DETERMINANTS FACTORS OF QUALITY INDICES, ANTIOXIDANT CAPACITY, PHENOLIC CONTENT, AND OXIDATIVE STABILITY OF LEBANESE OLIVE OIL

by

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Acknowledgment

"Alone we can do so little, together we can do so much" – Helen Keller

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List of Abbreviations

ABTS:	2,2'-azino-bis (3-ethylbenzothiazoline-6-sylphonic acid)
AC:	Antioxidant Capacity
ANOVA:	One-way analysis of variance
DAD:	Diode array detector
DPPH:	2,2-diphenyl-1-picrylhydrazyl
EFA:	Esterified fatty acids
EU:	European Union
EVOO:	Extra Virgin Olive Oil
Fatty Acid Methyl Esters:	FAMEs
FC:	Folin-Ciocalteu
FID:	Flame ionization detector
FA:	Free acidity
FRAP:	Ferric Reducing Ability of Plasma
GA:	Gallic acid
GAE:	Gallic Acid Equivalent
HPLC:	High performance liquid chromatography
IOC:	International Olive Council
KI:	Potassium iodide
KOH:	Potassium hydroxide
LLE:	Liquid-liquid extraction
MANOVA:	Multivariate analysis of variance
MUFA:	MonoUnsaturated Fatty Acid
OO:	Olive oil
ORAC:	Oxygen Radical Absorbance Capacity

OSI:	Oxidative Stability Index
PUFA:	Polyunsaturated fatty acid
PV:	Peroxide Value
RSA:	Radical Scavenging Activity
SD:	Standard Deviation
SFA:	Saturated fatty acid
TE:	Trolox Equivalent
TG:	Triglyceride
TPC:	Total Phenolic Content
VOO:	Virgin Olive Oil

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Abstract

Background:

Olive oil (OO) has been widely known as a main source of lipid in the Mediterranean diet. Over the years, the interest in virgin olive oil (VOO) consumption boosted due to its sensory properties characterized by its unique taste, flavor and color; and its health properties exerted by its monounsaturated fatty acids (MUFAs), tocopherols, and phenolic compounds. Phenols are minor compounds with antioxidant capacities that contribute positively to OO shelf life and stability to oxidation. Literature data have shown that several agro-industrial factors including harvesting time, processing system and geographical origin affect OO fatty acid composition, individual and total phenolic content (TPC), its antioxidant capacity (AC), its oxidative stability (OSI), and its quality indices. In Lebanon, few studies were conducted on olive cultivars, and due to the lack of homogenous agro-industrial conditions and appropriate sample size, little is known on Lebanese olive cultivars characteristics including TPC, AC and OSI, and on the suitable agro-industrial factors that could lead to the production of good quality OO.

Objectives:

The aim of the present work is to assess the effect of some agro-industrial factors on OO TPC, AC, and OSI, and to determine the correlation between the latter characteristics. This study also aims to assess the correlation between quality indices, and their impact on TPC, AC and OSI, and to determine practices that could lead to high quality OO.

Methods:

This cross-sectional study forms part of a project funded by the CNRS and implemented at LARI in collaboration with NDU, USJ and USAID/LIVCD project. Sampling was carried out on 108 VOO and extra virgin olive oil (EVOO) samples harvested from

Lebanese northern (Akkar and Zgharta-Koura) and southern (Hasbaya and Jezzine) regions. Samples were collected at three harvesting times (early, intermediate, late) and crushed using different types of mills (traditional, sinolea, two-phase and three-phase decanters). Three samples were processed at each harvesting time and for each processing system. Peroxide value, free acidity, K_{232}, and K_{270} were determined on the same samples in previous studies according to IOC methods of analysis (EEC reg 2568_91). High performance liquid chromatography (HPLC) was used to identify individual phenolic compounds, and gas chromatography to determine OO fatty acid composition. Also, OSI was determined using Rancimat instrument (El Riachy *et al.* 2017). During the present study, Folin-Ciocalteu assay was conducted to quantify total phenols content (TPC) after their extraction using liquid-liquid extraction technique (by methanol/water 60/40, v/v), and AC was identified using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method.

Results:

Peroxide value and UV absorbance were within the limits set by EU Commission Regulation 1989; however, FA exceeded those limits. Samples mean TPC was 157.0 ± 32.8 $mg.kg^{-1}$, mean DPPH was 12.1 ± 4.6%, and mean OSI was 8.2 ± 1.6 h. The obtained results show that early harvested OO samples obtained from Akkar using 3-phase decanters exhibited the highest TPC. Also, early harvested samples from Jezzine using 3-phase decanters showed the highest AC. As for resistance to oxidation, early harvested samples from Hasbaya using 3-phase decanters possessed the highest OSI. Furthermore, the obtained results also revealed a moderate positive correlation between TPC and AC, and a strong positive one between TPC and OSI. However, weak negative correlations were observed between OO TPC/AC/OSI and its quality indices, and weak to moderate ones between individual phenols and the latter parameters.

Conclusion:

Agro-industrial factors contribute to OO's quality indices and characteristics including individual and total phenolic content, antioxidant capacity, and thereby resistance to oxidation. It also determines the characteristics, composition, and nutritional properties of Lebanese OO samples, obtained from different areas, and undergoing various pedoclimatic, industrial and agricultural conditions.

Keywords: Olive Oil; Extra Virgin Olive Oil; Quality Indices; Fatty Acid Composition; Total Phenolic Content; Antioxidant Capacity; Oxidative Stability Index.

Chapter I – Literature Review

I.1. Introduction and Background

I.1.1. Olive Cultivars and Distribution

The presence of the olive tree (*Olea europaea L.*) dated back to the twelfth millennium BC (Shahidi and Kiritsakis, 2017). Traditionally, olive cultivation originated from countries of the Mediterranean basin due to its climate characterized by limited water availability (El Riachy *et al.* 2017), and has spread to areas outside the Mediterranean far from its origin (Figure I.1). Spain ranks first in olive and olive oil (OO) production, followed by Italy, Greece, Turkey, and Tunisia (IOC, 2013; FAOSTAT, 2014).

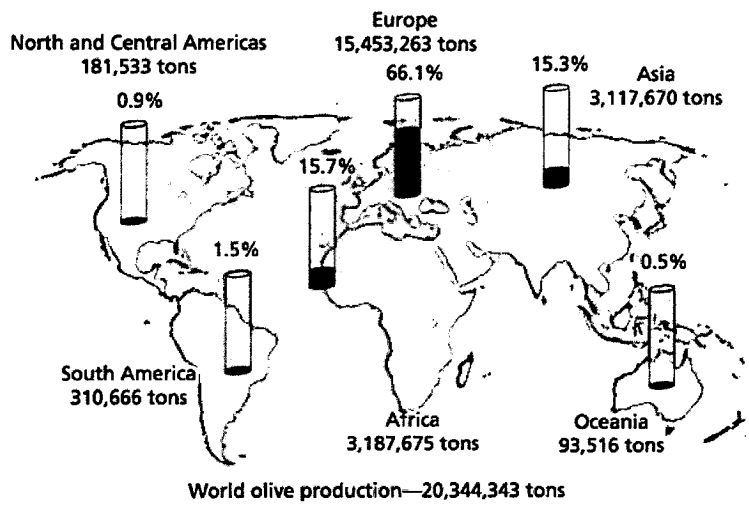

Figure I.1. World olive production according to Food and Agriculture Organization (FAOSTAT, 2013).

In Lebanon, OO production has been on the rise (IOC, 2012). Due to its moderate Mediterranean climate, Lebanon provides ideal conditions for cultivation of olive trees (Merchak *et al.* 2017) that occupy an area of about 58000 ha, representing approximately 23% of the total cultivated sector (El Riachy *et al.* 2017). They are raised in locations with altitudes ranging between 45 and 1200 m above sea level. The

Ministry of Agriculture (2010) reported that 41% of olive trees are located in northern regions, followed by Nabatieh (21%), Southern regions (15%), Bekaa (13%), and Mount Lebanon (10%) (Figure I.2).

Lebanese orchards are characterized by various cultivars. Baladi, Soury (originated from Tyr), Del, and Abou Chawkeh are the most common cultivars used for both table olive and OO production, Kalb el Tair and Jlot are exclusively used for table olive production, and Ayrouni and Teliani (Italien) are exclusively used for OO production (Chehade *et al.* 2012).

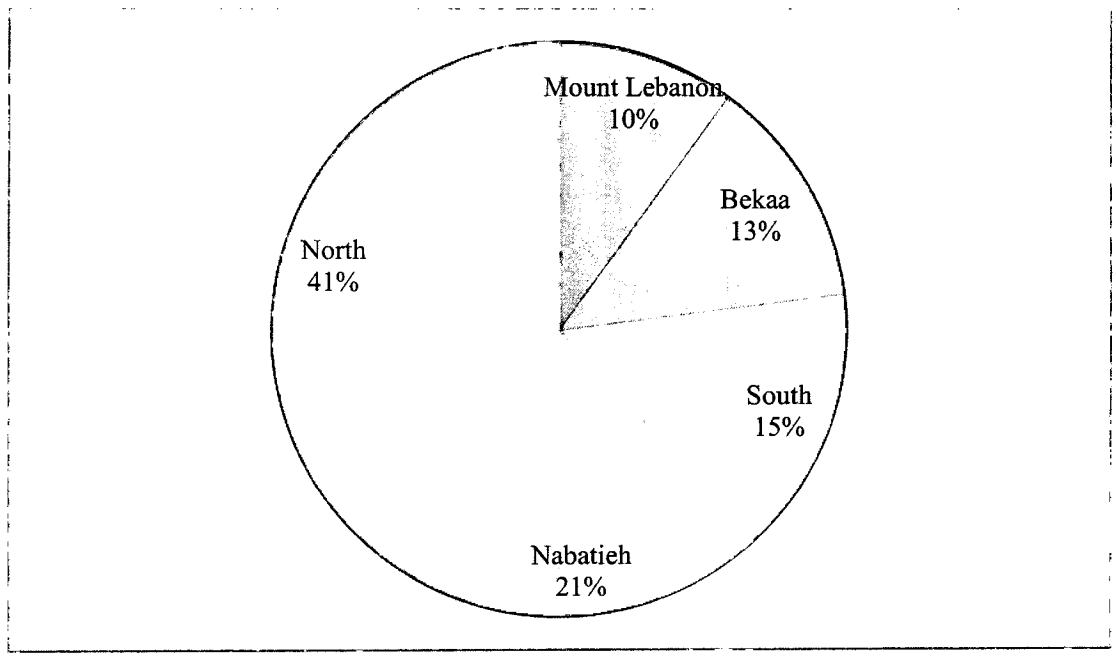

Figure I.2. Distribution of olive trees in Lebanon according to Ministry of Agriculture (2010).

I.2. Olive Oil

I.2.1. Production

After harvesting, both traditional and modern techniques are used to produce OO. In traditional techniques, olives are crushed into paste by granite stone mills equipped with a pressure system for around of 20-30 minutes. Afterwards, the paste is malaxed at a continuous and slow movement for 10-20 minutes allowing the release of free oil, which will be subsequently pressed on fiber disks. Then, a liquid-liquid separation is conducted through a vertical centrifuge by adding warm water (≤ 27°C) to the oil (Shahidi and Kiritsakis, 2017). In modern techniques, stone mills are being replaced by modern hammer mills, toothed disks, and cones to avoid the following disadvantages of the stone mills: 1) the extended contact of paste with air during the process, 2) its low working capacity, and 3) its reduced ability to release chlorophyll. Olives crushed using modern techniques exhibit better quality and oil yield (Di Giovacchino *et al.* 2002). After malaxation, phase separation occurs by using two- or three-phase horizontal centrifuges instead of traditional pressing systems to reduce 1) storage time, 2) processing time and 3) labor costs (Klen and Vodopivec, 2012).

I.2.2. Classification

The international olive council (IOC) has developed quality and purity criteria to classify OO as: 1) Virgin olive oil (VOO), 2) Refined Oil, and 3) Olive Oil (Figure I.3).

1) VOO is obtained by mechanical and physical means that do not change the oil's characteristics. This category of oil consists of two subgroups:
    1.1. Virgin olive oil for human consumption without further processing, including:
        1.1.a. Extra virgin olive oil (EVOO): which has excellent flavor and odor, and

a free fatty acid content of not more than 0.8 g oleic acid per 100 g oil

1.1.b. Virgin olive oil: which has fairly good flavor and odor, and a free fatty acid content of not more than 2 g oleic acid per 100 g oil

1.1.c. Ordinary virgin olive oil: which has more defects than previous subgroups and a free fatty acid content of less than 3.3 g oleic acid per 100 g oil. According to European Union (EU) laws, this type doesn't undergo bottling.

1.2. Virgin olive oil that doesn't fit for human consumption (Lampante): which has severe defects and a free fatty acid content of more than 3.3 g oleic acid per 100 g oil.

2) Refined olive oil is the oil obtained from refining virgin olive oil. It is flavorless, odorless, and has free fatty acid content of 0.3 g oleic acid per 100 g oil.

3) Olive oil is the oil obtained by mixing refined olive oil and VOO.

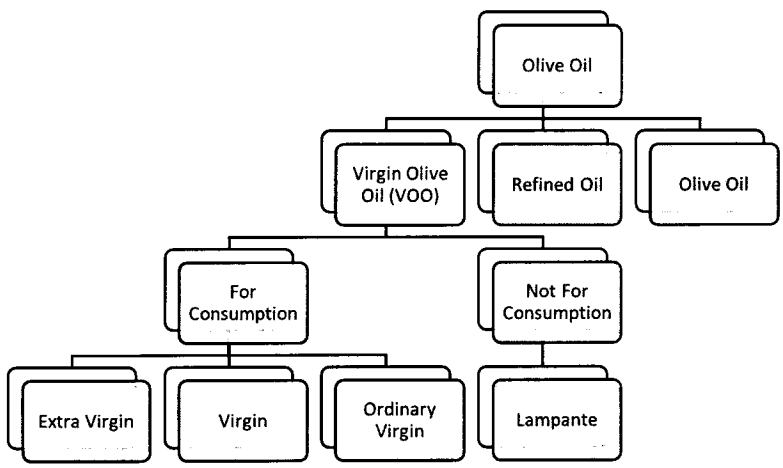

Figure I.3. Olive oil classification according to International Olive Council.

I.2.3. Composition

Major Components

OO consists mainly of: 1) triacylglycerols (TG), 2) diacylglycerol (DG), 3) monoacylglycerol (MG), and 4) free fatty acids (FA). Monounsaturated fatty acids (MUFAs) range between 55-83% of total composition, polyunsaturated fatty acids (PUFA) between 4-20%, and saturated fatty acids (SFAs) between 8-13%. The major fatty acids are: 1) palmitic (C16:0), 2) palmitoleic (C16:1), 3) stearic (C18:0), 4) oleic (C18:1), 5) linoleic (C18:2), and 6) linolenic (C18:3), while the minor ones are myristic (C14:0), heptadecanoic (C17:0) and eicosanoic (C20:0) acids (Boskou, 2015). Fatty acid composition is one of the most significant parameters that varies between OO and affects its quality. It can also be greatly affected by agronomical and environmental factors (Borges *et al.* 2017).

Minor Components

They are classified into two types: unsaponifiable (nonpolar) and saponifiable (polar) fractions. The nonpolar components, which are insoluble in aqueous medium after saponification, consist of: 1) squalene, 2) triterpenes, 3) sterols, 4) tocopherol, and 5) pigments; while the polar fraction includes phenolic compounds that vary distinctly between OOs, and exert nutritional and organoleptic properties (Boskou, 2015).

I.2.4. Health Benefits

The Mediterranean diet has dispersed throughout the world as synonymous of a healthy diet (Sofi *et al.* 2013). Adherence to this kind of diet, in which VOO is the main source of lipid, yielded beneficial effects (Bellavia *et al.* 2016), and it was inversely associated with the development of different types of cancer, cardiovascular diseases, diabetes, arthritis, and other chronic illnesses (Goulas *et al.* 2012; Boskou, 2015). The health

benefits of olives and olive oil are widely known due to their high: 1) total phenolic content (TPC), 2) antioxidant capacity (AC), and 3) MUFA content (Ghanbari *et al.* 2013). Several studies found that the protective effects of OO are attributed to: 1) reducing blood pressure, 2) protecting against endothelial dysfunction (Covas, 2007; Ghanbari *et al.* 2013), 3) lowering risk of several types of cancer (i.e. breast, pancreas, esophagus, rectum...) (Artajo *et al.* 2006), 4) inhibiting atherogenesis (Carluccio *et al.* 2003), 5) and controlling lipid profile (Ghanbari *et al.* 2013). Moreover, through its phenolic content, OO showed significant effects against cardiovascular diseases via its: 1) anti-platelet aggregation, 2) antioxidant, 3) anti-inflammatory, 4) antimicrobial, and 5) vasodilatory properties (Charoenprasert & Mitchell, 2012; Servili *et al.* 2014).

I.2.5. Quality Indices

According to EU Commission Regulation 1989, an OO must comply to the following set of fixed sensory and chemical parameters to be categorized as an EVOO: 1) FA (%) ≤0.8 g oleic acid/100g oil, 2) Peroxide Value (PV) ≤20 mEq $O_2.kg^{-1}$, 3) $K_{232} \leq 2.50$, 4) $K_{270} \leq 0.22$, 5) Median of defects = 0, and 6) Medium of fruity>0.

To date, several studies showed correlations between agro-industrial factors such as 1) olive cultivar, 2) ripening stage, 3) processing system, 4) crop year, and 5) irrigation regime and the mentioned quality indices.

Free Acidity (FA)

Free acidity is a measure of the concentration of FAs released through the hydrolysis of TG. This parameter demonstrates the quality of the olive fruit and its oil (United

States Standards for Grades of Olive Oil and Olive-Pomace Oil, 2010).

Several studies reported remarkable increase in FA with a progress in fruit ripening that can be explained by an elevation in endogenous enzymatic activity (Dag *et al.* 2011; Bengana *et al.* 2013; Koseoglu *et al.* 2013; Rivas *et al.* 2013). As for the processing system, traditional ones were found to yield oils with higher FA as compared to modern methods due to the increase in lipolitic enzyme that would favor TG hydrolysis, thus releasing FA (Tores and Maestri, 2006; Ammar *et al.* 2014; Serhan *et al.* 2016).

Peroxide Value (PV)

Upon fat and oil oxidation, peroxide molecules are produced. PV is a parameter that measures the concentration of unstable hydroperoxides (Pizzaro *et al.* 2013) and the degree of lipid oxidation (Rached *et al.* 2017). Thus, the higher the PV, the lower the oxidative stability (OSI) (Pizzaro *et al.* 2013).

