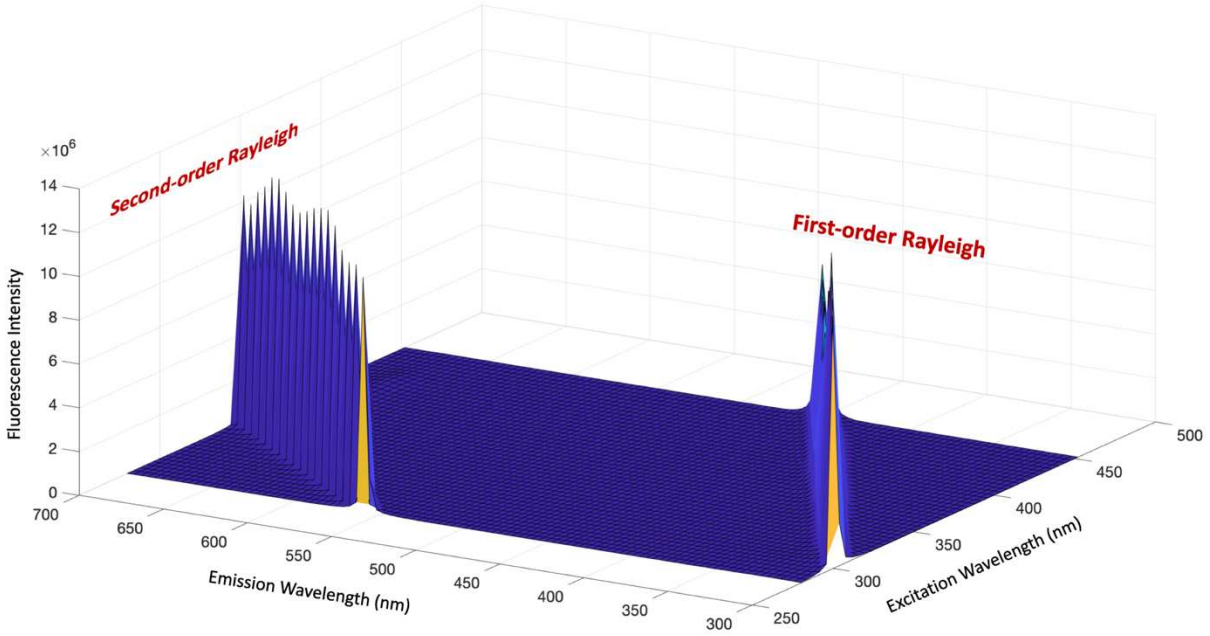

Figure 25. A spectrum showing the interference of first and second-order Rayleigh

4.3.4. Warping for chromatographic signal alignment (GC-FID)

Warping is the correction of misalignments for chemical measurements. These misalignments are caused by several factors such as instrument stabilization over time, minute changes in pressure and temperature or changes in the quality of the elution phase for liquid chromatography and others. So, warping becomes necessary when misalignment occurs at different positions along the retention time axis of a chromatogram.

Several warping methods exists such as icon-shift (Savorani et al., 2010) dynamic time warping (DTW) (Clifford et al., 2009), semi-parametric time warping and parametric time warping (PTW) (Eilers, 2004) and correlation optimized warping (COW) (Nielsen et al., 1998). The latter warping method was used as its commonly used for GC-FID chromatographs (Figure 26). To ensure the alignment of chromatograms, COW applies a piecewise (segement) linear stretching or compression to the studied and the reference chromatogram. The reference was divided in the same number of sections as the studied chromatogram. When both chromatograms have different lengths, the resulting segment length difference served as a default compression/elongation that caused the chromatograms to have the same length after warping (Nielsen et al., 1998) (Figure 26). This method's drawbacks are that it is time-consuming, and possible distortions of curves might happen due to the compression/elongation process.

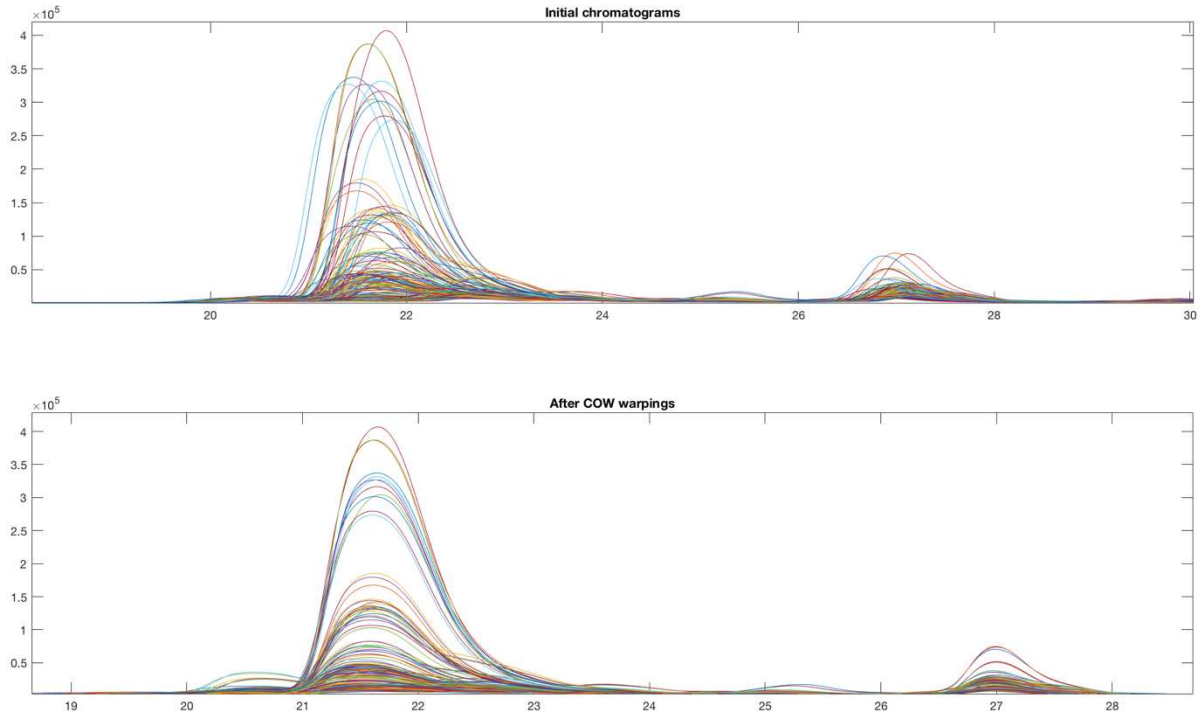

Figure 26. Alignment of chromatograms before and after warping

4.3.5. Outlier detection

Outlier samples in this thesis were detected using:

1. Exploratory analysis methods; which provide simple and easy tools such as the score-plot of PCA. Score plots reveals samples with similar measurement clustered together or placed close to each other. A sample far away from its cluster could be considered an outlier.
2. Residual analysis; it is also considered as one of the common methods for detecting outliers. Examining the residual plot for an observation with high residue than other observations reveals that the observation is an outlier.
3. Leverage plot; leverage measures how far an observation is from other observations.

Chapter 3: Impact of Growing Area and Technological Aspects on Lebanese Olive Oil: Characterization by unsupervised methods

Lebanon was off the chart when it comes to geographical characterization / indication. It is time to set the grounds for such possibility as this will improve the image of Lebanese olive oil and creates an access to international markets. There by, giving an opportunity for the Lebanese olive farmers for a better market access. For this to happen, the major quality affecting parameters need to be identified.

This chapter, under article form, demonstrates that geographical indication is possible in Lebanon, mostly attributed to the climatic effect and the altitude of the growing area. It also reveals the two key parameters affecting Lebanese olive oil quality: the improper storage practices and olive oil processing practices.

Impact of Growing Area and Technological Aspects on Lebanese Olive Oil: Characterization by unsupervised methods

Omar H. Dib[1,2*], Ali Bassal[2], Hussein Dib[2], Rita Yaacoub [2], Nathalie Locquet[1], Luc Eveleigh[3] and Christophe B. Y. Cordella[1]

[1] UMR PNCA, AgroParisTech, INRA, Université Paris-Saclay, Paris, France, 75005

[2] Department of Food Science and Technology, Lebanese University, Beirut, Dekweneh, Lebanon, 1003

[3] Ingénierie Procédés Aliments, INRA, AgroParisTech, Université Paris-Saclay, Massy, France, 91300

* Correspondance (Omar H. Dib)

UMR PNCA, AgroParisTech, INRA, Université Paris-Saclay, Paris, France, 75005.

Department of Food Science and Technology, Lebanese University, Beirut, Dekweneh, Lebanon, 1003.

Email address: dibhomar@gmail.com,

Tel: +961 3 821834 / +33 6 86 69 81 85

Abstract

The influence of growing-area and technological aspects on the Lebanese Soury variety is presented. The pedoclimatic conditions, quality parameters, and fatty acids of oil samples were examined using chemometric tools to demonstrate the typicality of Lebanese olive oil. Furthermore, the effect of several technological factors on olive oil quality was studied. Accordingly, olive oil samples were collected across Lebanon corresponding to two climatic zones (Zone A: Low-altitude regions; Zone B: High-altitude regions). Principal component analysis was capable of discriminating the two zones with distinct fatty acid profiles. Zone A exhibited a fatty acid profile mostly dominated by linoleic, linolenic, palmitic, and palmitoleic acid content. As for zone B, it showed a unique fatty acid profile distinguished by oleic acid. These results are mostly attributed to the climatic effect and the altitude of the growing area. Moreover, independent component analysis, coupled to one-way ANOVA, demonstrated that significant differences ($p<0.05$) were found in quality indices (acidity and peroxide value) as well as fatty acid contents (oleic and linoleic acid) when comparing uncontrolled to controlled extraction methods. This study provides a baseline for future intensive characterization of Lebanese olive oil and detects the non-compliances attributed to the disqualification of olive oil virginity.

Keywords: chemometric tools, fatty acid, growing area, olive oil, quality assessment

1. Introduction

Olive trees cover 5.4 % of the Lebanese territory or 8 % of total agricultural lands in Lebanon (Investment Development Authority in Lebanon [IDAL], 2017). Olive groves, almost solely rain-fed, are dispersed over the provinces of Lebanon, producing 24,000 tons of olive oil per year (International Olive Council [IOC], 2018). This production is being conducted by 485 registered mills scattered throughout Lebanon. About 85% of the mills still rely on traditional-extraction systems, particularly pressing method (Lebanese Ministry of Agriculture [MOA], 2012).

Granite millstones and nylon fiber mats, the basic components of the pressing mill, are still being used despite their high susceptibility to contamination, and most prominently, their effect on olive oil components (Giovacchino, 2000). Olive paste remnants on mats can initiate several biological and chemical processes affecting the virginity of olive oil. Olive quality may deteriorate by other technological variables occurring before and after processing. Irrational practices during fruit transport and olive and olive oil storage may lead to the lipolysis of triglycerides and to the chemical oxidation of fatty acids affecting the major constituents of olive oil (Gharbi et al., 2015).

Besides the technological aspect, a wide variety of factors such as olive cultivar (Aguilera et al., 2005), fruit maturity (Amanpour et al., 2019), and growing area (Arslan et al., 2013) have a significant influence on olive oil chemical composition. Among these factors, the effect of the growing area is undeniably of primary importance, especially on the fatty acid profile (Aguilera et al., 2005). In most studies, altitude and climate have a vast impact on the profile of fatty acids. For example, countries close to the equator tend to have a higher palmitic, palmitoleic, linoleic and alpha-linolenic concentrations in contrary to those with a very cool climate where a higher oleic level is observed (Aparicio et al., 1994). Furthermore, differences between regions with distinct altitudes were noticed, as higher altitudes increase monounsaturated fatty acids, and lower altitudes increase polyunsaturated and saturated fatty acids (Di Bella et al., 2007).

Lebanon’s diverse topographies, stretching mainly in a north-south direction, and Mediterranean climate establishes the potential for fatty acid profiles discrimination. Studies targeting the fatty acid composition of Lebanese olive oil originating from different growing areas are scarce. Still, some authors have indicated the possibility that the pedoclimatic conditions of some Lebanese olive growing areas can influence both saturated and monounsaturated fatty acids (Merchak et al., 2017; Riachy et al., 2018).

This study investigates the use of chemometrics to highlight the effect of growing area on the Lebanese olive oil based on the resulting physicochemical parameters (mainly fatty acids) and to detect the technological variables leading to disqualification of Lebanese olive oil virginity. The correlation between the micro and macro-components in olive oil and technological variables are examined using two main chemometric tools, principle component analysis (PCA), and independent components analysis (ICA).

2. Methodology

2.1 Sampling

A total of seventy-eight olive oil samples of Soury variety were collected during the years 2016 and 2017. The samples were divided into two groups, uncontrolled and controlled.

The uncontrolled group is composed of 58 extra virgin olive oil (EVOO) and virgin olive oil (VOO) samples (500 ml) collected from nine sampling-locations across Lebanon. These locations include Akkar, Hasbaya, Bint Jbail, Tyr, Marjaayoun, Batroun, Koura, Nabatiyeh, and Zgharta and are identified by the EU to have the potential for geographical indications (United Nations [UN], 2010). These samples are referred to as uncontrolled since the olive oil samples were directly collected from farmers (Table 1).

Table 1. Practices applied before, during and after extraction of olive oil

	Harvesting date	**Olive fruit storage**	**Fruit storage duration (days)**	**Extraction method**	**Olive oil storage**
Uncontrolled	Mid-End October	Plastic Bags	2-3 days	Granite Stone/Pressing mats/ Vertical Centrifuge	Stainless steel container
Controlled	Early – End October	Crates	< One day	Hammer/ thermomixer/Centrifuge	Dark Glass bottles

The remaining 20 samples were extracted from 5 kg of healthy Soury olive fruits of a maturity index of 1.76 based on the Uceda et al. (1975) procedure. The fruits were carefully hand-harvested, excluding infected or physically damaged fruits, from randomly selected olive trees in olive orchards located in the above-stated regions. The latter set of samples will be referred to as controlled.

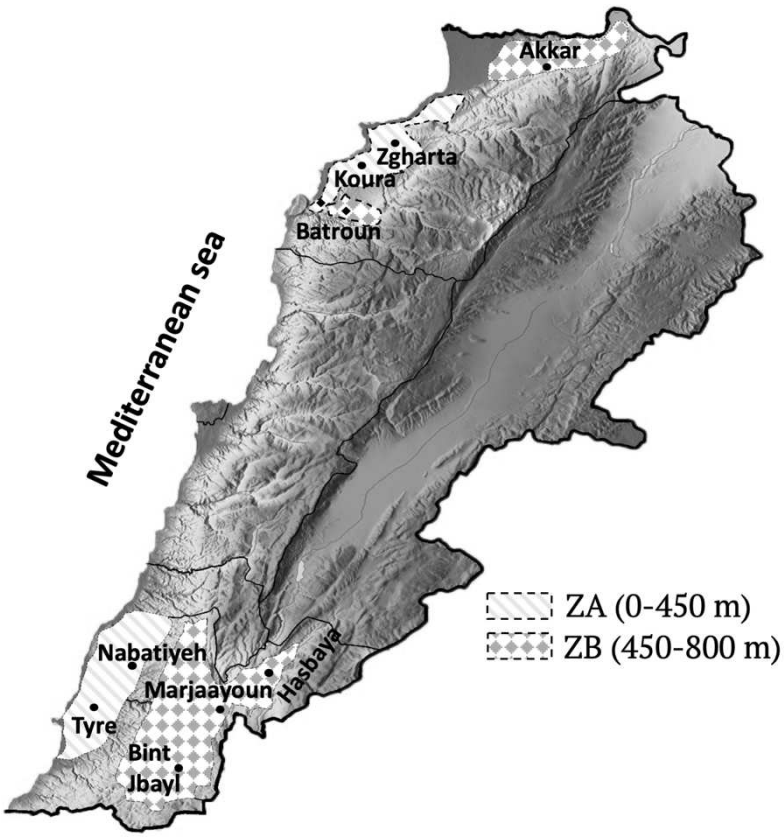

Figure 1. Lebanese map specifying the location of the two zones and the sites where olive fruits and oils were collected.

The controlled samples serve to highlight two hypotheses. Firstly, we are interested in proving that the variability emerging from uncontrolled samples does not affect the discrimination among the regions. Secondly, we want to evaluate the impact of technological factors on the chemical and quality parameters compared to those of the uncontrolled extraction.

In this study, regions with similar altitudes across the south and north of Lebanon were grouped into two zones (Figure 1). Zone A (ZA) contains areas with low altitudes ranging from 0 m to 450 m. Zone B (ZB) contains olive groves situated at high altitudes ranging from 450 m to 800 m. The climate conditions of the two zones were obtained from the Lebanese Agricultural Research Institute and are presented in Table 2.

Table 2. Sample distribution and climatic conditions of the two zones recorded over two years (2016 and 2017)

		Climatic conditions 2016			Climatic conditions 2017		
Zones	Altitude (m)	Mean Precipitation (mm)	Average Temperature (°C)	Mean Relative Humidity (%)	Mean Precipitation (mm)	Average Temperature (°C)	Mean Relative Humidity (%)
ZA[†]	0-450	578	19	61	501	20	62
ZB[‡]	450-800	781	16	48	702	17	54

[†] Number of samples collected from low-altitude regions over the two seasons: Koura (9), Zgharta (9), Tyre (6), Nabatiyeh (7), Batroun (2) and Akkar (4). [‡] Number of samples collected from high-altitude regions over the two seasons: Marjaayoun (9), Hasbaya (8), Bint Jbayl (9), Akkar (9) and Batroun (6)

2.2 Olive oil extraction

The oil was obtained by cold extraction within 24 h from harvesting using an Abencor analyzer (Mc2 Ingenieria y Sistemas, Seville, Spain), simulating commercial oil-extraction systems. Olives were crushed using a hammer mill equipped with a 5.5 mm sieve. The resulting olive paste was distributed into stainless steel containers and kneaded in a mixer at 50 rpm for 30 mins at 28°C. The obtained paste was then centrifuged at 3500 rpm for 2 mins. All oil samples were decanted and stored in glass bottles at 4°C without headspace until analysis.

2.3 Quality Indicators determination

Oil quality indexes including acidity as a percentage of oleic acid (%), peroxide value (PV) expressed in milliequivalents of active oxygen per kilogram of oil (meq O2/kg), and UV spectrophotometric indices (K232, K270) were determined according to the European Union Commission Regulation EEC No 2568/91 (European Union Commission [EEC], 2013). Spectrophotometric determinations were made using a UV spectrophotometer (Hitachi U-2900, Spectrophotometer, Japan).

2.4 Fatty acid analysis

The fatty acid methyl esters (FAME) of olive oil were extracted according to the International Olive Council (IOC) method COI/T.20/Doc. No 33 (IOC, 2017). The FAME was prepared according to Part I and Part II of this method.

A gas chromatograph Shimadzu GC-2025 (Kyoto, Japan) equipped with a capillary column SP-2380 (30 m × 0.32 mm i.d. x 0.20 μm film thickness; Supelco, Bellefonte, PA, USA) and a flame ionization detector was used for the determination of fatty acid profile. Helium was employed as the carrier gas at a flow rate of 1 mL/min. Injector and detector temperatures were

held at 230°C and 240°C, respectively. The injection volume into a split GC port was one μL, and a split injection mode (1/100) was used. The following oven temperature program was used: initial temperature 165°C held for 10 min; ramped at 1.5°C min^{-1} up to 200°C. Fatty acids were identified by comparing the retention time of experimental peaks with those obtained by the external standard mixture.

2.5 Statisitcal analysis

2.5.1 One-way Analysis

ANOVA was applied to the chemical parameters of olive oil to study the effect of the growing area on the oil characteristics. Also, ANOVA was carried out on the independent components scores to establish statistical interpretation. Tukey's test was used to establish the significance of difference at a 5 % level among means.

2.5.2 Principle Component Analysis

PCA was developed by Harold Hotelling in the 1930s (Hotelling, 1936). The core idea behind PCA is to represent the initial data matrix by a product of two smaller matrices, the scores matrix (T), and loadings matrices respectively (P) in such a way the product of:

$$TP^t + E = X \quad (1)$$

With P^t corresponds to the transposed matrix of P, and E is the residues matrix.

T and P matrices describe the original data in a lower-dimensional space to get a more readable overview of the data. Columns of T are factorial coordinates of samples (called scores), and lines of P^t are factorial contributions of variables (called loadings) (Cordella, 2012).

2.5.3 Independent Component Analysis

ICA was developed in the 1990s (Comon, 1994). ICA is known primarily as a method of blind source separation (BSS) and can recover N unknown source signals mixed together, in unknown proportions, in a set of P observed mixtures. A key point of ICA is that each observation is a mixture of these sources. In most cases, including the case of analytical chemistry data, it is assumed that the part related to the non-linear mixed signals is negligible compared to the linear portion, and we work with a linear mathematical model, which simplifies calculations. This BSS method relies on the assumption that the source signals are statistically independent.

The general model of ICA is:

$$X = AS + E \quad (2)$$

where X is the matrix of the recorded signals, S is the matrix of "pure" source signals, and A is the unknown mixing matrix corresponding to the "proportions" ("scores") of source signals in each mixture. In recent years, the number of applications of the ICA technique has increased significantly in analytical chemistry, and especially in the treatment of signals acquired during food analysis (Ammari et al., 2012).

Data were analyzed using Matlab version R2015b (The Mathworks Inc., MA, USA).

3. Results and Discussion

3.1 Growing area effect on Lebanese olive oil

3.1.1 Fatty acid composition & quality indices

Table 3 shows the fatty acid composition and quality indices of uncontrolled and controlled samples for ZA and ZB.

The main olive growing areas in Lebanon are mainly distributed along ZA and ZB (Figure 1). Regions belonging to ZA, located on low altitude areas, had higher palmitic, palmitoleic, linoleic, linolenic, and lower oleic acid content when compared to ZB high-altitude regions for both uncontrolled and controlled (Table 3). This is due to the growing area effect, as stated by studies published earlier (Stefanoudaki et al., 1999; Issaoui et al., 2010). For instance, ZA scored a linoleic acid content of 11.37%, a linolenic acid content of 0.64%, and an oleic acid content of 69.94 %. As for ZB, an increase in oleic acid (73.24%) and a decrease in linoleic acid and linolenic acid were observed (10.46 %, 0.59%). These results are per the outcomes described by other studies for oils extracted from olives grown at different altitudes. For instance, Aguilera et al. (2005) showed that oils obtained from high altitudes have a greater content of oleic acid, whereas, at low altitudes, the oils have higher linoleic content, which is the case for ZA and ZB for both controlled and uncontrolled.

In Lebanon, high-altitude areas have a cooler climate compared to low-altitude areas (Table 2). The temperature has an impact on the fatty acid profile of olive oil, as established by several authors (Di Bella et al., 2007; García-Inza et al., 2014). High temperatures increase the content of several fatty acids, such as palmitic, palmitoleic, linoleic and, linolenic, and decrease that of oleic acid. This observation is also noted in ZA and ZB of both groups.

Regarding the quality indices, only the acidity of the samples belonging to ZA showed a significant difference from ZB in the uncontrolled group (Table 3). However, the acidity of ZA and ZB of controlled samples showed no significant difference meaning that this quality parameter is not affected by the growing area. The increase in acidity noticed in ZA may be due

to several factors such infestation by pests (Daane & Johnson, 2010), fungal pathogens (Gomes et al., 2012), advanced ripening (Inglese et al., 2011), and tree fruit load (Trentacoste et al., 2010). Peroxide value, K232, and K270 showed no significant difference.

