Determinant Factors of Antioxidant Capacity and Phenolic Content of Lebanese Olive Oil

by

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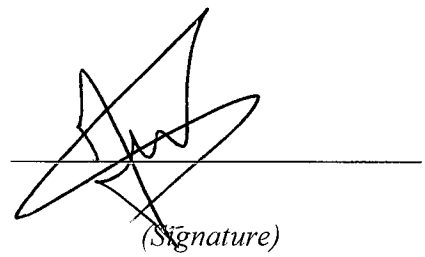

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Abstract

Determinants Factors of Antioxidant Capacity and Phenolic Content of Lebanese Olive Oil

Background: Olive Oil (OO) is premium oil which plays an important part of the Mediterranean cuisine due to its nutritional and sensory characteristics. Studies highlight the nutritional benefits associated with the consumption of OO including the low level of saturated fatty acids (SFA), the high level of monounsaturated fatty acids (MUFA) and the moderate level of polyunsaturated fatty acids (PUFA) along with minor constituents including phenolic compounds. Studies highlight the importance of phenolic compounds as they are the major bioactive of OO which contribute to its sensory, organoleptic and biological properties, in addition to their antioxidant capacity and their ability to provide oil stability to oxidation thus increasing its shelf life. However, several agro-industrial factors are reported to affect the types and quantity of phenolic and antioxidant content of OO.

Objectives: The purpose of this research is to identify the major agro-industrial factors that affect the antioxidant capacity and the phenolic content of Lebanese OO and to study the correlation between the antioxidant capacity and the phenolic content of OO

Methods: OO samples (n= 54) from 2016 crop, were originated from two areas: Zgharta-Koura (North) and Jezzine (South). These samples were collected in triplicate from 3 processing systems at 3 harvesting times by 3 different farmers at each harvesting. Liquid-Liquid extraction was used for the extraction of the phenolic compounds from OO followed by Folin-Ciocalteu method for their quantification and 2,2-diphenyl-1-picrylhydrazyl (DPPH) for the determination of their antioxidant capacity. The data collected were analyzed and used to calculate the concentration of antioxidant and total phenolic content (TPC) of OO samples. Moreover, the differences between means was assessed using Multivariate Analysis of Variances (MANOVA) and Three-way Analysis of Variance (ANOVA). The correlation between the antioxidant capacity and TPC was analyzed using the Spearman Correlation and Logistic Regression. The effect of OO categories on TPC and antioxidant capacity was assessed using Independent samples *t*-test and Mann Whitney U-test.

Results: Results indicated that the level of TPC of OO samples ranged between 78.92 and 202.97 mg GAE.kg^{-1} of oil and the level of the antioxidant capacity ranged between 0.28 and 27.14 %. Results also showed that the geographical origin, the harvesting time and the processing time had no significant effect on OO TPC content and the antioxidant capacity. The results also reported a mid, positive correlation between TPC and the antioxidant capacity. In addition to the high significant difference between TPC averages of virgin olive oil (VOO) and extra virgin olive oil (EVOO). TPC level was shown to be higher for EVOO as compared with those from VOO.

Keywords: Olive Oil; Phenolic Compounds; Antioxidant Capacity; Agro-Industrial Factors.

Table of Content

List of abbreviations

ABTS:	2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ANOVA:	One-Way Analysis of Variances
ATR:	Attenuated Total Reflectance
CE:	Capillary Electrophoresis
DES:	Deep Eutectic Solvents
DPPH:	2, 2-diphenylpicrylhydrazyl
EVOO:	Extra Virgin Olive Oil
FRAP:	Ferric Reducing Ability of Plasma Assay.
FTIR:	Fourier Transform Infrared Spectrometer
GC:	Gas Chromatography
HACCP:	Hazard Analysis Critical Control Point
HPLC:	High Performance Liquid Chromatography
IOC:	International Olive Council
IR:	Infrared
IRB:	Institutional Review Board
MeOH:	Methanol
MS:	Mass Spectroscopy
MUFA:	Monounsaturated Fatty Acids
NIR/MIR:	Near Infrared or Mid Infrared Spectroscopy
NMR:	Nuclear Magnetic Resonance Spectroscopy
OO:	Olive Oil

ORAC:	Oxygen Radical Absorbance Capacity Assay
PC:	Phenolic Compounds
PUFA:	Polyunsaturated Fatty Acids
SFA:	Saturated Fatty Acids
SPSS:	Statistical Package for Social Science
TPC:	Total Phenolic Compounds

List of tables

List of figures

CHAPTER I

1. Introduction:

1.1 Composition of Olive Oil (OO)

Olive oil (OO) is a vegetable oil obtained from the fruit of the olive tree (*Olea europaea L.*) by mechanical means (crushing, mixing and centrifugation) under thermal conditions (Gouvinhas et al., 2014; Genovese et al., 2015). It is a premium oil which plays an important part of the Mediterranean cuisine due to its nutritional and sensory (color, taste, odor, aroma, etc.) characteristics (Baiano et al., 2009; Mitsopoulos et al., 2016). Spain, Italy, and Greece are considered the main producers of OO (Sevim et al., 2013).

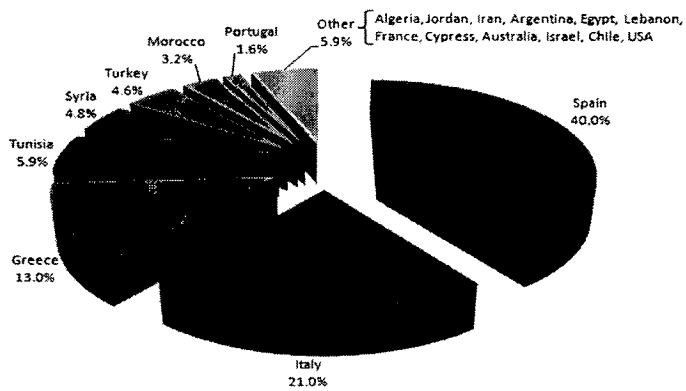

Figure 1.1 World Production of olive oil by countries (Vossen, P., 2013)

The high demand for OO is associated with the dietary habits linked to its composition, nutritional, and health benefits associated with high content of monounsaturated fatty acids (MUFA) (72%), specifically oleic acid (C18:1), moderate content of polyunsaturated fatty acids (PUFA) (14%), and its richness in natural antioxidants including sterols, polar phenolic compounds (PC), and other

non-polar PC, carotenoids and tocopherols (Boskou et al, 2006; Gouvinhas et al., 2016) in addition to its economic benefits.

1.2 Benefits of Olive Oil

1) Health Benefits

As per the international olive council (IOC), OO acts as 1) primary prevention of developing cardiovascular disease, due to the low level of saturated fatty acids (SFA), 2) protects the immune system against microbiological hazards and reduces the risk of infections, 3) enhances digestion and absorption thus helps to combat constipation (Aparicio-Ruiz and Harwood, 2013;Vossen, P., 2013); 4) has an impact on controlling blood sugar level and lowers the risk of type 2 diabetes; 5) favors absorption of some beneficial substances including omega-3 fatty acids in fish and lycopene in tomatoes and increase their bioavailability (Aparicio-Ruiz and Harwood, 2013); 6) acts as a good aid in treating skin disorders such as acne, eczemas etc. correlated with the presence of fat soluble vitamins (vitamins A, D, E and K) responsible for the protection against the free radicals (IOC); 7) helps to maintain the structure of the brain cells, due to the high level of MUFA; 8) exerts a positive influence on growth and bone mineralization during infancy (IOC) and 9) prevents the onset of gallstones by stimulating the digestion of lipids (IOC).

2) Economic Benefits

At international level, olive farming is a significant land use in the Mediterranean regions covering over 5 million ha in the EU member states. OO production showed a great increase in the last 50 years; from 1 million tons approximately in 1950s to around 1.5 million tons/ year (Aparicio-Ruiz and Harwood, 2013) especially in Spain, Italy, Greece and Portugal. It was being reported also that In 2017, olive oil production reached 23,000 tons growing by a CAGR of 4.66 % between 2011 and 2017.Similar movement is being seen in OO consumption especially in Syria, Turkey,

and Morocco accounting for over 20% of world production; in addition to USA which has become the leading world importer of OO. Exports from the Middle East are valued at almost $1 billion while EU exports of OO are worth about $2 billion based on IOC data. So, OO is an important contributor to economic development of agriculture because it has gained from media attention and this attention could be used to benefit exporters and importers from developing countries (Delisi et al., 2016).