According to the literature, significant differences in PV were observed among OO belonging to different cultivars (Nakbi *et al.* 2010; Koseoglu *et al.* 2013; Jolayemi *et al.* 2016). Moreover, ripening stage had a significant inverse relationship with PV due to a decrease in lipoxygenase enzyme activity as maturity progressed (Baccouri *et al.* 2008; Bengana *et al.* 2013; Rivas *et al.* 2013; Bajoub *et al.* 2014). As for processing system, studies have shown significantly higher PV in oils processed using traditional systems in comparison to those centrifuged, and this might be due to the contact of samples with metal surface of traditional equipment (Vekiari *et al.* 2007), or with light and air while agitating using a millstone (Serhan *et al.* 2016). Another explanation of the latter results could be attributed to the higher phenolic content obtained in OO processed using modern centrifuge (Vekiani *et al.* 2001).

UV Absorbance at 232 and 270 nm

UV spectrophotometric technique has been used to indicate OO quality, purity, state of preservation and changes brought about in it by technological processes (Puente, 2008). The IOC specified 2 wavelengths (232 and 270 nm) for the determination of UV absorbance of VOO. Absorption at both wavelengths is related to the formation of primary oxidation products (diene at 232 nm and trienes at 270 nm) in OO (Ammar *et al.* 2014), and low values confirm a good quality of the analyzed OO (Bajoub *et al.* 2014).

Conflicting results were reported on the effect of the following agro-industrial factors: 1) cultivar (Torres & Maestri, 2006; Ceci *et al.* 2017), 2) crop year (Salvador *et al.* 2003; Caruso *et al.* 2014), 3) processing system (Gimeno *et al.* 2002; Ammar *et al.* 2014), 4) fruit ripening (Bajoub *et al.* 2014; Dabbou *et al.* 2015), and 5) irrigation regime (Tovar *et al.* 2002; Dabbou *et al.* 2011; Caruso *et al.* 2014; Dabbou *et al.* 2015) on both K_{232} and K_{270}. Moreover, many authors showed greater effects of these factors on K_{232} when compared to K_{270}.

I.3. Olive Oil Polar Phenolic Compounds

I.3.1. Types

Phenols are classified into six main families consisting of: 1) phenolic acids (i.e. caffeic, vanillic, *p*-coumaric, *o*-coumaric, protocatechuic, sinapic, p-hydroxybenzoic and gallic acids), 2) phenolic alcohols (3,4-DHPEA or hydroxytyrosol, and p-HPEA or tyrosol), 3) flavonoids (i.e. flavones, flavonols, flavanones, flavanols, anthocyans and derived glucosides), 4) isochromans, 5) lignans, and 6) secoiridoids (i.e. oleuropein, demethyloleuropein, and ligstroside) (El Riachy *et al.* 2011).

I.3.2. Benefits

Phenolic compounds contribute positively to the VOO shelf life and freshness (Boskou; 2015), induce stability to oxidation; and exhibit desirable sensory properties through offering a pleasant bitter-pungent taste, and a unique aroma and color (Gomez-Rico *et al.* 2008; El Riachy *et al.* 2012; Ricciutelli *et al.* 2017). Additionally, phenols, mainly oleuropein and 3,4-DHPEA, are of considerable importance to human health due to their anti-carcinogenic (Charoenprasert and Mitchell, 2012; Talhaoui *et al.* 2016), anti-inflammatory (Parkinson and Keast; 2014), antioxidant (Servili *et al.* 2009; Charoenprasert and Mitchell, 2012; Boskou, 2015; Talhaoui *et al.* 2016), anti-microbial (Charoenprasert and Mitchell, 2012), anti-platelet aggregation (Inglese *et al.* 2011) and vasodilatory properties (Inglese *et al.* 2011; Boskou, 2015). They also inhibit the activity of oxygen reactive species, thereby reducing oxidative stress (Boskou, 2015) and the incidence of cardiovascular events and strokes (Estruch *et al.* 2013). Additionally, phenols were shown to exhibit significant inhibitory effect on LDL oxidation (Nakbi *et al.* 2010). Concerning 3,4-DHPEA, it was proven to exert protective effects against metabolic syndrome by reducing hyperlipidemia, hyperglycemia and insulin resistance (Cao *et al.* 2014; Peyrol *et al.* 2017).

I.3.3. Total Phenolic Content Quantification

The analytical methods of OO TPC quantification vary widely including: 1) Folin-Ciocalteu (FC), 2) Total Phenolic Index, 3) Near Infrared spectroscopy determination (NIR), 4) Fluorescence-based determination, and 5) Nuclear Magnetic Resonance Spectroscopy (NMR) (El Riachy *et al.* 2011). FC assay is a common, simple, and cheap procedure that involves a reaction between the FC reagent and the hydroxyl group

found in phenols leading to a change in the mixture's colorimetric properties. However, the limitations of this method are represented by its limited sensitivity and inability to differentiate between mono-phenols and *o*-diphenols (El Riachy *et al.* 2011; Reboredo-Rodriguez *et al.* 2016).

I.3.4. Antioxidant Capacity (AC) of Olive Oil Phenols - Mechanism and Determination

Oxidation, a reaction consisting of 3 steps (initiation, propagation and termination) leads to: 1) the development of unpleasant taste and odor, 2) loss of nutrients, 3) formation of toxic compounds, and 4) a reduction in shelf life and quality (Saldaña and Martinez-Monteagudo, 2013). Free radical, an unstable molecular species with an unpaired electron, is produced in the initiation step of oxidation and may be involved in a number of diseases involving the heart, lungs, kidneys, liver, eyes, brain and many others (Lobo *et al.* 2010).

Hydrophilic and lipophilic phenols, the most existing antioxidants in OO, exert their activity through: 1) decreasing oxygen concentration, 2) obstructing singlet oxygen, 3) preventing first chain initiation through electron donation and scavenging initial radicals, 4) inducing metal chelation, and 5) decomposing peroxides (Shahidi, 1995; Baiano *et al.* 2009).

Over the years, many researchers have evaluated OO antioxidant capacity using various assays such as: 1) ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), 2) DPPH (2,2-diphenyl-1-picrylhydrazyl), 3) ORAC (oxygen radical absorbance capacity) (Ninfali *et al.* 2001; Alvarez-Suarez *et al.* 2011; Baiano *et al.* 2013; Condelli *et al.* 2015), 4) FRAP (ferric reduced ability of plasma) (Kalogeropoulos *et al.* 2014),

and 5) beta-carotene bleaching assay (Condelli *et al.* 2015). DPPH and ABTS are the two most commonly applied methods due to their rapid and simple use. Each of these methods is characterized by its own selectivity, solubility, and colorimetric properties (Cerretani and Bendini, 2010).

I.4. Oxidative Stability Index (OSI)

OO has been widely known for its resistance to oxidation due to its FA composition characterized by a high MUFA/PUFA ratio, and its high TPC that prevent lipid oxidation in its initial stage (Velasco & Dobarganes, 2002; Nakbi *et al.* 2010; El Riachy *et al.* 2011; Rigane *et al.* 2012; Ceci *et al.* 2017). Oxidation is a slow process at the initial stage, proceeded by a sudden rise in the reaction rate. The induction time which is the period required for oxidation reaction to occur is used to measure the OO stability. In the literature, several methods were used to identify OO's resistance to oxidation including: 1) Rancimat, 2) automated oxidative stability instrument, 3) active oxygen method (AOM), 4) Schaal test, and 5) Oxygen uptake method (oxidograph) (Velasco & Dobarganes, 2002; Nakbi *et al.* 2010; Mancebo-Campos *et al.* 2014; Ceci *et al.* 2017). Over the years, the Rancimat method has been widely used because it is rapid, reproducible, inexpensive, accessible, and easy (Nakbi *et al.* 2010; Ceci *et al.* 2017). The rise in water's conductivity measured by this instrument allowed the determination of induction time (Farhoosh, 2007).

I.5. Agro-industrial factors

The literature data showed that individual and TPC, antioxidant capacity, and oxidative stability index of an OO depend on several agro-industrial factors, most importantly: 1)

harvesting time, 2) processing system, and 3) geographical origin (Boskou *et al.* 2015; El Riachy *et al.* 2018).

I.5.1. Effect on total phenolic content, antioxidant capacity, oxidative stability index (Appendix A)

Harvesting Time

Several studies demonstrated significant effects of olive fruits harvesting time on its TPC. In Tunisia, a study carried out by Hbaieb *et al.* (2017) on two Tunisian olive cultivars (Chetoui and Chemlali) resulted in an increase followed by a decrease in TPC as ripening progressed, due to a change in the olive's enzymatic expression. Similar results were obtained for Chetoui and Chemlali cultivars harvested at five different harvesting times (Baccouri *et al.* 2008). These results were in accordance with several studies conducted in Spain (Gambacorta *et al.* 2010; Nieto *et al.* 2010; Hbaieb *et al.* 2015), Turkey (Köseoglu *et al.* 2013; Jolayemi *et al.* 2016;), Argentina ($p \leq 0.01$) (Ceci *et al.* 2017), and Croatia (Lukic *et al.* 2017).

Additionally, significant differences were observed in radical scavenging activity of oils at different ripening indices. A study done by Gambacorta *et al.* (2010) on Spanish Coratina olive cultivar with different ripening indices showed significant decrease in antioxidant capacity with an advance in ripening. These results were in agreement with those described in Turkey (Koseoglu *et al.* 2013) and Tunisia (Hbaieb *et al.* 2017).

Likewise, oxidative stability was significantly affected by fruit harvesting time. In a study done by Hbaieb *et al.* (2015) on oil samples of Arbequina cultivar harvested at different times, induction time clearly decreased with a progression in ripening process. Identical results were reported in Argentina ($p \leq 0.01$) (Ceci *et al.* 2017), and Turkey (Koseoglu *et al.* 2013).

Processing System

In the literature, significant effects of different processing system on olive oil's TPC were reported. Salvador et al. (2003) conducted a study on 140 Spanish samples of Cornicabra cultivar and found significantly higher TPC in oils undergoing modern decanters, specifically 2-phases decanters (160 $mg.kg^{-1}$ oil), followed by 3-phases decanters (142 $mg.kg^{-1}$oil), and traditional press system (100 $mg.kg^{-1}$oil).

Other authors also found significantly higher TPC in oils processed using 2-phases compared to 3-phases decanters in studies conducted in Tunisia (251.64 $mg.kg^{-1}$ oil vs 210 $mg.kg^{-1}$ oil) (Ammar *et al.* 2014), Spain (123.82 $mg.kg^{-1}$ oil vs 72.93 $mg.kg^{-1}$ oil) (Gimeno *et al.* 2002), Greece (31.59 ±2.72 mg GAE.100 g^{-1} vs 37.33 ±3.22 mg GAE.100 g^{-1}) (Kalogeropoulos *et al.* 2014), and Italy (292 vs 197 $mg.L^{-1}$) (Di Giovacchino *et al.* 2001). Because of phenol's high solubility in water, the reported lower TPC in 3-phases compared to 2-phases processed OO was mainly due to the addition of water only while processing olives in 3-phases decanters.

The effects of processing system on OO AC were observed in the literature. In Tunisia, Ammar *et al.* (2014) reported significantly higher AC in OO processed using 2-phases decanters (2.30 ± 0.09 $\mu g.mL^{-1}$) compared to those processed by 3-phases decanters (4.40 ± 0.24 $\mu g.mL^{-1}$) and press system (13.59 ± 0.45 $\mu g.mL^{-1}$), stating that the low inhibition percentage (IC_{50}) indicates high AC. Additionally, in Greece Kalogeropoulos *et al.* (2014) found higher AC in OO processed using 2-phases (26.51 ± 2.00 mmol Trolox equivalent (TE)) compared to 3-phases decanters (25.52 ± 2.32 mmol TE) per 100 g of sample, with no significant difference.

Furthermore, OO oxidative stability was highly dependent on the processing system. Ammar *et al.* (2014) conducted a study in Tunisia on samples of Chemlali cultivar that were processed differently, and revealed significantly higher induction time at 120°C,

with a continuous air flow of 20 L/h in samples decanted using two-phase compared to three-phase decanters (6.48 h vs 4.38 h). Similar results were reported by Di Giovacchino *et al.* (2001) who evaluated OSI under same Rancimat conditions as the previous study (14.2 h vs 11 h), and Salvador *et al.* 2003 using different temperature (100°C) and airflow conditions (10 L/h) (65.8 h vs 57.2 h).

Geographical Origin

To date, several publications revealed strong and significant effects of geographical origin on OO TPC; which is clearly related to various environmental conditions. Five studies conducted in Tunisia showed higher TPC in samples obtained from areas with the highest altitude and mean annual rainfall (Guerfel *et al.* 2009; Issaoui *et al.* 2010; Youssef *et al.* 2011; Issaoui *et al.* 2013; Mansour *et al.* 2015). Additionally, the type of soil had a significant effect on TPC in both studies performed in Italy (Baiano *et al.* 2013) and Tunisia (Rached *et al.* 2017).

Moreover, literature data found that the influence of geographical origin on AC depended on OO TPC. In Turkey, two studies comparing samples cultivated in different locations found significantly higher AC in samples with the highest TPC (Arslan & Ozcan, 2011; Arslan *et al.* 2013). These findings were similar to the results obtained in Tunisia (Issaoui *et al.* 2010) and Italy (Baiano *et al.* 2012).

Furthermore, OO stability index was significantly influenced by the growing region. In Tunisia, oil samples obtained from northern regions of high altitude and mean rainfall had significantly higher stability to oxidation compared to samples from southern areas of lower altitude and rainfall (Mansour *et al.* 2015; Issaoui *et al.* 2010; Issaoui *et al.* 2013; Youssef *et al.* 2011). These findings were similar to the results obtained in Spain

by Borges *et al.* (2017). The results could be explained by the different TPC and antioxidant profiles of olive oil samples collected from different geographical areas.

I.5.2. Effect on individual phenolic content

Literature studies have shown a wide range of variation in individual phenols that could be attributed to numerous agro-industrial factors. Harvesting time significantly influenced both hydroxytyrosol and tyrosol, the most abundant phenolic alcohols that are products of oleuropein and ligstroside aglycone degradation. In Tunisia, a study conducted by Hbaieb e*t al.* (2017) on OO obtained from 12 different harvesting times found an increase in both phenolic alcohols. Hydroxytyrosol increased from 5.14 $mg.kg^{-1}$ to 8.05 $mg.kg^{-1}$, and tyrosol from 3.41 to 4.29 $mg.kg^{-1}$, with an advance in harvesting time. Those results were in line with those found in a study carried out in Turkey by Jolayemi *et al.* (2016).

Moreover, a slight increase followed by a significant decrease in both apigenin and luteolin were observed with a progress in harvesting time (Ben Brahim *et al.* 2017; Hbaieb *et al.* 2017).

Regarding processing system, it significantly affected specific individual phenols. A study conducted by Ammar *et al.* (2014) on samples of Chemlali cultivar processed using 2- and 3-phase decanters showed significantly higher mean content in total flavonoids in 2-phase decanted oils (14.5 $mg.kg^{-1}$) as compared 3-phase decanted ones (13.0 $mg.kg^{-1}$). On the other hand, conflicting results were obtained regarding both hydroxytyrosol and tyrosol. While both tyrosol and hydroxytyrosol were found higher in 2-phase decanted oils (hydroxytyrosol: 6990.2 $mg.kg^{-1}$, tyrosol: 499.4 $mg.kg^{-1}$) as compared to 3-phase decanted ones (hydroxytyrosol: 2018.6 $mg.kg^{-1}$, tyrosol: 42.7 $mg.kg^{-1}$) in a study conducted by Kalogeropoulos *et al.* (2014), they were shown higher in 3-phase decanted oils (hydroxytyrosol: 765.0 $mg.kg^{-1}$, tyrosol: 64.0 $mg.kg^{-1}$) as

compared to 2-phase decanted ones (hydroxytyrosol: 413.0 $mg.kg^{-1}$, tyrosol: 32.0 $mg.kg^{-1}$) in a study conducted by Klen & Vodopivec, (2012).

Concerning geographical origin, statistically significant difference was observed in individual phenolic constituents of OO obtained from different locations. A study conducted in Turkey by Arslan *et al.* (2013) found significant difference in various phenols such as secoiridoids, hydroxytyrosol, tyrosol and phenolic acids of OO obtained from three regions in Turkey. These findings were similar to those obtained by Italy (Baiano *et al.* 2013) and Turkey (Arslan & Ozcan, 2011).

I.6. Correlation between agro-industrial factors and TPC, AC, and OSI in Lebanon

Nationally, four studies were conducted on Lebanese OO cultivars to examine the effect of agro-industrial factors on the fruit and its oil's composition (TPC) and quality characteristics (AC, and OSI).

Serhan *et al.* (2016) conducted a study on 25 Lebanese OO samples obtained from different northern regions, and extracted using traditional and modern (three-phase) techniques. Insignificant difference in TPC was reported among samples collected from different geographical areas due to the greater effect of other factors such as velocity of crushing machine, volume of water added, duration of contact with water, and others. However, significantly higher OSI was observed in modern compared to traditionally pressed oils (24.4 h vs 17.2 h).

To investigate the effect of different irrigation regimes on fruit's quality and composition in two cultivars (Baladi and Edlbi), El Riachy *et al.* (2017) reported significantly higher TPC in the least irrigated samples of Edlbi cultivar compared to

Baladi cultivar; which was attributed to the activity of the enzyme L-phenylalanine ammonia-lyase that works best under water deficit conditions. The authors also showed significantly high OSI in OO obtained from Edlbi cultivar and from rain fed trees, highlighting the correlation between TPC and OSI.

Moreover, Chehade *et al.* (2016) conducted a study on five different Lebanese cultivars (Aayrouni, Abou Chawkeh, Baladi, Del, and Soury) and observed significant difference in TPC ranging between 208±41 and 430±61 $mg.kg^{-1}$. Similar significant results were observed by Chehade *et al.* (2012) in a study conducted on 8 Lebanese varieties from different regions and grown under different pedoclimatic conditions.

Authors of these studies ended their research by suggesting the: 1) application of more control on OO samples, 2) increase in number of sample size, 3) evaluation of OO varieties under similar agro-industrial conditions, and 4) analysis of other unstudied OO contents such as chlorophyll and OSI before and after extracting phenols.

I.7. Correlation between total phenolic content, antioxidant capacity, and oxidative stability index

OO is well known for its extended shelf life and resistance to oxidation due to its high content of antioxidants, determined by concentration and composition of its minor compounds (Nakbi *et al.* 2010), and low unsaturation level characterized by a high MUFA/PUFA ratio (Dabbou *et al.* 2010; Rigane *et al.* 2012). Berengeur *et al.* (2006) found a close correlation between OSI and TPC with $r^2 = 0.98$. Moreover, Ceci *et al.* (2017) observed higher OSI in OO cultivar with the highest 1) MUFA/PUFA ratio ($p < 0.001$), 2) TPC ($p = 0.001$), 3) tocopherols ($p < 0.001$), and 4) carotenoids ($p = 0.001$) as compared to cultivars with lowest values. Similar results were obtained

in Tunisia (Ammar *et al.* 2014; Mansour *et al.* 2015; Chtourou *et al.* 2013; Dabbou *et al.* 2009; Nakbi *et al.* 2010; Rigane *et al.* 2013; Youssef *et al.* 2011), Spain (Sena-Moreno *et al.* 2017; Tovar *et al.* 2002), Italy (Tura *et al.* 2013), and Lebanon (El Riachy *et al.* 2017; Serhan *et al.* 2016).