Table 3. Mean values ± standard deviation of fatty acids composition and quality indices of Lebanese Olive Oil

	Uncontrolled		Controlled	
Fatty Acids (%) and Quality indices	ZA[1]	ZB[2]	ZA	ZB
Palmitic acid	11.51 ± 1.00b	10.46 ± 1.29a	11.63 ± 0.99b	9.39 ± 0.76a
Palmitoleic acid	0.55 ± 0.09b	0.46 ± 0.06a	0.54 ± 0.08b	0.39 ± 0.06a
Stearic acid	4.21 ± 0.39a	4.10 ± 0.31a	4.10 ± 0.40a	4.11 ± 0.38a
Oleic acid	69.94 ± 1.44a	73.14 ± 1.77b	71.59 ± 0.98a	75.21±2.02b
Linoleic acid	11.37 ± 1.24b	9.83 ± 1.16a	8.97 ± 1.32b	8.14 ± 1.01a
Linolenic acid	0.64 ± 0.06b	0.59 ± 0.07a	0.63 ± 0.08b	0.53 ± 0.05a
Acidity† (g/100 g)	1.20 ± 0.38b	0.89 ± 0.43a	0.29 ± 0.04a	0.32 ± 0.06a
PV‡ (meq O_2/kg)	11.84 ± 5.20a	11.47 ± 3.65a	2.98 ± 0.87a	3.16 ± 1.27a
K232	2.07 ± 0.34a	1.94 ± 0.23a	1.82 ± 0.24a	1.81 ± 0.19a
K270	0.15 ± 0.15a	0.20 ± 0.14a	0.16 ± 0.03a	0.19 ± 0.05a

All determinations were carried out in duplicate. Different letters in the same row show significant differences (Tukey's test, $p \leq 0.05$). † Calculated as oleic acid; ‡ Peroxide value; [1] olive oil samples collected a low-altitudes; [2] olive oil samples collected at high-altitudes.

3.1.2 Exploring the regional growing effects on fatty acids by PCA

A PCA model with 58 uncontrolled olive oil samples and 10 variables, i.e., an initial X data matrix of 58 x 10 was conditioned. All data were normalized before running PCA, so all variables have the same standard deviation and thus the same weight. Six principal components (PCs) were extracted, covering 93.7 % of the total variance, where the first two components accounted for 63 % of the variability in the data set. The number of PC components was determined using the scree plot. The first two PCs were plotted showing a good differentiation between samples located in ZA and ZB associated to different altitudes, average temperatures (Tavg), relative humidity (RH), and precipitation as well as fatty acid profile (Figure 2).

PC1 clearly shows the discrimination of uncontrolled samples belonging to ZA and ZB (Figure 2). Most of the regions belonging to ZB are mostly correlated with oleic acid content and inversely correlated with RH. It has been reported by Ranalli et al. (1997) that the percentage of oleic acid is negatively correlated with the RH of the atmosphere. This coincides with the

fact that most of the regions in ZA are characterized by RH and thus less influenced by oleic content. Another reason for such low content of oleic acid in ZA is the altitude. Several authors demonstrated that olives grown at lower altitudes with high average temperatures showed lower oleic acid and higher polyunsaturated and saturated fatty acids in contrary to those grown at higher altitudes with cooler climate (Issaoui et al., 2010; Stefanoudaki et al., 1999). Also, García-Inza et al. (2014) studied the effect of high temperature by placing olive fruiting branches in transparent plastic chambers with individualized temperature control. The results showed that high temperatures decrease the oleic acid content and increase the palmitic, palmitoleic, linoleic, and linoleic acid contents. So, the scores and loadings of ZA and ZB on PC1 are in agreement with the information given above. ZA has positive scores and is correlated with the following variables: linoleic, linolenic, palmitic, palmitoleic, temperature, and RH, whereas ZB scores were negative and correlate d with oleic, and altitude parameters respectively (Figure 2). It is noteworthy to mention that the variable "precipitation" did not affect the discrimination between ZA and ZB since the distance between the latter and the origin is closer than all other variables.

As ZA and ZB represent the main olive growing areas in this study, the above PCA model needs to be validated since it was conducted on uncontrolled samples. So, a set of 20 samples (Controlled) with optimum conditions were projected on the above PCA model. The new observations seem to fit the above discrimination based on PC1 (Figure 3). ZB is still dominated by oleic acid content, whereas ZA is characterized by linoleic, linolenic, palmitoleic, and palmitic acid. The climatic conditions responsible for such discrimination are altitude, temperature, and RH. This is the same result obtained with the uncontrolled samples.

Apparently, the growing area had a significant impact on fatty acids, even in the presence of other forms of variability, such as processing techniques and different oil qualities.

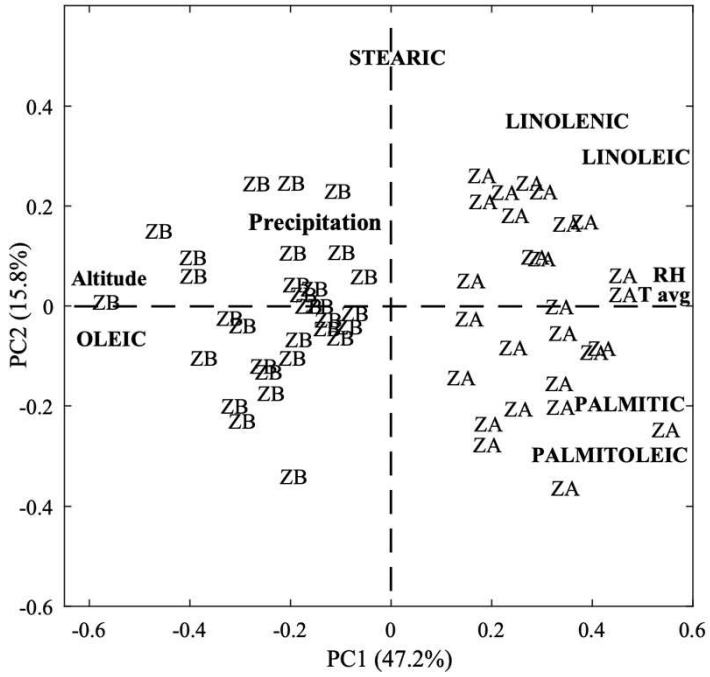

Figure.2 Principal component analysis of uncontrolled olive oil samples showing the distribution of the two zones based on fatty acid profiles. ZA: Zone A (olive oil samples collected at low altitudes); ZB; Zone B (olive oil samples collected at high altitudes);

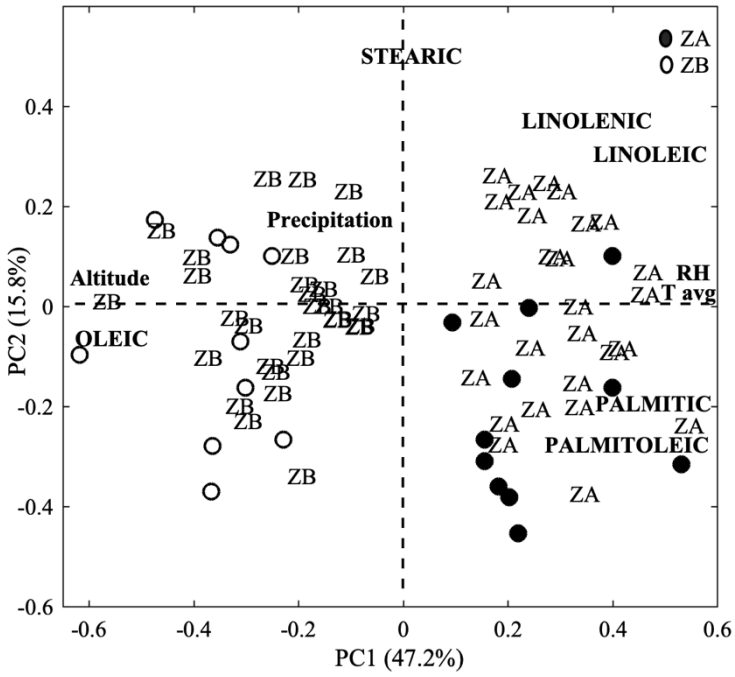

Figure 3. Bi-plot presentation showing the projection of the observations of controlled olive oil samples on the uncontrolled PCA. ZA: Zone A (olive oil samples collected at low altitudes), ZB: Zone B (olive oil samples collected at high altitudes)

3.2 Factors causing the disqualification of olive oil virginity by ICA

Several crucial parameters for obtaining a good quality olive oil were studied to detect the leading factor in the disqualification of Lebanese olive oil from being considered an extra virgin. In 2016, 25 thousand tons of olive oil were produced, and only 16 % (3929 tons) of total olive oil production consisted of EVOO (IDAL, 2017). To assess the above situation, a comparison was established between the uncontrolled group and the controlled group. It is noteworthy to mention that in this comparison, 20 samples that share the same altitude and climatic conditions as those of the controlled group were selected from the total number of uncontrolled samples. In this study, ICA combined with one-way ANOVA was chosen over PCA since the analysis of the loadings carried out by the latter is often not direct and may highlight mixtures of different phenomena explained by the data (Rutledge & Jouan-Rimbaud Bouveresse, 2013). Data were standardized and an ICA model with 4 independent components (IC) was calculated from the dataset. Then the proportions of each IC component were subjected to one-way ANOVA to establish the statistical inference.

The ICs corresponding to the separation of both groups based on the significance level ($p<0.05$) are IC1 (3.34E-10 < 0.05), IC2 (2.30E-06 < 0.05), IC3 (4.99E-06 < 0.05), IC4 (8.40E-08 < 0.05), which are in turn attributed to peroxide value, oleic acid, linoleic acid, and acidity,

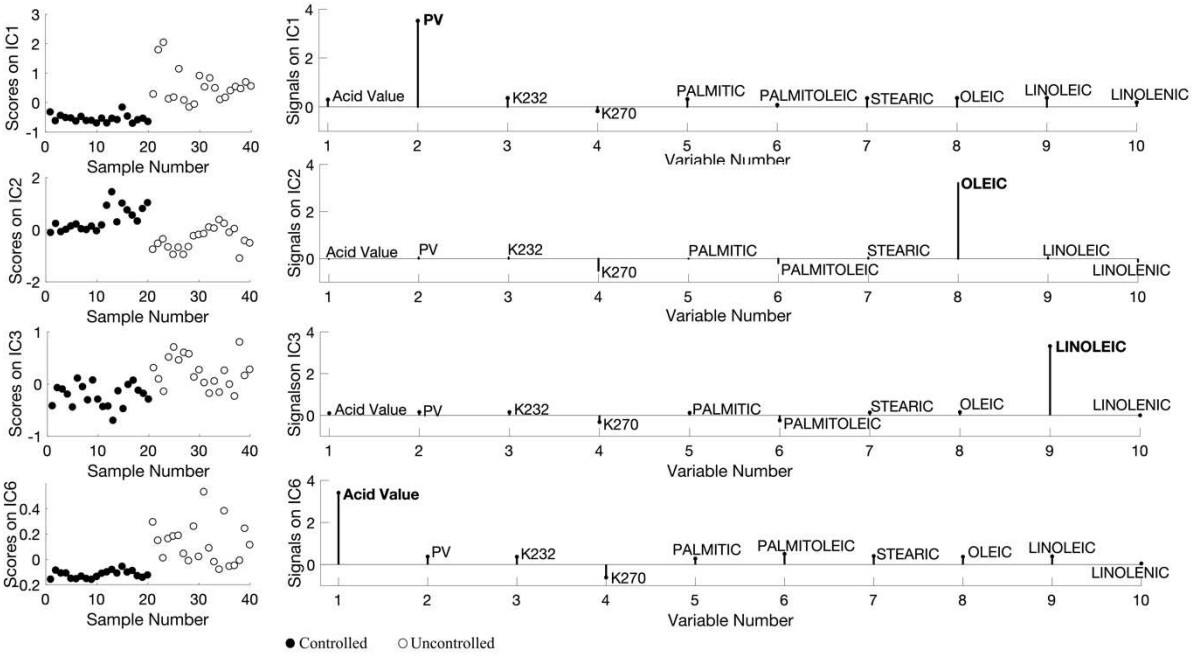

Figure 4. ICA scores and loadings of Lebanese olive oil chemical parameters; PV: Peroxide value.

respectively. Figure 4 shows the proportions of the significant IC components (equivalent scores), and their corresponding pure signals (unmixed loadings).

ANOVA was also conducted on the above groups as an extra measure of certainty for the ICA results. The key variables obtained by ICA for differentiating both groups are in coordination with those obtained with ANOVA, as shown in Table 4. The uncontrolled group, classified as VOO (according to IOC standards), exhibited significantly higher acidity and peroxide values when compared with oils extracted under optimum conditions. In addition, lower content of oleic and higher content of linoleic acid was observed in the uncontrolled group. As for the controlled group, they are classified as EVOO since all quality parameters are within the norms set by IOC for EVOO (Table 4). Several factors and practices interfere in affecting olive oil quality and composition, varying from agricultural to technological ones (Gharbi et al., 2015). Based on Table 1, the two critical factors/practices responsible for the quality deterioration of olive oil are fruit storage condition and duration, and extraction method.

Although in the comparison mentioned earlier, modern processing technique was compared against the traditional ones (Table 1), the latter technique should manage to produce EVOO. Several authors reported that pressing systems can produce EVOO if the machinery and the production site are clean (Di Giovacchino et al., 1994; Di Giovacchino et al., 1996). Besides, IOC, in its guide on quality management for the olive oil industry, recommends the application of good hygienic and manufacturing practices to ensure the best olive oil quality outcome (IOC, 2006). Most of the traditional mills in Lebanon do not apply any means for cleanliness or quality control. Olive paste in layers stacked on pressing mats, which are challenging to be kept clean and exempt from oxidized oil and fermentation defects possibly transferable to the oils. Besides, most of the farmers transport olive fruits in plastic sacks that stay for several days in the mill before being pressed. These harmful practices favor the enzymatic lipolysis of the fruit triacylglycerol, which strongly affects the quality indicators of olive oil (Kiritsakis et al., 1998).

Table 4. Fatty acid composition and quality indices of olive oil obtained either by controlled or uncontrolled extraction.

Fatty Acids (%) and Quality indices	Uncontrolled	Controlled
Palmitic acid	10.95 ± 1.27a	10.51 ± 1.40a
Palmitoleic acid	0.50 ± 0.09a	0.46 ± 0.10a
Stearic acid	4.11 ± 0.35a	4.10 ± 0.30a
Oleic acid	71.65 ± 2.28a	73.40 ± 2.36b
Linoleic acid	10.54 ± 1.43b	8.56 ± 1.19a
Linolenic acid	0.61 ± 0.07a	0.58 ± 0.08a
Acidity† (g/100 g)	1.04 ± 0.43b	0.30 ± 0.05a
PV‡ (meq O2/kg)	11.64 ± 4.40b	3.07 ± 1.07a
K232	1.94 ± 0.27a	1.81 ± 0.20a
K270	0.18 ± 0.07a	0.18 ± 0.04a
Percentage of samples classified as EVOO	33.30%	100%

All determinations were carried out in duplicate and mean value ± standard deviation. Different letters in the same row show significant differences ($p \leq 0.05$). † Calculated as oleic acid; ‡ Peroxide value.

Conclusion

PCA and one-way ANOVA were able to reveal a correlation between the fatty acid profile and the pedoclimatic conditions of the main olive growing regions in Lebanon. Low altitudes regions were characterized by palmitic, palmitoleic, linoleic, and linolenic acid, whereas high-altitude regions were represented by their high oleic acid content.

On the other hand, ICA facilitated the chemical interpretation by providing chemically pure components that crossed the parameters sets by IOC for EVOO. Fruit storage condition/duration and the non-hygienic conditions of the extraction method were identified as the major quality affecting parameters. The solution for such non-compliances would rely on good management/hygienic practices which include, ensuring suitable pre-processing and post-processing storage conditions and providing basic hygiene.

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Chapter 4: Conventional and Ultra-fast Analysis Exposing the Harvest Date Impact on Lebanese Olive Oil: The Soury Variety

Choosing the harvest date is the most important decision taken by the olive farmer every year. Picking up earlier than expected or delaying it may affect the chemical composition of the resulting olive oil. It sets the limits on the qualities of olive oil.

As known, delaying the harvest means that ripening is advancing and thus some physical changes will occur to the fruit. This including changing color, softening of the tissues and lastly falling of the fruits from the tree. As olive fruit ripens, several minor and major constituents of the olive oil change as for example and in some varieties the oleic acid content decrease. This particular monounsaturated fatty acid represents around 55-80 % of olive oil and it is known for its broad range of health benefits like protecting against cardiovascular diseases. Never to mention the decrease in total polyphenol content where they act as antioxidants.

This chapter shows the impact of harvest period on the quality parameters, polyphenols, fatty acids, sterols, and volatile compounds on Lebanese olive oil from the Soury variety. It also presents the appropriate period for the Lebnanese farmers to pick the olive fruit and thus obtain an olive oil with the highest quality grade

Conventional and Ultra-fast Analysis Exposing the Harvest Date Impact on Lebanese Olive Oil

The Soury Variety

Omar H. Dib[1,2*], Christophe B.Y. Cordella[1,4], Rita Yaacoub [2], Hussein Dib[2], Nathalie Locquet[1], Luc Eveleigh[3] and Ali Bassal[2]

[1] UMR PNCA, AgroParisTech, INRA, Université Paris-Saclay, Paris, France, 75005

[2] Department of Food Science and Technology, Lebanese University, Beirut, Dekweneh, Lebanon, 1003

[3] Ingénierie Procédés Aliments, INRA, AgroParisTech, Université Paris-Saclay, Massy, France, 91300

[4] C[2]B research group – Chimiométrie pour la Caractérisation de Biomarqueurs

*Corresponding author: Christophe B.Y. Cordella;

UMR PNCA, AgroParisTech, INRA, Université Paris-Saclay, Paris, France

C[2]B research group – Chimiométrie pour la Caractérisation de Biomarqueurs

Tel : +33(1)666416900 ;

Email : (christophe.cordella@inrae.fr)

Abstract

The impact of harvest period on the quality parameters, polyphenols, fatty acids, sterols, and volatile compounds of Lebanese olive oil from the Soury variety was investigated in this study. Two groups of olive oil were compared, each with a specific harvest date. HD1 was harvested in October, whereas HD2 was picked in November. The analysis of both olive oil categories showed that HD2 witnessed a significant increase in all quality parameters except K270 and a decrease in total polyphenol content from 138 mg/mL to 44 mg/mL. Oleic and linoleic acids had an inverse relation, where the former decreased and the latter increased with the harvest date's advancment. The relative amount of β-Sitosterol was mainly found to decrease, while those of stigmasterol, $\Delta^{5,24}$-stigmastadienol, Δ^{7}-stigmastenol, and Δ^{7}-avenasterol increased with delaying harvest time. As for the volatile compounds, principle component analysis was used on the flash GC data to differentiate HD1 from HD2. Ethanol was found mostly characterizing HD2, whereas HD1 was influenced by 1-hexanol and (E,E)-2,4-decadienal. It can be concluded that the Soury variety should be harvested early, and a delay would result in the declassification of Lebanese olive oil quality from extra virgin to virgin olive oil.

Keywords: chemical characterization, harvest date, Lebanese olive oil, soury variety

1. Introduction

Lebanon is home to the oldest olive trees dating back centuries (at least 1,500 years). It has been distinguished for its trade-in crop products, including olive and olive oil along the Mediterranean Basin (Thalman, 2000, Beayno, Mattar et al., 2002, Mahfoud, 2007). Lebanon produces around 24,000 tons of olive oil. For the last six years, the production ranged between 16,500 t and 25,000t (International Olive Council [IOC], 2018). This fluctuation in production can be induced by several agronomical (El Antari, Hilal et al., 2000, Stefanoudaki, Chartzoulakis et al., 2001) and technological factors (García, Seller et al., 1996, Koutsaftakis, Kotsifaki et al., 1999). Among these factors, an agronomical factor that is recognized as the one having the most detrimental effect on olive oil chemical composition is the harvest date (De La Torre, Lopez et al., 1985, Fiorino & Nizzi 1991, Koutsaftakis, Kotsifakis et al., 2000).

As olive fruit matures, the color of the fruit shifts from green at the start of the harvest period to small reddish-green spots to purple and lastly to black at the end of the harvest period (Motilva & Romero, 2010). Each stage imparts its chemical variations on the level of macro and minor components of olive oil, such as triglycerides, fatty acids, polyphenols, sterols, and chlorophylls, which in turn have an impact on olive oil quality (Gargouri, Rouina et al., 2016). Besides, these variations influence not only the quality but also the nutritional, the organoleptic characteristics, and the oxidative stability of olive oil (Maaitah, Al-Absi et al., 2009). However, the variation based on fruit maturity depends also on the cultivar in the study.

For instance, Issaoui, Flamini et al., (2010) related the increase of polyunsaturated and the decrease of monounsaturated fatty acid to fruit maturation. In other cases, the level of monounsaturated fatty acids increased, along with the level of saturated and polyunsaturated fatty acids, as in Barnea variety. As for the Soury variety, Lodolini, Polverigiani et al., (2017) showed that the delay of harvest has a detrimental effect on olive oil quality. As it ripens, oleic content declines, and linoleic increases, quality indices, mostly free fatty acids, are negatively affected, while polyphenol levels and oxidative stability drop sharply. Also, it has been reported that the sterol content decreases sharply from 2850 g/kg to 1644 g/kg (Noorali, Barzegar et al., 2014). Moreover, fruit maturation has also been shown to affect olive oil quality parameters such as free fatty acids, peroxide value, specific UV absorbances, and volatile compounds(Famiani, Proietti et al., 2002, Lazzez, Perri et al., 2008, Mailer, Ayton et al., 2010, Varzakas, Zakynthinos et al., 2010, Dag, Kerem et al., 2011, Gomez, Ruiz et al., 2011)

Because the macro and minor compounds of the Lebanese olive oil have not been studied extensively, we are interested in identifying some minor and macro components characterizing

the Soury variety and studying the harvest date's impact on their evolution. Also, this study stresses the volatiles as a research gap in Lebanese olive oil.

2. Methodology

2.1 Sampling

Sixty-three olive oil samples of Soury variety were used in this study. The samples were divided into two groups (HD1 and HD2) according to their harvest date.

HD1 includes 21 extra virgin olive oil samples (EVOO), each extracted from 5 kg of healthy Soury olive fruits picked from mid of October till the first week of November. The color of the olive fruits of this group was green with purple dots. Oil samples were obtained by cold extraction using an Abencor analyzer (Mc2 Ingenieria y Sistemas, Seville, Spain) with a hammer mill (5.5 mm sieve), a mixer (50 rpm for 30 mins at 28°C), and a centrifuge (3500 rpm for 2 mins). All oil samples were stored in glass bottles at 4°C without headspace till analysis.

HD2 is composed of 42 virgin olive oil (VOO) samples (500 mL) collected from Lebanese olive farmers. The olive fruits were harvested from mid of November till the end of November. The color of the olive fruits of this group was purple to black.