Whereas, at national level, around 6% of the Lebanese territory is occupied by olive trees and olive groves are grown in six major regions extending from North to South, including Batroun, Koura, Zgharta, Akkar, Rashaya El Foukhar, and Hasbaya. Around 70% of the olive trees are designed for the production of OO which showed 6.5% growth since 2000 reaching 11 thousand tons. Additionally and over the past 4 years, Lebanese OO exports have increased by around 83.6% reaching 59 thousand tons in 2014. All this progress is due to Lebanon's diverse topography, fertile soil, moderate climates, and olive varieties allowing farmers to produce different unique types of OO (IDAL, 2013).

Looking forward to the future, OO production will still increase by 8% and 10% to meet and exceed consumer needs (Vossen, P., 2013).

1.3 Different Types of Olive Oil

IOC (2016) has classified OO into several types, including:

a) Extra virgin olive oil (EVOO) which has a free acidity, expressed as oleic acid, of not more than 0.8 grams per 100 grams. It is also characterized for its fruity aroma and gold color.

b) Virgin olive oil (VOO) which has a free acidic content, expressed as oleic acid, of not more than 2 grams per 100 grams and it is known for its sharper and good taste.

c) Ordinary VOO which has an acidic content expressed as oleic acid, of not more than 3.3 grams per 100 grams.

d) VOO not fit for consumption which is intended for refining and not suitable for use and has a free acidity, expressed as oleic acid, of more than 3.3 grams per 100 grams.

e) Refined OO is a processed oil which has a free acidity, expressed as oleic acid, of not more than 0.3 grams per 100 grams.

f) OO is the oil consisting of refined OO and VOOs which can be consumed as they are. It has a free acidity, expressed as oleic acid, of not more than 1 gram per 100 grams.

g) Olive pomace oil which is rarely seen in the market.

h) Crude olive pomace which is intended for refining for use for human consumption.

i) Refined olive pomace oil which is the oil obtained from crude olive pomace oil by refining methods. It has a free acidity, expressed as oleic acid, of not more than 0.3 grams per 100 grams.

1.4 Production of Olive Oil

Lebanon is a small country in the Middle East, and its olive trees are dating back to an old era. Since it has a moderate climate and great water supplies, this makes ideal conditions for olive trees growth and cultivation (Merchak et al, 2017). As per IOC, the high amount of the bitter component (Oleuropein) of the olive, make it a fruit that cannot be consumed directly from the tree and it has to undergo several mechanical processes including (Figure 1.2):

a) Harvesting.

b) Transportation and Storage.

c) Leaf Removal and Washing.

d) Crushing.

e) Mixing.

f) Pressing.

g) Separation/Centrifugation.

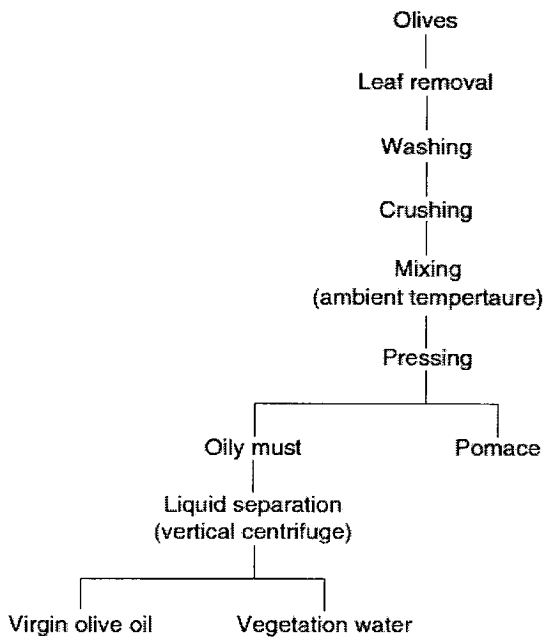

Figure 1.2 Flow process of olive oil production

a) Harvesting:

The best harvesting time for the production of oil is from October till December depending on the climate, weather and production area; and usually the harvesting methods are either by hand or shaking trees (Figure 1.8).

b) Transportation and Storage:

After olive fruits are picked in olive groves, and transported to oil mills in a bulk, using supporting packages. In order to avoid the risk of damaging the olives, the processing of oil in mills should be within 24 hours from harvesting. Otherwise olives should be stored under proper conditions by placing them in large containers with holes so that the air can circulate avoiding thick layers of drupes (Aparicio-Ruiz and Harwood, 2013) (Figure 1.3).

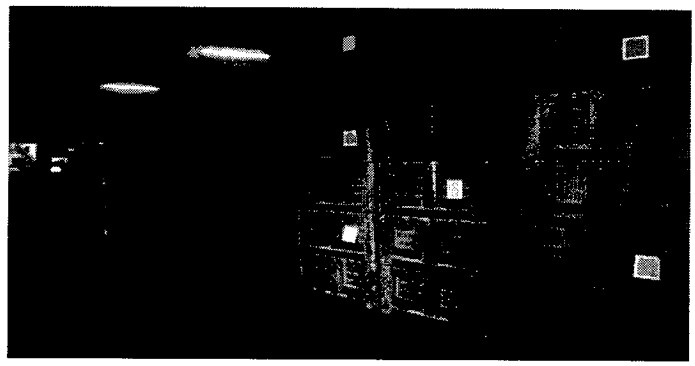

Figure 1.3 Storage of olive oil

c) Leaf removal and Washing:

Leaf removal and washing are necessary to remove leaves, and pollutants that could contaminate the oil and affect its quality. This process is done using 1) a vibrating screen and blower to remove the leaves (Vossen, P., 2013), 2) a washing machine with forced water circulation to wash the olives and 3) a metal detector to detect any physical hazard that could be harmful to the metal crusher (Figure 1.4).

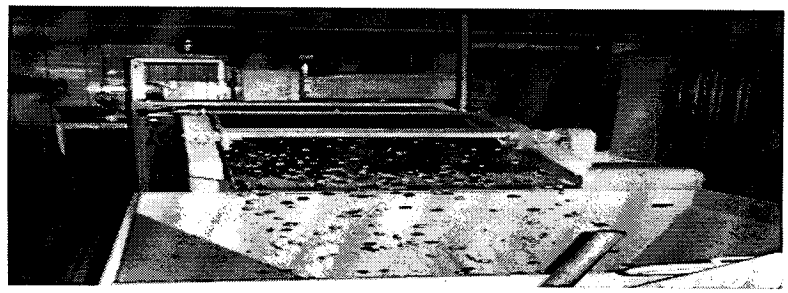

Figure 1.4 Washing the olives

d) Crushing:

At this point, crushing is continually carried out with two primary types of machines: either the stone mill or the hammer mill (Vossen, P., 2013) that crush the pulp and the olive seeds to ease the discharge of oil (Figure 1.5). The crushing method is a very gentle operation, producing a small increase in olive paste temperature (3-5°C); but avoids emulsions formation and increases the extraction yields. However, the millstones have a low load capacity making the process slow and discontinuous (Aparicio-Ruiz and Harwood, 2013).

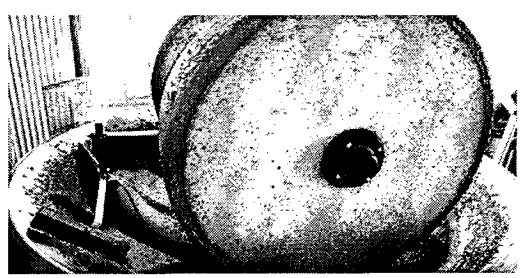

Figure 1.5 Millstone made of granite

e) Mixing:

The olive paste obtained after crushing needs to be mixed to reach maximum yield levels and for separation of the oil (Vossen, P., 2013). The mixing operation consists of 30 to 60 minutes of continuous thrilling of the paste. At this level, the olive paste is heated by hot water at a maximum temperature between 26.6 °C to 30 °C. It is important to mention that temperatures more than 30 °C can cause problems such as loss of fruit flavors, increases in bitterness, and increases in astringency (Vossen, P., 2013) (Figure1.6).