I.8. Correlation between quality indices and total phenolic content, antioxidant capacity, and oxidative stability index

Throughout the literature, studies related to correlation between different quality indices (PV, FA, UV absorbance) and TPC, AC, or OSI are limited. Only one study conducted by Gutiérrez *et al.* (2002) discussed the association between OSI and OO quality indices in 3 olive cultivars (Picual, Hojiblanca, and Arbequina), and found strong negative correlations between oxidative stability and: 1) PV ($r = -0.978$), 2) K_{232} ($r = -0.986$), and 3) K_{270} ($r = -0.982$). The findings implicate that oils with high OSI have low PV, and UV absorbance values (K_{232} and K_{270}). On the other hand, a correlation between OSI and FA was not clearly observed.

I.9. Rationale

The demand and consumption of OO continue to rise because of its sensory properties characterized by its distinct odor and flavor, and the beneficial health properties exerted by its chemical composition (Boskou, 2015). Internationally, many authors assessed the effect of various agro-industrial factors (i.e. harvesting time, processing system, geographical origin) on OO chemical composition (individual and TPC) and quality (AC, and OSI); while others reported the correlation between TPC, AC and OSI and

their effect on OO quality. Moreover, several studies highlighted the effects of agro-industrial factors on OO quality indices. However, studies conducted on the effects of OO quality indices on its TPC, AC and OSI are lacking.

In Lebanon, only four studies evaluated the effects of different agro-industrial factors on OO TPC, OSI and specific quality indices (Chehade *et al.* 2016; El Riachy *et al.* 2011; El Riachy *et al.* 2017; Serhan *et al.* 2016). Due to the lack of homogenous agro-industrial conditions and appropriate sample size, little is known on Lebanese olive cultivars characteristics including its fatty acid composition, individual phenolic content, TPC, AC and OSI, and no single study assessed 1) the effect of agro-industrial factors on AC, 2) agro-industrial factors that yield OO with highest TPC, AC and OSI and 3) the relationship between phenolic content, OSI, AC and quality and composition indices.

I.10. Objectives

Therefore, the main objectives of our study are:

1) to assess the effect of the agro-industrial factors (harvesting time, processing system, geographical origin) on individual phenols, TPC, AC, and OSI

2) to assess the correlation between quality indices (PV, FA, and UV absorbance), TPC, AC, and OSI

3) to determine the correlation between individual and total phenolic content, AC, and OSI

4) to define the agro-industrial factors that yield OO with the highest TPC, AC, and OSI

We hypothesize that OO quality indices, individual phenols, TPC, AC and OSI vary between samples obtained from different farmers, different Lebanese regions, different

processing system, and at different harvesting times. We also assume the existence of a correlation between TPC, AC, OSI, and OO grade defined by its quality indices.

Chapter II

II.1. Introduction

The presence of the olive tree (*Olea europaea L.)* dates back to the twelfth millennium BC (Shahidi and Kiritsakis, 2017). An olive fruit and its oil represent a heritage of most countries of the Mediterranean basin, and olive oil (OO) is the main source of fat in the Mediterranean diet (Oliveras-Lopez *et al.* 2014). OO is increasingly used in the Mediterranean diet and around the world for its delicious taste and specific aroma, as well as for its nutritional benefits (Boskou, 2015). The biological benefits of OO were attributed to its chemical composition constituted of high monounsaturated fatty acids (MUFAs) (oleic acid) content, a balanced presence of polyunsaturated fatty acids (PUFA), and minor components such as tocopherol and phenolic compounds (Ghanbari *et al.* 2013). MUFAs have a relative stability to oxidation; however, PUFAs are highly prone to oxidation leading to the deterioration of the OO and production of undesirable flavors. On the other hand, phenolic compounds composition, concentration and chemical structures as well as their antioxidant capacity (AC) and ability to scavenge free radicals play a significant role in the product stability and sensory characteristics (Aksoy *et al.* 2013; El Riachy *et al.* 2012; Hohmann *et al.* 2015). Phenolic compounds also play a significant role in human health due to their anti-carcinogenic, anti-inflammatory, antioxidant, anti-microbial, anti-platelet aggregation and vasodilatory properties (Boskou, 2015; Bulotta *et al.* 2014; Parkinson & Keast; 2014; Peyrol *et al.* 2017; Talhaoui *et al.* 2016). Rather than depending on OO TPC or stability to oxidation, the international olive council (IOC) has set quality and purity parameters to improve and control quality of OO once it is bottled.

The quality, composition and characteristics of OO depend on several environmental, agronomic, cultivation and technological factors (Ben Rached *et al.* 2017; El Riachy *et*

al. 2018). Specifically, harvesting time, processing system, and geographical origin are among the most contributing factors to the variation in OO quality, among all other factors (El Riachy *et al.* 2018). Regarding the effect of an advance in harvesting time on OO quality indices, FA was shown to remarkably increase due to an elevation in endogenous enzymatic activity, while PV decreased due to a decrease in lipoxygenase enzyme (Köseoğlu *et al.* 2016; Rivas *et al.* 2013; Bajoub *et al.* 2014). Ttraditional processing techniques (press system) were found to yield OO with higher FA due to the increase in lipolitic enzyme that would favor triglyceride hydrolysis (Ammar *et al.* 2014), and higher PV due to the contact of samples with metal surface of traditional equipment (Vekiari *et al.* 2007), or with light and air while agitating using a millstone (Serhan *et al.* 2016). Moreover, conflicting results were reported on the effect of harvesting time (Bajoub *et al.* 2014; Dabbou *et al.* 2015) and processing system (Gimeno *et al.* 2002; Ammar *et al.* 2014) on both K_{232} and K_{270}. Numerous authors also showed significant decrease in OO TPC, AC, and consequently oxidative stability index (OSI) with an advance in harvesting time that could be attributed to a change in olive's enzymatic expression (Ceci *et al.* 2017; Hbaieb *et al.* 2017; Lukic *et al.* 2017). Others revealed significantly higher phenolic composition, AC, and OSI in OO processed using modern techniques as compared to traditionally pressed ones, ascribing the findings to the high exposure to air in the latter techniques (Ammar *et al.* 2014; Kalogeropoulos *et al.* 2014). Several studies found that OO samples obtained from regions with high altitude and mean rainfall exhibited the highest TPC and OSI (Borges *et al.* 2017; Ben Rached *et al.* 2017). The correlations between phenolic composition and TPC in OO from one side and the AC and OSI from the other were also reported (Gambarcota *et al.* 2010; Hbaieb *et al.* 2015; Köseoğlu *et al.* 2016).

While several authors assessed the impact of the most important agro-industrial factors

on OO quality indices, fatty acid composition, phenolic compositions, AC and OSI, they fell short on investigating the multifactorial effects on OO composition and characteristics and on determining the most contributing factor leading to the most stable OO and that with high AC.

Therefore, the aim of this study was to characterize the OO produced in Lebanon from mono-cultivar 'Baladi' and to examine the influence of geographical origin, processing systems, and harvesting time on individual phenols, TPC, AC, OSI, fatty acid composition and quality indices. The study also assessed the correlations between OO intrinsic chemical composition, quality indices and quality characteristics. Finally, it aimed to determine the factors that could lead to the production of OO with the highest quality characteristics.

II.2. Materials and Methods

II.2.1. Materials

Powdered 2,2-diphenyl-β-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent (2M), sodium carbonate (Na_2CO_3), gallic acid (3,4,5-trihydroxybenzoic acid), sodium thiosulfate ($Na_2S_2O_3$), potassium dichromate ($K_2Cr_2O_7$), potassium iodide (KI), starch solution, phenolphthalein (1%), potassium hydroxide (KOH), acetic acid, diethyl ether, ethanol chloroform, methanol, cyclohexane and hexane (HPLC grade) were all purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were analytical grade and deionized water was prepared in the laboratory, using Labconco carbon water Pro-PS (Kansas USA).

II.2.2. Sampling

A total of 108 samples of olive oils from the 'Baladi' variety were collected as detailed in Table II.1 from the most important growing regions of Lebanon including Akkar and Zgharta-Koura in North Lebanon and Hasbaya and Jezzine from the South. The samples were obtained from the most common olive mills including press, sinolea, 2- and 3-phases. They were also collected at three harvesting times: early harvest (at the beginning of harvesting season in each region), intermediate harvest (at the middle of season) and late harvest (close to the end of harvesting season).

All samples were collected by three farmers at each processing technique and each harvesting time.

Table II.1. Sample characteristics of olive fruits and olive oil from different geographical origins and different processing systems.

Region	Rainfall[1]	Altitude	Latitude	Longitude	Processing system	Olive oil
Akkar	850 mm	300-700 m	34.5506°	36.0781°	Press	9
					2-phases	18
					3-phases	9
Zgharta-Koura	800 mm	200-350 m	34.2689°	35.7929°	Press	9
					3-phases	9
Hasbaya	650 mm	650-1050 m	33.3833°	35.6833°	Press	9
					Sinolea	9
					3-phases	9
Jezzine	750 mm	200-1000 m	33.5408°	35.5862°	Press	9
					2-phases	9
					3-phases	9
Total number of samples						108

[1]The rainfall corresponds to mean values collected between 2009 and 2015 from climatic stations of the Lebanese Agricultural Research Institute (LARI).

II.2.3. Analytical Methods

Quality Indices

Free acidity (FA)

In this study, the free acidity (FA) was determined according to the IOC methods of analyses (EEC reg 2568_91). A standardized KOH solution (0.1 N) was prepared by

dissolving 6.6 g in 1 L of ethanol. This solution was standardized and was used for titration.

For oil samples, an amount of 20 g of oil was mixed to 100 ml of a 50:50 (v/v) mixture of ethanol and diethyl-ether. Few drops of phenolphthalein (1%) were added as color indicator.

This light-yellow solution was then titrated with the standardized KOH solution until a light pink color is obtained. The volume of KOH was registered and the % FA was determined by applying the following formula:

$$\% \, Free \; acidity = \frac{(2.8245 \; x \; V \; x \; F)}{m}$$

Where,

V is the volume of KOH consumed during titration,

F is the factor obtained by the standardization procedure,

m is the weight of OO in g.

Peroxide Value (PV)

Peroxide value (PV) was determined according to IOC methods of analysis (EEC reg 2568_91). Briefly, a mother solution of sodium thiosulfate (0.1 N) was prepared by dissolving 15.81 g in 1 liter of distilled water. Then, a solution of sodium thiosulfate (0.002 N) was prepared by diluting 20 ml of the mother solution in a total of 1 L of distilled water. The real normality of the latter solution was determined by using a solution of potassium dichromate ($K_2Cr_2O_7$).

For peroxide value in oil samples, an aliquot of 2 to 2.5 g of oil was dissolved in a mixture of 15 ml of acetic acid glacial and 10 ml of chloroform. Then, 1 ml of previously prepared potassium iodide solution (30 g of solid KI dissolved in 21 ml of distilled water) was added. After manual shaking for one minute, the solution was

placed in obscurity for 5 minutes. Once over, 70 to 80 ml of distilled water and 2 ml of starch solution (1%) (as color indicator) were added to the solution, and then the titration by the standardized sodium thiosulfate solution was performed until the color changes from dark blue to transparent indicating the endpoint of the titration. The volume of consumed sodium thiosulfate was recorded and the peroxide value was determined by applying the following formula:

$$PV = \frac{(V\ x\ N_r x\ 1000)}{m}$$

Where,

V is the volume of sodium thiosulfate 0.002 N solution consumed during titration,

N is the real normality,

m is the weight of OO in g.

UV Absorbance at 232 and 270 nm

Both K_{232} and K_{270} were performed also according to IOC methods of analysis (EEC reg 2568_91) and using a UV-Visible spectrophotometer (6505 UV/Vis. spectrophotometer, Jenway scientific instruments, Stone, Staffordshire, ST15 OSA, UK).

For K_{232}, approximately 0.0812 g of oil where dissolved in 25 ml of cyclohexane, and the absorption was performed at 232 nm. Then, the following formula was applied to calculate K_{232}:

$$K_{232} = \frac{Absorbance\ at\ 232\ nm}{(4\ x\ m_{oil})}$$

Where,

A_{232} is the absorbance at 232 nm,

m_{oil} is the weight of OO in g.

For K_{270}, approximately 0.1 g of oil was dissolved in 10 ml of cyclohexane, and the absorption was accomplished at 270 nm. Then the following formula was applied:

$$K_{270} = \frac{Absorbance\ at\ 270\ nm}{(10\ x\ m_{oil})}$$

Where,

A_{270} is the absorbance at 270 nm,

m_{oil} is the weight of OO in g.

Liquid-Liquid Extraction of the Phenolic Compounds

The phenolic compounds in the OO were extracted using a modification of the procedure described by Montedoro *et al.* (1992). Hexane (2 mL) was added to an aliquots of OO (3 g), the flask was closed and shaken for 15 seconds using a Mini Vortex Scientific Product (VWR, Pennsylvania, USA). A volume of 2 mL of methanol/water (60/40, v/v) was added to the mixture and shaken for 2 min to undergo the first extraction. Samples were centrifuged for easier extraction for 2 min at 200 rpm, using the Thermo Electron Corporation centrifuge (Massachusetts, USA). For the second extraction, 2 mL of methanol/water (60/40) was added and shaken for 2 min. The two extracts were pooled together and stored at -18°C until further investigation.

Folin-Ciocalteu Assay of Total Phenolic Content

The TPC was assessed spectrophotometrically using the Folin-Ciocalteu colorimetric method described by Favati et al (1994). A solution of 20 g Na_2CO_3 in 80 mL of distilled water and a solution of 100 mg gallic acid in 100 mL methanol/water (60/40 w/v) were prepared. Solutions of different concentrations were prepared including a blank, 25, 50, 100, 200, 300, 400, 500, 600,700, 800, 900, and 1000 $\mu g.L^{-1}$. Samples were tested at

three different dilutions. The TPC in the standards and the OO was determined by adding to 100 µL of the phenolic oil purified extract, 675 µL distilled water, 25µL of Folin-Ciocalteu reagent and 200 µL of Na_2CO_3. The mixture was stirred and allowed to stand in the dark for 90 min. Then, the absorbance was assessed at 765 nm using a spectrophotometer (Analytic Jena Specord 250 Plus, Überlingen-Germany) and the software Winaspect Plus version 4.2. The total amount of phenols was expressed as mg gallic acid equivalent (GAE) per kg of oil.

DPPH Assay

The antioxidant capacity of the extracts was evaluated using the DPPH assay according to the procedure of Brand-Williams *et al.* (1995). Briefly, 250 µM DPPH: methanol solution was prepared. The blank solution was prepared by mixing 490 µL DPPH and 500 µL methanol (490:510, v/v) and its absorbance was controlled to be 1.2. Samples were assayed by using 10 µL or 20 µL of the phenolic extracts in presence of 490 or 480 µL methanol respectively and 500 µL DPPH. The mixtures were incubated for 15 minutes in the dark. Then they were tested at 515 nm and assayed using Helios Alpha UV-Vis spectrophotometer (UK). The percentage of inhibition was calculated by dividing the difference of the absorbance of the control and the absorbance of the sample, by the absorbance of the control.

$$\text{DPPH inhibition (\%)} = \frac{Ac - As}{Ac} \times 100$$

Where,

A_c is the absorbance of the control,

A_s is the absorbance of the sample.

Oxidative Stability

Oil oxidation stability was determined by a Rancimat apparatus (Model 892 Professional Rancimat, Metrohm SA, Herisau, Switzerland) following the procedures described by Tura *et al.* (2007): the induction time was determined by measuring the conductivity variation of 60 ml of water where the oxidative compounds were dissolved keeping 3 g of oil at 120°C under a constant air flow of 20 L/h. The results are expressed in hours.

Fatty acid composition

Esterified fatty acids (EFAs) from the OO were converted to their fatty acid methyl esters (FAMEs). With this objective, 0.1 g of oil were dissolved in 2 mL of *n*-hexane and then transmethylated with 200 μL of a methanolic solution of potassium hydroxide (2 M). The biphasic solution was shacked for 1 min in a Labinco minishaker and then left to settle for 5 min. An aliquot of 975 μL of the upper phase containing *n*-hexane and FAMEs were transferred to a test tube containing 25 μL of C19:0 as internal standard (IOOC, 2001). The resulting mixture was injected by duplicate into a Shimadzu GC-2010 Plus equipped with a split/split-less injector (250 °C) and a flame ionization detector (FID) (280°C). A fused silica capillary column (DB-wax; Agilent Technologies, Wilmington, DE; 60 m length x 0.25 mm i.d. and 0.25 μm of film thickness) was used as analytical column. The flow rate of nitrogen as carrier gas was 1.69 mL min^{-1}. The oven temperature was as follows: 15 min at 165 °C, from 165°C to 200°C at 5°C min^{-1}, 2 min at 200°C, from 200°C to 240°C at 5°C min^{-1}, and finally 5 min at 240°C. Identification of individual FAMEs was achieved by comparison of their

retention times with authentic commercial standards. The concentrations of EFAs were calculated as percentages.

Chromatographic analysis of individual phenols by HPLC-DAD

The produced extract was also used to determine the following eight individual phenolic compounds: hydroxytyrosol ($C_8H_{10}O_3$), tyrosol ($C_8H_{10}O_2$), vanillic acid ($C_8H_8O_4$), *p*-coumaric acid ($C_9H_8O_3$), oleacein ($C_{17}H_{20}O_6$), oleocanthal ($C_{17}H_{20}O_5$), luteolin ($C_{15}H_{10}O_6$) and apigenin ($C_{15}H_{10}O_5$).

The extracted phenolic fraction was injected in triplicate in a Shimadzu High Performance Liquid Chromatograph (HPLC) equipped with an automatic injector, a column oven and a diode array UV detector (DAD). Separation of individual phenols was achieved on a Microsorb-MV 100 C18 column (250 × 4.6 id mm, 5μ particle size), maintained at 40 °C.

The injection volume was 20 μL and the flow rate 1.0 mL/min. Mobile phases were 0.2 % o-phosphoric acid in water (mobile phase A) and a mixture methanol-acetonitrile (50:50, v/v) (B). The initial concentrations were 96% of A and 4% of B and the gradient was changed as follows: the concentration of B was increased to 50% in 40 min, increased to 60% in 5 min, and to 100% in 15 min, and maintained for 10 min. Initial conditions were reached in 7 min. The identification of OO phenols was performed at 280 nm, on the basis of their maximum absorption and retention times compared to those of commercial standard compounds.