2.2 Quality parameters

The determination of olive oil quality indices, such as acidity (calculated as oleic acid), peroxide value (PV), and UV spectrophotometric indices (K232, K270), was carried out according to the European Union Commission Regulation EEC No 2568/91 (European Union Commission [EEC], 2013).

2.3 Total polyphenols

The analysis of total polyphenol content was carried out based on Montedoro, Servili et al., (1992). The phenols were isolated from oil in hexane by double extraction with methanol-water (60:40, v/v). Total polyphenols were determined by a UV spectrophotometer (Hitachi U-2900, Spectrophotometer, Japan) at 765 nm using a Folin-Ciocalteu reagent. Caffeic acid standard solutions were used as a means of method calibration ($R^2 = 0.9995$).

2.3 Fatty acids

The fatty acid profile of olive oil was determined by gas chromatography (GC) after methyl esterification of the acids, according to the International Olive Council (IOC) method COI/T.20/Doc. No 33 (IOC, 2017). Chromatographic analysis was performed using Shimadzu GC-2025 (Kyoto, Japan) equipped with a capillary column SP-2380 (30 m × 0.32 mm i.d. x 0.20 µm film thickness; Supelco, Bellefonte, PA, USA) and an FID detector. Injector and

detector temperatures were held at 230°C and 240°C, respectively. The injection volume into a split GC port was 1μL, and a split injection mode (1/100) was used. Helium was employed as the carrier gas at a flow rate of 1 mL/min. The following oven temperature program was used: initial temperature of 165°C held for 10 min, ramped at 1.5°C min^{-1} up to 200°C. Fatty acids were identified by comparing the retention time of experimental peaks with those obtained by the standard external mixture.

2.4 Sterols

The sterols of olive oil were extracted according to the International Olive Council (IOC, 2013). 5g of olive oil were introduced to a 250 mL flask, already containing α-cholestanol solution (internal standard solution), and saponified with 2 N ethanolic 2M potassium hydroxide solution. After boiling the solution, 100 mL of distilled water was added, and three extractions of the same unsaponifiable fraction were carried out using diethyl ether (80ml, 70ml, 70 ml). The extracts were then washed with water (50 mL) until the wash water no longer gives a pink color upon the addition of phenolphthalein solution. The extracts were filtered on anhydrous sodium sulfate, and the filtered solvent was evaporated by distillation in a rotary evaporator at 30 ºC under vacuum.

Separation of the sterol and triterpene dialcohols fraction (erythrodiol + uvaol). 5% solution of the unsaponifiable was prepared in chloroform and using the 100 μL microsyringe, 0.3 mL of the solution was disposed on a narrow and uniform streak on the lower end (2 cm) of the TLC plate. The plate was then placed in the prepared developing chamber and allowed eluting until the solvent reaches approximately 1 cm from the plate's upper edge. The plate was then sprayed with 0.2% 2,7-dichlorofluorescein to identify the sterol area. The sterol band was then identified using UV light, and the silica gel on the marked area was scraped off using a metal spatula, dissolved in chloroform and diethyl ether, and evaporated to dryness. The obtained sterols and triterpene dialcohols were transformed into trimethylsilyl ethers by adding a 9:3:1 (v/v/v) mixture of pyridine/hexamethyldisilazane/trimethylchlorosilane (in the ratio of 50 □L for every milligram of sterols).

GC-MS Analysis. The mixture was analyzed using ITQ 900 GC-MS (Trace 1310 GC, Thermo Scientific, USA) system with a quadrupole ion trap mass analyzer supplied with split/splitless injection autosampler (Thermo Scientific AI/AS 1310). A DB-5 type 5% phenyl–95% methyl polysiloxane fused- silica capillary column, DB-5MS (30 m x 0.25 mm x 0.25 um, Agilent Technologies, USA), non-polar, low bleed, and with high-temperature limits (up to 350 ºC), was chosen for testing sterol samples. The helium carrier gas was used at a flow rate of 1.2

mL/min. The oven temperature was initially set at 100 °C for 2 mins, then gradually raised to 267 °C at 40 °C /min rate and held for 40 mins. The injection was spitless at 280 °C. The ion source temperature was set at 230 °C, and the transfer line was at 290 °C. No calibration ranges were needed, as all samples have an internal standard (α-cholestanol). Sterols were identified by comparing the retention time of the obtained peaks with the retention time of IOC reference peaks (similar experimental conditions were executed).

Sterol relative amounts (%) were expressed with respect to the internal standard and proportions of total sterols. The apparent β-Sitosterol was calculated as the sum of β-sitosterol, Δ^5-avenasterol, clerosterol, sitostanol, and $\Delta^{5,24}$-stigmastadienol.

2.5 Volatile compounds

Volatile compounds of olive oil samples were analyzed using FGC E-nose Heracles II (AlphaMos, Toulouse, France), equipped with two columns: a non-polar column (MXT5: 5% diphenyl, 95% methylpolysiloxane, 10 m length and 180 mm diameter) and a slightly polar column (MXT1701: 14% cyanopropylphenyl, 86% methylpolysiloxane, 10 m length and 180 mm diameter).

500 µL of olive oil was placed in a 20 mL vial and sealed with a cap. The vial present in Heracles' auto-samples was then transferred to a shaker oven for 15 min at 80 °C, shaken at 500 rpm. Next, a syringe pierced the cap's silicone septum and sampled 5 mL of the headspace, which were then adsorbed on a CARBOWAX trap, maintained at 30 °C for 36 s, while the carrier gas (H_2) flowed through it to concentrate the analytes by removing excess air and moisture. The analytes were then desorbed by increasing the trap's temperature to 240 °C in 90 s, and the sample was injected. The thermal program started at 40 °C (held for 2 s) and increased to 280 °C at 4 °C /s. The final temperature was held for 21 s. The total separation time was 100 s. At the end of each column, an FID detector was placed, and the acquired signal was digitalized every 0.01 s.

For calibration, an alkane solution (from n-hexane to n-hexadecane) was used to convert retention time in Kovats indices and identify the volatile compounds using specific software (AroChemBase, Alpha MOS, France).

3. Results and Discussion

3.1 Quality parameters and total polyphenol content

The results illustrated in Table 1 show the fundamental differences in the analyzed physicochemical parameters between olive fruits harvested in October and those in November.

The quality parameters of olive oils (acidity and peroxide value) were found to be significantly different during the two notable harvest dates in Lebanon. On average, acidity values were 0.31% and 1.21 % for HD1 and HD2, respectively, exceeding the limit established by the International Olive Council (IOC) for oils to be considered extra virgin (0.8 %) for the latter. The observed increase in November corroborates with other authors (Youssef, Zarrouk et al., 2010, Fuentes de Mendoza, De Miguel Gordillo et al., 2013). It can be explained by the increase in lipolytic activity as the olives become more sensitive to mechanical damage and pathogenic infections (Anastasopoulos, Kalogeropoulos et al., 2011).

Peroxide value of olive oils harvested in November showed a significant increase of about 9.34 meq O_2/kg compared to those picked in October. However, the delay in harvesting olive fruits should show a lower level of peroxide due to the decrease in lipoxygenase activity (Salvador, Aranda et al., 2001, Alowaiesh, Singh et al., 2016). The significant increase observed in HD2 was unexpected and required further examination. Although an increase was noticed, all olive oils presented peroxide values that did not exceed the maximum acceptable limit for their classification as extra virgin olive oils (< 20mEq O_2/kg).

K232 and K270 are the markers of olive oil alteration. K232 is in direct relation with poly-unsaturated fatty acids and peroxide value, whereas K270 relates to aldehyde and ketone substances. As the harvest date is delayed, the value of K232 increases, while K270 decreases (Hamidoghli, Jamalizadeh et al., 2008). However, a study by Bengana et al., (2013) showed an inverse pattern as olive fruit ripens, while in other studies, both indicators decreased with maturity and depended on olive cultivar and harvest date. In our case, a decrease in K232 and an increase in K270 were observed for the studied Soury variety shown in Table 1. All studied samples had K232 and K270 values consistent with the limit defined by IOC for extra virgin olive oil.

Phenolic compounds are of great organoleptic and nutritional interest. They are associated with the taste and the oil's stability due to their antioxidant properties (Maga 1978, Nergiz & Ünal 1991). As the harvest time is delayed, oleuropein, the main bitterness-producing component in olives, progressively decreases (Amiot, Fleuriet et al., 1986, Amiot, Tacchini et al., 1990). This was confirmed by our results (Table 1), as the total polyphenol content observed for HD1 was significantly higher than that for HD2. These findings suggest a decline in the oil's oxidative stability as harvesting is delayed (Gutiérrez, Jímenez et al., 1999, Trentacoste et al., 2020). The observed decline as ripening advance may be attributed to the polyphenol oxidase that dominates the final stage of the ripening process and to the fusion of the phenolic acids into the

cell walls as a fruit defensive mechanism against pathogens (Amira el, Behija et al., 2012, Rodríguez, Gómez et al., 2016).

Table 1. Quality parameters and total polyphenol content for both groups (HD1 and HD2)

	Acidity † (g/100g)	PV ‡ (meq O_2/kg)	K232	K270	Total polyphenols (mg/mL)
HD1	0.31 ± 0.06b	3.10 ± 1.06b	1.81 ± 0.22b	0.17 ± 0.04a	138.31 ± 43.44a
HD2	1.21 ± 0.37a	12.44 ± 4.54a	2.04 ± 0.30a	0.15 ± 0.04b	44.08 ± 21.34b
EVOO*	≤0.8	≤20	≤2.5	≤0.22	-

All determinations were carried out in duplicate. Different letters within the same column show significant differences (Tukey's test, $p \leq 0.05$). † Calculated as oleic acid; ‡ Peroxide value; *Standards of IOC for extra virgin olive oil (EVOO)

3.2 Fatty acid composition

Fatty acids are the major constituents of olive oil and are an essential factor in determining the authenticity and the quality of olive oil (Essiari, Zouhair et al., 2014). Changes in the fatty acid composition are presented in Table2. The major fatty acids included in this study are palmitic, oleic, and linoleic acid. The minor fatty acids are palmitoleic, stearic, linolenic.

Among fatty acids, oleic acid was high in HD1 and showed a slight decrease in HD2. On the contrary, the concentration of linoleic acid significantly increased from 9.09 % to 11.09 % (El Qarnifa et al., 2019). This increase may be related to the activity of oleate desaturase. Oleate desaturase is an enzyme responsible for the desaturation of Oleoyl-ACP (precursor of longer-chain unsaturated fatty acids) into linoleate-ACP, which might be the reason behind the notable difference in the oleic and linoleic concentrations between these two groups. This enzyme has been extensively discussed in seed oils; however, the information regarding the enzyme activity in olive oil is still limited (Hernandez, Padilla et al., 2011). Another factor for the existing difference between HD1 and HD2 is the continuing biosynthesis of triglycerides as the harvesting date is delayed (Flamini, 2010 & Guiterrez, 1999). Lodolini, Polverigiani et al. (2017) have also reported that the Soury variety should be harvested early. The postponement of olive fruit harvest has a detrimental effect on olive oil's fatty acid composition, mainly oleic and linoleic content.

All fatty acids were within the acceptable limit for their classification as extra virgin olive oils in both HD1 and HD2.

Table 2. Mean values ± standard deviation of fatty acids (%) composition of Lebanese olive oil at two harvest dates (October and November)

	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic
HD1	10.71 ± 1.37a	0.50 ± 0.17a	3.70 ± 0.71a	73.79 ± 2.20a	9.09 ± 1.53b	0.59 ± 0.07a
HD2	11.28 ± 1.3a	0.58 ± 0.17a	4.01 ± 0.5a	70.86 ± 2.34b	11.09 ± 1.45a	0.63 ± 0.08a
EVOO*	7.5-20	0.3-3.5	0.5-5	55-83	3.5-21	≤ 1

All determinations were carried out in duplicate. Different letters within the same column show significant differences (Tukey's test, $p \leq 0.05$). *Standards of IOC for extra virgin olive oil (EVOO).

3.3 Sterol content

The sterol content of olive oil samples for the two harvesting periods is recorded in Table3. The sterols with the highest amounts were β-sitosterol, Δ^5-avenasterol, and campesterol representing more than 90% of total sterol content, whereas other sterols like stigmasterol, clerosterol, sitostanol, $\Delta^{5,24}$-stigmastadienol, Δ^7-stigmastenol, Δ^7-avenasterol and two triterpene dialcohols (erythrodiol and uvaol) were within small amounts.

The total sterol content of HD2 exceeded the 1000 mg/kg threshold for HD2, as shown in Table 3. This result is in agreement with Lukić, Lukić et al., (2013), who stated that the levels of sterols continue to increase until the olive fruit is ripened. Apparent β-Sitosterol of both groups was higher than 93% as determined by the EU regulations.

The mean relative amount of β-sitosterol was mainly found to decrease, while those of stigmasterol, $\Delta^{5,24}$-stigmastadienol, Δ^7-stigmastenol, and Δ^7-avenasterol increased as per previous studies (Camera, Angerosa et al., 1975, Koutsaftakis, Kotsifaki et al.. 1999, Salvador, Aranda et al.. 2001, Vekiari, Oreopoulou et al., 2010). The percentage of clerosterol was relatively stable, which agrees with the findings of Lazzez, Perri et al., (2008). The same was observed for campesterol, sitostanol, Δ^5-avenasterol, and erythrodiol + uvaol.

Concerning the amount of β-Sitosterol, representing the most copious compound in the sterolic fraction, the maximum relative amount (about 91.49%) was observed for HD1, whereas the lowest content was about 89.67% for HD2. The Δ5-avenasterol, the second most abundant sterolic compound, reached 4.47% during the maturity process (HD2). Several authors had found a negative correlation between β-sitosterol content, Δ^5-avenasterol and Δ^7-avenasterol in different varieties (Koutsaftakis, Kotsifaki et al., 1999, Fernández-Cuesta, León et al., 2013, Lukić, Lukić et al., 2013, Yorulmaz, Erinc et al., 2013, Noorali, Barzegar et al., 2014). They stated that β-Sitosterol is minimum and Δ^5-avenasterol and Δ^7-avenasterol is maximum when olives are obtained at their optimum maturation stage. In this study, a decrease in β-Sitosterol

content and an increase in Δ^7-avenasterol was observed. This complements the results obtained by the latter authors. However, Δ^5-avenasterol content showed no significant differences between HD1 and HD2 in disagreement with the same research.

The third sterolic compound, the campesterol, reached the highest of 2.10% for HD2. Our samples presented low levels of this sterol (less than the 4% limit value), and no significant difference was observed between HD1 and HD2. As for the stigmasterol, the authors found its high content to be an indicator for lower olive oil/olive fruit quality (JM, S et al., 1996, Koutsaftakis, Kotsifaki et al., 1999, Gutiérrez and Fernández 2002, Temime, Manai et al., 2008). This is in agreement with the results shown in Table 1 and Table 3. HD2 (VOO) has a lower quality than HD1 (EVOO) and thus higher stigmasterol content. The campesterol/stigmasterol ratio, another parameter classified as a quality index of olive oils (Koutsaftakis, Kotsifaki et al., 1999), also decreased as the harvest date is delayed. The Δ^7-Stigmastenol and $\Delta^{5,24}$-stigmastadienol witnessed a significant increase in HD2. The primary factor of this increase is the delay in the harvest. Erythrodiol + uvaol content in all the oil samples studied was below the limit of 4.5% and was not affected by the delay of harvest time.

Table 3. Relative amounts (%) of sterols and triterpene diols determined in olive oils at two harvest dates (October and November)

	HD1	HD2	IOC standards
Relative amount (%)			
Cholesterol	0.05a ± 0.02a	0.04 ± 0.02a	≤0.5
Campesterol	2.06 ± 0.83a	2.10 ± 0.74a	≤4
Stigmasterol	0.44 ± 0.29b	0.72 ± 0.55a	<campesterol
Campesterol/Stigmasterol ratio	5.20 ± 1.29a	3.56 ± 1.52b	
Clerosterol	0.69± 0.24a	0.71 ± 0.20a	
β-Sitosterol	91.49 ± 3.51a	89.67 ± 3.33b	
Sitostanol	0.15 ± 0.09a	0.15 ± 0.13a	
Δ^5-Avenasterol	3.99 ± 2.04a	4.47 ± 2.21a	
$\Delta^{5,24}$-Stigmastadienol	0.35 ± 0.20b	0.50 ± 0.25a	
Δ^7-Stigmastenol	0.35 ± 0.20b	0.69 ± 0.38a	≤0.5
Δ^7-Avenasterol	0.49 ± 0.37b	0.71 ± 0.40a	
Apparent β-Sitosterol	96.59 ± 1.48a	95.52 ± 1.69a	≥93
Erythrodiol + Uvaol	2.55 ± 1.21a	2.18 ± 1.07a	4.5%
Total sterols (mg/Kg)	894.40 ± 211.84b	1077.48 ± 327.7a	≤1000

Results are denoted as mean value ± standard deviation of two replicates. Different letters in the same row show significant differences (Tukey's test, $p \leq 0.05$).

3.4 Volatile compounds

FGC was carried out to detect the volatile compounds that may arise due to the delaying of olive fruit harvest, particularly the Soury variety.

A PCA model with 21 olive oil samples composing the HD1 group and 42 olive oil samples, belonging to the group HD2 and 10201 data points (constituting the flash GC chromatogram), i.e., an initial X data matrix of 63 x 10201 was conditioned. The initial chromatograms were corrected for misalignments before any further analysis using correlation optimized warping (COW) (Nielsen, Carstensen et al., 1998). Then data were then normalized using standard normal variate (SNV) (Zeaiter and Rutledge 2009).

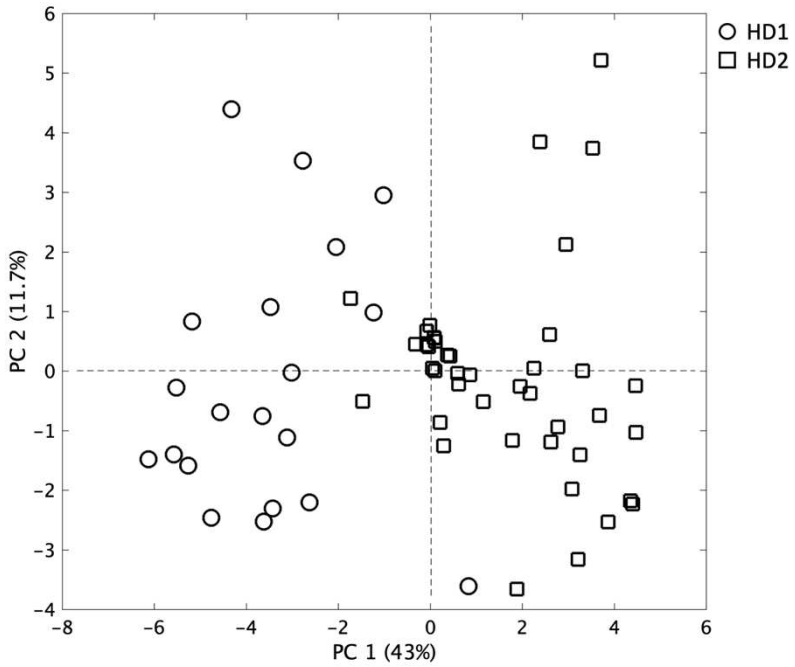

Figure 1. PCA scatter plots of the two groups (HD1 and HD2) obtained by analyzing the flash-GC chromatogram

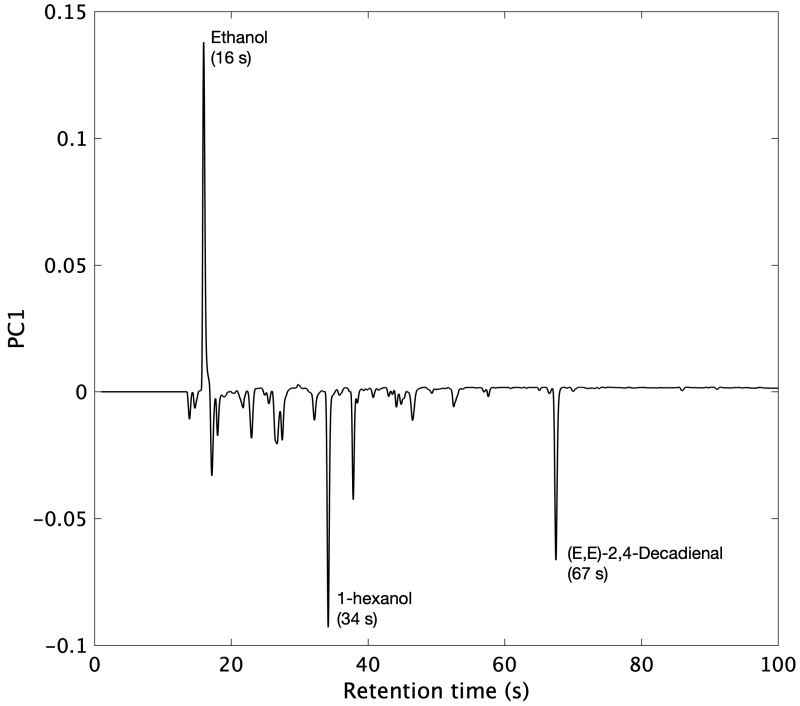

Figure 2. Loading plot showing the peaks differentiating HD1 from HD2

Six principal components (PCs) were extracted, covering 86.5 % of the total variance, where the first two components accounted for 54.7 % of the variability in the data set. The first two PCs were plotted, showing a good differentiation between HD1 and HD2 (Figure 1). PC1 clearly shows the discrimination between HD1 and HD2. One-way ANOVA was also conducted on PC1 scores as an extra measure of certainty for the PCA results. A significant difference was also noted between HD1 and HD2 ($p \leq 0.05$) as shown in Figure 3. In comparing HD1 and HD2, three peaks are identified as the determinant factor in the separation of the two latter groups (Figure 2). The first peak is ethanol, belonging to one of the major volatile compounds of olive oil, i.e., alcohols. The Kovats indices (KI) for this peak was identified through AroChembase software and compared with KI available in the literature (Table 4).