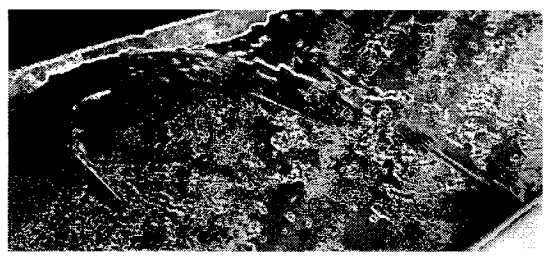

Figure 1.6 Mixing process

f) Pressing:

To extract oil from pressing method, the olive juice passes through filter mats that are piled to form a column, in which it will be placed in hydraulic pressure in order to extract the mixture of water (50%) and oil (20 to 25%) of the total olives. This method is being used to ensure that the olives are well ground, and allow the fruit enzymes to produce oil aromas and taste (Aparicio-Ruiz and Harwood, 2013).

g) Separation/Centrifugation:

The oily must extracted by pressing method is then collected in draining well and sent to a centrifuge (Figure 1.7). The centrifuge rotating at high speed features the differences between the weights of liquids and solids. In this way, OO and vegetation water are separated from the paste by continuous operation. The decanters used for this process have different load capacity of olive paste depending on their type and size (Figure 1.9).

A) When the three-phase system is being used, the olive paste is forced to the decanter by adding water to increase the liquidness of the mix. This paste undergoes centrifugation, in which solids are separated from oily liquids then; the liquid undergoes another centrifugation in which oil is separated from vegetable water.

B) However, in the two-phase system, the olive paste is being centrifuged without the addition of water in order to separate oil from pomace without the yield of vegetation water (figure 1.7).

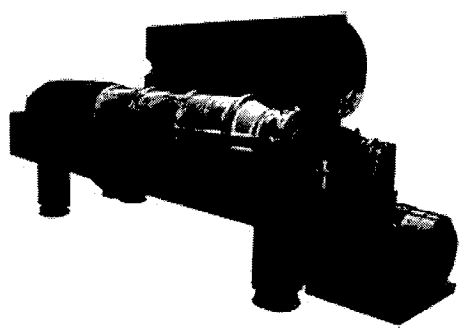

Figure 1.7 Two phase decanter centrifuge

h) Storage:

After processing, OO should be stored in bulk and in the dark for 1 to 3 months to resolve any remaining particulate and fruit water. Premium-quality oils should be stored in stainless steel and maintained at a constant temperature of between 7.2 °C to 18.3 °C (Vossen, P., 2013).

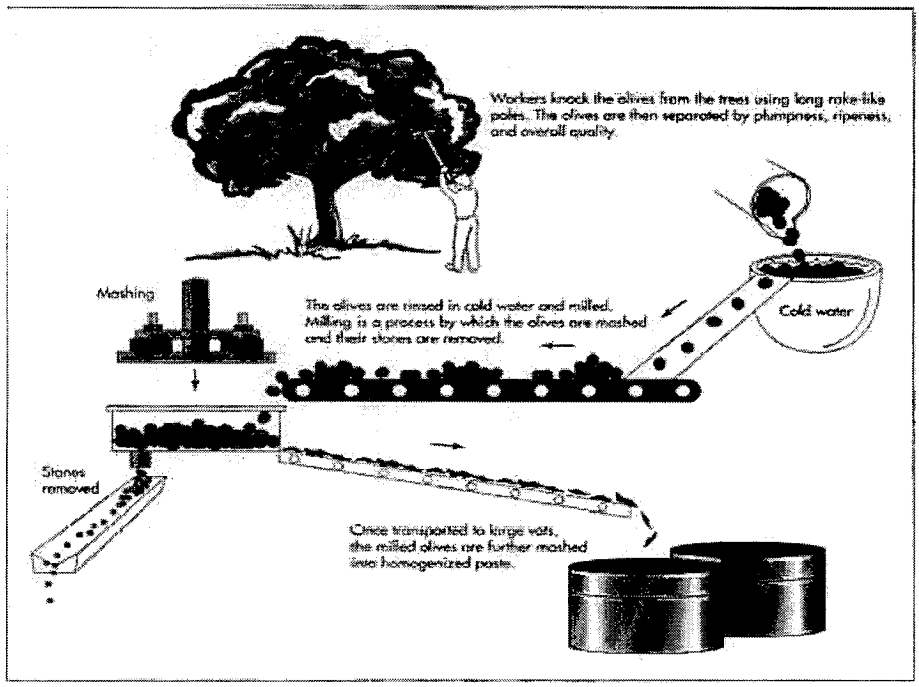

Figure 1.8 First steps of olive oil production in mills

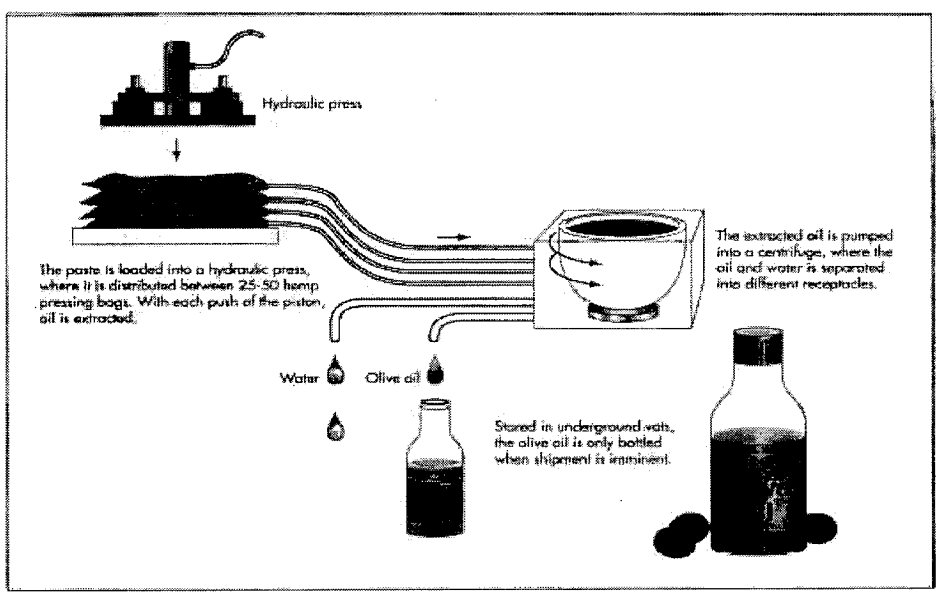

Figure 1.9 Second steps of olive oil production in mills

1.5 Comparison between Traditional and Modern Mills:

Broadly speaking, there are two methods for OO production, including 1) the traditional technique and 2) the modern technique (a.k.a continuous). Many differences can be detected between both mills.

1) In the traditional method, the olives are crushed using a millstone made of granite that helps in the mixing of olive paste and transforming the oil droplets into large ones. It also helps in reducing added water (easier to manage), and provides better grinding of the olives. However, it has many disadvantages because it is time consuming, requires more labor (Vossen, P., 2013), slow, non-continuous, difficult to clean, the filter mats become easily contaminated (Vossen, P., 2013) and expensive (Aparicio-Ruiz and Harwood, 2013). Also, the mixing method in the traditional technique needs 20 to 30 minutes however; it is automatic in the modern mixers. In the past, old mixers were not covered and placed in series, while the new mixers are placed separately and are closed for safety practices and HACCP (Hazard Analysis Critical Control Point) (Aparicio-Ruiz and Harwood, 2013).
2) Whereas, the modern centrifugal method showed many benefits, it is small compared to old ones thus does not require too much space. The modern method uses industrial decanter to separate all the phases, specifically the two phase decanter that works in an automated way, thus decreasing the cost, water consumption and waste water. Aparicio-Ruiz and Harwood (2013) reported that "the chances of obtaining an oil of good quality have been improved by the introduction of the two-phase centrifugal decanter". This produces more stable oil that has a higher content of TPC and nutritional properties. It is bitterer, pungent, and more stable during storage and produces almost no waste water but it requires more energy and technical labor (Vossen, P., 2013). However,

the three phase decanter is more expensive, and emits greater amount of vegetable water, making the oil less stable and with lower phenolic content (Vossen, P., 2013). Also, three-phase system processors use two centrifuges: one for the ''wet'' oil from the decanter and a second one to separate the oil from the fruit– water of the decanter (Vossen, P., 2013). Additionally, the press method can also be used as another modern method to extract oil from the olives. It is an automated method that requires less energy, less cost to establish but relatively more labor (Table 1.1) (Aparicio-Ruiz and Harwood, 2013).