Results were elaborated by Shimadzu LabSolution software. Phenolic compounds quantification was achieved using syringic acid as internal standard and 9 points calibration curves of authentic standards. Results were expressed as mg of the target analyte per kg of oil.

II.2.4 Statistical Analysis

The results were expressed as mean ± standard deviation using SPSS Statistics version 22. Both one-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were performed to compare mean values of all parameters. The correlation between OO grades and TPC/AC and oxidative stability was assessed using the Pearson correlation and logistic regression. The assumptions related to each statistical test used were checked before running it.

II.3. Results and Discussion

II.3.1. Descriptive analysis of olive oil quality indices

The quality characterization of OO from Baladi cultivar obtained from four regions, four processing systems and three harvesting times, had been investigated (Table II.2). The results showed that 52 out of 108 samples (48%) had free acidity above the maximum limit established by EU regulation 2013 (≤0.8 g oleic acid/100g oil). FA ranged between 0.3 ± 0.1 and 3.9 ± 1.4 g oleic acid/100g oil, with a mean value of 1.1 ± 0.5 g oleic acid/100g oil which exceeded the set parameter highlighting an advanced level of degradation (Issaoui *et al.* 2010).

Regarding PV, only 7 out of 108 samples (6.4%) had values that exceeded the limit established by EU regulation 2013 (≤20 mEq $O_2.kg^{-1}$). PV ranged between 6.9 ± 0.7 and 26.8 ± 1.5 mEq $O_2.kg^{-1}$, and the mean value of 13.4 mEq $O_2.kg^{-1}$ which was below the set limit.

Concerning spectrophotometric indices, only 1 sample out of 108 exceeded the limits set for K_{232} (≤2.50), and none of the samples exceeded those set for K_{270}. K_{232} ranged

between 1.1 ± 0.5 and 2.3 ± 0.3 and K_{270} ranged between 0.1 ± 0.0 and 0.2 ± 0.1, with respective mean values of 1.6 ± 0.2 and 0.1 ± 0.0.

II.3.2. Descriptive analysis of total phenolic compounds, antioxidant capacity, and oxidative stability index

The TPC, AC and OSI of the OO samples were also investigated (Table II.2). OO TPC ranged between 73.2 ± 14.3 and 265.5 ± 40.8 mg GAE.kg^{-1}, with a mean value of 157.0 ± 32.8 mg GAE.kg^{-1} of oil. In the literature, few of the obtained TPC were between the range 200 and 1500 mg.kg^{-1} which is considered one of the indications of a good quality OO (Hrncirik & Fritsche, 2005). The current results were in concordance with those obtained among samples collected during crop season 2013-2014 from five districts in North Lebanon by Serhan *et al.* (2016) with TPC ranging between 75.4 and 358.1 mg GAE.kg^{-1}. Internationally, significantly lower TPC ranges were obtained in studies conducted in Spain (42.1-123.8 mg GAE.kg^{-1}) (Gimeno *et al.* 2002) and Algeria (85-126 mg GAE.kg^{-1}) (Bengana *et al.* 2013); whereas higher ranges were found in ones conducted in Chile (208.76-493.25 mg GAE.kg^{-1}) (Romero *et al.* 2016), Tunisia (212.09 and 1167.03 mg GAE.kg^{-1}) (Ben Mansour *et al.* 2015), Italy (37.1 and 477.7 mg GAE.kg^{-1}) (Tura *et al.* 2013), and Morocco (216.8 and 668.7 mg GAE.kg^{-1}) (Bajoub *et al.* 2014). This significant variation could be explained by differences in: 1) water availability (Anastasopoulos *et al.* 2011), 2) irrigation regime, 3) maturity level (Baccouri *et al.* 2008), 4) crushing machine and its velocity (Serhan *et al.* 2016), 5) malaxation time and temperature (Serhan *et al.* 2016), 6) processing system (Dabbou *et al.* 2010), and 7) enzymatic activity (Machado *et al.* 2013).

As for OO antioxidant profile, DPPH value ranged between 1.6 ± 1.6 and 29.1 ± 11.5 %, with a mean DPPH value of 12.1 ± 4.6% among all samples. Following the same

DPPH method, a broad range of AC was observed in two studies conducted on 2 known Tunisian varieties: Chetoui and Chemlali. The first study was carried out during 2004-2005 crop season by Nakbi *et al.* (2010) and resulted in % DPPH ranging between 37.23 ± 0.92 and 78.56 ± 5.69%. The second study was conducted during 2014-2015 crop season by Hbaieb *et al.* (2017), and found %DPPH ranging between 11.9 and 88.5%. This wide variation in AC was attributed to the variation in TPC (Issaoui *et al.* 2010; Chtourou *et al.* 2013; Ammar *et al.* 2014; Ben Brahim *et al.* 2017; Hbaieb *et al.* 2017), and the presence of specific phenolic compounds including oleuropein and oleuropein derivatives (Ammar *et al.* 2014), vanillic and cinnamic acid (Arslan & Özcan, 2011; Ammar *et al.* 2014).

With regard to OO samples' resistance to oxidation, the studied OO OSI ranged between 3.8 ± 0.3 and 13.9 ± 4.1 h with an average value of 8.2 ± 1.6 h. The lowest stability to oxidation of 3.8 h was observed in late harvested OO samples obtained from Akkar using traditional press system, while the highest one was found in early harvested OO obtained from Hasbaya using 3-phase decanters. Those results highlighted that the oxidative stability of the oil obtained in the current study was similar to that reported in Tunisia (2.5–12.7 h) (Issaoui *et al.* (2010), and lower than that reported in Algeria (27–36 h) (Bengana *et al.* (2013). The reason of this high variability in OSI, especially in OO of the same country, could be either attributed to the variation in phenolic compounds (Ben Brahim *et al.* 2017) and tocopherols (Chtourou *et al.* 2013), or to the effect of cultivar (Ceci *et al.* 2017), processing system (Serhan *et al.* 2016), and geographical origin (Issaoui *et al.* 2010).

II.3.3. Descriptive analysis of fatty acid composition

The fatty acids composition including palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids, was assessed (Table II.3). Oleic, palmitic and linoleic acids were the most commonly present and ranged between 67.5-72.6%, 10.3-13.9% and 9.3-13%, respectively. The results showed that fatty acids content of all samples was within the standard range set by Olive Oil and Olive Pomace Oil Standards. Our results were also similar to previously reported data by Serhan *et al.* (2016) and Rigane *et al.* (2013).

Table II.2. Descriptive statistics for total phenolic content (mg GAE.kg^{-1} of oil), DPPH$^{\cdot}$ inhibition (%), oxidative stability index (h) and quality indices: free acidity (%), peroxide value (mEq O_2.kg^{-1}), K_{232}, and K_{270} of olive oil samples obtained from four geographical origins in Lebanon (Akkar, Zgharta-Koura, Hasbaya and Jezzine), extracted using different processing systems (2-phase, 3-phase, press, and sinolea), and harvested at different times (early, intermediate, late).

Region	Geographical Origin	Processing System	Harvesting Time[1]	Total Phenolic Content ± SD[2]	DPPH[3] ± SD	Oxidative Stability Index ± SD	Free Acidity	Peroxide Value	K_{232}	K_{270}
North	Akkar	2-phase decanter	Early	155.5 ± 11.4	15.7 ± 2.1	8.7 ± 1.4	1.1 ± 0.5	12.7 ± 1.6	1.3 ± 0.1	**0.2 ± 0.1**
			Intermediate	184.5 ± 49.3	17.2 ± 4.2	7.7 ± 0.6	2.6 ± 1.7	15.7 ± 2.6	1.9 ± 0.4	0.2 ± 0.0
			Late	136.7 ± 27.3	6.9 ± 3.1	6.3 ± 1.7	2.6 ± 1.0	18.7 ± 1.1	1.7 ± 0.2	0.1 ± 0.1
		3-phase decanter	Early	**265.5 ± 40.8**	16.7 ± 5.3	12.0 ± 0.7	0.6 ± 0.3	13.2 ± 1.6	1.7 ± 0.1	**0.1 ± 0.0**
			Intermediate	183.8 ± 14.2	10.8 ± 4.9	11.2 ± 2.2	0.6 ± 0.1	10.1 ± 0.6	1.7 ± 0.2	0.2 ± 0.0
			Late	194.5 ± 16.3	14.2 ± 5.9	13.7 ± 2.9	0.5 ± 0.0	12.7 ± 1.5	1.7 ± 0.1	0.2 ± 0.0
		Press	Early	112.8 ± 14.8	17.7 ± 1.2	5.4 ± 0.7	1.2 ± 0.5	16.8 ± 1.7	1.7 ± 0.1	**0.1 ± 0.0**
			Intermediate	103.0 ± 19.8	11.4 ± 8.6	4.5 ± 0.3	0.8 ± 0.0	9.5 ± 1.3	1.5 ± 0.1	0.1 ± 0.1
			Late	91.7 ± 20.5	8.7 ± 6.4	**3.8 ± 0.3**	0.9 ± 0.3	15.3 ± 3.1	1.6 ± 0.1	**0.1 ± 0.0**
	Zgharta-Koura	3-phase decanter	Early	151.6 ± 69.4	20.2 ± 13.2	9.2 ± 0.8	0.6 ± 0.1	9.5 ± 1.8	1.6 ± 0.3	0.2 ± 0.0
			Intermediate	172.7 ± 58.3	10.4 ± 6.3	8.4 ± 0.8	0.8 ± 0.1	9.5 ± 2.7	1.4 ± 0.4	**0.1 ± 0.0**
			Late	175.7 ± 7.9	13.2 ± 7.2	7.5 ± 1.1	2.2 ± 2.6	12.9 ± 7.2	1.7 ± 0.5	**0.2 ± 0.1**
		Press	Early	144.4 ± 8.4	19.0 ± 8.6	9.7 ± 2.2	0.7 ± 0.2	**6.9 ± 0.7**	1.7 ± 0.1	**0.1 ± 0.0**
			Intermediate	180.2 ± 21.4	2.5 ± 2.5	6.9 ± 1.0	2.1 ± 2.3	7.8 ± 2.6	1.5 ± 0.3	**0.1 ± 0.0**
			Late	146.3 ± 28.8	4.0 ± 3.4	6.4 ± 1.7	0.8 ± 0.2	9.4 ± 0.6	1.3 ± 0.8	0.1 ± 0.1
South	Hasbaya	3-phase decanter	Early	232.3 ± 32.8	19.9 ± 2.0	**13.9 ± 4.1**	0.4 ± 0.1	12.4 ± 3.7	1.7 ± 0.0	**0.1 ± 0.0**
			Intermediate	129.5 ± 19.6	4.6 ± 2.2	9.4 ± 0.6	0.4 ± 0.2	8.8 ± 0.4	1.6 ± 0.1	**0.1 ± 0.0**
			Late	160.2 ± 65.8	12.4 ± 6.4	10.3 ± 3.2	0.5 ± 0.2	**26.8 ± 1.5**	1.5 ± 0.0	**0.1 ± 0.0**
		Press	Early	175.6 ± 85.7	16.5 ± 1.1	7.8 ± 3.8	0.5 ± 0.1	11.4 ± 2.7	1.6 ± 0.1	0.1 ± 0.0
			Intermediate	201.6 ± 44.6	9.6 ± 3.5	11.3 ± 1.4	**0.3 ± 0.1**	9.8 ± 1.5	1.6 ± 0.0	0.1 ± 0.0
			Late	180.9 ± 53.0	11.2 ± 7.3	9.1 ± 0.8	0.8 ± 0.1	14.4 ± 2.4	**2.3 ± 0.3**	0.2 ± 0.0
		Sinolea	Early	122.1 ± 40.7	2.7 ± 2.6	10.8 ± 1.2	**0.3 ± 0.1**	11.5 ± 2.0	1.8 ± 0.2	0.2 ± 0.0
			Intermediate	182.7 ± 5.0	7.6 ± 4.1	8.7 ± 1.8	0.4 ± 0.0	13.4 ± 1.5	1.7 ± 0.2	**0.1 ± 0.0**
			Late	176.4 ± 39.5	14.1 ± 5.1	10.0 ± 1.4	0.4 ± 0.1	15.5 ± 2.0	2.2 ± 0.4	0.2 ± 0.0
	Jezzine	2-phase decanter	Early	189.6 ± 120.4	18.3 ± 13.1	8.4 ± 3.8	1.0 ± 0.3	16.2 ± 6.9	1.7 ± 0.4	0.2 ± 0.0
			Intermediate	157.1 ± 34.6	15.1 ± 0.5	4.8 ± 1.2	**3.9 ±1.4**	23.6 ± 7.0	1.8 ± 0.2	0.2 ± 0.0
			Late	127.7 ± 0.0	10.5 ± 0.0	5.4 ± 1.6	0.8 ± 0.3	10.9 ± 3.3	1.4 ± 0.2	0.1 ± 0.0
		3-phase decanter	Early	200.2 ± 24.0	**29.1 ± 11.5**	11.9 ± 1.4	0.7 ± 0.1	13.0 ± 3.9	1.5 ± 0.3	0.1 ± 0.1
			Intermediate	140.0 ± 0.0	7.8 ± 0.0	6.4 ± 1.3	0.9 ± 0.5	14.2 ± 2.4	1.7 ± 0.4	**0.1 ± 0.0**
			Late	106.5 ± 35.8	3.9 ± 0.0	4.5 ± 1.6	1.2 ± 0.2	13.6 ± 4.0	**1.1 ± 0.5**	**0.1 ± 0.0**
		Press	Early	91.9 ± 9.4	9.3 ± 6.8	3.9 ± 0.8	2.2 ± 0.3	14.9 ± 1.6	1.5 ± 0.5	0.1 ± 0.1
			Intermediate	128.7 ± 58.3	11.5 ± 6.2	8.0 ± 4.6	1.4 ± 0.6	15.3 ± 2.7	1.4 ± 0.4	**0.2 ± 0.1**
			Late	**73.2 ± 14.3**	**1.6 ± 1.6**	4.4 ± 1.3	1.3 ± 0.6	14.8 ± 5.5	1.4 ± 0.2	**0.1 ± 0.0**
Mean ± SD				**157.0 ± 32.8**	**12.1 ± 4.6**	**8.2 ± 1.6**	**1.1 ± 0.5**	**13.4 ± 2.6**	**1.6 ± 0.2**	**0.1 ± 0.0**

[1] Harvest period was conducted at different dates including early (September), intermediate (mid-November) and late (mid-December).

[2] SD: standard deviation.

[3] DPPH$^{\cdot}$: 2,2-diphenyl-1-picrylhydrazyl free radical used to assess the antioxidant activity of the oil and calculated as follows DPPH$^{\cdot}$ inhibition % $=\frac{Ac-As}{Ac} x$ 100 where Ac was the absorbance of the control and As was the absorbance of the sample.

Table II.3. Fatty acid composition (% fatty acid methyl esters) of olive oil samples obtained from four geographical origins in Lebanon (Akkar, Zgharta-Koura, Hasbaya and Jezzine), extracted using different processing systems (2-phase, 3-phase, press, and sinolea), and harvested at different times (early, intermediate, late).

Region	Geographical	Processing System	Harvesting	C16:0[1]	C16:1[2]	C18:0[3]	C18:1[4]	C18:2[5]	C18:3[6]
North	Akkar	2-phase decanter	Early	13.2 ± 0.4	0.6 ± 0.0	3.6 ± 0.0	69.8 ± 0.2	10.8 ± 0.2	0.7 ± 0.0
			Intermediate	13.4 ± 0.7	0.7 ± 0.1	3.2 ± 0.2	69.3 ± 0.6	11.6 ± 0.4	0.5 ± 0.1
			Late	13.2 ± 0.4	0.6 ± 0.1	3.4 ± 0.2	69.3 ± 0.8	11.6 ± 0.8	0.7 ± 0.1
		3-phase decanter	Early	13.7 ± 0.2	0.7 ± 0.1	2.3 ± 0.2	71.2 ± 0.8	10.1 ± 1.1	0.7 ± 0.1
			Intermediate	12.9 ± 1.0	0.5 ± 0.1	3.1 ± 0.7	70.9 ± 0.2	10.5 ± 0.2	0.7 ± 0.1
			Late	12.4 ± 0.7	0.7 ± 0.2	3.1 ± 0.6	**72.6 ± 0.4**	9.6 ± 0.4	0.4 ± 0.2
		Press	Early	**13.9 ± 0.2**	0.6 ± 0.0	3.0 ± 0.4	**67.5 ± 0.2**	**13.0 ± 0.0**	0.6 ± 0.0
			Intermediate	13.8 ± 0.3	0.5 ± 0.0	3.6 ± 0.1	68.3 ± 0.7	11.8 ± 0.5	0.6 ± 0.0
			Late	12.5 ± 0.7	0.4 ± 0.0	3.6 ± 0.6	69.8 ± 0.5	11.7 ± 0.3	0.6 ± 0.0
	Zgharta-Koura	3-phase decanter	Early	13.0 ± 0.3	0.4 ± 0.0	3.7 ± 0.5	69.6 ± 0.5	11.2 ± 0.2	0.6 ± 0.0
			Intermediate	12.3 ± 0.6	0.4 ± 0.0	4.4 ± 0.3	69.5 ± 1.2	11.1 ± 1.4	0.6 ± 0.0
			Late	11.6 ± 0.0	0.4 ± 0.0	4.5 ± 0.0	68.6 ± 0.8	12.6 ± 0.8	0.7 ± 0.0
		Press	Early	13.5 ± 0.3	0.5 ± 0.0	3.5 ± 0.4	69.0 ± 0.0	11.3 ± 0.6	0.6 ± 0.1
			Intermediate	13.7 ± 0.3	0.5 ± 0.0	4.1 ± 0.2	69.4 ± 1.0	10.3 ± 1.2	0.5 ± 0.0
			Late	12.6 ± 0.1	0.4 ± 0.0	3.0 ± 1.1	70.3 ± 0.2	11.6 ± 0.7	0.5 ± 0.0
South	Hasbaya	3-phase decanter	Early	13.0 ± 0.6	0.4 ± 0.1	3.4 ± 0.3	71.6 ± 1.6	9.6 ± 1.8	0.6 ± 0.0
			Intermediate	11.9 ± 0.0	0.4 ± 0.0	3.2 ± 0.5	70.8 ± 0.3	11.5 ± 0.2	0.6 ± 0.0
			Late	11.8 ± 0.1	0.3 ± 0.1	3.0 ± 0.0	71.6 ± 0.8	11.2 ± 0.9	0.6 ± 0.1
		Press	Early	12.7 ± 0.7	0.4 ± 0.1	3.4 ± 0.5	71.4 ± 1.0	10.1 ± 1.4	0.6 ± 0.0
			Intermediate	12.6 ± 0.6	0.6 ± 0.1	3.4 ± 0.3	72.0 ± 0.8	9.5 ± 0.9	0.6 ± 0.1
			Late	11.0 ± 0.4	0.3 ± 0.1	4.0 ± 0.2	71.8 ± 0.6	10.8 ± 0.5	0.6 ± 0.1
		Sinolea	Early	12.7 ± 0.3	0.5 ± 0.0	3.3 ± 0.6	72.1 ± 1.3	**9.3 ± 0.9**	0.6 ± 0.0
			Intermediate	13.6 ± 2.2	0.5 ± 0.1	3.1 ± 1.0	69.4 ± 4.0	11.3 ± 2.7	0.6 ± 0.1
			Late	10.7 ± 0.1	0.3 ± 0.0	4.0 ± 0.2	72.0 ± 0.5	10.8 ± 0.5	0.6 ± 0.0
	Jezzine	2-phase decanter	Early	12.3 ± 0.3	0.4 ± 0.0	3.9 ± 0.1	71.9 ± 1.9	9.4 ± 1.9	0.5 ± 0.1
			Intermediate	12.1 ± 0.2	0.5 ± 0.1	3.7 ± 0.3	71.6 ± 0.8	10.2 ± 0.8	0.5 ± 0.0
			Late	11.9 ± 0.5	0.4 ± 0.2	3.8 ± 0.3	71.3 ± 1.0	10.6 ± 1.2	0.5 ± 0.0
		3-phase decanter	Early	13.0 ± 1.0	0.5 ± 0.1	3.4 ± 1.0	71.2 ± 1.6	10.0 ± 0.9	0.6 ± 0.1
			Intermediate	12.7 ± 0.9	0.5 ± 0.1	3.9 ± 0.5	69.4 ± 0.7	11.4 ± 1.0	0.6 ± 0.1
			Late	11.2 ± 0.6	0.4 ± 0.0	4.1 ± 0.1	70.7 ± 0.6	11.4 ± 0.2	0.6 ± 0.1
		Press	Early	11.3 ± 0.5	0.3 ± 0.0	4.3 ± 0.4	70.5 ± 0.4	11.2 ± 0.4	0.7 ± 0.0
			Intermediate	11.6 ± 1.6	0.6 ± 0.3	3.5 ± 0.7	71.6 ± 1.6	10.5 ± 2.1	0.6 ± 0.1
			Late	**10.3 ± 0.3**	0.3 ± 0.0	4.1 ± 0.6	72.2 ± 1.1	11.0 ± 1.0	0.6 ± 0.1
Olive Oil and Olive Pomace Oil Standard Limits				**7.5-20.0**	**0.3-3.5**	**0.5-5.0**	**55.0-83.0**	**3.5-21.0**	**≤1.5**