Table 4. Olive oil volatile compounds based on Kovats indices (KI) calculated on retention time (RT) for two columns

RT DB5 (sec)	RT DB1701 (sec)	KI (DB5)	KI (DB1701)	Possible chemical candidate	Reference
16	39	440	883	Ethanol	(Ivanova-Petropulos, Mitrev et al., 2015) (Silva, Freitas et al., 2012) (Silva, Freitas et al.. 2012) (Brkić Bubola, Koprivnjak et al., 2012)
34	59	835	1340	1-hexanol	(Ben Mansour, Chtourou et al., 2017) (Pouliarekou, Badeka et al., 2011) (Silva, Freitas et al.. 2012)
67	92	1320	1730	(E,E)-2,4 -Decadienal	(Kesen, Kelebek et al., 2013) (Reiners and Grosch 1998)

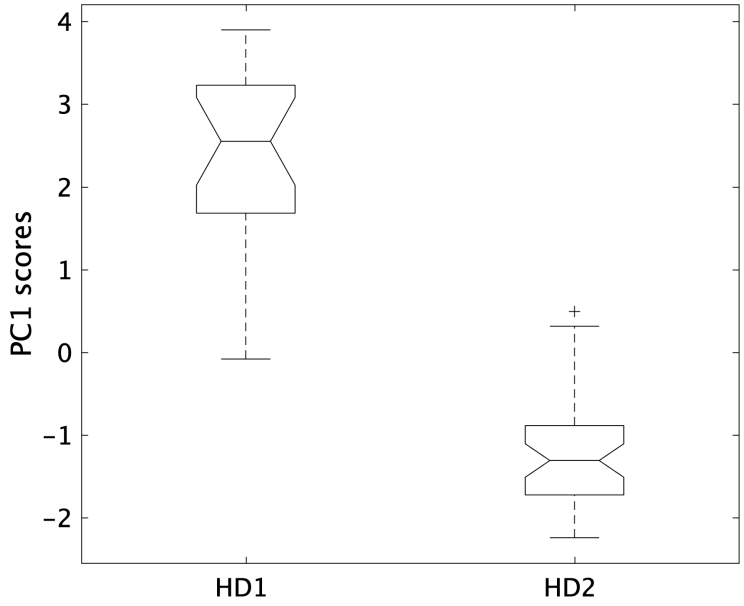

Figure 3. Boxplot showing the discrimination of both groups HD1 and HD2 ($p \leq 0.05$)

Ethanol is a major component, which is related to the fermentation activity occurring before olive oil extraction (Beltrán, Bejaoui et al., 2015), and it is responsible for the sensory descriptor "Alcohol" (Morales, Luna et al., 2005).The possible source of ethanol can be from the olive fruit itself. Beltrán, Bejaoui et al.. (2015) showed that ethanol content increase during the ripening process. This increase in directed by alcohol dehydrogenase activity where its levels were high due to advanced stages of maturation. The remaining two high-intensity peaks, mostly characterizing HD1, are 1-hexanol and (E, E)-2,4-decadienal. 1-hexanol appears in HD1 as a sign of unripe olives (Aparicio and Morales 1998). It is known that at a later harvest date (HD2), the aromatics, like 1-hexanol, describing the term "fruity-grassy" are lower while other negative aromatic notes increase like ethanol (Salvador, Aranda et al.. 2001). As for (E, E)-2,4-decadienal, its presence in HD1 may be due to the long incubation period of olive oil just before injection into the FGC, which led to such a volatile compound. This peak is also present in HD2; however, its presence is hidden by the ethanol high-intensity peak (Figure 4).

This is backed up by several studies whose results indicate the presence of (E,E)-2,4-decadienal in seed oil after applying heat (Andrikopoulos, Chiou et al., 2004, Boskou, Salta et al., 2006).

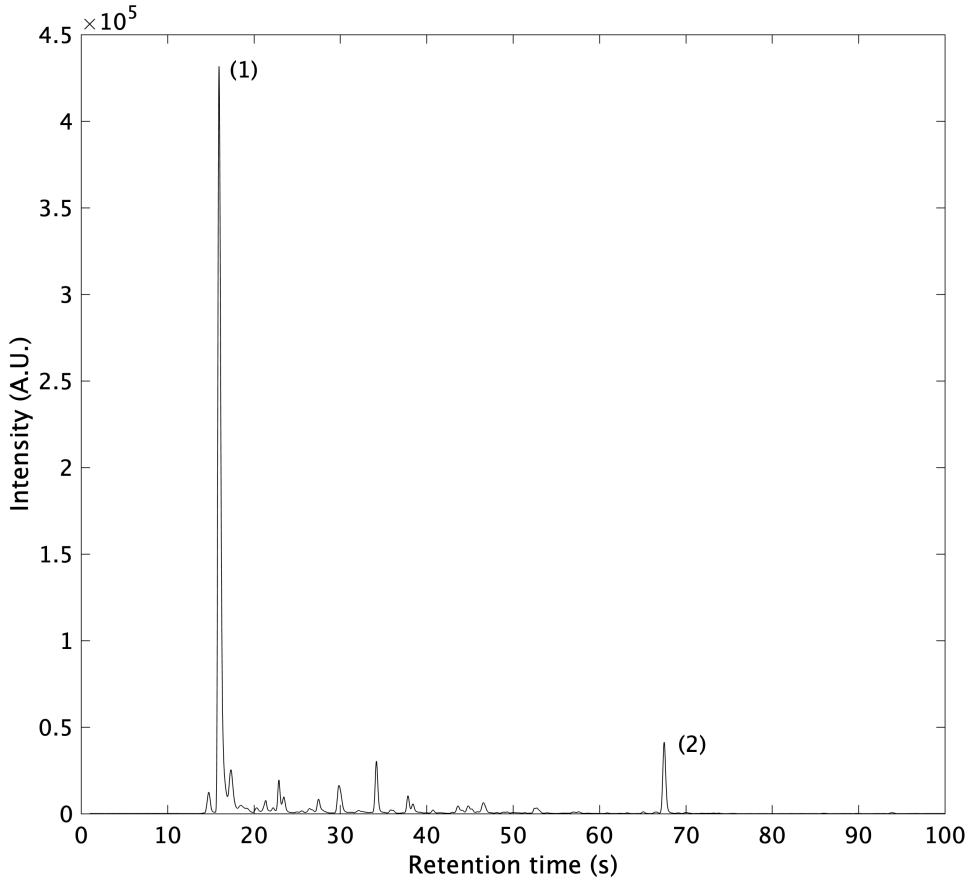

Figure 4. Flash GC chromatogram of olive oil sample belonging to HD2. (1): Ethanol; (2): (E, E)-2,4-decadienal.

Conclusion

The harvest date appears to have a decisive role in the qualitative characteristics, saponifiable and non-saponifiable fraction of Lebanese olive oil. All results obtained showed that the harvest date strongly influenced acidity and total polyphenols. Besides, a change in the fatty acid profile characterized by a higher linoleic and lower oleic content, an increase in Δ^7-stigmastenol exceeding the limit set by the IOC standards, and a dominating aromatic compound (ethanol) was noticed for olive fruits picked at a later date

Generally, it can be concluded that the harvest from mid-October to the first of November could be a criterion to obtain olive oil of the highest quality grade. Harvesting at this period is preferred for the Soury variety, as it ripens most of the macro and minor components exceed the limits set by the international olive council for extra virgin olive oil.

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Chapter 5: Does Variability Affect the Performance of Front-Face Fluorescence Spectroscopy? A Study Case on Commercial Lebanese Olive Oil

Fluorescence spectroscopy is known as a rapid, non-invasive and highly sensitive technique for analysis of food. It is known to be more cost-efficient where analysis can be carried out directly on the intact samples without any-pretreatment or usage of chemical. This type of spectroscopy is useful for routine quality control.

This chapter presents the potential of front-face fluorescence spectroscopy coupled with chemometric techniques, namely multiple linear regression (MLR) applied on parallel factor (PARAFAC) scores and partial least squares (PLS), to predict Lebanese olive oil samples possessing a wide range of variability. This variability includes samples harvested at different dates and from two seasons, processed using different extraction methods, collected from different altitudes and other factors. Would fluorescence spectroscopy prove its usefulness again in the grading of olive oil samples with significant savings of analytical measurements?

Does Variability Affect the Performance of Front-Face Fluorescence Spectroscopy? A Study Case on Commercial Lebanese Olive Oil

Omar H. Dib[1,2*], Jad Rizkalah[2], Rita Yaacoub[2], Hussein Dib[2], Nathalie Locquet[1,4], Luc Eveleigh[3] and Christophe B. Y. Cordella[1,4], Ali Bassal[2]

[1] UMR PNCA, AgroParisTech, INRA, Université Paris-Saclay, Paris, France

[2] Department of Food Science and Technology, Lebanese University, Beirut, Dekweneh, Lebanon

[3] Ingénierie Procédés Aliments, INRA, AgroParisTech, Université Paris-Saclay, Massy, France

[4] C[2]B research group – Chimiométrie pour la Caractérisation de Biomarqueurs

*Corresponding author: Christophe B.Y. Cordella;

UMR PNCA, AgroParisTech, INRA, Université Paris-Saclay, Paris, France

C[2]B research group – Chimiométrie pour la Caractérisation de Biomarqueurs

Tel: +33(1)666416900;

Email: (christophe.cordella@inrae.fr)

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Omar H. Dib, Jad Rizkalah, Rita Yaacoub, Hussein Dib, Nathalie Locquet, Luc Eveleigh, Christophe B. Y. Cordella, and Ali Bassal. The first draft of the manuscript was written by Omar H.Dib and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Abstract

The potential of front-face fluorescence spectroscopy coupled with chemometric techniques, namely multiple linear regression (MLR) applied on parallel factor (PARAFAC) scores and partial least squares (PLS), was tested on Lebanese olive oil samples possessing natural variability within their chemical parameters. Ninety-six olive oil samples have been harvested at different dates and from two seasons, processed using different extraction methods, collected from different altitudes and other factors that can increase the variability of the samples' chemical composition. Fluorescence excitation-emission matrices (EEM) of the collected samples were measured, and the relationship between them and the chemical parameters was examined. Twenty-two MLR regression models based on PARAFAC scores were generated, the majority of which showed a good correlation coefficient ($R>0.7$ for ten predicted variables). A second model using PLS on the unfolded EEM was also conducted to improve the regression and to assess if it can handle the variability in hand. However, similar results, with a slight improvement over the MLR model, were obtained. In a non-experimental design, such variability may hinder the potentials of front-face fluorescence; however average to good MLR and PLS models were obtained, predicting the Lebanese olive oil deterioration quality parameters and fatty acid content.

Keywords: Front-face fluorescence; PARAFAC; Lebanese olive oils; Multiple Linear Regression; Partial Least Square; Variability;

Introduction

Lebanese olive oil holds the highest commercial prospects amongst all the other crops covering about 21% of the cultivated area and 69% of the land cultivated with fruits. Although Lebanon is considered a small-scale producer of olive oil compared to other Mediterranean countries, it has the reputation of producing high-quality artisanal oils. This high-end product is increasingly being exported to countries like Saudi Arabia (2,635 tons), United States (1,315 tons), and others [1].

To keep up with such high-quality product, particularly olive oil's nutritional and sensory properties, specialized institutions such as the International Olive Council (IOC) have set explicit olive oil quality controls and regulations [2]. These strict controls require diverse analytical methods of different complexities, ranging from simple procedures (UV spectrophotometric indices determination) to more complex ones (e.g., determination of the composition and content of sterols and triterpene dialcohols). The complexity of some of the procedures is also reflected in the ample time and materials required to determine olive oil genuineness and quality [3]. Nowadays, the presence of new sample-holder accessories, software, and numerous chemometrics applications gave way for faster and simpler analytical tools, i.e., spectroscopic techniques, principally front-face fluorescence spectroscopy.

Front-face fluorescence spectroscopy has been successfully used as a rapid, non-invasive, and highly sensitive technique for analysis of olive oil quality and showed to be more cost-efficient compared to other analytical procedures [4]. This type of spectroscopy can be particularly useful as routine quality control since the analysis is carried out directly on the intact samples without any-pretreatment or usage of chemical reagents [5]. These advantages have rendered front-face fluorescence as an important tool in the evaluation of olive oils properties. There are several published studies showing the diversity of fluorescence applications [6]. These include studies on the detection of olive oil adulteration with vegetable oils or pomace oil [7,8], on monitoring thermal oxidation [9-13], on the prediction of origin [14], and quality parameters evaluation [3,15]. As for the latter application, several studies have showed that the fluorescence excitation-emission spectra of olive oils contain information on quality parameters such as acidity, peroxide value, and UV absorbance at 232 nm (K232) and 270 nm (K270).

Acidity, based on IOC, is one of the crucial quality parameters for classification of olive oil into different categories. The increase in acidity is due to free fatty acids (palmitic, oleic, and linoleic acids) resulting from triglyceride decomposition. Studies on this parameter have focused on the fluorescence band at 429-545 nm, which is due to oleic acid [16]. Kyriakidis and Skarkalis [17] also suggested that the low fluorescence intensities at 445 and 475 nm are attributable to the

high content of monounsaturated fatty acids (oleic acid). The study by Poulli, Mousdis, and Georgiou [16] also showed the link between the fluorescent bands at 273 and 325 nm and palmitic and linoleic acids, respectively. Other important quality parameters are peroxide value and K270. These two precursors of primary and secondary oxidation products have also been linked to high fluorescent intensities at 450-460 nm and 470 nm, respectively [3,18]. The increase in the fluorescence intensity in these regions is due to the oxidation of fatty acids into conjugated hydrogen peroxides and conjugated triene and diene systems. The fluorescence emission spectra also contain information on fluorescent molecules such as chlorophyll (600-700 nm), Vit E (525 nm), polyphenols, and α-tocopherol (300–390 nm) [17].

A lot of emphasis has been put on the evaluation of olive oil quality using fluorescence spectroscopy. However, most of the samples in the studies have been altered so that mostly two groups of olive oil are present: natural state and oxidized state. Thus, a controlled experimental design, whether in the extraction of olive oil or oxidizing the oil at an incremental temperature, is consistent throughout the studies [3,9,10,13,19]. Such patterns have probably highlighted the utmost fluorescence role in predicting olive oil quality (R^2 as high as 0.99). However, in a non-experimental design, several variables exist, such as mode of extraction, harvest time, season effect, maturity index, storage conditions, etc. that significant affect olive oil quality. The lack of consistency or fixed pattern might alter the predictive models' capability of olive oil quality by fluorescence spectroscopy.

In light of the above, this study offers a practical case in which the efficiency of fluorescence spectroscopy combined with multi-way and multivariate chemometric tools is used for rapid quantitative and qualitative characterization of un-altered samples of Lebanese olive oil.

Materials and Methods

Samples

Ninety-six samples of Lebanese olive oil were evaluated in this study. The first two groups of samples, namely, uncontrolled-2016 (n= 54) and uncontrolled-2017 (n = 20) compromised of different olive oil qualities, were obtained directly from the farmers during the harvesting seasons 2016 and 2017. These two groups are referred to as "Uncontrolled" since all the variables involved in producing olive oil starting from harvesting date to olive oil storage, are based on the farmers' practices (Online Resource 1).

The third group (n=22), referred to as controlled, included olive oil that was extracted using an Abencor analyzer (Mc2 Ingenieria y Sistemas, Seville, Spain) from healthy olive fruits (collected in 2017 harvesting season) of a maturity index of 1.76 [20]. Olives were crushed using a hammer mill where the resulting olive paste was distributed into stainless steel

containers and kneaded in a mixer at 50 rpm for 30 mins at 28°C. The obtained paste was then centrifuged at 3500 rpm for 2 mins.

All oils were stored in dark glass bottles at 4°C without headspace until analysis. The characteristics of all of the studied samples are presented in Table 1.

Physico-chemical parameters determination

Quality indicators

Acidity defined as a percentage of oleic acid (%), peroxide value expressed in milliequivalents of active oxygen per kilogram of oil (meq O2/kg), and UV spectrophotometric indices (K232, K270) were determined according to the European Union Commission Regulation EEC No 2568/91 [21]. Spectrophotometric determinations were made using a UV spectrophotometer (Hitachi U-2900, Spectrophotometer, Japan).

Total polyphenols

The total polyphenol content of olive oil was determined according to Montedoro, Servilli and Baldiolo et al. [22] at 765 nm by a UV spectrophotometer with a slight modification (Hitachi U-2900, Spectrophotometer, Japan).

Extraction was carried out on a 5g of oil by adding 10 ml hexane and 15 ml methanol/water (60:40 v/v). 0.2 ml of the aqueous phase was collected after vortexing for 3 minutes and centrifuged at 1900 rpm for 12 minutes at 5°C. The aqueous phase was mixed with 5 ml of Folin–Ciocalteu (1:10 V/V in distilled water) reagent and after 3 mins, with 1 ml of saturated Na_2CO_3. The final solution was maintained in a dark place at room temperature for 60 minutes. Caffeic acid standard solutions were used to calibrate the method ($R^2 = 0.9995$).

Chlorophyll

Chlorophyll concentrations of the samples were determined according to Isabel, Rejano-Navarro, and Gandul-Rojas et al. [23] with a slight modification in the procedure. 5 g of olive oil was dissolved in 25 mL of acetone, and the absorbance of the samples at 670 nm and 642,5 nm were measured with a UV spectrophotometer (Hitachi U-2900, Spectrophotometer, Japan).

Fatty acid

The fatty acid methyl esters (FAME) of olive oil were extracted according to the International Olive Council (IOC) method COI/T.20/Doc. No 33. [24]. The FAME was prepared according to Part I and Part II of this method.

The fatty acid profile was determined using a gas chromatograph (GC) Shimadzu GC-2025 (Kyoto, Japan) equipped with a capillary column SP-2380 (30 m × 0.32 mm i.d. x 0.20 μm film thickness; Supelco, Bellefonte, PA, USA) and a flame ionization detector (FID). Injector and

detector temperatures were held at 230°C and 240°C, respectively. The injection volume into a split GC port was 1 µL, and a split injection mode (1/100) was used. The following oven temperature program was used: initial temperature 165°C held for 10 min; ramped at 1.5°C min^{-1} up to 200°C. Helium was employed as the carrier gas at a flow rate of 1 mL/min. Fatty acids were identified by comparing the retention time of experimental peaks with those obtained by the external standard mixture.

All physicochemical analyses were performed in duplicates for each sample.

3D front-face fluorescence spectroscopy

3D front-face spectra were measured directly on the olive oil samples without prior preparation using a fluorescence spectrophotometer (Hitachi, F-7000 FL, 2240-001) equipped with a xenon lamp, excitation and emission monochromators, and a front-face cell holder. Measurements were carried out on the samples filled in quartz cuvettes. The front-face angle was set at 56° to maximize signal to noise ratio. The excitation wavelengths ranged from 280 to 540 nm and emission wavelengths from 280 to 700 nm, with 20 and 5 nm intervals in excitation and emission acquisitions, respectively.

Excitation and emission monochromator slit widths were set at 4 nm, and the scan rate was 240 $nm.min^{-1}$. The photomultiplier (PMT) detector voltage of 350 V was used. All samples were analyzed in duplicates on two different sides of the cuvette. The excitation-emission matrices (EEMs) were placed in a 3-way cubic array with the sample in the first mode. [96 x 14 x 85] x 2 duplicates.

Data Processing

Spectral Pretreatment

Rayleigh signals were removed by inserting zero values at $\lambda_{emission} \leq \lambda_{excitation}$ (since no emission is present at wavelengths below the excitation wavelength) and by inserting missing values at $\lambda_{emission} = \lambda_{excitation} + 5$ to $\lambda_{emission} = \lambda_{excitation} + 25$ [25,26]. The insertion of zeros and missing values were performed with homemade algorithms.

Unfold scaling pretreatment

All the EEMs in the pretreated cube were unfolded according to the excitation mode (transformed into a matrix of the concatenated emissions). Then for each sample, the norm of all the emissions is used to scale the data (dividing each emission by the norm). The scaled emissions of all the samples are finally refolded back to reform the three-way array (Fig. 1).

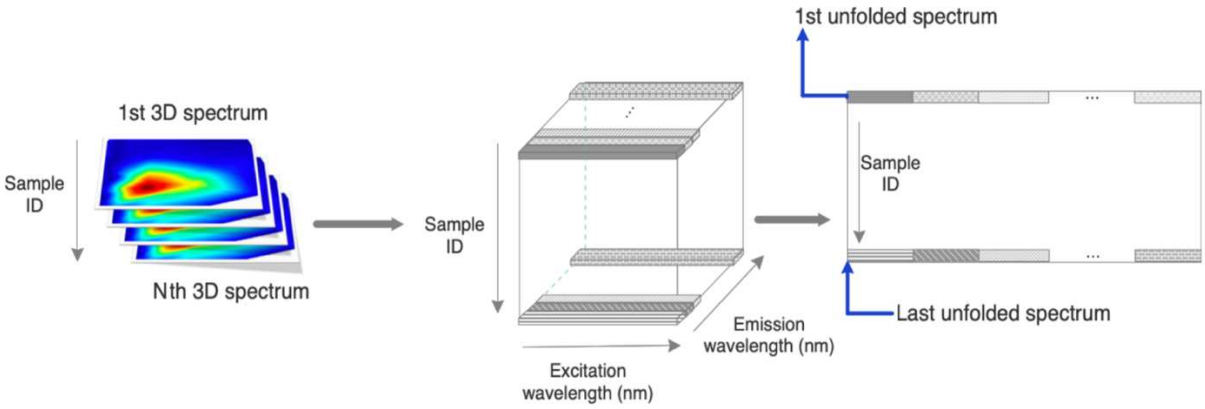

Fig. 1 Graph representing the folding of the spectra data into a cube (where PARAFAC is applied) and then unfolding into a two-way data (where PLS is applied).

Statistics

One-Way ANOVA

One-way ANOVA was conducted to highlight the variability (in terms of physicochemical parameters) between the means of the three sample groups. Tukey's test was used to establish the significance of difference at a 5 % level among means. Although the sample size of the three groups is not the same, this tool's sole purpose is to display the variation present between the three groups.

Parallel factor analysis

Parallel factor analysis (PARAFAC) models with 1 - 8 components were fitted on the front-face fluorescence data using the N-way toolbox for MATLAB [26,27]. Non-negativity constraint was applied in all three modes since concentrations, and spectral values are always positive. Corcondia (core consistency diagnosis) was used to measure the percentage of agreement of the PARAFAC models with ideal tri-linearity. The number of PARAFAC factors to retain and the models' validity were assessed through the residual analysis, the physical appropriateness of the spectral decomposition parameters and Corcondia [26].

Split-half analysis was also performed for validation. The randomized data was divided into two halves, and a PARAFAC model was created on both halves. The result of this test was to ensure that more or less identical excitation and emission loadings are obtained [28].

Parallel factor analysis - Multiple linear Regression

Regression models using multiple linear regression (MLR) were performed between conventionally measured quality parameters (**y**) and the scores of the PARAFAC factors. Correlation coefficients (R) were used to evaluate the regression models' quality after stepwise removal of insignificant factors.

Elimination of outliers was achieved by inspection of leverages and residues, where observations having high residues and leverages were crossed out to improve the models.

Partial least squares

Partial least squares regression (PLS) is a generalization of the above method (MLR) [29]. However, PLS was used to test the correlation on the unfolded EEM (192 x 1192), i.e., unfolding the cube structure from 3-way to 2-way array, unlike the case in MLR where PARAFAC scores were generated based on the original 3-way array. The unfolded EEM was then linked to the chemical parameters of the olive oil samples. Data treatment involved mean centering, and outliers were eliminated by inspecting leverages and residues to improve the PLS model [30].

Data were analyzed using Matlab version R2015b (The Mathworks Inc., MA, USA).