Nowadays, the high demand for OO makes it difficult, to produce all oil using the traditional methods because these methods can't tolerate the production of large quantities. The advocates of modern machines methods highlights that the centrifugation is quicker, faster and more hygienic. In addition, OO produced with traditional methods has a fruity flavor but bitter at the same time. However, the oil produced with modern method is less fruity and high in PC (Aparicio-Ruiz and Harwood, 2013). Modern methods use different substances in various steps of the production of oil in order to control production "from farm-to-fork" thus to create a standard taste and aroma. In addition to the temperature which can influence the quality of OO; in modern system, olive paste temperature never goes above 27°C during all the oil process (Aparicio-Ruiz, and Harwood, 2013) (Table 1.1)

Table 1.1 Comparison between traditional and modern mills

System	Advantages	Disadvantages
Traditional	Lower water consumption Pomace with low water content	Time consuming Difficult to clean Non continuous More labor
2-phase system	Higher PC level More stable oil Lower water and energy consumption Continuous	Very wet pomace Lower working capacity Bitter and pungent oil

	No production of vegetation water	
3-phase system	High working capacity Dry pomace Continuous	Lower PC level Less stable oil Higher water and energy consumption Production of vegetable water
Press	Dry pomace Lower energy consumption Less cost	Lower working capacity and more labor More paste and oil contact with oxygen Difficult to clean

2. Phenolic Compounds of Olive Oil

OO has been used throughout ages in many fields from food flavoring to pharmaceutical and cosmetics due to their PC and their antioxidant properties that show a wide range of health benefits and great importance in biological system (Baiano et al., 2009).

2.1 Benefits and Types:

a) Benefits:

PC are one of the most widely occurring phytochemicals and major bioactive, responsible for the stability and organoleptic properties of OO (Favati et al., 1994). They are group of secondary metabolites which structurally comprise an aromatic ring and hydroxyl groups (Balasundram et al., 2005). These PC play an important role in 1) providing protection against pathogens, 2) improving the physiological properties including antioxidant, anti-microbial, and anti-allergenic effects (Balasundram et al., 2005; Baiano et al.,2009); and 3) providing stability to oxidation due to their ability of donating hydrogen atoms (Zamora, 2016). The beneficial effects derived from the PC have been correlated with their antioxidant activity (Table 1.2).

Table 1.2 The correlation between the total phenolic content and antioxidant activity

Author	Title	Objective	Study approach	Samples	Results
Mitsopoulos et al., 2016	Total Phenol Content and Antioxidant Activity of Leaves and Drupes in Major Greek Olive Varieties	Comparison between the major Greek olive varieties in regards to their phenol content and total antioxidant activity	Experimental Design	The Greek olive varieties	A positive correlation between total phenol content and antioxidant activity.
Gorinstein et al., 2003	The contents of the main biochemical compounds and the antioxidant activity of some Spanish olive oils	Comparison of the contents of the main biochemical compounds and the antioxidant capacity of five Spanish olive oils by four different antioxidant tests	Experimental Design	The Spanish olive oils	The high TPC increases antioxidant activity. A linear correlation between PC and antioxidant activity

b) Types:

PC can be categorized into two classes:

A) The non-polar PC such as Tocopherols, Sterols, and Triterpene compounds (Zamora, 2016).

B) The polar PC classified as 1) phenolic acids that act as growth regulators and antioxidants (Zamora, 2016) such as Hydroxybenzoic acids (Gallic acid, Vanillic, Syringic acids etc.)

and Hydroxycinnamic acids (Cinamic, and Coumaric, etc.) 2) the phenolic alcohols including Hydroxyphenyls that play a role in fighting inflammation and 3) the Flavonoids in which Flavones and Flavonols are most widely occurring and have antimicrobial, antioxidant and organoleptic properties (Balasundram et al., 2005).

2.2 Factors Affecting the Phenolic Content and Antioxidant Capacity of Olive Oil

The antioxidant capacity and phenolic content in OO depend on several factors, including:

1) The nature of the cultivar (genetics):

a) El Riachy et al, 2012 reported a strong positive correlation between the different olive cultivars in Spain (Arbequina, Arbosana, and Sikita) and their genetic factors associated with phenolic composition variability. Significant difference among crosses was shown for TPC ($p<0.05$) ranging between 78 $g.kg^{-1}$ and 1008 $g.kg^{-1}$ in Arbequina and Arbosana and between 60 $g.kg^{-1}$ and 816 $g.kg^{-1}$ in Sikitia and Arbosana.

b) Baiano et al., 2013 also showed the positive effect of cultivar types including Coratina, Nocellara, Ogliarola, and Peranzana in four locations of Apulya region in Italy on OO phenolic content. Results showed that the highest and the lowest TPC was detected in Peranzana from location C and in Nocellara; whereas the TPC of Peranzana differ in each of the three locations.

2) The geographical conditions:

The phenolic content in OO varies with temperature, altitude, cultivation zone, precipitation and nature of the soil. The warmer the temperature and the higher the altitude of the orchard, the less

the TPC level in oil exists (Aparicio-Ruiz, & Harwood, 2013). Kiritsakis (1998) agreed on this point by reporting that the lower the altitude, the higher the TPC level. This is mainly due to the fact that lower altitudes have more suitable temperature and sun exposure compared to higher ones. Additionally, fruits from the same cultivar when cultivated from different regions have different TPC composition. It was mentioned in a study conducted in Portugal that TPC in oil varied from 6.1 $g.kg^{-1}$ in olive pulp when produced in the North to 17.5 $g.kg^{-1}$ in the central region (Zamora, 2016).Talking about soil, it showed no clear effect on OO composition but studies highlight that when nitrogen level increases in soil, this will result by lowering the oil stability and TPC in OO (Aparicio-Ruiz and Harwood, 2013). Another study conducted by Sevim et al., (2013) concluded that the OO from early harvest fruits of 2008 crop had better antioxidant capacity compared to the 2009 crop. It also reported that the difference between the two crops may be due to the dry conditions of 2008 year.

3) The maturity stage of olives:

The effect of fruit maturity stage on TPC is very significant. As fruits ripen, the PC decreases:

a) Kiritsakis (1998) talked about the relationship between the maturity of the fruit and the flavor components of the oil, but this study showed no correlation between fruit pigmentation and the PC in OO.
b) However, Jimenez et al., (2014) reported that the early ripening stage improves the sensory characteristics of OO.
c) In Spain, the level of Oleuropein content varies due to the difference in the nature of the following cultivars Arbequina, Cornicabra, Morisca, Picolimón, Picudo and Picual (Gómez-Rico et al., 2008). This difference was shown to be much higher for the Cornicabra and Picual

cultivars and accounted for about 95% of the TPC at the unripe stage and about 50–60% at overripe stages. The study showed that the levels of Oleuropein in the Arbequina cultivar decreased from 2230 mg/kg to 60 mg/kg **(3% of its initial content)** during fruit ripening and from 11600 mg/kg to 6340 mg/kg for the Cornicabra variety **(55% of initial content)** due to its extensive degradation. However, the Oleuropein content increased in the spotted fruits in the Picual and Picolimón cultivars and then decreased significantly in black olives due to the turnover of the phenolic moieties into new conjugates. In the case of the Morisca variety, the levels of Oleuropein increase were not statistically significant.