[1]C16:0, Palmitic; [2]C16:1, Palmitoleic; [3]C18:0, Stearic; [4]C18:1, Oleic; [5]C18:2, Linoleic; [6]C18:3, Linolenic

Table II.4. Mean ± Standard deviation (SD) of individual phenols (mg GAE.kg^{-1} of oil) determined in olive oil samples.

Region	Geographical	Processing	Harvesting	Hydroxytyrosol	Tyrosol	Vanilic	*o*-coumaric	Oleacin	Oleocanthal	Luteolin	Apigenin
North	Akkar	2-phase	Early	5.4 ± 1.3	4.6 ± 1.7	3.0 ± 0.1	2.5 ± 0.3	49.3 ± 24.5	87.6 ± 18.0	3.1 ± 0.2	**8.8 ± 2.8**
			Intermediate	10.8 ± 5.3	6.6 ± 2.4	3.0 ± 0.1	**3.1 ± 0.7**	63.4 ± 16.1	112.6 ± 23.0	2.4 ± 0.7	6.6 ± 2.5
			Late	7.6 ± 3.6	6.2 ± 2.3	**3.0 ± 0.3**	2.5 ± 0.8	28.5 ± 27.1	54.1 ± 23.1	**1.9 ± 0.2**	5.9 ± 1.0
		3-phase	Early	4.5 ± 0.5	2.3 ± 0.3	2.8 ± 0.1	1.5 ± 0.2	**64.8 ± 36.1**	**114.3 ± 55.1**	3.1 ± 0.8	8.1 ± 3.2
			Intermediate	4.9 ± 1.8	2.1 ± 0.2	2.7 ± 0.2	**1.3 ± 0.1**	36.5 ± 4.7	79.3 ± 21.4	2.3 ± 0.9	5.1 ± 1.3
			Late	4.6 ± 3.1	2.6 ± 1.6	2.7 ± 0.2	1.5 ± 0.1	33.5 ± 7.3	53.9 ± 43.0	2.5 ± 0.2	7.0 ± 1.9
		Press	Early	1.8 ± 0.0	2.9 ± 0.1	2.8 ± 0.1	2.2 ± 0.3	12.1 ± 2.2	63.3 ± 19.7	2.2 ± 0.1	3.1 ± 0.2
			Intermediate	**1.6 ± 0.1**	2.5 ± 0.9	2.9 ± 0.1	2.0 ± 0.3	7.0 ± 2.8	58.1 ± 14.7	2.1 ± 0.5	**1.6 ± 0.2**
			Late	1.7 ± 0.1	5.3 ± 3.7	2.8 ± 0.0	1.7 ± 0.1	6.6 ± 4.6	39.0 ± 5.4	2.1 ± 0.4	2.3 ± 0.9
	Zgharta-Koura	3-phase	Early	5.6 ± 1.1	4.1 ± 0.2	2.8 ± 0.4	2.4 ± 0.8	8.5 ± 3.8	43.2 ± 16.7	3.4 ± 0.4	6.8 ± 1.8
			Intermediate	4.2 ± 2.3	3.8 ± 3.5	2.7 ± 0.1	1.9 ± 0.5	6.4 ± 1.6	38.4 ± 19.6	3.7 ± 2.2	5.8 ± 3.5
			Late	3.5 ± 0.8	3.3 ± 0.8	2.7 ± 0.0	1.5 ± 0.0	7.1 ± 2.5	41.0 ± 4.0	3.7 ± 2.3	8.2 ± 0.5
		Press	Early	2.1 ± 0.1	2.5 ± 0.2	3.0 ± 0.0	2.6 ± 0.2	7.0 ± 2.3	30.3 ± 0.8	3.7 ± 2.4	4.1 ± 0.1
			Intermediate	3.2 ± 1.2	5.7 ± 1.1	3.0 ± 0.1	3.1 ± 0.1	8.1 ± 4.6	37.6 ± 7.8	3.7 ± 2.5	4.5. ± 1.4
			Late	2.5 ± 0.4	2.8 ± 0.4	2.7 ± 0.1	2.1 ± 0.6	8.9 ± 4.5	39.5 ± 20.7	3.7 ± 2.6	6.1 ± 4.0
South	Hasbaya	3-phase	Early	4.3 ± 0.6	1.7 ± 0.5	**2.6 ± 0.0**	1.5 ± 0.1	16.5 ± 12.1	66.0 ± 18.3	3.7 ± 2.7	6.1 ± 1.6
			Intermediate	3.1± 0.4	1.4 ± 0.5	2.6 ± 0.1	1.4 ± 0.0	9.1 ± 4.1	60.1 ± 10.4	3.7 ± 2.8	5.8 ± 1.9
			Late	3.1± 1.0	1.4 ± 0.2	2.7 ± 0.1	1.4 ± 0.2	24.5 ± 34.5	85.0 ± 60.2	**3.7 ± 2.9**	7.0 ± 3.3
		Press	Early	3.4 ± 1.6	2.8 ± 1.1	2.8 ± 0.2	2.0 ± 0.7	7.7 ± 3.3	27.1 ± 9.9	3.7 ± 2.10	3.7 ± 2.0
			Intermediate	2.6 ± 1.1	1.6 ± 0.9	2.8 ± 0.1	1.6 ± 0.2	6.6 ± 3.1	31.9 ± 20.4	3.7 ± 2.11	3.2 ± 1.6
			Late	3.0 ± 0.5	2.9 ± 1.0	3.0 ± 0.2	1.6 ± 0.2	8.5 ± 3.8	21.2 ± 3.0	3.7 ± 2.12	4.2 ± 0.2
		Sinolea	Early	2.6 ± 0.6	1.8 ± 0.1	2.6 ± 0.1	1.6 ± 0.1	17.7 ± 13.8	68.7 ± 0.8	3.7 ± 2.13	4.2 ± 2.0
			Intermediate	2.4 ± 0.2	1.5 ± 0.4	2.6 ± 0.1	1.5 ± 0.1	12.3 ± 0.9	37.5 ± 14.7	3.7 ± 2.14	3.9 ± 0.9
			Late	2.9 ± 0.4	1.1 ± 0.2	**2.6 ± 0.0**	1.5 ± 0.1	11.5 ± 9.5	28.8 ± 5.0	3.7 ± 2.15	4.0 ± 0.6
	Jezzine	2-phase	Early	5.9 ± 4.8	4.8 ± 5.5	2.7 ± 0.0	2.0 ± 0.5	15.6 ± 10.7	44.6 ± 19.2	3.7 ± 2.16	4.8 ± 1.6
			Intermediate	**17.1 ± 0.8**	**18.8 ± 3.1**	2.9 ± 0.2	1.6 ± 0.2	6.5 ± 0.9	26.5 ± 4.3	3.7 ± 2.17	7.3 ± 0.9
			Late	16.8 ± 0.7	18.5 ± 3.2	2.6 ± 0.3	1.4 ± 0.0	6.2 ± 0.9	26.2 ± 4.2	3.7 ± 2.18	7.1 ± 1.0
		3-phase	Early	4.8 ± 2.5	2.5 ± 1.6	2.8 ± 0.3	1.5 ± 0.2	17.9 ± 15.8	53.6 ± 37.3	3.7 ± 2.19	8.1 ± 2.2
			Intermediate	2.2 ± 0.0	1.5 ± 0.2	**2.6 ± 0.0**	1.6 ± 0.3	9.5 ± 3.9	54.0 ± 5.5	3.7 ± 2.20	3.3 ± 0.3
			Late	2.1 ± 0.6	4.5 ± 2.2	2.8 ± 0.2	1.4 ± 0.2	6.6 ± 4.2	29.8 ± 2.6	3.7 ± 2.21	2.6 ± 0.9
		Press	Early	1.8 ± 0.1	1.2 ± 0.0	2.7 ± 0.1	1.7 ± 0.1	6.8 ± 1.3	33.0 ± 3.7	3.7 ± 2.22	3.0 ± 0.5
			Intermediate	2.2 ± 0.9	1.3 ± 0.6	2.6 ± 0.1	1.5 ± 0.4	12.5 ± 13.5	39.6 ± 17.5	3.7 ± 2.23	4.7 ± 3.5
			Late	1.7 ± 0.2	**0.9 ± 0.4**	2.6 ± 0.1	1.5 ± 0.2	**4.8 ± 1.2**	**19.8 ± 1.9**	3.7 ± 2.24	2.3 ± 1.0
Mean ± SD				**4.4 ± 1.2**	**3.7 ± 1.2**	**2.8 ± 0.1**	**1.8 ± 0.3**	**16.6 ± 8.6**	**49.9 ± 16.1**	**3.7 ± 2.3**	**5.1 ± 1.6**

II.3.4. Descriptive analysis of individual phenolic composition

The phenolic composition of the oils was assessed (Table II.4). The most abundant compounds were oleocanthal and oleacein. These phenolic compounds, whenever present abundantly, contribute in obtaining high quality OO due to their anti-inflammatory, anti-carcinogenic, anti-microbial, anti-proliferative and antioxidant properties (Parkinson & Keast, 2014; Naruszewicz *et al.* 2015). Oleocanthal ranged between 19.8 and 114.3 $mg.kg^{-1}$ of oil while oleacein ranged between 4.8 and 64.8 $mg.kg^{-1}$. Simple phenolic acids, including vanillic acid and *o*-coumaric acids were detected in low amounts ranging between 2.6 and 3.0 $mg.kg^{-1}$ and between 1.3 and 3.0 $mg.kg^{-1}$, respectively. Similar results were also reported previously (Rigane *et al.* (2013), Franco *et al.* (2014), Romero *et al.* (2016).

Both hydroxytyrosol and tyrosol were the most abundant phenolic alcohols. Their levels fluctuated between 1.6 and 18.8 $mg.kg^{-1}$. Compared to the results observed in several Mediterranean countries, the current results were similar to those described in Spain (Hydroxytyrosol, 1.9-6.7 $mg.kg^{-1}$) (Hbaieb *et al.* 2015), and higher than those in Italy (Hydroxytyrosol, 0.4-0.9 $mg.kg^{-1}$; Tyrosol, 0.2-1.0 $mg.kg^{-1}$) (Baiano *et al.* 2013) and Turkey (hydroxytyrosol, 0.08-2.29 $mg.kg^{-1}$) (Jolayemi *et al.* 2016). This variability in OO phenolic content could be due to the harvesting time or fruit maturity (Arslan & Ozcan, 2011), and OO storage duration that could lead to an increase in phenolic alcohols concentration as a result of secoiridoids hydrolysis with time (Dabbou *et al.* 2009).

Regarding the flavonoids, both luteolin and apigenin ranged between 1.9 and 3.7 $mg.kg^{-1}$ of oil and between 1.6 ± 0.2 and 8.8 ± 2.8 $mg.kg^{-1}$, respectively. These results were higher than those obtained by Arslan *et al.* (2013) for both luteolin (1.2-2.2 $mg.kg^{1}$) and apigenin (0.06-0.1 $mg.kg^{-1}$) in Turkish OO, and lower than those obtained by Florez *et al.* (2017) only for luteolin (0.71-16.4 $mg.kg^{-1}$) in Spanish OO. Being two of the most anti-oxidative phenolic

compounds, both flavonoids affect OO quality and stability (Arslan *et al.* 2013). Overall variance in individual phenolic composition could be attributed to many parameters such as cultivar, geographical origin, climate, and storage conditions (Arslan & Ozcan, 2011).

II.3.5 Effect of agro-industrial factors on olive oil composition and quality

MANOVA

A multivariate analysis of variance (MANOVA) was performed to examine the effects of different agro-industrial factors (geographical origin, processing system and harvesting time) on OO composition and antioxidant parameters (individual phenols, TPC, DPPH, OSI). All corresponding assumptions were checked before running this test, and adequate actions were taken to control the existing violations. Results showed that the 2-way interaction (processing system*harvesting time) had a very high significant effect on the antioxidant parameters of OO ($wilk's\ \Lambda = 0.219;\ F\ (48; 304.208) = 2.291, p < 0.001$). Moreover, very high significant effects of the 2-way interactions (geographical origin*processing system) and (geographical origin*harvesting time) were also shown on OO antioxidant parameters, with respective values ($wilk's\ \Lambda = 0.499;\ \mathrm{F}(16; 122.00) = 3.171, p < 0.001$) and ($wilk's\ \Lambda = 0.258;\ \mathrm{F}(48; 304.208) = 2.008, p < 0.001$). As per each factor's effect, all three agro industrial factors (geographical origin, harvesting time and processing system) affected significantly the set of antioxidant parameters ($p < 0.001$). The latter results are documented in Table II.5.

It is essential to note that the power of the results obtained were all greater than 0.8; which indicated the efficiency and accuracy of the selected test MANOVA.

Table II.5. MANOVA results on the effects of different agro industrial factors and their interactions with antioxidant parameters of olive oil.

Interaction	Wilk's Λ	F	Power
Origin*System*Harvest	0.574	1.150	0.893
System*Harvest	0.219	2.291***	1.000
Origin*System	0.499	3.171***	0.998
Origin*Harvest	0.258	2.008***	0.999
Origin	0.160	6.518***	1.000
System	0.099	9.029***	1.000
Harvest	0.422	4.116***	1.000

*** $p < 0.001$

To go further through the analysis, and to check the effect of different agro industrial factors on each of the antioxidant parameters, the tests of Between-Subjects effects were used. To counteract the problem of multiple comparisons, Bonferroni correction which consists of dividing p value over the number of variables was calculated (0.05/8= 0.00625). Therefore, the effect of different factors on any of the antioxidant parameters was considered significant for a p value less than 0.00625. Results obtained showed significant effects of the 2-way interaction (geographical origin*processing system) specifically on OO TPC and OSI ($p < 0.00625$). This implied that whatever the geographical origin was, the processing system affected significantly OO TPC and OSI (Figure II.1).

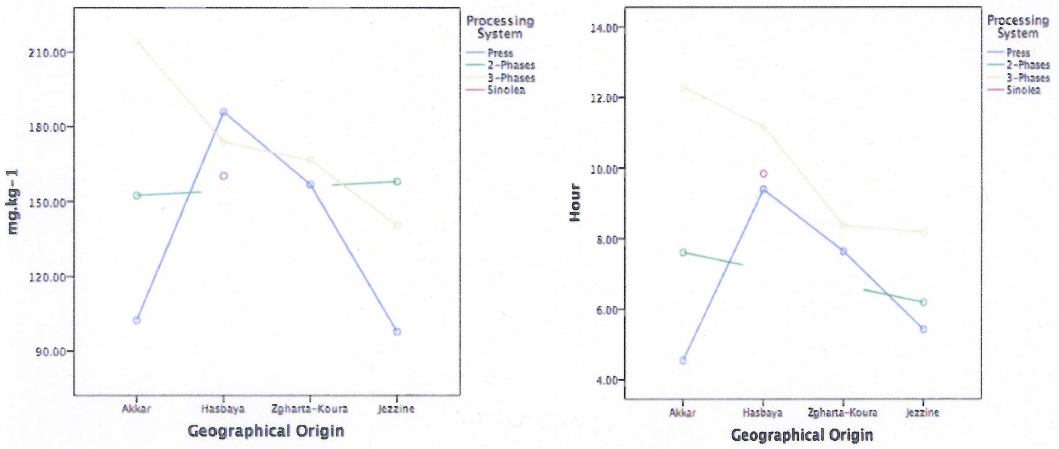

Figure II.1. The significant effect of 2-way interaction geographical origin*processing system on total phenolic content and oxidative stability index.

Regarding tyrosol, it was significantly affected by both 2-way interactions (geographical origin*harvesting time) and (processing*harvesting time) ($p < 0.00625$) (Figure II.2). This revealed that whatever were the geographical origins and the processing systems, harvesting time had significantly contributed to tyrosol levels.