Table 1. Chemical properties of the samples presented in this study over two harvesting seasons, 2016 and 2017

Fatty acids (%) and quality indices	Uncontrolled (2016)			Controlled 2017			Uncontrolled (2017)		
	Min	Median	Max	Min	Median	Max	Min	Median	Max
Acidity (g/100 g)	0.26	1.17	8.40	0.20	0.30	0.48	0.34	0.73	1.73
Peroxide value (meq O_2/kg)	6.70	12.76	59.22	1.75	2.82	6.28	2.89	9.19	15.30
Total polyphenols (mg/ml)	6.49	31.70	82.79	68.57	139.18	222.47	25.77	64.07	106.05
K232	1.39	2.06	3.26	1.51	1.76	2.22	1.54	1.87	2.56
K270	0.11	0.16	0.25	0.12	0.16	0.25	0.10	0.16	0.63
K266	0.12	0.16	0.25	0.13	0.18	0.26	0.11	0.17	0.60
K274	0.11	0.14	0.24	0.12	0.17	0.26	0.10	0.17	0.62
Delta K	0.00	0.00	0.04	-0.01	0.00	0.00	-0.05	0.00	0.01
Total chlorophyll (mg/L)	0.73	1.93	10.56	0.92	1.94	6.61	0.66	1.88	3.93
Beta carotene (mg/L)	0.40	0.74	3.11	0.06	0.53	8.87	0.12	0.58	3.87
Palmitic acid	9.84	11.74	14.01	8.12	10.48	13.76	7.61	9.71	13.04
Palmitoleic acid	0.41	0.57	1.39	0.30	0.44	1.01	0.32	0.42	0.80
Decenenic acid	0.07	0.18	0.31	0.04	0.17	0.26	0.08	0.19	0.26
Decenoic acid	0.10	0.21	0.41	0.06	0.20	0.33	0.11	0.20	0.27
Stearic acid	2.09	4.00	5.08	2.18	3.91	4.72	3.23	4.23	4.72
Oleic acid	66.00	70.34	76.10	69.40	73.94	78.74	70.61	73.54	77.88
Linoleic acid	7.48	11.27	14.73	6.24	8.76	12.12	7.74	9.65	12.46
Arachidic acid	0.22	0.58	0.70	0.33	0.60	0.76	0.50	0.69	0.83
Linolenic acid	0.49	0.64	0.84	0.41	0.58	0.77	0.48	0.59	0.70
Eicosenic acid	0.22	0.32	0.44	0.24	0.35	0.41	0.26	0.36	0.43
Behenic acid	0.04	0.15	0.19	0.08	0.17	0.22	0.11	0.19	0.24
Lignoceric acid	0.04	0.06	0.08	0.04	0.08	0.20	0.06	0.09	0.15

Results and Discussion

One-Way ANOVA

Olive oil quality is usually based on chemical parameters such as acidity, peroxide value, fatty acids, total polyphenol content and many others. The IOC lay down trade standards and policies to improve the overall quality of the end product and to ensure the fairness of the competition [31]. For instance, acidity is one of the leading parameters in classifying olive oil into different quality categories. This parameter is calculated based on free fatty acids that result from the triglyceride hydrolysis. Samples with an acidity of ≤0.8 are recognized as the best olive oil quality (EVOO), as in the case of controlled 2017. The remaining groups have a higher acidity, thus lower quality, and are recognized as VOO (Table 2). Peroxide value is the second most important quality parameter and it is used to determine the rancidity of the samples subjected to oxidation. This parameter is linked to most olive oil chemical parameters like K232, total polyphenols (antioxidants), and fatty acids. For instance, the increase in peroxide value coefficient lead to the decrease of polyphenol content in olive oil. This is clearly presented in Table 2, as the controlled group has the highest total polyphenol content and the lowest PV.

As for fatty acids, the mean concentration of behenic acid, arachidic, and palmitic acid are different for each of the three groups. As noticed in Table 2, the mean concentration of oleic, eiconsenic and lignoceric acids is the highest in olive oil samples belonging to controlled and uncontrolled 2017, and the remaining fatty acids (except for stearic) are the highest in olive oil samples from uncontrolled 2016. Among the remaining fatty acids are the oleic and linoleic acids. These two have an inverse relation, where the deterioration in olive oil quality lead to the increase in linoleic and decrease in oleic acid content, as observed in the uncontrolled group 2016.

According to Table 2, controlled 2017 represents the samples with the best olive oil quality (EVOO) followed by uncontrolled 2017 and uncontrolled 2016, based on IOC trade standards for olive oil [31]. For the uncontrolled groups, the variation in the quality indices and fatty acids results from various factors affecting all stages, from oil formation up to storage. They can be divided into factors affecting oil before extraction (environmental and climatic conditions, olive ripeness), or those affecting during oil extraction (milling, oil extraction system) or variables acting after oil extraction (oil storage, temperature, light) [32]. These factors affect the minor and major chemical constituents of olive oil, each in its own way. For example, the harvest date has a determinantal effect on both quality indices and fatty acid profile. In general, as fruit matures, total polyphenol content decrease, and the amount of polyunsaturated fatty acids like linoleic and linolenic acids increases [33]. This kind of variation presented within the three

groups, especially in the uncontrolled groups, should affect MLR and PLS models' predicting abilities, especially that the measured chemical properties of each group are assembled in one matrix (Y).

PARAFAC

A PARAFAC model was used over other multiway decomposition tools like Tucker3 model due to its simplicity and uniqueness of its decomposition parameters [9]. Before applying PARAFAC, treatment of the EEMs by eliminating 1st and 2nd order Rayleigh (in-house written algorithm) was conducted. The fluorescence landscapes of the olive oil sample with Rayleigh removal and after replacement of missing values are given in Figures 2a & 2b. Then the sample's fluorescence patterns were analyzed by the application of PARAFAC.

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk} \quad (1)$$

The objective is to resolve the fluorescence signal into individual contributions of fluorescence bilinear profiles varying only in intensity amongst the samples. Modes 2 (b_jf) and 3 (c_kf) obtained by the PARAFAC decomposition allowed the description of the emission and excitation profiles of each factor corresponding to the homogenous fluorescence profiles composing the bi-dimensional fluorescence landscapes. Mode one obtained by the PARAFAC decomposition i.e., the PARAFAC scores (a_if), was used to build the calibration models of conventionally analyzed physicochemical parameters [15,26,34]. PARAFAC models on fluorescence landscapes were estimated with one to eight factors. They were compared based on Corcondia, split-half validation, and visual inspection of both the residuals and the loadings.

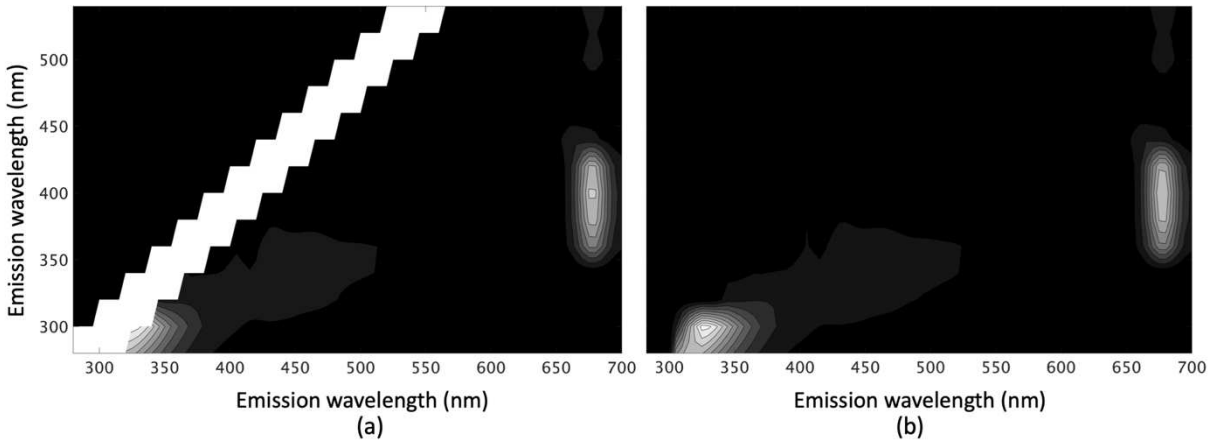

Fig. 2 Fluorescence landscapes of (a) pretreated EEM by removal of 1st and 2nd order Rayleigh scattering and (b) reconstituted EEM by replacing missing values

Table 2. Mean values and standard deviations of quality parameters and fatty acids composition (%) of the three groups.

Fatty Acids (%) and Quality indices	**Uncontrolled 2016**	**Uncontrolled 2017**	**controlled 2017**	**EVOO***
Acidity (g/100 g)	1.49 ± 1.29c	0.86 ± 0.38b	0.30 ± 0.05a	≤0.8
Peroxide value (meq O_2/kg)	15.90 ± 10.78c	9.39 ± 3.53b	3.06 ± 1.03a	≤20
Total polyphenols (mg/ml)	35.19 ± 15.94a	64.46 ± 23.63b	142.13 ± 44.97c	
K232	2.14 ± 0.37b	1.93 ± 0.26a	1.82 ± 0.21a	≤2.5
K270	0.16 ± 0.03a	0.23 ± 0.16b	0.18 ± 0.04a	≤0.22
K266	0.16 ± 0.03a	0.23 ± 0.15b	0.19 ± 0.04a	
K274	0.15 ± 0.01a	0.23 ± 0.16b	0.18 ± 0.04a	
Delta K	0.00 ± 0.01b	0.00 ± 0.01b	0.00 ± 0.00a	
Total chlorophyll (mg/L)	2.29 ± 1.63a	1.94 ± 0.93a	2.51 ± 1.60a	
Beta carotene (mg/L)	0.85 ± 0.42a	0.90 ± 0.93a	1.50 ± 2.34b	
Palmitic acid	11.79 ± 1.05c	9.61 ± 1.13a	10.59 ± 1.42b	2.0-7.5
Palmitoleic acid	0.63 ± 0.20b	0.45 ± 0.10a	0.49 ± 0.17a	0.3-3.5
Decenenic acid	0.18 ± 0.05a	0.18 ± 0.04a	0.16 + 0.04a	
Decenoic acid	0.21 ± 0.05a	0.20 ± 0.04a	0.20 ± 0.05a	
Stearic acid	3.89 ± 0.60a	4.22 ± 0.36b	3.72 ± 0.69a	0.5 – 5
Oleic acid	70.19 ± 2.10a	73.54 ± 1.99b	73.93 ± 2.19b	55-83
Linoleic acid	11.29 ± 1.47b	9.75 ± 1.33a	9.04 ± 1.48a	3.51-21
Arachidic acid	0.56 ± 0.08a	0.69 ± 0.07c	0.61 ± 0.1b	
Linolenic acid	0.64 ± 0.08b	0.59 ± 0.07a	0.59 ± 0.08a	≤1
Eicosenic acid	0.33 ± 0.04a	0.36 ± 0.04b	0.35 ± 0.04b	
Behenic acid	0.14 ± 0.02a	0.19 ± 0.03c	0.17 ± 0.03b	
Lignoceric acid	0.06 ± 0.01a	0.09 ± 0.02b	0.09 ± 0.03b	

Results are denoted as mean value ± standard deviation of two replicates. Different letters within the same row show significant differences (Tukey's test, $p < 0.05$); *IOC standards representing the important chemical parameters defining extra virgin olive oil (EVOO)

All determinations were carried out in duplicate. Different letters in the same row show significant differences (Tukey's test, $p < 0.05$). The PARAFAC model with seven components, i.e., seven different fluorescence profiles, was considered as optimal (explained variance 99.64%, Corcondia 78.65). The number of iterations that led to the 7-factor model was equal to 38. The emission and excitation plots (modes 2 and 3, respectively) are shown in Figure 3. The components were ordered based on the explained variance of (**X**).

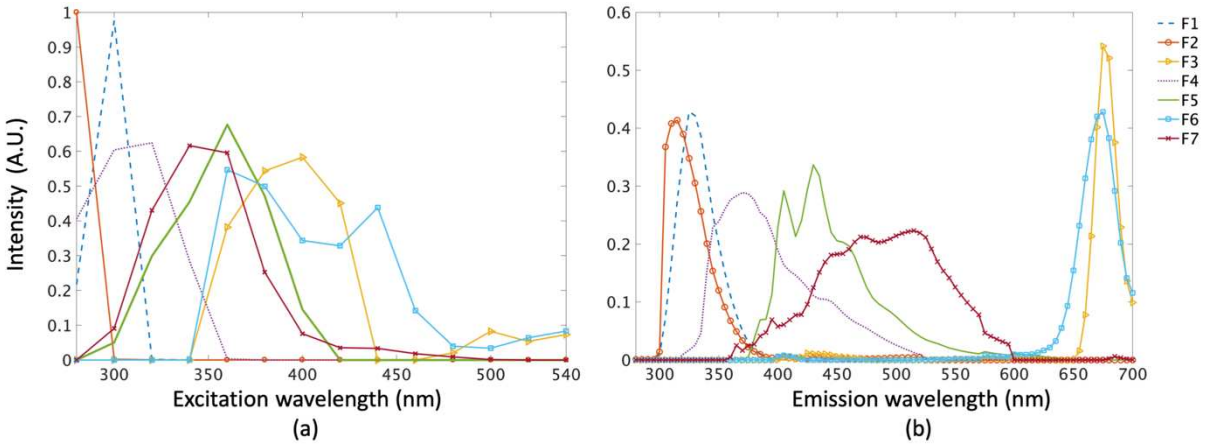

Fig. 3 (a) Excitation and, (b) emission loadings of a seven component PARAFAC model, based on the fluorescence landscapes of 96 olive oil

Several studies have appointed the PARAFAC factors excitation and emission maxima presented in figure 3 to tocopherol, polyphenols, oxidation products, and chlorophyll compounds [9,11,17,35-38]. Guimet, Ferré and Boqué et al.[36] applied PARAFAC in the evaluation of complete fluorescence landscapes of olive oils where its decomposition revealed four different fluorophores. The far dominating one was assigned to chlorophyll, and two of the derived fluorophores were assumed to be oxidation products with an excitation around 350 nm. This is verified because the fluorescence zone around 415-600 nm related to oxidation products (F4, F5, F7) hardly appears in virgin olive oils, as in Fig. 4a, whereas it is quite obvious in deteriorated olive oil samples as shown in Fig. 4b. As for chlorophyll (F3, F6) and polyphenols bands (F1, F2), both are noticed at an emission of 675 and 325 nm in both oxidized and fresh olive oil, however at different intensities. This difference is due to the increase of the oxidation products at the expense of both chlorophyll and polyphenols (Fig. 4a & 4b).

It is noteworthy to mention that PARAFAC profile scores ($\boldsymbol{a}_{if}$) are disproportional to the true fluorophore concentrations since they are profoundly disturbed by the samples' scattering and quenching properties. Nevertheless, the PARAFAC scores represent an amount of variability in the front-face fluorescence signal presented in terms of parallel profiles [39]. Given such variability, the scores are used in calibration over quality parameters measured using conventional physicochemical methods.

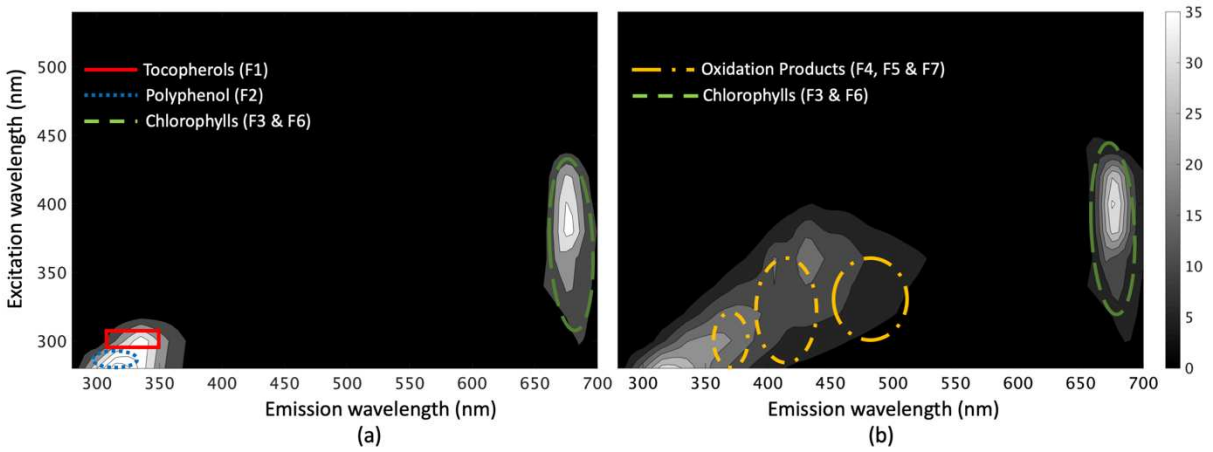

Fig. 4 Fluorescence contour map showing the spectral properties of (a) fresh and (b) oxidized olive oil

Multiple linear regression calibration models

Several regression modes were developed using multiple linear regression method were developed to verify the correlation between acquired front-face fluorescence signal and spectrophotometrically or chromatographically measured olive oil quality parameters. Calibration models are thus obtained via systematic information present in the front-face fluorescence signal. MLR models were fitted on PARAFAC scores, where seven components were used in the prediction. However, only significant factors ($p<0.05$) were kept for each model and were used to rebuild a new model with the remaining factors. Being given that several variables of $\boldsymbol{a}_{if}$ were used for the prediction of variable **y** (one at each time: acid value, peroxide value, delta K, total chlorophyll, beta-carotene, total polyphenols, and fatty acids), the regression was built according to the following equation:

$$\boldsymbol{y} = b_1\boldsymbol{a}_1 + b_2\boldsymbol{a}_2 + \ldots + b_7\boldsymbol{a}_7 + b_0 + \varepsilon \quad (2)$$

Thus, 22 calibrations models were generated; one for each physicochemical parameter and their fitting quality was interpreted in terms of correlation coefficient (R). The different regression models' parameters, together with the number of factors included in the model after outlier removal, are given in Table 3. As it is shown, the different regression models have R values higher than 0.6 (0.6-0.89) except for beta-carotene and middle Ks, which were excluded. The highest prediction was achieved for acid value, peroxide value, and delta K, as well as the fatty acids (linoleic and oleic). Total polyphenols and total chlorophyll were also well predicted with correlation coefficients of 0.73 and 0.71, respectively. Predicted values for acid value and peroxide values are plotted against measured values and are given in figure 5a & 5b.

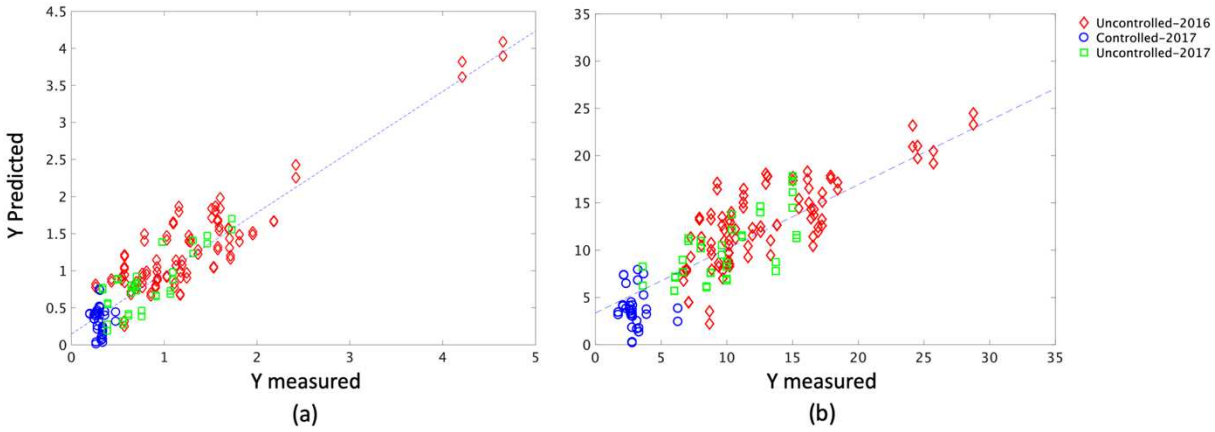

Fig. 5 The results of MLR on the PARAFAC scores in the emission range of 280- 700 nm, and the (a) acidity, (b) peroxide value of olive oil: predicted vs. measured plots

These results are concomitant with those of Engelsen [40] on oils. Tocopherols, polyphenols, chlorophyll/pheophytin and oxidation products related fluorophores contribute most to the acid value, peroxide value and delta K models prediction ability. These quality parameters are related to the oxidation/hydrolysis products and interrelate with levels of tocopherols, polyphenols, and chlorophyll, which are sensitive products to oxidation and act as antioxidants [12]. On the other hand, almost all fluorophores explained the MLR models of oleic and linoleic acids. Chlorophylls were also correlated with factors F3 and F6 related to chlorophyll fluorescence range zone, which makes sense. Similarly, polyphenols are best predicted with F2 related to polyphenols fluorescence zone.

Partial least square regression models

Partial least square regression was also used for olive oil quantification. Cross-validation was applied for all regression models based on leave two out method [41]. Two observations (duplicates of each sample) were systematically omitted at a time from the regression model, and the remaining samples were used to recalculate the model. The latter model was used to predict the response for the omitted observations. This procedure was repeated until all samples are omitted and fit [42].

Then the optimum number of latent variables was selected based on avoiding overfitting and high standard errors of both calibration and validations sets. The predictability of the models was examined by calculating the residual standard error of calibration (RMSEC) for the calibration data set, the residual standard error of validation (RMSEV) for the validation data set, and the correlation coefficient (R). The statistics of PLS of calibration and validation data sets are given in Table 3. A good predictive model should present a high validation R and a low RMSEC and RMSEV**.** PLS model ability was examined with the validation set.

The PLS regression models representing quality indices have validation R values higher than 0.6 (0.6-0.81) with low RMSEC and RMSEV values except for total chlorophyll, beta-carotene, and middle Ks (Table 3). The highest prediction was achieved for acid value, peroxide value, and delta K with a high validation R-value indicating a good prediction model. Whereas the regression model of total polyphenols showed medium prediction models (R<0.8). As for fatty acids, Linoleic and oleic acid were best predicted with a validation R of 0.8. The remaining models showed adequate prediction capabilities except for palmitoleic acid with a validation correlation coefficient as low as 0.05, which is not used for quantitative predictions. Predicted values for oleic and linoleic acids are plotted against measured values and are given in figure 6a & 6b.

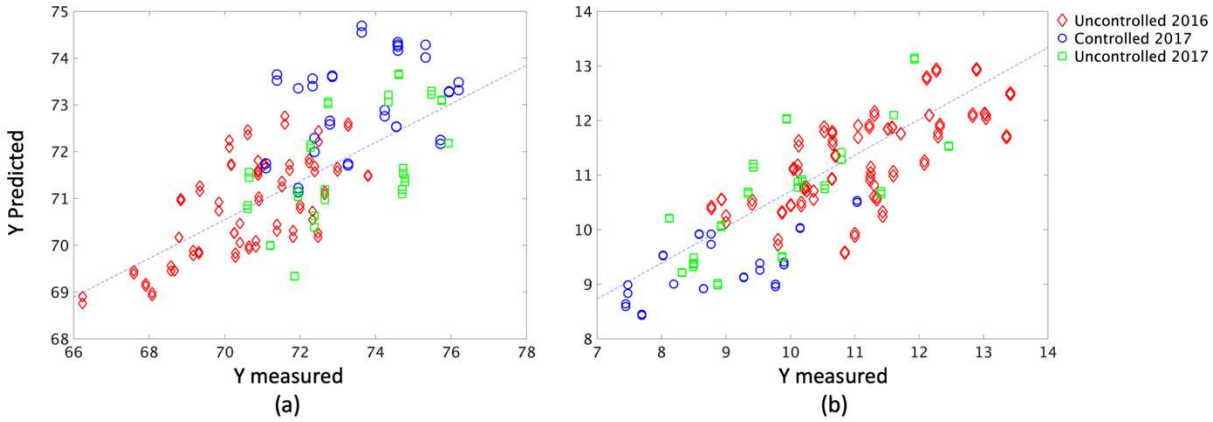

Fig. 6 The results of PLS of the entire EEMs in the emission range of 280- 700 nm, and the (a) Oleic acid, (b) linoleic acid contents of olive oil: predicted vs. measured plots for the validation sets, in a model with two PLS latent variables

PLS regression can handle the collinearity problem present in MLR regression allowing the former to use the maximum information, and therefore higher regression coefficients [43]. However, in this study, the comparison between the outcomes of PLS on unfolded EEM and those of MLR on PARAFAC scores is quite hard. For instance, multiway is more robust than multivariate analysis. In PARAFAC, the data was reduced, and the decomposition was done only on **X,** then the scores were linked to **Y**. Whereas in PLS, the decomposition was executed on both **X** and **Y,** then the results were linked to **Y**. In addition, the number of latent variables was determined based on sample empirical cross validation whereas in MLR the factors were selected based on statistical significance [44,45]. Despite these differences, each of the two models was able to predict the major parameters involved in the deterioration of olive oil, i.e. acidity, peroxide value, delta K, oleic and linoleic acids. This is due to the fact that these parameters are interconnected as they are major indicators informing on the level of lipid

lipolysis or oxidation either when oil is inside the fruit or outside during the extraction process and storage [29].