4) Processing Techniques:

a) Extraction: Samples from Greece, Italy and Iran were studied for their PC and were divided into 3 and 4 groups according to the methods of extraction. Difference in PC level was found between 2 samples from Greece and Italy only as a modern extraction method was being used (Zadeh et al., 2008).

b) Cooling: It was reported by Veneziani et al., (2017) that rapid cooling of OO at 15°C showed a negative correlation with OO PC due to the thermal inhibition of the main enzymes responsible for a process of degradation.

c) Heating: Goulas et al., 2015 showed that heating using microwave can retain the antioxidant properties of OO whereas frying up to 75% causes degradation of PC. This study highlighted that baking was most suitable thermal processing technique. A positive correlation has been also shown between processing temperature and PC level ($p=0.001$) (Aguilera et al., 2015). This may be explained because the partition depends on temperature only.

d) Mixing: Aguilera et al., 2015 also reported that the PC level was more significant when the mixing temperature was between 25 and 30°C however no significant increase in PC concentration was being shown when temperature was between 30 and 35°C.

5) Pressing technique:

A significant difference in PC of OO obtained from different pressing systems was shown. OO from traditional mills show lower PC content compared to other mill types. Also, OO obtained from the three-phase system, have a lower content of PC because additional water was provided to dilute the olive paste. The best olive oil quality is obtained from the two-phase system, as water consumption and wastewater production decrease, thus the PC level increases (Khdair et al., 2015).

6) The storage and packaging of OO:

a) Dabbou et al., (2011) showed a positive correlation between the PC ($p<0.05$) and packaging material mentioning that glass bottles provide better protection from oxidation for OO than do polyethylene plastic bottles. Also, the study indicated that the exposure of OO samples to light and high temperatures caused deterioration in OO quality.

b) Also, Gargouri et al., (2015) highlighted that tin containers and dark glass bottles showed the greatest stability against oxidation as OO can be stored up to 6 months at ambient conditions without any changes in its quality.

7) Irrigation:

Water level has a major negative effect on TPC level. Studies indicate that 20% loss of PC was observed in olive that receives good amount of water (Zamora, 2016). So, usually the lower the water content, the higher the tree growth is seen (Aparicio-Ruiz, & Harwood, 2013).

a) Aguilera et al., 2015 reported that the concentration of PC in oil is being decreased during processing of olive fruits with higher water amount.

b) In California, an experiment was done showing the positive correlation between irrigation level, TPC and oxidative stability. This study showed three to four times more or less TPC in a single cultivar (Arbequina) based on the amount of water the trees received. The heavily irrigated trees produced oils that were less fruity (Aparicio-Ruiz, & Harwood, 2013).

c) In Italy, low PC level was noticed as the trees were given more water (Servili et al., 2007). Because all these agro-industrial factors affect the TPC and antioxidant capacity of OO, it is highly important to control these variables in order to increase the quality, stability and sensory characteristics of OO as well as extend its shelf life. For this reason, OO should be produced in a safe environment.

2.3 Extraction of Phenolic compounds from Extra Virgin Olive Oil (EVOO)

Among the various components of OO, PC are of major importance, thus, many analytical methods have been suggested for their extraction including a) Solid Phase Extraction, and b) Liquid-Liquid Extraction c) Ultra High Performance Liquid Chromatography coupled with Mass Spectroscopy (HPLC-MS) (Alarcón Flores et al., 2012) d) Deep Eutectic Solvents (DES) (Garcia et al., 2016) e) Ultrasonic techniques (Rashed et al., 2016) etc; however, the commonly used extraction method for TPC is the Liquid-Liquid extraction because it is a quick (Favati et al.,1994), inexpensive, readily available and easy to operate technique (Heffernan et al., 2014).

The quantification of PC is carried out by using several methods including: a) Capillary electrophoresis (CE) b) Colorimetric Folin-Ciocalteu method (Favati et al., (1994) and Zamora, (2016)) c) High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) d) Near Infrared (NIR) or Mid Infrared (MIR) spectroscopy e) Nuclear Magnetic Resonnance (NMR) spectroscopy f) Transform Infrared Spectroscopy (FTIR) equipped with Attenuated Total

Reflectance (ATR) etc.; However, Folin-Ciocalteu method (Favati et al., (1994) and Zamora, (2016)) is the best method used for quantification.

Folin-Ciocalteu is invented by Otto Folin, Vintilă Ciocalteu , and Willey Glover Denis. It is used as a reagent in chromatographic and spectrophotometric procedures. The method is based on the reduction of the complex by PC to form a blue color reaction. The Folin-Ciocalteu assay is being chosen over other techniques because it is rapid, easy to perform, cheap, and requires no sample preparation. It only needs UV-VIS spectrophotometry for the measurement of the absorbance which is available in all laboratories.

2.4 Determination of Antioxidant Capacity (DPPH)

Antioxidants are important because they prevent lipid oxidation and protect the body against free radicals that are elaborated in the development of chronic diseases including cancer, cardiovascular diseases and inflammation (Locatelli et al., 2008). Several chemical models have been developed to assess the ability to prevent oxidative changes including a) ABTS assay 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) b) Beta-carotene bleaching assay, c) The Radical Scavenging Assay using (2,2-diphenylpicrylhydrazyl) (DPPH) d) Oxygen Radical Absorbance Capacity (ORAC) assay and e) the Ferric Reducing Ability of Plasma (FRAP) assay (Thaipong et al., 2006). DPPH method is one of the most widely employed methods that were developed by Blois in 1958 in order to estimate the antioxidant activity by using a stable free radical (DPPH). The principle is based on the disappearance of purple color of the stable free radical solution after mixing DPPH solution with a polar PC. This is done through scavenging reactions with antioxidants, measured at a specific absorbance using Analytic Jena Specord 250 plus Spectrophotometer double beam along with the software Winaspect Plus version 4.2 (Del Monaco

et al., 2015). The percentage of discoloration will be used as the measure of the antioxidant activity that will be expressed as the IC_{50} (Inhibition concentration, ppm) which explain the amount of sample required to reduce the blank DPPH by 50% (Mitsopoulos et al., 2016).

DPPH inhibition % will be calculated using this formula:

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

Ac: is the absorbance of the DPPH solution in methanol.

As: is the absorbance of the sample.

DPPH technique is rapid, easy to perform, simple and inexpensive as it only needs a spectrophotometer to perform.

3. Rationale/Purpose of the study

"Olive trees occupy an area of 563 km^2 in Lebanon which represents 5.4% of the country's territory (IDAL, 2013). The oil productivity ranges between 18-25%. The main cultivars produced are Aayrouni, Abou Chawkeh, Baladi, Del, Jlot, and Soury and there are approximately 500 oil-processing mills" (Aparicio-Ruiz and Harwood, 2013) (Figure 1.10). Since the olive production in Lebanon is spreadable and to our knowledge no previous studies were performed about the different factors that affect the quantity, the antioxidant capacity and the TPC of OO, this study will be the first to highlight the effects of the following major environmental and agro-industrial factors:

1) Geographical conditions
2) Maturity stage of olives
3) Pressing technique

of North and South areas during early, intermediate and late harvesting at 3 different kinds of mills including Press, two-phase and three-phase systems; as well as to study the correlation between the antioxidant capacity and the phenolic content of OO.