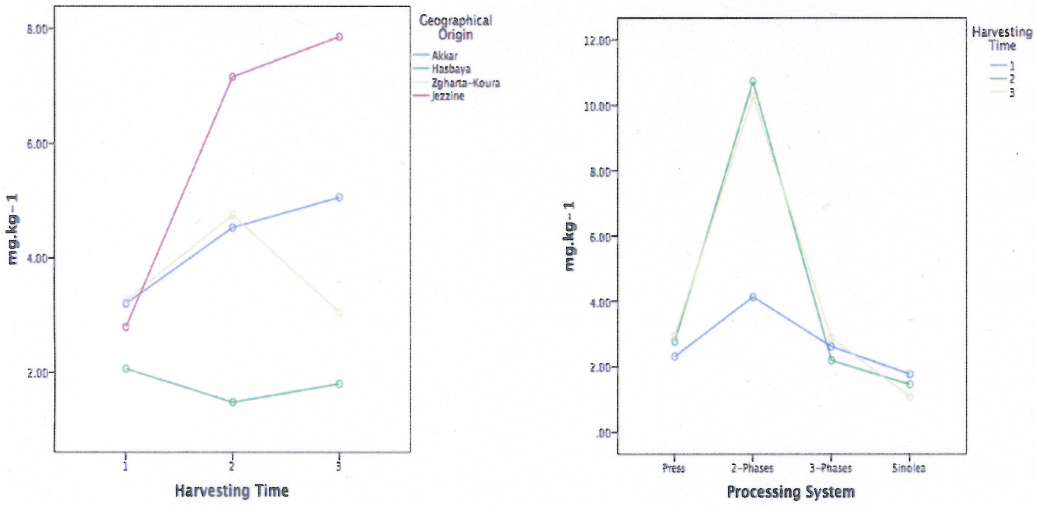

Figure II.2. Tyrosol (mg.kg^{-1}) as affected by both 2-way interactions geographical origin*harvesting time and processing system*harvesting time.

Similar to tyrosol, hydroxytyrosol was significantly affected by the 2-way interaction (processing system*harvesting time), also implying significant influence of harvesting time on its concentration, independently of the processing system (Figure II.3).

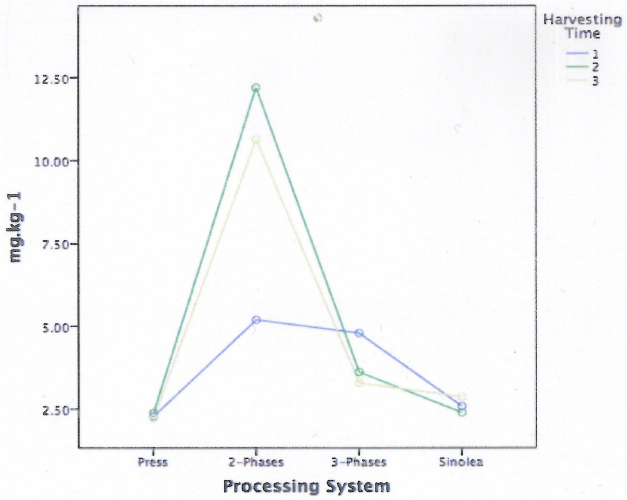

Figure II.3. Hydroxytyrosol (mg.kg^{-1}) as affected by 2-way interaction processing system*harvesting time.

Agro-industrial factors effect on antioxidant parameters

To determine each factor effect and its significance on the antioxidant parameters (TPC, DPPH, OSI, and individual phenols), Duncan test in post hoc was performed.

Geographical Origin

Geographical origin significantly affected both phenolic alcohols hydroxytyrosol and tyrosol, oleacein, oleocanthal, and TPC ($p < 0.00625$) (Table II.6).

Results showed that OO obtained from Hasbaya, situated in southern Lebanon, contained the highest TPC (173.48 mg.kg^{-1}); followed by Zgharta-Koura (161.81 mg.kg^{-1}), Akkar (157.07 mg.kg^{-1}), and Jezzine (136.24 mg.kg^{-1}). These results were similar to those determined in Lebanon by Chehade *et al.* (2015) (224.0 ± 27.0 vs 394.0 ± 13.0 mg.kg^{-1}, $p \leq 0.05$) and in Tunisia by Guerfel *et al.* (2009) (225.0 vs 304.72 mg GAE.kg^{-1}, $p < 0.001$), that ascribed their results to the different climatic conditions among regions. On the other hand, they were not in line with those found in Tunisia by Issaoui *et al.* (2010) where northern samples presented higher TPC compared to southern ones (228.25 vs 561.8 mg GAE.kg^{-1}, $p < 0.05$). The

variability in phenolic content obtained at both national and international levels could be attributed to differences in type of soil (Ben Rached *et al.* 2017; Baiano *et al.* 2013), rainfall level (Arslan *et al.* 2013), climate conditions (Issaoui *et al.* 2013; Issaoui *et al.* 2010; Guerfel *et al.* 2009), and altitude (Arslan *et al.* 2013; Youssef *et al.* 2011).

Regarding individual phenols, oleocanthal, the most abundant phenolic compound, was highest in OO obtained from Akkar (74.42 $mg.kg^{-1}$) situated in northern Lebanon and lowest in Jezzine (36.78 $mg.kg^{-1}$), situated in the south. OO obtained from Hasbaya and Zgharta-Koura recorded oleocanthal values of 47.37 and 38.35 $mg.kg^{-1}$; respectively.

The highest concentrations of Oleacein were obtained in Akkar (33.69 $mg.kg^{-1}$), followed by Hasbaya (12.71 $mg.kg^{-1}$), Jezzine (9.75 $mg.kg^{-1}$), and Zgharta-Koura (7.67 $mg.kg^{-1}$). The phenolic alcohol hydroxytyrosol was highest in Jezzine (6.37 $mg.kg^{-1}$), followed by Akkar (5.21 $mg.kg^{-1}$), Zgharta-Koura (3.52 $mg.kg^{-1}$), and Hasbaya (3.05 $mg.kg^{-1}$). With respect to tyrosol concentration, it was also highest in Jezzine (6.08 $mg.kg^{-1}$) and lowest in Hasbaya (1.78 $mg.kg^{-1}$); while OO obtained from Akkar and Zgharta-Koura recorded tyrosol values of 4.20 $mg.kg^{-1}$ and 3.69 $mg.kg^{-1}$; respectively. The significant difference of individual phenolic concentrations obtained from different regions was in line with previous studies (Guerfel *et al.* 2009; Arslan *et al.* 2013; Baiano *et al.* 2013).

AC was shown to be highest in samples obtained from Akkar (12.85%), and lowest in those obtained from Hasbaya (10.94 %); however, with insignificant difference. Several studies conducted on OO obtained from various countries also showed higher AC in northern OO as compared to southern ones, but with significant effect that was attributed to the difference in altitude and temperature among different geographical origins (Issaoui *et al.* 2010; Arslan & Ozcan, 2011; Baiano *et al.* 2013). The insignificant difference in AC observed among samples in the present study could have been exerted by a type of fruit fly that attacked a batch of the

analyzed samples (Mraicha *et al.* 2010).

Regarding OO stability, OSI was highest in samples obtained from Hasbaya (10.14 h), and lowest in Jezzine (6.64 h); however, difference was insignificant. With respect to northern regions, OO samples obtained from both Akkar and Zgharta-Koura exhibited close OSI (8 h). Incompatible results were obtained in two studies conducted in Tunisia showing higher OSI in samples obtained from the north as compared to those from the south (Issaoui et al. 2010; Youssef *et al.* (2011). The opposing results obtained in the present study might be assigned to the high TPC in samples obtained from Hasbaya in the south as compared to samples obtained from the remaining regions (Guerfel *et al.* 2009; Ben Mansour *et al.* 2015).

Table II.6. Effect of four regions of different geographical origin on olive oil antioxidant parameters.
Different letters in the same row show significant differences ($p < 0.05$)

	Geographical Origin			
	Akkar	Hasbaya	Jezzine	Zgharta-Koura
Total Phenols (mg/kg)	157.07 ^{ab}	173.48 ^{a}	136.24 ^{b}	161.81 ^{a}
DPPH (%)	12.85	10.94	12.52	11.56
Oxidative Stability (h)	8.14	10.14	6.64	8.01
Individual Phenols ($mg.kg^{-1}$)				
Hydroxytyrosol	5.21 ^{b}	3.05 ^{c}	6.37 ^{a}	3.52 ^{c}
Tyrosol	4.20 ^{b}	1.78 ^{c}	6.08 ^{a}	3.69 ^{b}
Oleacein	33.69 ^{a}	12.71 ^{b}	9.75 ^{b}	7.67 ^{b}
Oleocanthal	74.42 ^{a}	47.37 ^{b}	36.78 ^{b}	38.35 ^{b}
Apigenin	5.47	4.67	5.00	5.90

Processing System

Concerning the type of processing technique, results showed that it significantly affected OO TPC and OSI; along with all the analyzed individual phenols: hydroxytyrosol, tyrosol, oleacein, oleocanthal and apigenin ($p < 0.00625$).

The results in Table II.7 showed that OO processed using 3-phases decanters significantly

exhibited the highest TPC (179.4 $mg.kg^{-1}$), followed by sinolea (160.4 $mg.kg^{-1}$), 2-phases decanters (158.6 $mg.kg^{-1}$), and pressing system (135.8 $mg.kg^{-1}$). Ammar et al. (2014) and Kalogeropoulos et al. (2014) also reported a significant effect of processing systems on the OO's TPC; however, the highest concentrations were detected in OO processed by 2-phases decanters. On the other hand, Serhan et al. (2016) reported that among 25 samples obtained using traditional press system and 3-phases decanters, the TPC was identical ($p > 0.05$). The differences in TPC between samples obtained from different processing systems could be attributed to many factors such as velocity of crushing machine (Serhan *et al.* 2016), volume of water added and time of oil contact with water (Ammar *et al.* 2014).

With respect to individual phenols, the significant difference in both phenolic alcohols could be attributed to 2-phases decanters where the corresponding OO showed highest values of hydroxytyrosol (9.7 ± 1.0 $mg.kg^{-1}$) and tyrosol (8.5 ± 1.2 $mg.kg^{-1}$) as compared to ones processed by 3-phases decanters (4.0 ± 0.3 vs 2.5 ± 0.2 $mg.kg^{-1}$), Sinolea (2.6 ± 0.1 vs 1.4 ± 0.1 $mg.kg^{-1}$), and press system (2.3 ± 0.1 vs 2.7 $mg.kg^{-1}$), respectively. Oleacein, oleocanthal and apigenin also exhibited highest concentrations (34.5 ± 5.4 $mg.kg^{-1}$; 67.3 ± 7.3 $mg.kg^{-1}$; 6.8 ± 0.4 $mg.kg^{-1}$, respectively) among oils processed using 2-phases decanters as compared to other types of mills. Comparable results were obtained by Kalogeropoulos *et al.* (2014) attributing the results obtained to the absence of water addition whenever using 2-phases decanters.

As for AC, it was shown to be the highest in OO produced using 3-phases decanters (14.2%), followed by 2-phases decanters (13.7%), press (10.2%), and sinolea (8.1%); however, with insignificant difference. Kalogeropoulos *et al.* (2014) also found insignificant results; however, with higher DPPH values in oils obtained by 2-phases decanters (26.5 ± 2.00 mmol Trolox Equivalent (TE)) as compared to those obtained by 3-phases decanters (25.5 ± 2.32 mmol TE)

($p > 0.05$). This insignificant difference was explained by the use of DPPH assay which is more suitable to samples with lipophilic antioxidants. On the other hand, many other authors found significantly higher AC in oils obtained from 2-phases decanters due to their higher TPC which could make current results clearer, since oils processed using 3-phase decanters were found to have the highest TPC. Regarding OO stability to oxidation, OO from 3-phases decanters significantly exhibited the highest induction time (10.2 h), followed closely by ones from sinolea (9.8 h), 2-phases decanters (7.1 h), and finally press systems (6.8 h). Likewise, Serhan *et al.* (2016) showed comparable significant difference with OO from 3-phases decanters presenting higher OSI (24.4 h) as compared to traditionally pressed ones (17.2 h) ($p < 0.05$). On the other hand, significant differences were observed in induction time among samples processed differently; however, OO processed by 2-phases decanters presented the highest resistance to oxidation (Ammar *et al.* 2014; Salvador *et al.* 2003; Giovacchino *et al.* 2001). The latter results were justified by the absence of water while processing OO using 2-phases decanters.

Table II.7. Effect of different processing systems on olive oil antioxidant parameters.
Different letters in the same row show significant differences ($p < 0.05$)

	Processing System			
	Press	2-Phases Decanters	3-Phases Decanters	Sinolea
Total Phenols (mg/kg)	135.8^{b}	$158.6^{a,b}$	179.4^{a}	$160.4^{a,b}$
DPPH (%)	10.2	13.7	14.2	8.1
Oxidative Stability (h)	6.8^{b}	7.1^{b}	10.2^{a}	9.8^{a}
Individual Phenols ($mg.kg^{-1}$)				
Hydroxytyrosol	2.3^{c}	9.7^{a}	4.0^{b}	2.6^{c}
Tyrosol	2.7^{b}	8.5^{a}	2.5^{b}	1.4^{b}
Oleacein	8.0^{c}	34.5^{a}	20.8^{b}	13.8^{bc}
Oleocanthal	36.7^{b}	67.3^{a}	61.6^{a}	45.0^{b}
Apigenin	3.6^{b}	6.8^{a}	6.4^{a}	4.0^{b}

Harvesting time

Harvesting time was shown to significantly affect OO DPPH and OSI, along with both

individual phenols tyrosol and oleocanthal ($p < 0.00625$).

Regarding TPC, table II.8 showed that samples collected at early harvesting period (end October) had the highest TPC (166.4 mg GAE.kg^{-1}), followed by a slight decrease at intermediate (mid-November) (162.34 mg GAE.kg^{-1}) and late (beginning December) (143.5 mg GAE.kg^{-1}) periods; however, this difference was insignificant ($p = 0.125$). The insignificant decrease in TPC with fruit ripening is consistent with earlier findings observed by Hbaieb *et al.* (2015) and Dag *et al.* (2011) ($p > 0.05$). On the other hand, many authors found compatible results; however, difference in TPC was found significant with a progression in harvesting time (Ceci *et al.* 2017; Gouvinhas *et al.* 2015; Gambarcota *et al.* 2010; Nieto *et al.* 2010). This decrease in TPC with an advance in harvesting time could be attributed to the difference in polysaccharides in the cell wall that might affect the release of phenols during fruit crushing (Köseoğlu *et al.* 2016), the presence of high concentration of phenylalanine ammonia lyase responsible for phenol synthesis during early harvesting dates (Machado *et al.* 2013), malaxation time (Lukic *et al.* 2017; Gambarcota *et al.* 2015), and the increased level of precipitation (Machado *et al.* 2013; Gomez-Rico *et al.* 2007).

Regarding individual phenols, the most abundant compound oleocanthal significantly decreased with a progress in harvesting time (59.9 mg.kg^{-1} vs 41.6 mg.kg^{-1}). Phenolic alcohol, tyrosol, significantly increased with a progress in harvesting time; where it was lowest at early harvesting time (2.9 mg.kg^{-1}) and highest at late time (4.6 mg.kg^{-1}). Similar results were obtained by Jolayemi *et al.* (2016) where the increase in phenolic alcohol concentration with a progression in harvesting time was attributed to the presence of hydrolytic enzymes, ß-glucosidase and esterase, that are responsible for hydrolysis of oleuropein into hydroxytyrosol and tyrosol.

Concerning AC, it significantly decreased with harvesting time, where OO obtained from early harvested olives were characterized by the highest AC (16.7%) as compared to those from intermediate (7.9%) and late time (9.3%). Similar significant results were reported by Ben Ibrahim *et al.* (2017) Franco *et al.* (2014), Köseoğlu *et al.* (2016), Gambarcota *et al.* (2010) ($p < 0.05$), and the strongest AC was obtained in samples at the first ripening stage. The current significant reduction in antioxidant activity with an advance in ripening stage was in line with TPC that was highest in samples at early harvesting time (Ben Ibrahim *et al.* 2017). With respect to OO induction time, the highest OSI was observed in samples harvested at early time (9.2 h), decreasing significantly in those harvested at intermediate (7.9 h) and late (7.5) time. Compatible results were obtained by Köseoğlu *et al.* (2016), Hbaieb *et al.* (2015), Ben Brahim *et al.* (2017), and Ceci *et al.* (2017), and were explained by the richness of early harvested samples in TPC, tocopherols, chlorophyll and carotenoids (Nieto *et al.* 2010).

Table II.8. Effect of different harvesting times on olive oil antioxidant parameters.
Different letters in the same row show significant differences ($p < 0.05$)

	Harvesting Time		
	Early	Intermediate	Late
Total Phenols (mg/kg)	166.4	162.3	143.5
DPPH (%)	16.7^{a}	10.4^{b}	9.3^{b}
Oxidative Stability (h)	9.2^{a}	7.9^{b}	7.5^{b}
Individual Phenols ($mg.kg^{-1}$)			
Hydroxytyrosol	3.9	5.4	4.9
Tyrosol	2.9^{b}	4.4^{a}	4.6^{a}
Oleacein	22.8	20.1	15.0
Oleocanthal	59.9^{a}	57.4^{a}	41.6^{b}
Apigenin	5.8	4.8	5.4

II.3.6. Correlation between olive oil composition and its quality indices

Correlation between total phenolic content, antioxidant capacity and oxidative stability index

To detect the effect of the two predictors (TPC and DPPH) on OSI, a stepwise multiple linear regression (MLR) was conducted. The assumptions were checked as follows. A linear relationship was found between the two predictors (TPC and DPPH) and OSI (Tested graphically). According to Kolmogorov-Smirnov test, all variables were normally distributed with respectively $(D(104) = 0.074;\ p > 0.05), (D(104) = 0.070;\ p > 0.05),\ (D(104) = 0.084;\ p > 0.05)$. No significant outliers were detected.

As presented in table II.9, the matrix of Pearson showed a strong correlation between TPC and OSI $(r = 0.676, p < 0.001)$ and a relatively weak, but extremely significant correlation between DPPH and OSI $(r = 0.362, p < 0.001)$. Otherwise, the relation between the two predictors (TPC and DPPH) is extremely significant, but weak $(r = 0.334, p < 0.001)$, respecting the assumption of multicollinearity.

Table II.9. Matrix of Pearson showing correlation between TPC, DPPH and OSI.
*** $p < 0.001$

	OSI	TPC	DPPH
OSI	1	0.676***	0.362***
TPC		1	0.334***
DPPH			1

When the correlation between TPC and AC was evaluated in the literature, positive results with stronger correlation between latter factors were found with $r = 0.89$ (Köseoğlu *et al.* 2016), and $r = 0.7166$ (Gambarcota *et al.* 2010) indicating that samples with highest TPC exhibit highest AC and radical scavenging capacity (Issaoui *et al.* 2010; Arslan *et al.* 2013).