It is worth mentioning that the variability presented within the samples (Table 1 & 2) inflicted both models' prediction capabilities. Several studies have conducted MLR and PLS prediction models to show the suitability of fluorescence in detecting the quality parameters of olive oil, and a correlation coefficient between 0.8 and 0.99 was obtained. However, most of these studies have examined one or two variables while keeping other variables fixed. For instance, Guimet, Ferré, and Boqué et al.[18] studied the relation between fluorescence EEM and some quality parameters (peroxide value, K232, and K270) of different oil qualities (13 extra virgin, 2 virgin, 16 pure and 2 pomace olive oils), keeping the harvest year and storage conditions as fixed variables. Sikorska, Khmelinskii, and Sikorski et al. [11] demonstrated the use of fluorescence spectroscopy for monitoring the effect of light on the chlorophyll and total polyphenols content of extra virgin olive oil, while maintaining the extraction method and harvesting date as constant variables. Also, Tena, Aparicio, and García-Gonzàlez [12] showed the potential of EEM to study virgin olive oils thermo-oxidized at a fixed pattern (190°C for 94 h, where a sample of 40 ml was collected every 2 h). The incremental variability aims to add more quality variations, however, based on the core asset (virgin olive oil). The difference between this study and other studies is that numerous factors, ranging from agricultural to technological ones, have affected the minor and major constituents of olive oil in a natural way (Online Resource 1, Tables 1 & 2). In a non-experimental design, this variability cannot be controlled, and thus a random pattern may appear as a result, which leads to the increase in the number of outliers and the lowering of the prediction capacity of multivariate models, as shown by MLR and PLS.

Table 3. Results of MLR on PARAFAC scores and PLS on unfolded EEM cube of olive oil quality indices and fatty acids .

Fatty acids (%) and quality indices	MLR		PLS				
	PARAFAC factors*	Multiple R	RMSEC	Calibration R	RMSEV	Validation R	latent variables
Acidity (g/100 g)	F1, F2, F4, F7	0.89	0.35	0.88	0.68	0.84	2
Peroxide value (meq O_2/kg)	F2 to F6	0.84	3.38	0.87	3.95	0.82	2
Total polyphenols mg/ml (765nm)	F2, F7	0.75	23.51	0.71	29.33	0.66	2
K232	F3 to F6	0.69	0.19	0.64	0.20	0.58	2
K270	-	0.31	0.03	0.33	0.04	0.11	2
K266	-	0.44	0.03	0.47	0.05	0.28	2
K274	-	0.41	0.08	0.40	0.09	0.25	4
Delta K	F2 to F5	0.81	0.0017	0.82	0.0023	0.78	2
Total chlorophyll (mg/L)	F1 to F6	0.71	0.66	0.59	0.83	0.53	2
Beta carotene (mg/L)	-	0.35	1.26	0.18	1.42	0.37	2
Palmitic acid	F1 to F7	0.68	1.01	0.61	1.10	0.56	2
Palmitoleic acid	F1 to F6	0.67	0.30	0.28	0.33	0.05	2
Decenenic acid	F1 to F7	0.74	0.02	0.65	0.03	0.60	2
Decenoic acid	F1 to F7	0.70	0.02	0.60	0.03	0.55	2
Stearic acid	F1 to F6	0.75	0.28	0.72	0.29	0.68	2
Oleic acid	F1, F2, F4, F5, F6	0.76	1.47	0.75	1.64	0.70	2
Linoleic acid	F1, F2, F4, F5, F6	0.80	0.84	0.83	0.93	0.80	2
Arachidic acid	F2, F3, F4, F6	0.59	0.06	0.55	0.06	0.51	2
Linolenic acid	F1 to F3, F5 to F7	0.63	0.05	0.70	0.06	0.67	2
Eicosenic acid	F1 to F6	0.62	0.02	0.68	0.03	0.63	2
Behenic acid	F2, F3, F4, F7	0.60	0.02	0.73	0.02	0.71	5
Lignoceric acid	F2, F3, F4, F7	0.56	0.01	0.59	0.02	0.55	2

*PARAFAC factors were selected based on statistical significance ($p < 0.05$).

Conclusion

This study was dedicated to demonstrating the capabilities of front-face fluorescence coupled to chemometric tools such as PARAFAC, MLR, and PLS to predict inconsistent qualities of Lebanese olive oil. Despite the challenging variability present in the Y matrix, certain deterioration quality parameters and fatty acids were predicted using front-face fluorescence spectroscopy. Again, this technique proves its usefulness in grading of olive oil samples with significant savings of analytical measurements specified in the regulations. It makes it possible to easily link the fluorophores content with the degradation of the samples' main physicochemical parameters.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Online Resource 1. Practices applied by the farmer from harvesting olive fruits till storage of olive oil

Province	Olive Pressing Date	Altitude (m)	Material used for fruit storage	Fruit storage duration (hours)	Material used for olive oil Storage	Type of the mill
Hasbaya	Nov-16	643	Nylon	> 48	Plastic	3-phase
	Oct-16	774	Crates	< 24	Plastic	Traditional
	Nov-16	597	Nylon	24	Plastic	Traditional
	Nov-16	464	Nylon	48	Plastic	3phase
	Nov-16	712	Nylon	12	Plastic	3phase
Marjaayoun	Oct-16	585	Nylon	48	Plastic	Traditional
	Oct-16	509	Nylon	48	Stainless	Traditional
	Oct-16	416	Crates	12	Stainless	2-Phase
Tyre	Oct-16	182	Nylon	12	Plastic	Traditional
	Nov-16	235	Crates	24	Glass	Traditional
	Nov-16	442	Nylon	12	Plastic	3-phase
	Nov-16	393	Nylon	24	Plastic	Traditional
	Oct-16	379	Crates	12	Iron	3-phase
Zgharta	Nov-16	319	Crates	> 48	Stainless	Traditional
	Nov-16	183	Crates	48	Stainless	Traditional
	Dec-16	301	Crates	24	Plastic	3-phase
	Dec-16	446	Nylon	12	Plastic	Traditional
Tyre	Oct-17	500	Nylon	> 48	Glass	Traditional
	Nov-17	450	Nylon	> 48	Plastic	Traditional
Marjaayoun	Oct-17	590	Crates	< 24	Plastic	Traditional
	Oct-17	610	Crates	< 24	Stainless	3-phase
Hasbaya	Nov-17	450	Crates	> 48	Plastic	Traditional
	Nov-17	550	Nylon	> 48	Plastic	3-phase
Zgharta	Dec-17	140	Crates	24	Plastic	Traditional
	Dec-17	300	Crates	24	Plastic	3-phase

Chapter 6: General Results and Discussion

The purpose of this work is to search and demonstrate the link between environmental aspects (region, climate, altitude and soil conditions) in Lebanon and cultivation/harvesting techniques (processing, harvest date, time, cultivar) and the resulting physico-chemical characteristics of Lebanese olive oil. The three main research questions addressed are:

(1) Investigate and identify potential specific molecular markers reflecting oil geographical typicality.

(2) Development of a rapid assessment tool in order to replace the conventional analytical methods, as well as,

(3) Identifying sources and causes of non-conformities in the cultivation and/or processing of Lebanese olive oil

1. Results

1.1. Impact of growing area and technological aspects on Lebanese olive oil

In this part, the effect of growing area (altitude and temperature) and technological factors (practices applied to olive fruit just before processing, and extraction method) on Lebanese olive oil chemical composition is highlighted.

Principal component analysis was able to show that altitude is an important influencer, especially when it comes to fatty acids. For instance, low altitude areas, had higher palmitic, palmitoleic, linoleic, linolenic, and lower oleic acid content when compared to high-altitude regions. These results are per the outcomes described by other studies for oils extracted from olives grown at different altitudes (Aguilera et al. 2005). Temperature and relative humidity also played an important part in discriminating both regions as high-altitude areas in Lebanon have a cooler climate compared to low-altitude areas. Olive oil collected from low altitude, exhibited a fatty acid profile mostly dominated by linoleic, linolenic, palmitic, and palmitoleic acid content. Whereas, high altitude areas, showed a unique fatty acid profile distinguished by oleic acid (Di Bella et al., 2007; García-Inza et al., 2014). It is noteworthy to mention that precipitation did not affect the discrimination between high and low altitude regions.

Moreover, independent component analysis, coupled to one-way ANOVA, demonstrated the sources and causes of non-conformities in Lebanese olive oil specifically unproper fruit storage, and bad hygienic manufacturing practices. Significant differences ($p<0.05$) were found in

quality indices (acidity and peroxide value) as well as fatty acid contents (oleic and linoleic acid) when comparing olive oil extracted under controlled condition to those extracted under farmers supervision. The increase in the latter chemical parameters would disqualify olive oil grade from extra virgin to virgin olive oil.

1.2 Harvest Date effect on the Lebanese Olive Oil from the Soury Variety

This study shows the impact of the harvest period on the major and minor chemical components of Lebanese olive oil from the Soury variety. The major components included total polyphenols and fatty acids whereas the minors were sterols in addition to volatile coumpounds.

The analysis of olive oil with different harvest dates has proved that delaying harvest is critical and has determental effects on Lebanese olive oil. For instance, acidity had increased from 0.31% to 1.21 % exceeding the limit established by the International Olive Council (IOC) for EVOO (0.8 %). Peroxide value and K232 showed a significant increase whereas K270 and total polyphenols values decreased as harvest is delayed. All olive oils presented peroxide and K coefficients values that did not exceed the maximum acceptable limit for their classification as extra virgin olive oils.

As for fatty acids, monounsaturated fatty acids decreased, polyunsaturated fatty acids increased whereas saturated fatty acids remained stable with increased maturity. The concentration of linoleic acid significantly increased from 9.09 % to 11.09 % whereas the concentration of oleic acid decreased from 73.79 % to 70.86 %. All fatty acids were in the acceptable limit for their classification as extra virgin olive oils except for palmitic acid. Palmitic acid concentration remained the same with the advancement of harvest date, however, it exceeded the standards set by IOC for extra virgin olive oil.

The relative amount of β-Sitosterol was mainly found to decrease, while those of stigmasterol, $\Delta^{5,24}$-stigmastadienol, Δ^{7}-avenasterol and Δ^{7}-stigmastenol, increased with delaying harvest time.

As for the volatile compounds, principle component analysis was used on the flash GC data to study the impact of harvest date and its delay. Ethanol was found to mostly characterize olive oil sampled at an advanced harvest date. On the other hand, olive fruits harvested at an early date were mostly dominated by 1-hexenol. (E,E)-2,4-decadienal was found in all samples due to the high temperature in the incubation period lying just before injection of the volatiles into the FGC columns.

1.3 A rapid technique replacing the conventional analytical methods

In this study, the potential of front-face fluorescence spectroscopy coupled with chemometric techniques, namely multiple linear regression (MLR) applied on parallel factor (PARAFAC) scores and partial least squares (PLS), was tested on Lebanese olive oil samples. The objective was to check if this technique can prove its usefulness again in grading olive oil samples possessing natural variability within their chemical parameters

Fluorescence excitation-emission matrices (EEM) of the collected samples were measured, and the relationship between them and the chemical parameters was examined. Two models were created. The first model was based on MLR performed on PARAFAC scores while the second was based on PLS regression applied directly on the unfolded EEM.

Twenty-two MLR regression models were generated (one for each physicochemical parameter), the majority of which showed a good correlation coefficient ($R>0.7$ for 10 predicted variables). The highest prediction was achieved for acid value ($R = 0.89$), peroxide value ($R = 0.84$), and delta K ($R = 0.81$), as well as linoleic ($R = 0.80$) and oleic acids ($R = 0.76$). Total polyphenols and total chlorophyll were also well predicted with correlation coefficients of 0.73 and 0.71. The remaining models showed adequate prediction capabilities except for palmitoleic acid with a validation correlation coefficient as low as 0.05, which is not useable for quantitative predictions.

As for PLS, similar results with slight improvement over the MLR models were obtained. The PLS regression models over quality indices showed validation R values higher than 0.6 (0.6-0.81) with low RMSEC and RMSEV values except for total chlorophyll, beta-carotene and middle Ks. The highest prediction was achieved for acid value, peroxide value and delta K with a high validation R value ($R>0.8$) indicating a good prediction model; whereas the regression model for total polyphenols showed lower prediction quality ($R<0.8$). As for fatty acids, linoleic and oleic acids were best predicted with a validation R of 0.8.

The flowchart below gives an overall image on how all elaborated studies were approached (Figure 27).

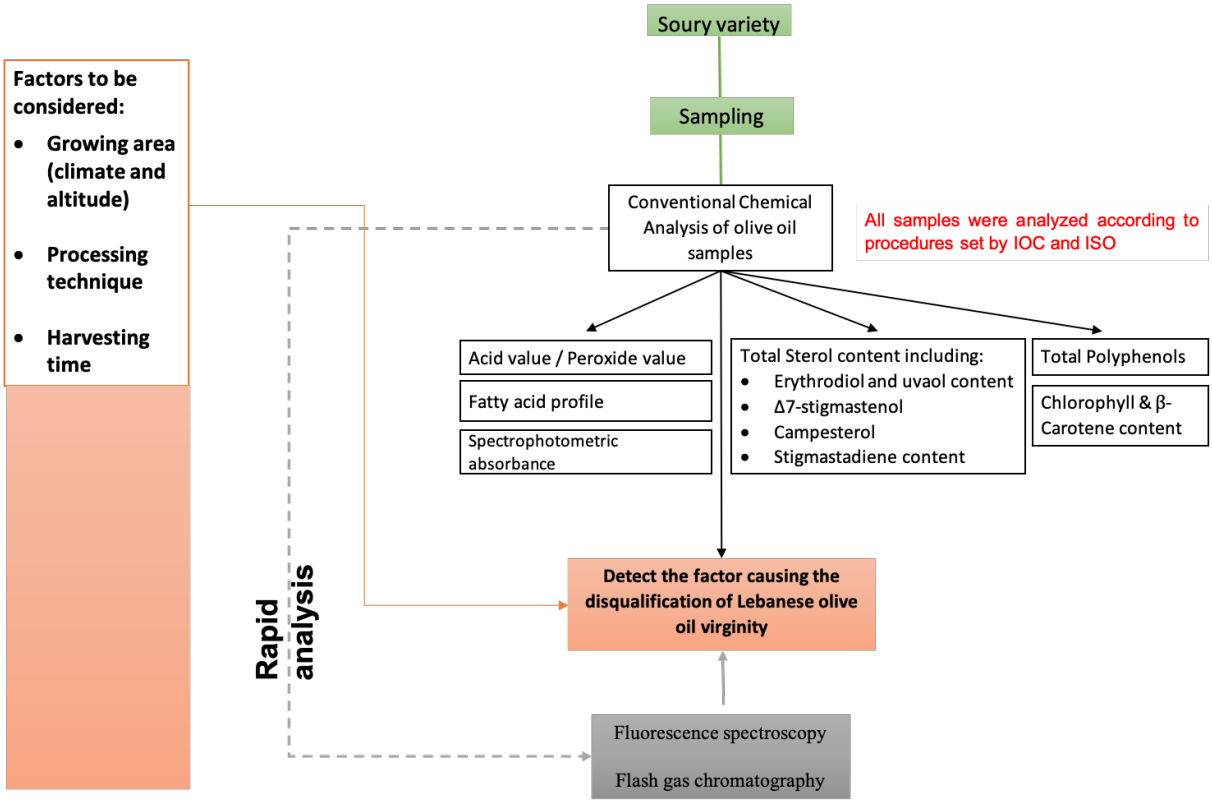

Figure 27. Flowchart detailing the control plan for all conducted studies

2. Discussion

Lebanon's diverse topographies, stretching mainly in a north-south direction, and Mediterranean climate have shown its effect on the major constituents of olive oil, i.e., fatty acids. Although studies targeting the fatty acid composition of Lebanese olive oil originating from different growing areas are scarce (Merchak et al., 2017; Riachy et al., 2018), still this work has indicated the main pedoclimatic conditions that can influence saturated, polyunsaturated and monounsaturated fatty acids.

Three main pedoclimatic conditions, altitude, temperature and relative humidity, were the major influencers and the reason for the distinctive fatty acid profile of the Lebanese olive oil. For instance, Lebanese areas with the high altitudes (450 m to 800 m) and lower average temperature (16 ºC) have increased oleic acid content of olive oil. As for areas with low altitudes (0 m – 450 m) and higher average temperature (19 ºC), the fatty acid profile was characterized by linoleic, linolenic, palmitoleic, and palmitic acids. This fluctuation in fatty acids profile between areas of different altitudes, is caused by several chemical reactions and enzymes happening at the level of the olive fruit.

For instance, going from low to high or high to low altitudes corresponds to a decrease or increase in the environment temperature and relative humidity, respectively. This will certainly

impact the underlying physiological metabolic processes, in particular, the process of fatty acid synthesis (Arslan et al., 2013; Çetinkaya et al., 2017; Issaoui et al., 2010; Nergiz et al., 2009; Stefanoudaki et al., 1999). For example, García-Inza et al. (2014) studied the effect of high temperature by placing olive fruiting branches in transparent plastic chambers with individualized temperature control. The results showed that high temperatures decrease the oleic acid content and increase the palmitic, palmitoleic, linoleic, and linoleic acid contents. The latter is in accordance with the fatty acid profile of regions with low altitude and high temperatures.

These preliminary results will help set the ground for advanced work to be done in this field. It also can present a guide for Protected Geographical Indication (PGI). This will improve the image of Lebanese olive oil and creates an access to international markets, thereby giving an opportunity for the Lebanese olive farmers for a better market access (See Chapter 3 for in-depth discussion).

However, several challenges face the application of PGI. One of these critical challenges is the inferior quality of olive oil in Lebanon indicated by the low rate of exportable grade (about 30% of total olive oil production) (IDAL, 2017b). The quality of oil varies according to and within geographical designation due to variables such as agricultural practices (Gargouri et al., 2016), pre and post-harvest operations (Kiritsakis et al., 1998) and processing (Di Giovacchino et al., 1994; Di Giovacchino et al., 1996). The negligence of such practices is strongly related to the marketability of the oil.

A control plan was established to link the cultivation/harvesting techniques (processing, maturity index, time, cultivar) to the resulting physicochemical of olive oil from the Soury variety with the aid of several chemometric techniques like ICA and PCA. This plan generated more than one agronomical and technological factor causing the disqualification of Lebanese EVOO starting with the first factor which is harvest date and ending with the extraction process. Delaying the harvest of the Soury variety beyond mid-November had an intense effect on Lebanese olive oil quality. It has showed that the main quality parameter such as acidity responsible for grading olive oil had exceeded the standards set by the IOC for EVOO and that the olive oil has lost its oxidative stability due to the drop in total polyphenol content.

Oleic and linoleic acids were also affected by the delay of harvest. The increase of linoleic on the expense of oleic acid is due to two main factors. The first factor may be related to the activity of oleate desaturase enzyme which desaturates the Oleoyl-ACP (precursor of longer-chain unsaturated fatty acids) into linoleate-ACP (Hernandez et al., 2011). The second factor lies in the continuing biosynthesis of triglycerides as the harvesting date is delayed (Flamini, 2010 &

Guiterrez, 1999). Lodolini et al. (2017) have also reported that the Soury variety in particular, should be harvested early. As it ripens, oleic content declines, and linoleic increases, quality indices, mostly free fatty acids, are negatively affected, while polyphenol levels and oxidative stability drop sharply.

Δ^7-stigmastenol is one of the causes of the virginity disqualification of Lebanese olive oil. It's obvious that the factor responsible for the increase of this sterol content is the delay in harvest time where it increased dramatically in November. As for other sterols, the relative amount of β-Sitosterol was mainly found to decrease, while those of stigmasterol, $\Delta^{5,24}$-stigmastadienol, and Δ^7-avenasterol increased with delaying harvest time.

Never to mention that ethanol was the volatile compound dominating most samples harvested in November. The increase in ethanol content is directed by the increase of alcohol dehydrogenase activity during advanced stages of maturation (Beltrán et al., 2015). It is worthy to mention that (E,E)-2,4-decadienal was present in olive oil harvested at optimum and at a delayed harvest date. Its presence is due to the long incubation period of olive oil just before injection into the FGC, which led to the appearance of such a volatile compound (See Chapter 4 for in-depth discussion).

In addition to harvest date, two major factors are linked to this disqualification which are the unproper fruit storage condition/duration practices and the lack of good hygienic and manufacturing practices during oil extraction. Both factors favor the enzymatic lipolysis of the fruit triacylglycerol strongly affecting the quality indicators and fatty acid content of olive oil (Kiritsakis et al., 1998). Generally, it can be concluded that picking the olive fruit from mid-October to the first of November could be a criterion to obtain olive oil of the highest quality grade. Also adhering to good hygienic / storage practices while extracting olive oil would preserve its virginity (See Chapter 3 for in-depth discussion).

As for the olive oils extracted from Soury olive fruits harvested at an optimum date (mid-Oct to mid-Nov) and under optimum extraction conditions, all quality parameters were in range of EVOO category. The extracted oil has a high content of polyphenol and monounsaturated fatty acids and low polyunsaturated and unsaturated fatty acids. As for sterols, all were within the standards set by the IOC, even the Δ^7-stigmastenol content. Also this oil was dominated by the volatile compound 1-hexanol, describing the term "fruity-grassy", which appears as a sign of unripe olives (Aparicio & Morales, 1998) (See Chapter 4 for in-depth discussion).