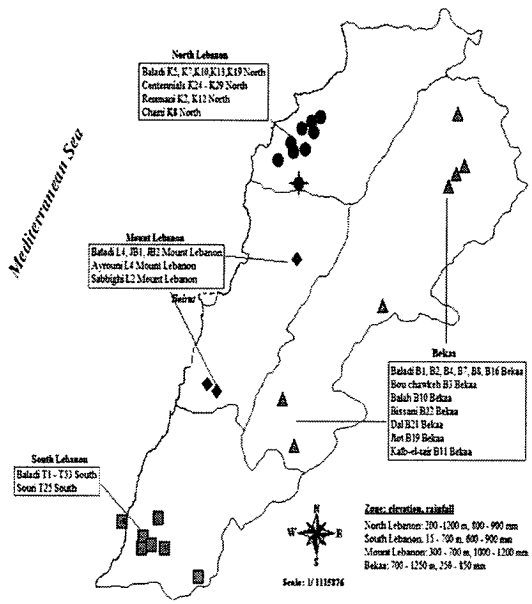

Figure 1.10 Lebanese cultivars

Chapter II

1. Introduction

Olive Oil (OO) is the oldest crop in history and it is one of the most expensive edible oils in the Mediterranean countries (Aparicio-Ruiz and Harwood, 2013). OO production using mechanical processes (including harvesting, transportation and storage, leaf removal and washing, crushing, mixing, pressing, and centrifugation) showed a great increase in the last 50 years especially in Spain, Italy, Greece and Portugal (Aparicio-Ruiz and Harwood, 2013). OO plays an important role in the human diet and its consumption is associated with the prevention of cardiovascular diseases; protection of the immune system and reduction of the risk of infections; control blood sugar level and lowering the risk of type 2 diabetes among others (Aparicio-Ruiz and Harwood, 2013). Those health benefits are highly associated with the OO chemical composition of monounsaturated fatty acids (MUFA), specifically oleic acid (C18:1), polyunsaturated fatty acids (PUFA), and natural antioxidants including sterols, polar phenolic compounds (PC), and other non-polar PC, carotenoids and tocopherols (Boskou et al, 2006; Gouvinhas et al., 2016). Studies highlight the importance of polar PC (such as phenolic acids, phenolic alcohols and flavonoids) as they are the major bioactive which contribute to the beneficial sensory, organoleptic and biological properties of OO, in addition to their antioxidant capacity and their ability to provide oil stability to oxidation (Favati et al., 1994). Also, Phenols are important compounds because of their contribution to human health such as anti-mutagenic and/or anti-carcinogenic activities, anti-viral and anti-inflammatory action (Karakaya, 2014). Moreover, several epidemiological studies have shown other beneficial effects of phenols including the protective effect against insulin sensitivity and diabetes, Parkinson, Alzheimer, and liver diseases (El Riachy et al., 2013). The concentration of

phenols in OO is strongly affected by several agro-industrial factors such as the nature of the cultivar and the harvesting time (El Riachy et al., 2013), the geographical conditions, the maturity stage of olives, the processing techniques, the pressing techniques, the storage and packaging of OO, as well as the irrigation (Gomez et al., 2008; Servili et al., 2013).

At Lebanese level, and over the past 4 years, OO exports have increased by around 83.6%. All this progress is due to Lebanon's diverse topography, fertile soil, moderate climates, and olive varieties allowing farmers to produce different unique types of OO (IDAL, 2013). However, and to my knowledge there is no Lebanese studies that are performed about the different factors that affect the quantity, the antioxidant capacity and the PC of OO. New study conducted by El Riachy et al., (2013) about the variability of phenolic compounds according to the nature of cultivars and ripening showed a decrease in phenolic content along ripening due to hydrolysis. Another study conducted by Serhan et al, (2016) showed a strong positive correlation between the total phenolic and the olive growing area in Baladi cultivars. In addition this study also exposed the negative correlation between the high altitude level and TPC as well as the extraction systems. However, there is a lack of studies on Lebanese OO TPC and antioxidant capacity; thus the purpose of this research is to identify the effect of agro-industrial factors on the PC content and antioxidant capacity.

2. Materials and Methods

2.1 Materials

The following materials were used for conducting the experiment: test tubes, volumetric flask, glass bottles, beaker, pipettes, pipettes tips, pipette Pasteur sterile, glass cuvettes, Eppendorf,

analytical balance (Mettler Toledo, Switzerland), centrifuge (Thermo Electron Corporation, USA), rotary shaker (Mini Vortex, China), UV-VIS spectrophotometer (Analytic Jena Specord 250 Plus, Germany).

The following chemicals were used for the analysis: hexane, methanol (Sigma-Aldrich, 32213-2.5 L, EC France), Folin-Ciocalteu reagent, powdered 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), Gallic acid (97.5-102.5% (titration) Sigma, G7384-100G) and sodium carbonate (Na_2CO_3) (Sigma-Aldrich, 13418-1Kg-R, Germany). Deionized water was prepared using Labconco carbon water Pro-Ps (Kansas USA).

2.2 Samples Collection

Fifty four (n=54) OO samples from 2016 crop originating from North and South regions from Lebanon were stored in dark containers and transported to the laboratory. These samples were from 3 processing systems including press, 2-phase and 3-phase systems and collected in triplicate at 3 harvesting time including early, intermediate, and late harvesting by 3 different farmers at each harvesting. All samples were collected and processed in duplicate. Samples were stored in the dark at a temperature less than -18°C.

2.3 Extraction of the Phenolic Fraction

The PC were isolated using Liquid-Liquid extraction with a 60:40 v/v methanol-water mixture. OO (3 g) were weighed in a test tube using the analytical balance followed by the addition of 2 mL of hexane then agitated for 15 seconds using the rotary shaker (Mini Vortex). Later, 1.75 mL of $MeOH/H_2O$ (60:40, v/v) were added to the mixture and shaken for 2 minutes using also the

rotary shaker (Mini Vortex). Directly, the first lower phase containing the polar PC was separated from the oily phase after placing the samples in the centrifuge for 2 minutes using the Thermo Electro Corporation at 800 rpm. The remaining mixture experienced a second extraction by adding again 2 mL of MeOH/H_2O (60:40, v/v) and shaken for 2 minutes then placing it in the centrifuge for an additional 2 minutes. Finally, the 2 extracts were mixed and stored in the freezer at a temperature of -18°C until further analyses (El Riachy et al. 2012).

2.4 Folin-Ciocalteu Assay of Phenolic Compounds

TPC were quantified according to the Folin-Ciocalteu colorimetric method and Gallic acid was used for the preparation of the standard curve.

First for the samples, Na_2CO_3 solution was prepared by diluting 20 g of Na_2CO_3 with 80 mL of water until dissolution. Also, Gallic acid solution was prepared by diluting 100 mg of Gallic acid with 100 mL of distilled water. A set of standard solution of Gallic acid were obtained including a blank 25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 µg/L Gallic acid solutions respectively. Finally for the phenolic standard preparation, 100 µL of the phenolic oil purified extract, 675 µL distilled water, 25 µL of Folin-Ciocalteu reagent were mixed with 200 µL of Na_2CO_3. The mixture then was shaken properly and placed in the dark for 90 minutes. After 90 minutes, all samples were removed and placed in cuvettes glass in order to read the absorbance at 765 nm on the UV-VIS. The method was based on the reduction of the complex by PC to form a blue color reaction. The absorbance of the standard solutions and samples were measured at 765 nm in relation to a Gallic acid standard curve using spectrophotometer double beam (Analytic Jena Specord 250 Plus) and the computer software. All measurements were performed in duplicate. According to the Beer-Lambert Law, the absorbance (the intensity of the color) was directly

proportional to the Gallic acid concentration. The results were expressed as milligrams of Gallic acid equivalents per gram of sample (mg GAE/ kg of oil).

2.5 DPPH Assay of Antioxidant Activity Determination

The antioxidant capacity was estimated using DPPH assay following the procedure described by Mitsopoulos et al., (2016). DPPH stock solution was prepared by solubilizing 0.0099 g in 100 mL MeOH/H_2O (60:40, v/v). The solutions were prepared by adding 10 µL of the extract to 490 µL and 500 µL of DPPH solution. Then, the mixture was shaken and placed at room temperature in the dark for 15 minutes. The absorbance was measured at 515 nm using Analytic Jena Specord 250 plus Spectrophotometer double beam (Germany) using the software Winaspect Plus version 4.2. Duplicate trials are tested as follow: 20 µL of the extract was added to 480 µL of methanol and 500 µL of DPPH. Methanol solution was incubated for 15 minutes and read at 515 nm. The percentage of discoloration after 15 minutes was used as the measure of the antioxidant capacity that was expressed as the IC_{50} (Inhibition concentration, ppm) which explain the amount of sample required to reduce the blank DPPH by 50%.

DPPH inhibition % was calculated using the following formula:

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

(Where, Ac: is the absorbance of the DPPH solution in methanol and As: is the absorbance of the sample).