Comparable positive results were obtained in many countries of the Mediterranean basin such as in Tunisia (Ammar *et al.* 2014; Issaoui *et al.* 2013; Rigane *et al.* 2013; Youssef *et al.* 2011; Nakbi *et al.* 2010), Turkey (Arslan *et al.* 2013), Italy (Condelli *et al.* 2015; Baiano *et al.* 2013; Baiano *et al.* 2012), and Spain (Sena-Moreno *et al.* 2017; Gambarcota *et al.* 2010). However, the weak correlation obtained in the present results could be explained by factors that might contribute to antioxidant other than TPC such as tocopherols (Ceci *et al.* 2017) hydroxytyrosol, luteolin, total secoiridoids (Arslan *et al.* 2013; Issaoui *et al.* 2010), carotenoids and chlorophylls (Nieto *et al.* 2010).

Also, numerous studies have reported similar positive correlation between phenols and OO resistance to oxidation (Hbaeib *et al.* 2015; Berengeur *et al.* 2006). Ben Brahim *et al.* (2017) stated that the high stability of OO against oxidation corresponds to the antioxidant effect of phenolic compounds, specifically oleuropein aglycone and hydroxytyrosol. Likewise, Ceci *et al.* (2017) found higher induction time in OO samples with highest: 1) TPC ($p < 0.001$), 2) oleuropein and its derivatives, 3) tocopherols ($p < 0.001$), 4) carotenoids ($p < 0.001$), 5) MUFA/PUFA ratio ($p < 0.001$). On the other hand, Franco *et al.* (2014) found that OO samples with lowest TPC exhibited the highest induction time, attributing the results to the high concentration of hydroxytyrosol derivatives which are known for their AC. Therefore, the high correlation in the current study could be influenced by the presence of specific individual compounds, high TPC, along with other compounds with antioxidant abilities such as chlorophyll, carotenoids, and tocopherols.

To go further through the analysis of the results, both predictors (TPC and DPPH) were used by the MLR to create the prediction model, presented by the below summary (table II.10).

Table II.10. Prediction model of both predictors (TPC and DPPH) obtained by Multiple Linear Regression.

R	R^2	Adjusted R^2	Standard error of the estimate	Durbin-Watson
0.692	0.478	0.468	2.39	1.472

The R value, shown in table 6, was approximately equal 0.7; indicating that the data fits the model. The adjusted R^2=0.468 showed that 47% of OSI value can be explained by TPC and DPPH. In order to report how well the regression equation predicts a certain value, the ANOVA table in SPSS is usually relied on. According to the obtained results, our regression model predicted the OSI significantly well ($p < 0.001$).

Based on the table of coefficient (table II.11), the regression model that can be used to predict OSI from TPC and DPPH was as follows:

$$OSI = (0.037x\ TPC) + (0.063x\ DPPH) + 1.691$$

To check which factor had a higher effect on OSI, table II.11 showing both the unstandardized and the standardized coefficient was also used. TPC, with the highest standardized coefficient (ß+= 0.625) affected OSI more than DPPH (ß+= 0.153).

Table II.11. Table of Coefficient of both predictors TPC and DPPH.
TPC: total phenolic content, OSI: oxidative stability index, ß*: unstandardized coefficient, ß+: standardized coefficient

	ß*	Standard Error	ß+
Constant	1.691	0.722	
TPC	0.037	0.005	0.625
DPPH	0.063	0.031	0.153

Correlation between individual phenolic content and total phenolic content, antioxidant capacity and oxidative stability index

To detect the effect of different individual phenols on TPC, DPPH and OSI, a stepwise MLR was conducted, and all assumptions were checked. Pearson's matrix (table II.12) showed that apigenin was the most significantly correlated and contributing phenol to TPC ($r = 0.425, p < 0.001$). Concerning phenolic alcohols, while hydroxytyrosol exhibited a significant positive weak correlation with TPC ($r = 0.184, p < 0.05$), tyrosol showed an insignificant negative weak one represented by an $r = -0.045$. Both oleacein and oleocanthal exhibited weak-moderate highly significant correlations with TPC; with respective *r* values equal to 0.376 and 0.305 ($p < 0.001$).

With respect to AC, all individual phenols had weak correlations with DPPH. Apigenin was the most contributing phenol ($r = 0.274, p < 0.01$). As for phenolic alcohols, while hydroxytyrosol showed a significant positive weak correlation with DPPH ($r = 0.162, p < 0.05$), tyrosol presented an insignificant negative weak one ($r = -0.002$). Both individual phenols oleacein and oleocanthal showed a significant; however, weak correlation with DPPH with respective r values equal to 0.248 and 0.216 ($p < 0.05$).

In terms of correlation between OSI and individual phenols, moderate associations were observed in the obtained results. Apigenin showed the highest correlation ($r = 0.416$). Weak, but highly significant correlations were found between OSI and oleacein ($r = 0.273, p < 0.01$), and oleocanthal ($r = 0.258, p < 0.01$). Regarding phenolic alcohols, only tyrosol showed an extreme highly significant negative correlation with induction time, represented by $r = -0.337, (p < 0.001)$.

On both national and international levels, studies lacked data on oleocanthal and oleacein and their correlation with TPC/AC/ and OSI. However, previous investigations showed incompatible results regarding hydroxytyrosol, apigenin and luteolin that were known for their

antioxidant activities and were shown to exhibit a strong correlation with TPC, and OSI (Arslan *et al.* 2013; Ceci *et al.* 2017). This incompatibility could be attributed to the effect of agricultural practices and agro-industrial factors that can induce negative impacts on OO AC and OSI (Machado *et al.* 2013). On the other hand, tyrosol results in the current study were in line with earlier findings which reported that tyrosol does not possess an antioxidant capacity (Allalout *et al.* 2009).

Table II.12. Matrix of Pearson showing correlation between individual phenols and total phenolic content, antioxidant capacity and oxidative stability.
TPC: total phenolic content, OSI: oxidative stability index, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	TPC	DPPH	OSI	Hydroxy-tyrosol	Tyrosol	Oleacein	Oleocanthal	Apigenin
TPC	1			0.184*	-0.045	0.376***	0.305***	0.425***
DPPH		1		0.162*	-0.002	0.248*	0.216*	0.274**
OSI			1	-0.056	-0.337***	0.273**	0.258**	0.428***
Hydroxy-tyrosol				1	0.824	0.339	0.176	0.471
Tyrosol					1	0.024	-0.056	0.269
Oleacein						1	0.800	0.575
Oleocanthal							1	0.445
Apigenin								1

Since "stepwise" method was selected while conducting the analysis, apigenin, among all other individual phenols, was the selected predictor affecting TPC and DPPH, and both apigenin and tyrosol were selected while analyzing OSI. Going further through the analysis of the current results, the obtained adjusted R^2 (table II.13) indicated that 17% of TPC and 6.6% of DPPH could be explained by apigenin, and 38% of OSI could be explained by both apigenin and tyrosol. The tables of ANOVA obtained in SPSS were evident that the regression models predict TPC, DPPH, and OSI significantly well ($p < 0.001$).

Table II.13. Prediction model of total phenolic content, antioxidant capacity and oxidative stability index obtained by Multiple Linear Regression.
TPC: total phenolic content, OSI: oxidative stability index

Dependent Variable	R	R^2	Adjusted R^2	Standard error of the estimate	Durbin-Watson
TPC	0.425	0.181	0.173	49.58	1.347
DPPH	0.274	0.075	0.066	7.57	1.4
OSI	0.030	0.397	0.386	2.54	1.103

Based on table II.14, it is important to highlight the fact that the individual phenol apigenin showed the highest effect on all parameters: TPC, AC and OSI. Additionally, the regression models that can be used to predict TPC, DPPH and OSI from individual phenols were as follows:

$$TPC = 9.041\ x\ Apigenin + 109.373$$

$$DPPH = 0.837\ x\ Apigenin + 7.729$$

$$OSI = (0.692\ x\ Apigenin) + (-0.362\ x\ Tyrosol) + 5.927$$

Table II.14. Table of coefficient of individual phenols along with their effect on total phenolic content, DPPH, and oxidative stability index.
TPC: total phenolic content, OSI: oxidative stability index, ß*: unstandardized coefficient, ß+: standardized coefficient

Parameter	Individual phenol with highest effect	ß*	Standard Error	ß+
Total Phenolic Content Constant = 109.373	Apigenin	9.041	1.876	0.425
DPPH Constant = 7.729	Apigenin	0.837	0.288	0.274
OSI Constant = 5.927	Apigenin	0.692	0.098	0.551
	Tyrosol	-0.362	0.059	-0.479

Correlation between total phenolic content, antioxidant capacity and oxidative stability index and quality indices

To detect the effect of the predictors TPC, DPPH and OSI on quality indices, a stepwise MLR was performed, and all assumptions were checked. Table II.15 revealed extremely significant negative weak-moderate correlations between TPC and FA ($r = -0.335, p < 0.001$) and between OSI and FA ($r = -0.458, p < 0.001$). Concerning both UV absorbance K_{232} and K_{270}, while the matrix of Pearson showed a significant positive weak correlation between K_{232} and TPC ($r = 0.213, p < 0.05$), and a highly significant one with OSI ($r = 0.253, p < 0.01$), it revealed a significant weak correlation between K_{270} and DPPH ($r = 0.210, p < 0.05$). It is important to note that PV didn't exhibit any significant correlation with any of the composition and quality characteristics. The obtained results were incompatible with the only study found in the literature on the association between TPC/AC/OSI and OO quality indices in 3 olive cultivars, resulting in strong negative correlation between oxidative stability and: 1) PV ($r = -0.978$), 2) K_{232} ($r = -0.986$), and 3) K_{270} ($r = -0.982$). The correlation between OSI and FA was not clearly observed in the latter study (Gutiérrez *et al.* 2002). The present obtained weak correlations showed that the quality of OO, whether VOO or EVOO, does not necessarily determine or affect its TPC, AC, and OSI.

Table II.15. Matrix of Pearson showing correlation between quality indices (PV, FA, K_{232}, K_{270}) and TPC, DPPH and OSI.

TPC: total phenolic content, OSI: oxidative stability index, PV: peroxide value, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	Free Acidity	Peroxide Value	K_{232}	K_{270}	TPC	DPPH	OSI
Free Acidity	1				-0.335***	-0.136	-0.458***
PV		1			-0.223	-0.080	-0.206
K_{232}			1		0.213*	0.123	0.253**
K_{270}				1	0.098	0.210*	0.171
TPC					1		
DPPH						1	
OSI							1

Since "stepwise" method was selected while performing the analysis, SPSS selected one predictor out of the 3 (TPC, DPPH, and OSI) that could have a better effect on the quality indices. TPC was the chosen predictor while analyzing PV, OSI was chosen for FA and K_{232} analysis, and DPPH for K_{270}. To go further through the analysis of the present results, and based on the adjusted R^2 obtained in table II.16, it was obvious that 4.1% of the PV value could be explained by TPC, 20% of FA value and 5.5% of K_{232} value could be explained by OSI, and 3.5% of K_{270} value could be explained by DPPH. It was also evident, depending on ANOVA tables, that the regression model significantly predicted PV ($p < 0.05$), FA ($p < 0.001$), K_{232} ($p < 0.01$), and K_{270} ($p < 0.05$).

Table II.16. Prediction model of all predictors obtained by Multiple Linear Regression.

Dependent Variable	R	R^2	Adjusted R^2	Standard error of the estimate	Durbin-Watson
Peroxide Value	0.223	0.050	0.041	4.45	1.167
Free Acidity	0.458	0.210	0.202	0.59	0.921
K232	0.253	0.064	0.055	0.31	1.502
K270	0.210	0.044	0.035	0.04	1.208

Depending on table II.17, induction time was shown to have the greatest effect on both FA and K_{232}, with respective standardized coefficients ß+= -0.458 and ß+= 0.253. While TPC was shown to have the greatest effect on PV; DPPH exhibited the highest one on K_{270}; with respective ß+= -0.223 and ß+= 0.210.

Table II.17. Table of coefficient of all predictors showing their effect on the different quality indices.

TPC: total phenolic content, OSI: oxidative stability index, ß*: unstandardized coefficient ß+: standardized coefficient

Quality Index	Predictor with highest effect	ß*	Standard Error	ß+
Peroxide Value	TPC	-0.018	0.008	-0.223
Constant = 15.928				
Free Acidity	OSI	-0.094	0.018	-0.458
Constant = 1.701				
K_{232}	OSI	0.025	0.010	0.253
Constant = 1.400				
K_{270}	DPPH	0.001	0.001	0.210
Constant = 0.124				

Also based on the coefficient table, the regression model that can be used to predict quality indices from TPC, DPPH and OSI were as follows:

$$PV = -0.018\,x\,TPC + 15.928$$

$$FA = -0.094\,x\,OSI + 1.701$$

$$K232 = 0.025\,x\,OSI + 1.400$$

$$K270 = 0.001\,x\,DPPH + 0.124$$

The present study was characterized by numerous advantages. First, its large sample size was rarely observed on the international and national levels. Second, it was one of the few studies that assessed the effect of three different agro-industrial factors on OO quality and composition. Third, this study was the first to report OO individual phenolic profile and AC at the national level.

II.4. Conclusion

To conclude, this study was conducted to determine the composition and the quality characteristics of Lebanese OO obtained from different geographical origins, processed differently, and picked at different harvesting times. Overall, the study's outcomes showed that OO samples obtained from Akkar at early harvesting time using 3-phase decanters exhibited the highest TPC. Those obtained from Jezzine at early harvesting time using 3-phase decanters showed the highest AC. As for resistance to oxidation, samples obtained from Hasbaya at early harvesting time using 3-phase decanters possessed the highest OSI. Furthermore, the obtained results also revealed weak positive correlations between TPC and AC and AC and OSI, a strong positive correlation between TPC and OSI; however weak correlations between OO TPC/AC/OSI and its quality indices. The latter findings suggest the fact that processing early harvested olive fruits using 3-phase decanters yield OO with the highest TPC, AC, and consequently OSI. The findings of this study revealed previously unknown composition and quality characteristics of Lebanese OO such as AC and individual phenol composition, as well as it determined ideal conditions for OO production. Additionally, it could assist in fighting against the fraud in EVOOs, and ensuring its compliance with the corresponding regulations and parameters of its category.

APPENDIX A

Title/Authors/ Country	Samples and Conditions	Methods	Results
Changes in phenolic compounds and Rancimat stability of olive oils from varieties of olives at different stages of ripeness. Nieto *et al.* (2010) – **Spain**	Olive samples from 3 cultivars: - Arbequina - Picual - Hojiblanca Different olive oil mills were used Different harvesting stages (beginning, middle, and end of the harvest)	Scavenging activity Folin-Ciocalteu Rancimat HPLC Sensory Analysis	TPC: • Individual and TPC declined with a progress in maturation stage.
Gambacorta *et al.* (2010) – **Spain**	Coratina olives Handpicked from an orchard in South Italy during season 2006/07 2 different ripening stages	Free acidity Peroxide Value Extraction	TPC: • Higher phenolic content was observed in oils picked earlier. • Significant difference in TPC was found between the 2 studied ripening indices ($p<0.001$) Antioxidant capacity: • Significant decrease in antioxidant capacity observed with a progression in ripening indices ($p<0.05$)
Influence of time of harvest and ripening index on olive oil yield and quality. Dag *et al.* (2011) – **Israel**	2 cultivars: Soury and Barnea different growing locations Different harvesting dates and ripening stages	Free acidity Peroxide value Extraction Folin-Ciocalteu Gas chromatography (GC)	TPC: • TPC was negatively correlated with increasing levels of ripening of olive fruits
Quality characteristics and antioxidant properties of Turkish monovarietal olive oils regarding stages of olive ripening Köseoğlu *et al.* (2013) – **Turkey**	2 olive cultivars: 1) Memecik, 2) Gemlik 2015 crop season Same growing area Three different ripening stages	Peroxide value Free acidity Extraction Folin-Ciocalteu Rancimat	TPC: • Significant difference was observed in TPC of a single cultivar at different ripening stages ($p<0.05$) • Significantly, TPC increased from ripening stage 1 till 2, and then decreased in stage 3 for both cultivars Antioxidant capacity: • Antioxidant capacity significantly decreased with the increase of ripening index ($p<0.05$) OSI: • Oxidative stability significantly decreased with the progression of ripening stage ($p<0.05$)

Phenolic compounds and antioxidant capacity of virgin olive oil. Franco *et al.* (2014) – **Spain**	Olives of 7 cultivars: 1)Arbequina, 2) Carrasquena, 3) Corniche, 4) Manzanilla Cacerena, 5) Manzanilla Sevillana, 6) Picual, 7) Verdial de Badajoz Same geographical area Same agronomic and pedoclimatic conditions During season 2011/12 in Spain	Reversed phase high performance liquid chromatography DPPH	DPPH: • The strongest antioxidant capacity was found in the first ripening stage (IM<2).
Discrimination and characterisation of extra virgin olive oils from three cultivars in different maturation stages using Fourier transform infrared spectroscopy in tandem with chemometrics. Gouvinhas *et al.* (2015) – **Portugal**	Monovarietal EVOO from 3 cultivars: - Cobrancosa - Galega - Picual) Obtained from an olive orchard in Portugal Crop season 2012-2013 Olives collected from 10 trees with the same age and growing area Handpicked at three ripening stages	Folin-Ciocalteu ABTS assay	TPC: • TPC declined with the progress of ripening. Antioxidant activity: • The antioxidant activity decreased with the progress of ripening stage. (Green>Ripe)
Monitoring endogenous enzymes during olive fruit ripening and storage: Correlation with virgin olive oil phenolic profiles Hbaieb *et al.* (2015) - **Spain**	Samples of Arbequina variety Harvested at: 1) green ripening stage (October 2012); 2) 24 weeks after flowering	Extraction Rancimat	TPC: • Significant quantitative differences were observed in TPC • The content of total phenolics, o-diphenolics and secoiridoids derivatives decreased about 20–25% with progress in ripening stage OSI: • Oil stability index decreased with a progress in ripening stage.
Effects of malaxation temperature and harvest time on the chemical characteristics of olive oils. Jolayemi *et al.* (2016) – **Turkey**	N=36 samples of 2 Turkish varieties: - Ayvalik - Memecik Different ripening stages Different growing locations (South vs. North)	Extraction Folin-Ciocalteu Chlorophyll and carotenoid content Peroxide value Free Acidity Color parameters HPLC Gas chromatography	TPC: • TPC significantly declined with the progress of ripening • At 27°C, TPC ranged between 116.7 ± 23.0 to 94.65 ± 14.6
LC–MS phenolic profiling combined with multivariate analysis as an approach	Olives from 4 Tunisian cultivars: - Toffehi	Extraction DPPH	TPC: • Significant quantitative differences in phenolic fraction were obvious between different cultivars at different ripening

for the characterization of extra virgin olive oils of four rare Tunisian cultivars during ripening Ben Ibrahim *et al.* (2017) – **Tunisia**	- Fakhari - Jemri - Zalmati Same agronomic and environmental conditions Handpicked during season 2015/16 at three harvesting stages	Rancimat apparatus	stages. DPPH: • Significant decrease in antioxidant capacity of oils with an increase in ripening index was observed • The reduction in phenolic content during ripening caused a decrease in antioxidant activity.
Chemical quality and oxidative stability of extra virgin olive oils from San Juan province (Argentina) Ceci *et al.* (2017) – **Argentina**	N=30 Three Argentinian cultivars (Changlot Real, Arbequina, Coratina) Harvested from different locations Different ripening stages	Free acidity Peroxide value Extraction Folin-Ciocalteu	TPC: • Fruit ripening caused negative effects on TPC of olive oil. ($p<0.01$) • TPC was highest in oils of olives with low ripening index OSI: • OSI significantly increased with a decrease in stage of ripening
Complex interactive effects of ripening degree, malaxation duration and temperature on Oblica cv. virgin olive oil phenols, volatiles and sensory quality Lukic *et al.* (2017) – **Croatia**	Oblica cultivar predominant in Croatia Harvested in 2015 3 ripening degrees (green (RD1), semi ripe (RD2), ripe (RD3)	Extraction	TPC: • Fruits ripening had the greatest influence on quality of olive oil • Phenols were found to decrease during ripening
Effect of Tunisian olive ripeness on endogenous enzymes and virgin olive oil phenolic composition Hbaieb *et al.* (2017) – **Tunisia**	Monovarietal VOOs from two main Tunisian cultivars, (Chetoui and Chemlali) Harvested during crop season 2014/15 Different non-irrigated growing areas Same extraction, agronomic and pedoclimatic conditions	Extraction of olive oil HPLC DPPH Enzyme extraction	TPC: • Significant difference in TPC observed between cultivars at different ripening stages • Chetoui cultivar had higher TPC at all ripening stages • An increase of TPC was observed at the beginning followed by a gradual decrease DPPH: • Significant difference in radical scavenging activity was observed between cultivars of different ripening stages • Chetoui cultivars had a higher radical scavenging activity than Chemlali ($p<0.05$)