Although in the above studies conventional analytical methods were used, a rapid technique was tested to demonstrate its capabilities for predicting the inconsistent qualities of Lebanese olive. A front-face fluorescence coupled to chemometric tools such as PARAFAC, MLR, and

PLS proved its usefulness in the grading of olive oil samples with significant savings of analytical measurements specified in the regulations. On the qualitative side, this technique with the help of some chemometric tools like PARAFAC made it possible to easily link the fluorophores content with the degradation of the main physicochemical parameters of the samples especially that olive oil contains fluorophores that are connected directly and indirectly to some of the physicochemical properties like polyphenols, free fatty acids, chlorophylls etc (Ammari et al., 2012; Cheikhousman et al., 2005a; Guimet et al., 2004b; Kyriakidis et al., 2000; Sikorska et al., 2005; Sikorska et al., 2008; Zandomeneghi et al., 2005). Whereas, on the quantitative side, good prediction models were obtained. In both models, acid value, peroxide value and delta K models were highly predicted since these quality parameters are related to the oxidation/hydrolysis products and interrelate with levels of tocopherols, polyphenols, and chlorophyll, which are sensitive products to oxidation and act as antioxidants (Tena et al. (2012). The same was applied to oleic and linoleic models where almost all fluorophores contributed to the two latter prediction models. However, it is worthy to mention that the variability presented within the samples inflicted on some level the prediction capabilities of both models (MLR and PLS) as the challenge itself was to overcome the variability present in the oil samples that were harvested at different dates and from two seasons, processed using different extraction methods, collected from different altitudes and other factors. Several studies have conducted MLR and PLS prediction models to show the suitability of fluorescence in detecting the quality parameters of olive oil, and a correlation coefficient between 0.8 and 0.99 was obtained. However, most of these studies have examined one or two variables while keeping other variables fixed. For instance, Sikorska et al. (2008) demonstrated the use of fluorescence spectroscopy for monitoring the effect of light on the chlorophyll and total polyphenols content of extra virgin olive oil, while maintaining the extraction method and harvesting date as constant variables. Guimet, Ferré, and Boqué et al., 2005 studied the relation between fluorescence EEM and some quality parameters (peroxide value, K232, and K270) of different oil qualities (13 extra virgin, 2 virgin, 16 pure and 2 pomace olive oils), keeping the harvest year and storage conditions as fixed variables. In a non-experimental design, this variability cannot be controlled, and thus a random pattern may appear as a result, which leads to the increase in the number of outliers and to the lowering of the prediction capacity of multivariate models, as shown by MLR and PLS.

In addition to fluorescence spectroscopy, flash GC, an analytical process which depends on the volatilization of olive oil volatile compounds and then their separation through chromatographic analysis was utilized as another rapid test. The results of this analysis gave an overview on the

main volatiles characterizing the Soury variety in matter of seconds. Also, it clearly presented the impact of delaying harvest date on olive oil quality by identifying a specific volatile compound, i.e. ethanol [3].

The impact of both rapid techniques is unquestionable as they strongly contribute to the enhancement of the analytical productivity, such as reducing costs and time, and to the minimization of risks for the analyst. The real deal here was to create a dependable bypass to the exhaustive conventional analysis operations. Difficulties has been encountered with both techniques and future work should rely on, adapting new chemometric models like PLSDA, and on utilizing standards to identify and quantify using the resulting olive oil volatile compounds to improve the quantitative aspect of fluorescence spectroscopy and flash gas chromatography, respectively (See Chapter 5 for in-depth discussion).

Conclusion & Perspectives

Conclusion

Below summarize the conclusions of the work presented in this thesis:

1. Principal component analysis and one-way ANOVA were able to set the ground for Protected Geographical Indication by revealing a correlation between the fatty acid profile and the pedoclimatic conditions of the main olive growing regions in Lebanon. Altitude was the primary influencer on the Lebanese olive oil's chemical composition, particularly the fatty acids. For instance, the fatty acid profile of low-altitude regions was characterized by palmitic, palmitoleic, linoleic, and linolenic acid. Whereas the fatty acid profile of high-altitude regions was mostly dominated by oleic acid.
2. The disqualification of Lebanese olive oil virginity is caused by two variables:
    a. *Agronomical (Harvest date).* The harvest date appears to have a decisive role in the qualitative characteristics, saponifiable and non-saponifiable fraction of Lebanese olive oil. Picking the olive fruit beyond the first week of November has negatively affected the chemical compositions of Lebanese olive oil. All results obtained showed that as the Soury variety ripens, most of the macro and minor components exceed the limits set by the international olive council for extra virgin olive oil.
    b. Technological. Independent component analysis and one-way ANOVA were able to identify the technological disqualification factors through facilitating the chemical interpretation and providing chemically pure components that crossed the parameters sets by IOC for EVOO. The two major factors are the unproper fruit storage condition/duration and the lack of good hygienic and manufacturing practices during oil extraction. The solution for such non-compliances would rely on good management/hygienic practices which include, ensuring suitable pre-processing storage conditions and providing basic hygiene.
3. Rapid techniques such as FFFS and Ultra-FGC, and with the aid of chemometric tools like PARAFAC, MLR, PLS and PCA created a dependable bypass to the exhaustive conventional analysis operations. The results of their analysis gave an overview on how it is possible to easily link the fluorophores content with the degradation of the samples' main physicochemical parameters and on the main volatiles characterizing the variety

under study in matter of seconds. Both techniques showed that they strongly contribute to the enhancement of the analytical productivity by reducing costs and time, and by minimization of risks for the analyst.

Perspectives

Several perspectives are possible for the rest of this research work:

- This work will set the ground for Protected Geographical Indication (PGI).
    - Better market access for olive producers in Lebanon
- Communicate with the responsible entities in Lebanon to disseminate the causes of non-conformities to olive oil producers in Lebanon
    - Improve the exports of Lebanese EVOO
- Improve the quantitative / qualitative aspect of fluorescence spectroscopy and flash gas chromatography, respectively.
    - Adapt new chemometric models like PLSDA to improve the models' predictability
    - Utilize external standards to identify and quantify the rest of olive oil volatile compounds

Résumé en français

1. Introduction

Les oliviers couvrent 5,4 % du territoire libanais et 8 % de l'ensemble des terres agricoles du Liban, et sont destinés à la fois à la production d'huile et d'olives de table (Chehade et al., 2016). Le germoplasme de l'olivier libanais se caractérise par une diversité assez importante. Quatre variétés locales, à savoir Abou-Chawki, Baladi, Del et Soury, sont les plus cultivées dans les plantations commerciales. Les oliveraies sont dispersées dans les cinq provinces du Liban et produisent 90 307 000 tonnes d'olives par an, dont environ 70 % de la production totale est transformée en huile d'olive. En 2011, 11,3 mille tonnes d'huile d'olive ont été produites et seulement 30 % de la production totale d'huile d'olive était constituée d'huile d'olive extra vierge (IDAL, 2017b).

La qualité inférieure de l'huile d'olive au Liban est reflétée par un faible taux d'huiles de classes exportables (environ 30 % de la production totale d'huile d'olive) (IDAL, 2017b). Dans le cadre de la recherche scientifique et du développement, un travail important a été effectué pour améliorer la qualité de l'olive et de l'huile d'olive et, par la suite, améliorer les moyens de subsistance et l'économie de la communauté libanaise. Mais peu a été fait en ce qui concerne l'amélioration des caractéristiques de la qualité commerciale. Le faible pourcentage d'huiles d'olive extra-vierges libanaises est un indicateur de ces limitations qui sont principalement dues à la grande variabilité de la qualité et de la composition chimique de l'huile. Les travaux de recherche précédents, bien que limités, ont attribué cette qualité inférieure à la non-conformité des micro-composants tels que les stérols aux normes de la COI. Il a été rapporté que le stigmasténol et certains acides gras polyinsaturés entravaient la commercialisation internationale de l'huile d'olive libanaise (Youssef, 2002 ; Mireille, 2003 ; Claude, 2005 ; Rabih, 2005 ; Breidi, 2011).

Cette incohérence est due à plusieurs facteurs parmi lesquels la situation géographique (composition du sol, altitude et latitude), les conditions climatiques, le processus d'extraction de l'huile, le cultivar, la date de récolte, etc. (Zamora et al., 2001 ; Temime et al., 2006 ; Rotondi et al., 2004 ; Kiritsakis et al., 1998 ; Franco et al., 2015 ; Dag et al., 2011 ; Baccouri et al., 2007 ; Abaza et al., 2005). Chacun des facteurs mentionnés ci-dessus a son poids sur les paramètres qui définissent la virginité de l'huile d'olive. Ces paramètres comprennent les acides gras libres, l'indice de peroxyde, l'absorbance dans UV, ainsi que d'autres paramètres de qualité tels que le profil des acides gras et la fraction insaponifiable.

Bien que le Liban soit considéré comme un petit producteur d'huile d'olive par rapport aux autres pays méditerranéens, il a la réputation de produire des huiles artisanales de haute qualité. Ce produit haut de gamme est de plus en plus exporté vers des pays comme l'Arabie saoudite (2 635 tonnes), les États-Unis (1 315 tonnes) et d'autres pays (IDAL, 2017a).

Pour suivre ce produit de haute qualité, notamment en termes de propriétés nutritionnelles et sensorielles de l'huile d'olive, des institutions spécialisées telles que le Conseil oléicole international (COI) ont mis en place des contrôles explicites de la qualité de l'huile d'olive. L'application de ces derniers se révèle parfois complexe en termes de temps et de matériaux nécessaires pour déterminer l'authenticité et la qualité de l'huile d'olive (Guzman et al., 2015). Aujourd'hui, la disponibilité de nouveaux accessoires pour les porte-échantillons, de logiciels et de nombreuses applications chimiométriques a fait place à des techniques d'analyse plus rapides et plus simples, notamment la spectroscopie de fluorescence frontale et la chromatographie en phase gazeuse ultra-rapide (Flash-GC). La spectroscopie de fluorescence a été utilisée avec succès comme une technique rapide, non invasive et très sensible pour l'analyse de la qualité de l'huile d'olive et s'est révélée plus rentable que d'autres procédures analytiques (Lleo et al., 2016) ; sans parler de la diversité de ses applications, en particulier dans le domaine de l'analyse de l'huile d'olive (Locquet et al., 2019). Il s'agit notamment d'études sur la détection de l'adultération de l'huile d'olive avec des huiles végétales ou de l'huile de grignons (Dankowska & Małecka, 2009 ; Sayago et al., 2007), le suivi de l'oxydation thermique (Cheikhousman et al, 2005a ; Poulli et al., 2009 ; Sikorska et al., 2008 ; Tena et al., 2012 ; Tena et al., 2009), l'authentification de l'origine (Dupuy et al., 2005), et l'évaluation des paramètres de qualité (Guimet et al., 2004a ; Guzman et al., 2015). Quant à la GC flash, il s'agit d'une technique très sélective et sensible utilisée pour analyser en quelques secondes les composés volatils présents dans l'huile d'olive. Ce type de chromatographie a été utilisé pour la discrimination des origines géographiques (Melucci et al., 2016), la détection de l'adultération (Jabeur et al., 2014) et la détermination des grades de qualité de l'huile d'olive (Barbieri et al., 2020). Ces deux types d'outils analytiques rapides peuvent être particulièrement utiles pour le contrôle de qualité de routine, notamment à l'aide d'outils chimiométriques.

Ce travail étudie l'utilisation de la chimiométrie comme la PCA, la PARAFAC, l'ICA, la MLR et la PLS pour mettre en évidence l'effet de la zone de culture sur l'huile d'olive libanaise sur la base des paramètres physico-chimiques résultants, pour détecter les facteurs technologiques et agricoles conduisant à la disqualification de la virginité de l'huile d'olive libanaise et pour tester l'utilité des techniques d'analyse rapide dans le classement des échantillons d'huile d'olive.

Objectifs

Les objectifs généraux peuvent être résumés comme suit :

- Elaboration d'un plan de contrôle pour évaluer les caractéristiques physico-chimiques et les niveaux de qualité des OVE par rapport à une référence internationale
- Améliorer la production, la qualité et, à terme, la commercialisabilité et la compétitivité de l'HOVE produite au Liban sur les marchés internationaux

Les objectifs spécifiques comprennent :

(1) Identifier les sources et les causes des non-conformités au niveau de la culture et/ou la transformation de l'huile d'olive libanaise tout en trouvant des solutions applicables afin de s'adapter aux exigences internationales,

(2) Étude de l'effet de la zone de culture sur la composition chimique de l'huile d'olive libanaise.

(3) Développement d'un outil d'évaluation rapide afin de remplacer les méthodes d'analyse conventionnelles.

2. Résultats

2.1 Impact de la zone de culture et des aspects technologiques sur l'huile d'olive libanaise

Dans cette partie, l'effet de la zone de culture (altitude et température) et des facteurs technologiques (pratiques appliquées aux fruits de l'olivier juste avant la transformation, et méthode d'extraction) sur la composition chimique de l'huile d'olive libanaise est mis en évidence.

L'analyse en composantes principales a pu montrer que l'altitude est un facteur important, surtout en ce qui concerne les acides gras. Par exemple, les huiles des régions de basse altitude présentent une teneur plus élevée en acides palmitique, palmitoléique, linoléique, linolénique et oléique que celles des régions de haute altitude. Ces résultats sont conformes aux résultats décrits par d'autres études pour les huiles extraites d'olives cultivées à différentes altitudes (Aguilera et al. 2005). La température et l'humidité relative ont également joué un rôle important dans la différenciation des deux régions, car les régions de haute altitude du Liban ont un climat plus frais que les régions de basse altitude. L'huile d'olive recueillie à basse altitude, présentait un profil d'acides gras principalement dominé par une teneur en acide linoléique, linolénique, palmitique et palmitoléique. En revanche, les zones de haute altitude présentaient un profil d'acides gras unique, caractérisé par la présence d'acide oléique (Di Bella et al., 2007 ; García-Inza et al., 2014). Il convient de mentionner que les précipitations n'ont pas affecté la discrimination entre les régions de haute et de basse altitude.

De plus, l'analyse en composantes indépendantes, couplée à une ANOVA à un facteur, a démontré les sources et les causes des non-conformités dans l'huile d'olive libanaise, notamment un stockage inadapté des fruits et des mauvaises pratiques de fabrication et d'hygiène. Des différences significatives ($p<0,05$) ont été constatées au niveau des indices de qualité (acidité et indice de peroxyde) ainsi que dans les teneurs en acides gras (acide oléique et linoléique) en comparant l'huile d'olive extraite dans des conditions contrôlées à celle extraite sous la supervision des agriculteurs. L'augmentation des valeurs de ces derniers paramètres chimiques aurait pour effet de disqualifier l'huile d'olive de la catégorie "huile d'olive vierge extra" à la catégorie "huile d'olive vierge".

2.2 Effet de la date de récolte sur l'huile d'olive libanaise de la variété Soury

Cette étude montre l'impact de la période de récolte sur les composants chimiques majeurs et mineurs de l'huile d'olive libanaise de la variété Soury. Les composants majeurs sont les polyphénols totaux et les acides gras, tandis que les composants mineurs sont les stérols, en plus des composés volatils.

L'analyse de l'huile d'olive issues de dates de récolte différentes a prouvé que le retard de la récolte est critique et a des effets déterminants sur l'huile d'olive libanaise. Par exemple, l'acidité est passée de 0,31 % à 1,21 %, dépassant la limite fixée par le Conseil oléicole international (COI) pour l'HOVE (0,8 %). D'autre part, l'indice de peroxyde et le K232 ont connu une augmentation significative, tandis que le K270 et les teneurs en polyphénols totaux ont diminué suite à un retard de la récolte. Toutes les huiles d'olive présentaient des valeurs de peroxyde et de coefficients K qui ne dépassaient pas la limite maximale acceptable pour leur classification en tant qu'huiles d'olive vierges extra.

En ce qui concerne les acides gras, les acides gras mono-insaturés ont diminué, les acides gras polyinsaturés ont augmenté alors que les acides gras saturés sont restés stables avec l'évolution de la maturité. La concentration d'acide linoléique a augmenté de manière significative, passant de 9,09 % à 11,09 %, tandis que la concentration d'acide oléique a diminué, passant de 73,79 % à 70,86 %. Tous les acides gras se trouvaient dans la limite acceptable pour la classification des huiles en tant qu'huiles d'olive vierges extra.

D'un autre côté, la quantité relative de β-Sitostérol a principalement diminué, tandis que celles de stigmastérol, Δ5,24-stigmastadiénol, Δ7-avenastérol et Δ7-stigmasténol, ont augmenté avec l'avancement de la date de récolte.

En ce qui concerne les composés volatils, l'analyse en composantes principales a été utilisée sur les données de GC flash pour étudier l'impact de la date de récolte et de son retard. On a constaté que l'éthanol caractérisait surtout les huiles d'olive échantillonnées à une date de récolte

avancée. D'autre part, les fruits d'olive récoltés à une date précoce étaient principalement dominés par le 1-hexénol. Du (E,E)-2,4-décadiénal a été trouvé dans tous les échantillons en raison de la température élevée de la période d'incubation qui se situe juste avant l'injection des volatiles dans les colonnes de CGF.

2.3 Une technique rapide remplaçant les méthodes d'analyse classiques

Dans cette étude, le potentiel de la spectroscopie de fluorescence frontale couplée à des techniques chimiométriques, à savoir la régression linéaire multiple (MLR) appliquée sur les scores de l'analyse factorielle parallèle (PARAFAC) et la régression au sens des moindres carrés partiels (PLS), a été testé sur des échantillons d'huile d'olive libanaise. L'objectif était de vérifier si cette technique peut à nouveau prouver son utilité pour classer les échantillons d'huile d'olive présentant une variabilité naturelle dans leurs paramètres chimiques.

Les matrices d'excitation et d'émission de fluorescence (EEM) des échantillons collectés ont été mesurées et la relation entre celles-ci et les paramètres chimiques a été examinée. Deux modèles ont été créés. Le premier modèle était basé sur la MLR réalisée sur les scores PARAFAC, tandis que le second était basé sur la régression PLS appliquée directement sur l'EEM dépliée.

Vingt-deux modèles de régression MLR ont été générés (un pour chaque paramètre physico-chimique), dont la majorité a montré un bon coefficient de corrélation ($R>0,7$ pour 10 variables prédites). La prédiction la plus élevée a été obtenue pour l'indice d'acide ($R = 0,89$), l'indice de peroxyde ($R = 0,84$) et le delta K ($R = 0,81$), ainsi que pour les acides linoléique ($R = 0,80$) et oléique ($R = 0,76$). Les polyphénols totaux et la chlorophylle totale étaient également bien prédits avec des coefficients de corrélation de 0,73 et 0,71, respectivement. Les autres modèles ont montré des capacités de prédiction adéquates, sauf pour l'acide palmitoléique avec un coefficient de corrélation de validation aussi bas que 0,05, non valide pour les prédictions quantitatives.

Quant à la PLS, des résultats similaires avec une légère amélioration par rapport aux modèles MLR ont été obtenus. Les modèles de régression PLS sur les indices de qualité ont montré des valeurs R de validation supérieures à 0,6 (0,6-0,81) avec des erreurs de prédiction RMSEC et RMSEV faibles, sauf pour la chlorophylle totale, le bêta-carotène et le Ks moyen. La prédiction la plus élevée a été obtenue pour l'indice d'acide, l'indice de peroxyde et le delta K avec une valeur R de validation élevée ($R>0,8$) indiquant un bon modèle de prédiction ; tandis que le modèle de régression pour les polyphénols totaux a montré une qualité de prédiction inférieure ($R<0,8$). En ce qui concerne les acides gras, les acides linoléique et oléique ont été les mieux prédits avec une valeur R de validation de 0,8.

3. Discussion

La topographie diverse du Liban, s'étendant principalement dans une direction Nord-Sud, et le climat méditerranéen ont montré leur effet sur les principaux constituants de l'huile d'olive, c'est-à-dire les acides gras. Bien que les études ciblant la composition en acides gras de l'huile d'olive libanaise provenant de différentes zones de culture soient rares (Merchak et al., 2017 ; Riachy et al., 2018), ces travaux ont tout de même indiqué les principales conditions pédoclimatiques pouvant influencer les acides gras saturés, polyinsaturés et monoinsaturés.

Les trois principales conditions pédoclimatiques, l'altitude, la température et l'humidité relative, ont été les principaux facteurs d'influence et à l'origine d'un profil d'acides gras distinctif de l'huile d'olive libanaise. Par exemple, les zones libanaises de haute altitude (450 m à 800 m) et de basse température moyenne (16 °C) ont augmenté la teneur en acide oléique de l'huile d'olive. Quant aux zones de faible altitude (0 m à 450 m) et de température moyenne plus élevée (19 °C), le profil des acides gras était caractérisé surtout par les acides linoléique, linolénique, palmitoléique et palmitique. Cette fluctuation du profil des acides gras entre les zones de différentes altitudes est due à plusieurs réactions chimiques et enzymatiques qui se produisent au niveau du fruit de l'olivier.

Par exemple, le passage de basse à haute ou de haute à basse altitude correspond à une diminution ou une augmentation de la température et de l'humidité relative de l'environnement, respectivement. Cela aurait certainement un impact sur les processus métaboliques physiologiques sous-jacents, en particulier le processus de synthèse des acides gras (Arslan et al., 2013 ; Çetinkaya et al., 2017 ; Issaoui et al., 2010 ; Nergiz et al., 2009 ; Stefanoudaki et al., 1999). Par exemple, García-Inza et al. (2014) ont étudié l'effet de la température élevée en couvrant les branches d'olivier fructifiant dans des chambres en plastique transparent avec un contrôle de température individualisé. Les résultats ont montré que les températures élevées diminuent la teneur en acide oléique et augmentent les teneurs en acides palmitique, palmitoléique, linoléique et linoléique. Ceci est conforme au profil des acides gras des régions de basse altitude et de haute température.

Ces résultats préliminaires permettront de jeter les bases de travaux avancés dans ce domaine. Cette étude pourrait également servir de guide pour les Indications Géographiques Protégées (IGP). Cela permettra d'améliorer l'image de l'huile d'olive libanaise et de créer un accès aux marchés internationaux, offrant ainsi aux oléiculteurs libanais la possibilité d'un meilleur accès au marché (voir le chapitre 3 pour une discussion approfondie).

Toutefois, l'application de l'IGP se heurte à plusieurs difficultés. L'un de ces défis majeurs est la qualité inférieure de l'huile d'olive au Liban, comme en témoigne le faible taux de qualité

exportable (environ 30 % de la production totale d'huile d'olive) (IDAL, 2017b). La qualité de l'huile varie en fonction de la désignation géographique et au sein de celle-ci en raison de variables telles que les pratiques agricoles (Gargouri et al., 2016), les opérations avant et après récolte (Kiritsakis et al., 1998) et la transformation (Di Giovacchino et al., 1994 ; Di Giovacchino et al., 1996). La négligence de ces pratiques est fortement liée à la qualité marchande de l'huile.

Un plan de contrôle a été ainsi établi pour relier les techniques de culture/récolte (traitement, indice de maturité, temps, cultivar) à la physico-chimie de l'huile d'olive de la variété Soury qui en résulte, à l'aide de plusieurs techniques chimiométriques comme l'ICA et l'ACP. Ce plan a généré plus d'un facteur agronomique et technologique entraînant la disqualification de l'EVOO libanaise en commençant par le premier facteur qui est la date de récolte et en terminant par le processus d'extraction.