2.6 Statistical Analysis of Data

The data collected were analyzed and used to calculate the concentration of the antioxidant capacity and TPC content of OO. The differences between means were assessed using Multivariate

Analysis of Variances (MANOVA) and Three-way Analysis of Variance (ANOVA). The correlation between the antioxidant capacity and the TPC was analyzed using the Spearman's rank-order and logistic regression. The effect of OO categories on TPC and antioxidant capacity was assessed using Independent samples *t*-test and Mann Whitney U-test.

All statistical analysis were performed using the SPSS for windows v.22 software (SPSS Inc., IBM Corporation) and statistical significance level was set at $p < 0.05$.

3. Results and Discussions:

3.1 Total Phenolic Content and Antioxidant Capacity of Lebanese OO

Table 2.1 lists the results of the amount of the TPC and Antioxidant Capacity of Lebanese OO according to their region (North and South), processing techniques (2-phases, 3-phases and Press) and harvesting stages (early, intermediate and late harvesting). The level of TPC ranged between 78.92 and 202.97 mg GAE.kg^{-1} of oil. The mean TPC between samples was 138.8 ± 13.22 mg GAE.kg^{-1} of oil. These results can be compared with other results showed on the literature for Lebanese and non-Lebanese OO. Starting with the local studies, a study conducted by El Riachy et al., (2012) showed a high variability of phenolic compounds according to the nature of cultivars ranging between 7.33 mg GAE.kg^{-1} and 77.8 mg GAE.kg^{-1} for 1.5 kg of olive fruits picked from 3 different cultivars including Arbequina, Arbosana, and Sikitita. Also Serhan et al., (2016) reported that TPC level varied between 76 mg GAE.kg^{-1} and 358 mg GAE.kg^{-1} among 25 OO samples from Baladi cultivars. Merchak et al., (2015) also reported a huge variability of TPC level among Lebanese OO (n=234) samples collected from different regions during the 2012 harvest. It was found that TPC level ranged between 6.74 and 6.764 ppm with a mean of 0.12 ppm. Also total phenols, determined by the F-C method, showed high variability between genotypes from 494 to 131 mg GAE kg^{-1} of oil (EL Riachy et al., (2012). Asli et al., (2011) reported that the concentration

of TPC, evaluated calorimetrically, ranged between 374.00–855.36 mg/kg and 641.79–810.98 mg/kg for two Turkish varieties (Memeck and Edremit) during maturation. Another study done by Talhaoui et al., (2016) on the phenolic composition in six major cultivars grown in the same orchard under the same agronomical and environmental conditions reported the mean of TPC that ranged between 35.92 and 2699.89 mg GAE.kg^{-1} oil. The profile of the phenolic content was also reported by Galvano et al., (2007) and showed that the content of total phenols ranged from 148 mg GAE.kg^{-1} to 1212 mg GAE.kg^{-1}, with a mean value of 537.2 mg GAE.kg^{-1} for olives harvested in the period of September-December 2005 in the production area of the province of Siracusa (Sicily, Italy). Furthermore, De Fernandez et al., (2014) reported that the concentrations of phenols, expressed as Caffeic acid, in the studied OO in Argentina ranged from 171 mg CAE. kg^{-1} and 514 mg CAE. kg^{-1}. So, the phenolic contents of these monovarietal OO showed significant differences between different varieties ($p < 0.05$). Also, Kalogeropoulos et al., (2014) reported that the TPC level showed great variability, reported to range from 50 mg CAE. kg^{-1} to 1000 mg CAE. kg^{-1} in VOO from Greece, Israel, Italy, Spain, and Turkey, with usual values between 100 mg CAE. kg^{-1} and 300 mg CAE. kg^{-1}. So, the amount of polyphenols in OO vary, depending on several factors such as geographical conditions, climatic conditions, maturation stage and processing techniques (Serhan et al.,2016). Additionally, the phenolic fraction of olive oil can greatly vary among cultivars. Nakbi et al., (2010) reported that cultivar used to produce oil is an important factor in determining its content in phenolic compounds. Kalogeropoulos et al., (2014) confirmed in his study that the cultivar, climate and other environmental factors, such as harvesting time, the extraction process, and the conditions of packing, distribution, and storage are critical factors affecting the final phenolic content of OO.

For the antioxidant capacity, our results showed that the level varied between 0.28 % and 27.14% with a mean value of 10.4 ± 4.28%. These results also can be compared with other results showed on the literature including a study by Laincer et al., (2014) which reported significant differences ($p < 0.05$) in the antioxidant capacity among the varieties founded. The concentration of the antioxidant capacity for the different Algerian OO extracts ranged between 36.57 ± 1.71 % and 72.20 ± 2.19 %. The extracts of Limli, Akerma and Chemlal Tazmalt oils recorded the lowest percentages (39.96, 38.20 and 36.57% respectively). These values were similar to those reported by Nakbi et al. (2010) for Tunisian cultivars in which the antioxidant capacity values were 78.56% and 37.23% for Chetoui and Chemlali varieties, respectively. Another study conducted by Xiang et al., (2014) showed that the results of the antioxidant capacity of four oils including Barnea, Coratina, Koreniki, and Manzallini were 49.66, 21.33, 20.00 and 25.33 mg/ml respectively. Also, findings obtained in a study conducted by De Fernandez et al., (2014) demonstrated that Arauco olive oil, autochthonous for Argentina, possesses the highest antioxidant scavenging properties, which are very likely due to the presence of high contents of phenolic compounds.

The antioxidant capacity of the extracts reported by several studies might depend on the composition and profile of PC. Their antioxidant properties can be related to hydrogen donation and their ability to improve radical stability by forming an intra-molecular hydrogen bond between the free hydrogen of their phenoxyl radicals or the different radical scavenging techniques used to detect the antioxidant capacity (Xiang 2014). Moreover, the different oxidative stability can also be related to the fatty acid composition and the effect of various antioxidant compounds (Nakbi et al.,(2010)).

Table 2.1 Characterization of OO samples regarding their TPC content (mg GAE.kg^{-1} of oil) and DPPH$^{\bullet}$ inhibition (%).

Region	Processing Technique	Harvesting Stage[1]	TPC[2] ± SD[3](mg GAE.kg^{-1} of oil)	DPPH$^{\bullet}$[4] Inhibition ± SD (%)
North	2-phases decanter	Early	110.185 ± 8.4	10.22 ± 5.57
		Intermediate	202.97 ± 23.72	27.14 ± 23.3
		Late	118.55 ± 12.67	7.43 ± 2.36
	3-phases decanter	Early	127.9 ± 19.52	16.70 ± 5.96
		Intermediate	196.47 ± 10.15	10.92 ± 0.89
		Late	137.18 ± 13.88	6.81 ± 2.54
	Press	Early	140.81 ± 8.78	12.68 ± 1.41
		Intermediate	146.26 ± 18.87	5.26 ± 4.62
		Late	180.22 ± 13.96	0.28 ± 0.32
South	2-phases decanter	Early	189.55 ± 21.11	18.28 ± 6.76
		Intermediate	87.54 ± 13.88	4.87 ± 0.73
		Late	147.32 ± 10.02	13.04 ± 5.20
	3-phases decanter	Early	172.41 ± 12.16	19.29 ± 4.47
		Intermediate	147.67 ± 2.50	14.65 ± 1.54
		Late	106.47 ± 7.67	3.89 ± 4.54
	Press	Early	84.91 ± 11.18	6.20 ± 1.11
		Intermediate	122.88 ± 24.17	7.26 ± 4.25
		Late	78.92 ±5.32	2.28 ± 1.53
			138.8 ± 13.22	**10.4 ± 4.28**

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

[2] TPC: stands for Total Phenolic Content which is quantified based on mg of gallic acid equivalence (GAE) per kg of oil.

[3]SD: stands for standard deviation.

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

3.2 Factors affecting the Total Phenolic Content and Antioxidant Capacity of Lebanese OO

In order to check the reason behind the different level of TPC and the antioxidant capacity of our OO samples, the geographical origin, the harvesting time and the processing time factors were studied as a way of checking their possible significance on the TPC and antioxidant capacity averages. A Multivariate Analysis of Variances (MANOVA) was conducted in order to compare multiple dependent variables between independent groups after checking all the required assumptions.