Table I.1. Literature data on the effect of harvesting time on total phenolic content, antioxidant capacity, and oxidative stability index of olive oil samples

Title/Author/Country	Samples and Conditions	Methods	Results
Natural antioxidants and volatile compounds of virgin olive oils obtained by two or three-phases centrifugal decanters Giovacchino *et al.* (2001) – **Italy**	600 kg of leafless olives of 10 different cultivars 2-phase decanter and 3-phase decanter	Peroxide Value Free acidity Spectrophotometric absorption TPC Rancimat	TPC: • Significant difference in TPC observed between the 2 methods. • Oils extracted using 2-phase decanter had higher TPC than oils extracted using 3-phase (292 mg/l vs 197 mg/l, $p<0.05$) OSI: • Significant difference in induction time was observed. • 2-phase method led to a higher induction time (14.2 h vs. 11 h, $p<0.05$)
The effects of harvest and extraction methods on the antioxidant content (phenolics, α-tocopherol, and β-carotene) in virgin olive oil Gimeno *et al.* (2002) – **Spain**	N=60 samples of Arbequina cultivar and from two crops (years 1997–1998 and 1998–1999) 2 extraction methods	Free acidity Peroxide value Extraction Folin-Ciocalteu	TPC: • Significant difference ($p= 0.016$) observed in TPC between different processing system • TPC of green olives: I. Using 2 phase decanter: 123.82 $mg.kg^{-1}$ II. Using 3-phase decanter: 72.93 $mg.kg^{-1}$
Influence of extraction system, production year and area on Cornicabra virgin olive oil: a study of five crop seasons Salvador *et al.* (2003) – **Spain**	N= 140 (Cornicabra cultivar) Five crop seasons Five production areas Extracted using: - 2-phase decanter: N= 68 - 3-phase decanter: n= 63 - Press System: n=9	Free acidity Peroxide value UV absorption Extraction Folin-Ciocalteu	TPC: • TPC was significantly higher in oils extracted using modern decanters than oils extracted traditionally • Between both decanter systems, oils extracted using 2-phase had higher TPC content • TPC in: ($p<0.05$) I. 2-phase decanter: 160 mg/kg II. Traditional system: 100 mg/kg • The decrease in phenols might be due to their high solubility in water OSI: • OSI significantly differed between extraction systems • Oils extracted using decanter systems had higher OSI than oils extracted traditionally • OSI in: ($p<0.05$) I. 2-phase decanter: 65.8 h II. Traditional system: 46.3 h
Composition, volatile profiles and functional properties of virgin olive oils produced by two-phase vs three-phase centrifugal decanters Kalogeropoulos *et al.* (2014) – **Greece**	400 kg of olive fruits of Korneiki cultivar Hand-picked during January 2012 2-phase decanter and 3-phase decanter	Peroxide value Free acidity GC Extraction Folin-Ciocalteu DPPH	TPC: • Significant difference found between 2 extraction methods • 3-phase decanter: 31.59 ±2.72 mg GAE/kg *vs* 2-phase: 37.33 ±3.22 mg GAE/kg ($p<0.003$) DPPH: • Oils extracted using 2-phase decanter had a higher antioxidant capacity • Difference in antioxidant capacity between 2 extraction methods wasn't significant.

			I. 2-phase decanter: 26.51 ± 2.00 mmol Trolox equivalents II. 3-phase decanter: 25.52 ± 2.32 mmol Trolox equivalents
Effect of Processing Systems on the Quality and Stability of Chemlali Olive Oils Ammar *et al.* (2014) – **Tunisia**	Chemlali cv. olive samples 3 processing system: - 2-phase decanter - 3-phase decanter - repassed oil; obtained from second extraction of two-phase paste	Peroxide Value Free acidity Extraction Folin-Ciocalteu DPPH	Quality indices significantly differed in oils with different processing system TPC: • 2-phase decanter retained phenols more than three phase decanter (251.64 mg/kg vs 210 mg/kg) ($p<0.001$) • Significant differences in TPC were observed between the 3 types of processing systems • The addition of water in 3-phase and in repassed oil contributed to the lower TPC DPPH: • Significant difference in antioxidant capacity observed between the 3 types of processing system ($p<0.001$) • Oils decanted using 2-phase systems had the highest antioxidant capacity (2.30 ± 0.09 μg/mL) as compared to 3-phase (4.40 ± 0.24 μg/mL) and repassed oils (13.59 ± 0.45 μg/mL) ($p<0.001$) OSI: • Significant difference in OSI was found between the 3 systems • Oils processed using 2-phase decanter had the highest OSI due to its high phenolic content • OSI: ($p<0.001$) 1. 2 phase decanted oil: 6.48 h 2. 3-phase decanted oil: 4.38 h 3. Repassed oil: 3.69 h

Table I.2. Literature data on the effect of processing system on total phenolic content, antioxidant capacity, and oxidative stability index of olive oil samples

Author/Title/Year	Samples and conditions	Methods	Results
Effect of location on virgin olive oils of the two main Tunisian olive cultivars Guerfel *et al.* (2009) – **Tunisia**	Chemlali and Chetoui olive cultivars Same ripening stage 2 crop seasons (2006-2007) Different climatic conditions 3 locations: 1) Sfax (South of Tunisia), 2) Sousse (center of Tunisia), 3) Zaghouan (north of Tunisia)	Fatty acid composition Rancimat TPC HPLC Chlorophyll and carotenoids Free acidity Peroxide Value UV absorption	TPC: • Within the same cultivar, TPC significantly differed among the three different locations ($p<0.001$) • Chemlali cultivar obtained from Sousse (Center) had the highest TPC (330.8 mg/kg) (South: 304.72 mg/kg, North: 255.03 mg/kg) • Chetoui cultivar obtained from Zaghouan (North) location had the highest TPC (1003.71 mg/kg) (South: 158.01 mg/kg, Center: 256.02 mg/kg) OSI: • For the same cultivar, significant difference in OSI among 3 different locations was observed ($p<0.001$) • For Chemlali cultivar, samples obtained from Sousse (Center) had the highest induction time (45.5 h) (South: 41.8h, North: 27.4h) • For Chetoui cultivar, samples obtained from Zaghouan (North) had the highest induction time (77.3 h) (Center: 34.8h, South: 16.1h)
Effect of the growing area conditions on differentiation between Chemlali and Chetoui olive oils Issaoui *et al.* (2010) – **Tunisia**	Chetoui and Chemlali cultivars Same ripening degree North and South of Tunisia North: 650 mm rainfall; South: 190 mm rainfall Different pedoclimatic conditions	Free Acidity Peroxide Value UV absorbances Folin-Ciocalteu DPPH	TPC: • Significant difference observed in TPC between North and South growing areas ($p<0.05$) • Samples obtained from north produced oils with significantly higher TPC compared to southern samples • For Chemlali cultivar: North vs South: 573, 173 mg/kg respectively • For Chetoui cultivar: North vs South: 551, 274 mg/kg respectively Antioxidant capacity: • Antioxidant capacity varied significantly between samples obtained from different locations ($p<0.05$) • For Chemlali cultivar, northern samples had higher antioxidant capacity than the corresponding samples from the South (EC_{50}: lower values indicate higher scavenging activity: 177.4 mg/kg vs 793.4 mg/kg, respectively) • For Chetoui cultivar, northern samples had higher antioxidant capacity than the corresponding samples from the South (EC_{50} = 6.9 mg/kg vs 297.4 mg/kg) OSI: • Induction time differed significantly within the same cultivar obtained from different locations ($p<0.05$) • Within the same cultivar, northern samples had significantly higher induction times than the southern ones I. Chemlali cultivar: North: 6.4h vs 2.5 in South II. Chetoui cultivar: North: 12.7h vs 7.2 h in South

Phenolic profile and antioxidant activity of olive fruits of the Turkish variety "Sariulak" from different locations Arslan & Ozcan, (2011) – **Turkey**	Seriulak cultovar 4 locations: 1)Alanya (in Antalya), 2) Silifke (in Mersin), 3) Bucakkisla (in Karaman, 4) Ceyhan (in Adana) 2 experimental years (2006-2007) 3 harvesting dates	Extraction Folin-Ciocalteu DPPH	Antioxidant capacity: • Significant difference observed in antioxidant capacity between four different locations ($p<0.05$)
Irrigation Regimes and Growing Area Effects on Chetoui Olive Oil Quality Issaoui *et al.* (2013) – **Tunisia**	Chetoui irrigated cultivar Tunisia: 1) samples from North (rainfall: 600mm) 2) Samples from South (rainfall 250 mm) 2008-2009 crop season Different pedoclimatic conditions	Extraction Free Acidity Peroxide Value TPC Rancimat GC	TPC: • TPC strongly differed with respect to growing area • Oils with higher TPC content were obtained from North region OSI: • Oils produced in North had a higher induction time compared to samples from South ($p<0.05$) • Growing area had a stronger effect on OSI compared to irrigation regime
Volatile compounds and compositional quality of virgin olive oil from Oueslati variety: Influence of geographical origin Youssef *et al.* (2011) – **Tunisia**	Samples of Oueslati cultivar from different locations of the growing area Kairouan: - Ala - Jbel Rihan - Khit el Oued - Ain Jloula - Menzel Rais - Haffouz - and from growing area Sfax Kairouan: 379 m a.s.l; 320 mm rainfall; Sfax: 21 m a.s.l; 290 mm rainfall 2008-2009 crop season	Free acidity Peroxide Value UV absorption Folin-Ciocalteu Rancimat	TPC: • TPC significantly varied between different regions ($p<0.005$) • Jbel Rihan showed the highest value of TPC (859.81 mg/kg, while Menzel Rais showed lowest values (334.81 mg/kg) OSI: • Oxidative stability significantly differed between different locations ($p<0.005$) • Samples from Jbel Rihan had the highest induction time (103.62 hr) compared to Menzel Rais which had the lowest induction time (32.78 hr)
Effects of Cultivars and Location on Quality, Phenolic Content and Antioxidant Activity of Extra-Virgin Olive Oils Baiano *et al.* (2012) – **Italy**	EVOO (year 2011) Four different locations: 1) Colavecchia (A), 2) Casone (B), 3) Mezzanola (C), 4) Tatozzo (D) Different type of soil and cultivars Same agronomic techniques	Free acidity Peroxide Value UV absorbance Extraction Folin-Ciocalteu HPLC ABTS/DPPH/β-carotene linoleate model system	TPC: • Within the same cultivar (i.e: Peranzana cv.), significant difference was observed in TPC among 3 different locations ($p<0.05$) DPPH: • For all cultivars, antioxidant capacity varied with location ($p<0.05$) • Significant difference in DPPH was observed within Peranzana cultivar obtained from 3 different regions

Variations of phenolic compounds, fatty acids and some qualitative characteristics of Sariulak olive oil as induced by growing area Arslan *et al.* (2013) – **Turkey**	Sariulak cultivar 2007 crop season 3 locations from South region of Turkey: 1) Antalya (26m a.s.l), 2) Karaman (400 m a.s.l), 3) Mersin (20 m a.s.l)	Extraction of olive oil Extraction of phenols (liquid-liquid) Free acidity Peroxide Value	TPC: • Statistically significant difference in TPC observed between the different locations • Karaman location had the highest TPC, due to lower rainfall levels Antioxidant capacity: • Karaman oil had the highest antioxidant capacity (because of its highest TPC)
Effect of Agricultural Sites on Differentiation between Chemlali and Neb Jmel Olive Oils Ben Mansour *et al.* (2015) – **Tunisia**	2 Tunisian cultivars: 1) Neb Jmel, 2) Chemlali 2 regions: 1) Kairouan (in center:381 m a.s.l; 385.7 mm rainfall), 2) Gabes (in south: 224 m a.s.l; 217.3 mm rainfall) Rain-fed trees Crop season 2013-2014 Same ripening stage	Free acidity Peroxide Value Extraction Folin-Ciocalteu Rancimat	TPC: • Within the same cultivar, TPC significantly differed between different regions ($p<0.05$) • Cultivars from the center produced oils with higher TPC compared to South • For Chemlali cultivar, TPC in center was higher compared to that in South (364.58±12.4 *vs* 212.09±7.35 mg/kg) • For Neb Jmel cultivar, TPC in center was higher than that in South (1167.03±31.94 vs 513.57±18.9 mg/kg) OSI: • Induction time significantly varied according to the growing area ($p<0.05$) • Oils obtained from the center had a higher OSI compared to ones obtained from south • For Chemlali cultivar, center *vs* south: (7.27±0.5 h vs 5.61±0.38 h) • For Neb Jmel cultivar, center *vs* south: (15.38±0.18 h vs 8.34±0.15 h)
Pedologic Factors Affecting Virgin Olive Oil Quality of "Chemlali" Olive Trees (Olea europaea L.) Ben Rached *et al.* (2017) – **Tunisia**	Chemlali olive cultivar Same stage of ripening 2 crop seasons (2014-2015) Same growing area: Sousse 5 olive orchards with 6 different soil types: 1) sandy, 2) clay, 3) stony, 4) brown, 5) limestone, 6) gypsum	Free acidity Peroxide value UV absorbance Folin-Ciocalteu	TPC: • Significant difference in TPC was observed among the different types of soil ($p<0.05$) • TPC ranged from 123-284 mg/kg • Oils from soil clay had the highest amount of TPC (284±91.74 mg/kg), oils from soil brown recorded lowest TPC (123±15.83 mg/kg)

Characterization of Arbequina virgin olive oils produced in different regions of Brazil and Spain: Physicochemical properties, oxidative stability and fatty acid profile Borges *et al.* (2017) – **Spain**	Arbequina cultivar Two regions in Brazil (Minas Gerais and Rio Grande do Sul) and nine regions in Spain (Granada, Jaén, Málaga, Cádiz, Sevilla, Albacete, Toledo, Valladolid and Lérida). two-phase extraction system 2014/2015 crop season	Free acidity Peroxide Value UV absorbance Oxidative Stability	OSI: • Significant difference in OSI was found between samples of different regions ($p<0.05$) ranging between 5.32 ± 0.01h and 19.73 ± 0.67 h

Table I.3. Literature data on the effect of geographical origin on total phenolic content, antioxidant capacity, and oxidative stability index of olive oil samples

Title/Author/Country	Study Rationale & Aim(s)	Methods	Results
Characterization of the main Lebanese olive germplasm Chehade *et al.* (2012) – **Lebanon**	Aim: To characterize the morphological, bio-agronomical, phenological, sanitary, oil features of main Lebanese olive varieties	Extraction of phenols Folin-Ciocalteu	TPC: • Significant difference in phenolic content was observed between 6 studied cultivars. • Del cultivar had the highest total phenolic content (486.6 ± 56.76 mg/kg oil), followed by Soury cultivar (414 ± 3.78 mg/kg oil)
In situ evaluation of the fruit and oil characteristics of the main Lebanese olive germplasm Chehade *et al.* (2016) – **Lebanon**	Few studies have been carried out to evaluate the commercial characteristics of the fruit and oil quality Aim: to study the fruit and oil characteristics during normal harvesting time in areas where they have the largest distribution	Extraction Folin-Ciocalteu	TPC: • Cultivar greatly affected TPC • TPC ranged: 208±41 and 430±61 mg/kg oil The lowest oleic content was due to high temperature in South
Physicochemical Changes in Baladi Olive Oil as a Function of Production Area and Extraction System in North Lebanon Serhan *et al.* (2016) – **Lebanon**	No previous study showed the influence of extraction system on quality of Baladi OO Aim: examine effect of extraction and production area on OO properties	Extraction of phenols Folin-Ciocalteu Rancimat	TPC: • Difference was observed in TPC between oils extracted using different types of mills, but not statistically significant OSI: • Oil's OSI differed significantly between the two types of mills (OSI = 17.2 h for traditional system vs. 24.4 h for 3 phase system)
Influence of Irrigation Regimes on Quality Attributes of Olive Oils from Two Varieties Growing in Lebanon El-Riachy *et al.* (2017) – **Lebanon**	The lack of information on performance of local varieties under different irrigation regimes OO centrifuged used continuous systems are exhibiting a bitter taste, that may be reduced by irrigation Aim: Investigate the effect of different irrigation regimes of fruit quality, oil yield, and composition in 2 cultivars (Baladi and Edlbi)	Extraction Folin-Ciocalteu Rancimat	TPC: • In Edlbi cultivar, higher TPC in samples that were rainfed • No reduction in TPC was observed with irrigation among Baladi cultivar • Higher TPC was due to enzyme activity responsible for phenol synthesis OSI: • Samples from rain-fed trees had the highest induction time • The effect of irrigation on OSI was only significant for Edelbi variety This could be explained by the significant decrease in TPC in this variety

Table I.4. Literature data on effect of agro-industrial factors on total phenolic content, antioxidant capacity, and oxidative stability index of Lebanese olive oil samples

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