Le fait de retarder la récolte de la variété Soury au-delà de la mi-novembre a eu un effet énorme sur la qualité de l'huile d'olive libanaise. Il a été montré que le principal paramètre de qualité, à savoir l'acidité, responsable du classement de l'huile d'olive avait dépassé les normes fixées par le COI pour l'HOVE et que l'huile d'olive avait perdu sa stabilité oxydative en raison de la baisse de la teneur totale en polyphénols. Les acides oléique et linoléique ont également été affectés par un délai de la récolte. L'augmentation du linoléique au détriment de l'acide oléique est due à deux facteurs principaux. Le premier facteur peut être lié à l'activité de l'enzyme oléate désaturase qui désature l'Oleoyl-ACP (précurseur des acides gras insaturés à longue chaîne) en linoléate-ACP (Hernandez et al., 2011). Le deuxième facteur réside dans la poursuite de la biosynthèse des triglycérides, la date de récolte étant retardée (Flamini, 2010 & Guiterrez, 1999). Lodolini et al. (2017) ont également signalé que la variété Soury, en particulier, devrait être récoltée tôt. À mesure qu'elle mûrit, sa teneur en acide oléique diminue et sa teneur en acide linoléique augmente, les indices de qualité, principalement les acides gras libres, sont affectés négativement, tandis que les niveaux de polyphénols et la stabilité oxydative diminuent fortement.

Le Δ7-stigmastenol est l'une des causes de la disqualification de la virginité de l'huile d'olive libanaise. Il est évident que le facteur responsable de l'augmentation de cette teneur en stérols est le retard de la récolte où elle a augmenté de façon spectaculaire en novembre. Comme pour les autres stérols, la quantité relative de β-Sitostérol a principalement diminué, tandis que celles de stigmastérol, Δ5,24-stigmastadiénol, et Δ7-avenastérol ont augmenté avec le retard de la récolte.

Sans parler du fait que l'éthanol était le composé volatil qui dominait la plupart des échantillons récoltés en novembre. L'augmentation de la teneur en éthanol est dirigée par l'augmentation de l'activité de l'alcool déshydrogénase pendant les stades avancés de la maturation (Beltrán et al., 2015). Il convient de mentionner que le (E,E)-2,4-décadiénal était présent dans l'huile d'olive récoltée à la date optimale et à une date de récolte retardée. Sa présence est due à la longue période d'incubation de l'huile d'olive juste avant l'injection dans la MGF, qui a conduit à l'apparition d'un composé aussi volatil (voir le chapitre 4 pour une discussion approfondie).

Outre la date de récolte, deux facteurs majeurs sont aussi liés à cette disqualification, à savoir les mauvaises pratiques en matière de conditions et de durée de stockage des fruits et le manque de bonnes pratiques d'hygiène et de fabrication lors de l'extraction de l'huile. Ces deux facteurs favorisent la lipolyse enzymatique du triacylglycérol du fruit, ce qui affecte fortement les indicateurs de qualité et la teneur en acides gras de l'huile d'olive (Kiritsakis et al., 1998). De manière générale, on peut conclure que la cueillette des olives de la mi-octobre au premier novembre pourrait être un critère pour obtenir une huile d'olive de la meilleure qualité. Le respect des bonnes pratiques d'hygiène/de stockage lors de l'extraction de l'huile d'olive permettrait également de préserver sa virginité (voir le chapitre 3 pour une discussion approfondie).

Quant aux huiles d'olive extraites des olives de la variété Soury récoltées à une date optimale (mi-octobre à mi-novembre) et dans des conditions d'extraction optimales, tous les paramètres de qualité se situaient dans la catégorie EVOO. L'huile extraite a une teneur élevée en polyphénols et en acides gras monoinsaturés et une faible teneur en acides gras polyinsaturés et insaturés. Quant aux stérols, tous étaient conformes aux normes fixées par le CIO, même la teneur en Δ7-stigmasténol. Cette huile était également dominée par le composé volatil 1-hexanol, décrivant le terme "fruité-grassé", qui apparaît comme un signe d'olives non mûres (Aparicio & Morales, 1998) (Voir le chapitre 4 pour une discussion approfondie).

Bien que dans les études ci-dessus, des méthodes d'analyse conventionnelles aient été utilisées, une technique rapide a été testée pour démontrer ses capacités à prédire les qualités irrégulières de l'olive libanaise. Une fluorescence frontale couplée à des outils chimiométriques tels que PARAFAC, MLR et PLS a prouvé son utilité dans le classement des échantillons d'huile d'olive avec une économie significative au niveau mesures analytiques spécifiées dans les règlements. Sur le plan qualitatif, cette technique, associée à certains outils chimiométriques comme le PARAFAC, a permis de relier facilement la teneur en fluorophores à la dégradation des principaux paramètres physico-chimiques des échantillons, d'autant plus que l'huile d'olive contient des fluorophores qui sont directement et indirectement liés à certaines propriétés

physico-chimiques comme les polyphénols, les acides gras libres, les chlorophylles, etc (Ammari et al, 2012 ; Cheikhousman et al., 2005a ; Guimet et al., 2004b ; Kyriakidis et al., 2000 ; Sikorska et al., 2005 ; Sikorska et al., 2008 ; Zandomeneghi et al., 2005). Alors que, sur le plan quantitatif, de bons modèles de prédiction ont été obtenus. Dans les deux modèles, l'indice d'acidité, l'indice de peroxyde et le delta K ont fait l'objet de bonnes prédictions, car ces paramètres qualitatifs sont liés aux produits d'oxydation/hydrolyse et sont en corrélation avec les niveaux de tocophérols, de polyphénols et de chlorophylle, qui sont des produits sensibles à l'oxydation et agissent comme agents antioxydants (Tena et al., 2012). De manière similaire, presque tous les fluorophores ont contribué à l'élaboration des modèles de prédiction des acides oléique et linoléique. Cependant, la plupart de ces études ont examiné une ou deux variables tout en gardant d'autres variables fixes. Par exemple, Sikorska et al. (2008) ont démontré l'utilisation de la spectroscopie de fluorescence pour surveiller l'effet de la lumière sur la teneur en chlorophylle et en polyphénols totaux de l'huile d'olive vierge extra, tout en maintenant la méthode d'extraction et la date de récolte comme variables constantes. Guimet, Ferré, et Boqué et autres, 2005 ont étudié la relation entre la SEE par fluorescence et certains paramètres de qualité (indice de peroxyde, K232, et K270) de différentes qualités d'huile (13 huiles d'olive vierges extra, 2 vierges, 16 pures et 2 de grignons), en gardant l'année de récolte et les conditions de stockage comme variables fixes. Dans un modèle non expérimental, cette variabilité ne peut être contrôlée, et un modèle aléatoire peut donc apparaître, ce qui entraîne une augmentation du nombre de valeurs aberrantes et une diminution de la capacité de prédiction des modèles multivariés, comme le montrent les modèles MLR et PLS.

En plus de la spectroscopie de fluorescence, la GC flash, un processus analytique qui dépend de la volatilisation des composés volatils de l'huile d'olive puis de leur séparation par analyse chromatographique, a été utilisée comme autre test rapide. Les résultats de cette analyse ont donné un aperçu des principaux composés volatils caractérisant la variété Soury en quelques secondes seulement. En outre, elle a clairement présenté l'impact du délai de la date de récolte sur la qualité de l'huile d'olive en identifiant un composé volatil spécifique, à savoir l'éthanol 3. L'impact des deux techniques rapides est incontestable car elles contribuent fortement à l'amélioration de la productivité analytique, comme la réduction des coûts et du temps, et à la minimisation des risques pour l'analyste. Il s'agissait en fait de créer un contournement fiable des opérations d'analyse conventionnelles fastidieuses. Des difficultés ont été rencontrées avec les deux techniques et les travaux futurs devraient s'appuyer sur l'adaptation de nouveaux modèles chimiométriques comme la PLSDA, et sur l'utilisation de standards pour mieux identifier et quantifier les composés volatils de l'huile d'olive qui en résultent afin d'améliorer

l'aspect quantitatif de la spectroscopie de fluorescence et de la chromatographie en phase gazeuse flash, respectivement (voir le chapitre 5 pour une discussion approfondie).

4. Conclusion

Les conclusions des travaux présentés dans cette thèse sont résumées ci-dessous :

1. L'analyse en composantes principales et l'ANOVA à un facteur ont permis de poser les bases pour une Indication Géographique Protégée en révélant une corrélation entre le profil des acides gras et les conditions pédoclimatiques des principales régions oléicoles du Liban. L'altitude a été le principal facteur d'influence sur la composition chimique de l'huile d'olive libanaise, en particulier les acides gras. Par exemple, le profil des acides gras des régions de basse altitude était caractérisé par les acides palmitique, palmitoléique, linoléique et linolénique. Alors que le profil des acides gras des régions de haute altitude était principalement dominé par l'acide oléique.
2. La disqualification de la virginité de l'huile d'olive libanaise est causée par deux variables :

a. Agronomique (date de récolte). La date de récolte semble avoir un rôle déterminant dans les caractéristiques qualitatives, la fraction saponifiable et non saponifiable de l'huile d'olive libanaise. La cueillette des olives au-delà de la première semaine de novembre a eu un effet négatif sur les compositions chimiques de l'huile d'olive libanaise. Tous les résultats obtenus ont montré qu'à mesure que la variété Soury mûrit, la plupart des composants macro et mineurs dépassent les limites fixées par le Conseil oléicole international pour l'huile d'olive vierge extra.

b. Technologique. L'analyse indépendante des composants et l'ANOVA à un facteur ont permis d'identifier les facteurs de disqualification technologique en facilitant l'interprétation chimique et en fournissant des informations sur les composants chimiques purs qui depassent les limites des paramètres fixés par le COI pour l'EVOO. Les deux principaux facteurs sont les conditions/durée de stockage des fruits non appropriées et le manque de bonnes pratiques d'hygiène et de fabrication lors de l'extraction de l'huile. La solution à ces non-conformités reposerait sur de bonnes pratiques de gestion et d'hygiène, notamment la garantie de conditions de stockage appropriées avant le traitement et la garantie d'une hygiène de base.

3. Les techniques rapides telles que la FFFS et l'Ultra-FGC, et à l'aide d'outils chimiométriques comme le PARAFAC, le MLR, le PLS et le PCA ont créé un contournement fiable des techniques d'analyse conventionnelles laborieuses. Les résultats de leur analyse ont donné un aperçu sur la manière dont il est possible de relier facilement la teneur en fluorophores à la dégradation des principaux paramètres physico-chimiques des échantillons et sur les principaux

volatils caractérisant la variété étudiée en quelques secondes. Les deux techniques ont montré qu'elles contribuent fortement à l'amélioration de la productivité analytique en réduisant les coûts et le temps, et en minimisant les risques pour l'analyste.

5. Perspectives

Plusieurs perspectives sont possibles pour la suite de ces travaux de recherche :

- Ce travail préparera le terrain pour l'Indication Géographique Protégée (IGP).
    - Meilleur accès au marché pour les producteurs d'olives au Liban
- Communiquer avec les entités responsables au Liban pour faire connaître les causes des non-conformités aux producteurs d'huile d'olive au Liban
    - Améliorer les exportations de l'HOVE libanaise
- Améliorer l'aspect quantitatif / qualitatif de la spectroscopie de fluorescence et de la chromatographie en phase gazeuse flash, respectivement.
    - Adapter de nouveaux modèles chimiométriques comme la PLSDA pour améliorer la capacité prédictive des modèles
    - Utiliser des standards purs externes pour identifier et quantifier la totalité des composés volatils de l'huile d'olive.

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Annexes

Annex 1. Olive oil sampling filling form

Harvesting Season (2016-2017)			
Number of the sample	Date of collection	Extraction Date	
Name of the village	Altitude	Type of soil	
Material used for fruit storage	Fruit storage duration	Sample code	
Material used for oil storage	Fruit storage duration		
Olive fruit information			
Fruit variety	Fruit maturity		
Type of Irrigation	Variety		
Fruit fly infectoin (%)	Peacock infection rate (%)	Others	
Olive mill Information			
Name of the mill			
Mill Type	Traditional	3-phase	2-phase
Grinding method	Stone	Hammer	Disk
Malaxation method	Stone	Malaxer	
	Malaxation period	Malaxation temperature	
Amount of water added during extraction process			

Annex 2. Olive fruit sampling filling form

Harvesting Season (2017-2018)	
Location	Village
Name of the Farmer	Phone Number
Field Information	
Field Coordinates	Harvest Date
Type of Irrigation	Variety
Altitude	Soil Type
Sample Info	
Maturity Index	Sample Code
Oil Extraction Date	
Notes	

Annex 3. Olive oil data (Sample)

Sample No	Qaza	Region	Sampling Date	Olive Pressing Date	Coordinates	Altitude (m)	Material used in transferring olive drupes to the mill	Drupes storage duration before oil extraction (days)	Type of the mill
83	Bnt Jbeil	Bnt Jbeil	12/11/17	2/11/17	33 7 14 35 25 56	750	Khaysh	5	3 Phase
84		Maroun	12/11/17	22/10/2017	33 6 27 35 27 3	910	Nylon	2	3 Phase
85	Tyre	Srifa-Al ramla	12/11/17	15/10/2016	33.2871595 35.3960278	500	Nylon	3	Traditional
86		El Dahr	12/11/17	12/11/17	NA	450	Khaysh	3	Traditional
87	Marjaayoun	Khiam	26/11/17	20/10/2016	33 21 5 35 35 59	590	Crates	Same day	Traditional
88		Klayaa	26/11/17	15/10/2016	34 14 15 35 46 49	610	Crates	Same day	3 Phase
89	Hasbaya	El Mari	26/11/17	12/11/17	33 19 22 35 38 37	450	Crates	4	traditional
90		Hasbaya	26/11/17	19/11/2017	33 24 12 35 39 53	550	Nylon	4	3 Phase
91	Nabatiyeh	Zawtar EL Gharbiyeh	26/11/17	15/10/17	33 19 28 35 26 58	480	Khaysh	2	Traditional
92		Dwayr	26/11/17	6/11/17	35 23 29 N 35 35 22 E	380	Crates	2	2 Phase
93	Zgharta	Kfardlekos	10/12/17	2/12/17	34 23 11 35 54 40	140	Crates	1	Traditional
94		Bsabael	10/12/17	5/12/17	NA	300	Crates	1`	3 Phase
95	Koura	Nakhle	10/12/17	26/11/17	34 22 9 35 49 45	270	Nylon	1`	3 Phase
96		Bziza	17/1/18	15/11/2017	34 16 09 35 49 19	452	Nylon	1	Traditional

Annex 4. Olive Fruit Data (Sample)

Sample No	Qaza	Region	Coordinates	Altitude (m)	Variety	Harvest date	Sample Wt. (Kg)	Oil extraction date	Volume Extracted (ml)	Maturity Index	Irrigation
61	Nabatiyeh	Doueir	35 23 29 N 35 35 22 E	380	Abou Chawke	15-Oct	4.70	3-Oct	900	0	NA
62	Nabatiyeh	Zatar El Gharbiyi	33 19 28 35 26 58	480	Souri	15-Oct	4.60	3-Oct	1100	3.15	NA
63	Nabatiyeh	Sarba	33 28 54 35 37 44	420	Souri/Baladi	15-Oct	2.75	3-Oct	650	0	NA
64	Tyre	Deir Qanoun El Nahr	33.3009986 35.3098346	180	Souri	15-Oct	3.00	4-Oct	800	0	NA
65	Tyre	Srifa	33.2871595 35.3960278	500	Souri/Baladi	15-Oct	4.00	4-Oct	700	0	NA
66	Marjaayoun	Qlaya	33 19 19 35 33 6	660	Souri	16-Oct	4.00	9-Oct	750	3.3	NA
67	Marjaayoun	Deir Mimas	33 18 27 35 33 37	530	Souri	16-Oct	4.50	9-Oct	1150	1.4	NA
68	Marjaayoun	Khiam	33 21 5 35 35 59	590	Souri	16-Oct	4.30	9-Oct	1000	1.15	NA
69	Batroun	Douma	34 12 17 35 50 28	1050	Souri	17-Oct	2.90	13-Oct	510	0.32	A
70	Batroun	Halta	34 14 15 35 46 49	610	Souri	18-Oct	3.20	13-Oct	850	1.58	NA
71	Zgharta	Kfardlekus	34 23 11 35 54 40	140	Souri	19-Oct	3.30	13-Oct	690	1.35	NA

Titre : Analyse physico-chimique couplée à une démarche par empreintes pour la caractérisation d'huiles d'olive extra-vierges libanaises

Mots clés : Huile d'olive Chimiométrie Indication géographique Caractérisation physicochimique Qualité Analyse rapide

Résumé : L'huile d'olive est une composante vitale du régime méditerranéen en raison de sa valeur nutritionnelle et économique bien connues. Plusieurs facteurs environnementaux, agricoles et technologiques jouent un rôle important dans la définition de la qualité de l'huile d'olive. Au Liban, des études préliminaires ont montré que certains critères de qualité dépassent les valeurs seuils des normes éditées par le Conseil Oléicole International (COI) pour les huiles d'olive extra-vierges, dont les causes n'ont pas été identifiées. En conséquence, quatre-vingt-seize échantillons d'huile d'olive ont été récoltés sur deux saisons, traités en utilisant différentes méthodes d'extraction et collectés sur huit sites (Akkar, Chouf, Hasbaya, Koura, Tyr, Nabatiyeh, Zgharta et Hermel) à fort potentiel pour l'obtention d'une indication géographique protégée (IGP). Dans cette optique, les huiles extraites et/ou collectées, ont été soumises à une analyse chimique conventionnelle comme suggéré par le COI et à une analyse rapide en utilisant la spectroscopie de fluorescence 3D en mode frontal (3D-FFFS) et la chromatographie en phase gazeuse ultra-rapide (Ultra-Fast GC).

Une corrélation entre le profil en acides gras et les conditions pédoclimatiques des principales régions oléicoles du Liban a été constatée. L'altitude, la température et l'humidité relative sont les principaux facteurs d'influence du profil d'acides gras. Les régions libanaises à haute altitude, à température moyenne basse et à faible humidité relative ont une teneur élevée en acide oléique. Les zones à basse altitude, à température moyenne plus élevée et avec une humidité relative plus élevée ont un profil en acides gras caractérisé par les acides linoléique, linolénique, palmitoléique et palmitique. Les facteurs agricoles, en particulier la date de récolte, affectent également les constituants majeurs et mineurs de l'huile d'olive. En effet, l'acidité et les polyphénols totaux étaient fortement influencés par celle-ci. De plus, une modification du profil d'acides gras caractérisée par une teneur en linoléique plus élevée, une teneur en oléique plus faible, une augmentation du Δ^7-stigmasténol dépassant la limite fixée par le CIO et la présence de composés malodorants (dont l'éthanol) ont été observés lors de récoltes plus tardives. En outre, deux facteurs technologiques, notamment un stockage inadéquat des fruits et de mauvaises pratiques hygiéniques de fabrication, ont favorisé la lipolyse enzymatique du triacylglycérol du fruit modifiant de manière significative les profils d'arômes et d'acides gras de l'huile d'olive.

La 3D-FFFS et l'Ultra-FGC ont toutes deux montré de très bonnes performances. La 3D-FFFS couplée à des techniques chimiométriques a été appliquée sur des qualités hétérogènes et dégradées d'échantillons d'huile d'olive libanaise afin de prédire les principaux paramètres physicochimiques de qualité. Ainsi, vingt-deux modèles de régression MLR basés sur les scores PARAFAC ont été générés, dont la majorité a montré un bon coefficient de corrélation (R>0,7). Un deuxième modèle, utilisant la PLS sur les matrices d'émission-excitation (EEM) dépliées, a conduit à des résultats similaires, avec une légère amélioration par rapport au modèle MLR. D'autre part, l'Ultra Flash GC a permis d'identifier en quelques minutes seulement (< 2 min) l'éthanol, le (E,E)-2,4-décadiénal (défaut organoleptique) et le 1-hexanol (fruité, herbeux) comme principaux volatils caractérisant la variété Soury.

Cette étude offre la possibilité d'établir au Liban un plan de contrôle analytique qui lie les aspects environnementaux et les techniques de culture/récolte aux caractéristiques physico-chimiques de l'huile d'olive qui en résultent. Une telle matrice monitorée à l'aide de techniques d'analyse rapide facilitera la vérification de la conformité du produit final aux normes internationales. En outre, ce travail préparera le terrain grâce à une fiche d'identification détaillée pour l'IGP.

Title : Implementation of a physio-chemical approach coupled with a data fingerprinting methodology for the characterization of the Lebanese extra-virgin olive oils

Keywords : Olive oil Chemometrics Geographical indication Physicochemical characterization Quality Rapid analysis

Abstract : Olive oil is a vital component of the Mediterranean diet, hence Lebanese, owed to its well-known economic and nutritional value. Several environmental, agricultural, and technological factors play an essential role in defining olive oil's quality. In Lebanon, preliminary studies on the quality of extra virgin olive oil have shown that certain quality criteria exceed the International Olive Council's (IOC) standards. However, the causes of such non-conformities have not been clearly identified. Accordingly, ninety-six olive oil samples have been harvested from two seasons, processed using different extraction methods, and collected from eight locations (Akkar, Chouf, Hasbaya, Koura, Tyr, Nabatiyeh, Zgharta, and Hermel). These locations are identified by the European Union to have potentials for Protected Geographical Indications (PGI). In this perspective, and to meet the European framework's requirements, the analyzed oil will be subjected to conventional chemical analysis as suggested by the IOC and to ultra-fast analysis using 3D-front face spectroscopy (3D-FFFS) and ultra-flash gas chromatography (Ultra-FGC).

A correlation between the fatty acid profile and the pedoclimatic conditions of the main olive growing regions in Lebanon was noticed. Three main pedoclimatic conditions, altitude, temperature, and relative humidity, were the major influencers and the reason for the distinctive fatty acid profile of the Lebanese olive oil. Lebanese areas with high altitudes, low average temperature, and low relative humidity have high oleic acid content. As for areas with lower altitudes, higher average temperature, and higher relative humidity, the fatty acid profile was characterized by linoleic, linolenic, palmitoleic, and palmitic acids. In addition to the environmental factors, agricultural ones, particularly the harvest date, had affected the chemical constituents of olive oil. The results obtained showed that the harvest date strongly influenced acidity and total polyphenols. A change in the fatty acid profile characterized by a higher linoleic and lower oleic content, an increase in Δ^7-stigmastenol exceeding the limit set by the IOC standards, and a dominating off-flavor compound (ethanol) was noticed as a result of delaying the harvesting time. Besides, two technological factors, particularly improper fruit storage, and bad hygienic practices, significantly affected olive oil's quality parameters and fatty acid content.

3D-FFFS and Ultra-FGC were used in-line with conventional analysis, and they both showed an undeniable performance. 3D-FFFS coupled with chemometric tools, namely multiple linear regression (MLR) applied on parallel factor (PARAFAC) scores and partial least squares (PLS), was tested on inconsistent qualities of olive oil samples to predict quality parameters. Twenty-two MLR models were generated, the majority of which showed a good correlation coefficient (R>0.7). A second model using PLS on the unfolded emission-excitation matrices was also conducted to improve the regression and assess whether the variability can be handled successfully. However, similar results, with a slight improvement over the MLR model, were obtained. As for Ultra Flash GC, it made it possible to identify, in only a few minutes (< 2 min), ethanol, (E,E)-2,4-decadienal (organoleptic defect), and 1-hexanol (fruity, grassy) as the main volatiles characterizing the Soury variety.

This study offers the potential to disseminate an analytical control plan that links environmental aspects in Lebanon and cultivation/harvesting techniques to olive oil's resulting physicochemical characteristics. Such a matrix incorporating rapid analysis techniques will facilitate governance over the end product's final quality and, subsequently, conformity to IOC standards. Furthermore, this work will set the ground through a detailed identification fiche for PGI.