Table 2.2 The effect of geographical origin, the processing time and harvesting time on the DPPH averages

Factors	Statistical Test	df	P value
Regions	MANOVA	(2 ; 27)	2.093
Processing	MANOVA	(4 ; 54)	1.019
Harvesting	MANOVA	(4 ; 54)	1.503

The results shown on Table 2.2 indicate no significant effect of the geographical origin, the harvesting time and the processing system on the OO antioxidant capacity ($p>0.05$). Note that the three-way interaction (geographical origin*harvesting time*processing system) as well as the two-way interactions (geographical origin*harvesting time, geographical origin*processing system and harvesting time*processing system) didn't show also a significant effect on the OO antioxidant capacity with respectively$(F(8;54) = 1.257; p > 0.05)$,$(F(4;54) = 1.700; p > 0.05)$,$(F(4;54) = 0.533; p > 0.05)$ and $(F(8;54) = 0.307; p > 0.05)$

Table 2.3 The effect of geographical origin, the processing time and harvesting time on the TPC averages

Factors	Statistical Test	df value	P value
Regions	Three- way ANOVA	(1 ; 28)	3.620
Processing	Three- way ANOVA	(4 ; 28)	0.417
Harvesting	Three- way ANOVA	(2; 28)	0.712

Furthermore, table 2.3 lists the results of the effect of the three studied factors (geographical origin, harvesting time and processing system) on the TPC average using three-way ANOVA. The three-way ANOVA was used in order to determine if there is an interaction effect between three independent variables on a continuous dependent variable. The obtained results confirm the one's given by MANOVA. No significant difference between TPC averages regarding the main factors (geographical origin, harvesting time and processing system) was also observed. Also, no significant effect of the three-way interaction (geographical origin*harvesting time*processing system) and of all the two-way interactions (geographical origin*harvesting time, geographical origin*processing system and harvesting time*processing system) on the TPC average with respectively $(F(4; 28) = 2.511; p > 0.05), (F(2; 28) = 3.308; p > 0.05), (F(2; 28) = 0.712; p > 0.05)$ and$(F(4; 28) = 0.417; p > 0.05)$.Some authors in the literature showed close findings including a study done by Serhan et al., (2016) that showed no statistical significance for TPC and antioxidant capacity among production areas and extraction systems. However, other studies were not consistent with our findings including a study conducted by Salvador et al., (2003) that demonstrated the influence of the extraction system, crop season and production area on the chemical composition and quality of Cornicabra virgin olive oils (n=152) from five successive crop seasons (1994-1995 and 1998-1999). Results showed that the extraction system (two-phase, three-phase and press systems) had statistically significant differences ($P<0.05$) in antioxidant

content and TPC as well as oxidative stability. It was shown that the crop season is a critical variable, since the chemical composition of the OO vary considerably from one year to the next. OO quality varied among the five production areas considered, in the provinces of Toledo and Ciudad Real. As it was reported that OO from the South and Southeast of the province of Toledo are of higher quality. These results were different from our findings due to the difference in the profile of PC, and the oxidative stability before and after extracting phenols (Serhan et al., (2016). It could also be due to other factors including high temperature, prolonged malaxation time, type of mills etc. (Khdair et al. 2015; and Serhan et al., 2016).

However, although no significant differences of the main factors (geographical origin, harvesting time and processing system) were found on the OO TPC and the antioxidant capacity, a greater sample size may increase the power to detect significant differences between the groups. However and since the standard deviations around the means for each time point is quite low, which shows that it is unlikely that variation within a group is contributing to lack of significance, a means comparison was conducted to highlight the determinant factors involved in obtaining high TPC and antioxidant capacity. The results are shown below:

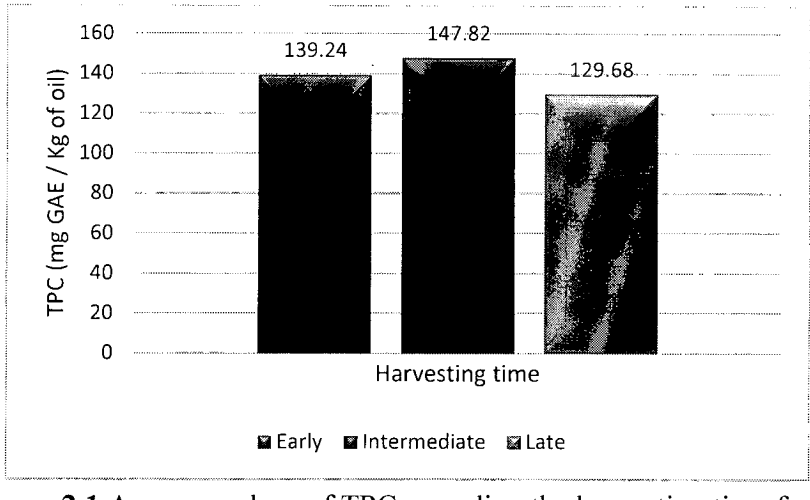

Figure 2.1 Average values of TPC regarding the harvesting time factor

It could be noted that the TPC increased from the early harvest to the intermediate harvest but later, it decreased drastically (figure 2.1). This is similar to the described pattern in a study done by Gomez-Rico et al., (2008) which reported that the level of Oleuropein decreased during the course of fruit ripening especially between the spotted and black drupes in almost all the varieties studied. Oleuropein content increased until reaching its maximum level in the spotted fruits in the Picual and Picolimón cultivars and then decreased significantly in black olives. This behavior could be due to the turnover of the phenolic moieties into new conjugates; on the other hand, the steady decrease in Oleuropein contents may be due to its extensive degradation. Also Gouvinhas et al., (2015) reported that for Cobrançosa and Galega cultivars, the samples presented an increase of TPC from the first to the second stage of ripening, then a decrease was observed in the third maturation stage. In addition, our findings are found to be non-consistent with other studies in the literature such as the study conducted by Trapani et al., (2018) that showed a decreasing trend in TPC content during ripening.

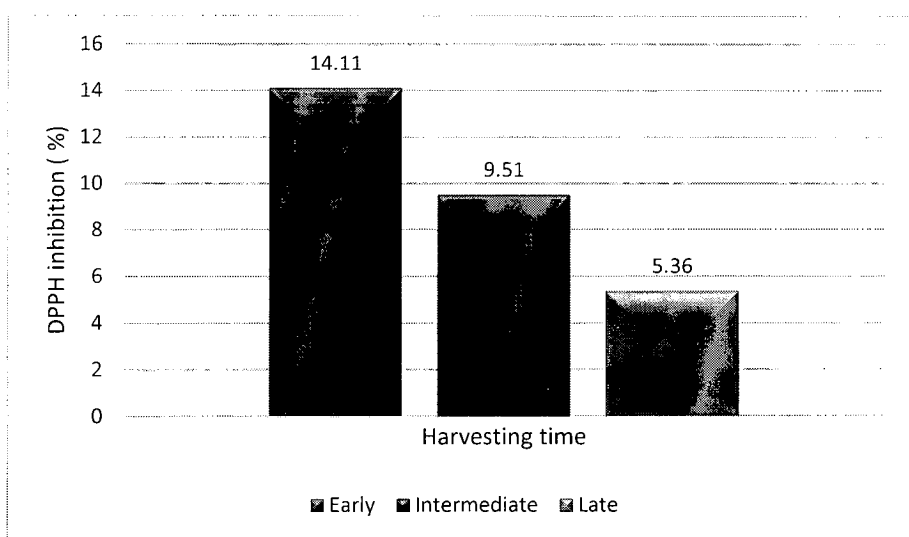

Figure 2.2 Average values of DPPH regarding the harvesting time factor

However, the antioxidant capacity showed a constant decrease along olive fruits ripening (figure 2.2). This is also similar to the described pattern in Sevim et al., (2013) study which concluded that the OO from early harvest fruits of 2008 crop had better antioxidant capacity compared to the 2009 crop. It also reported that the difference between the two crops may be due to the dry conditions of 2008 year.

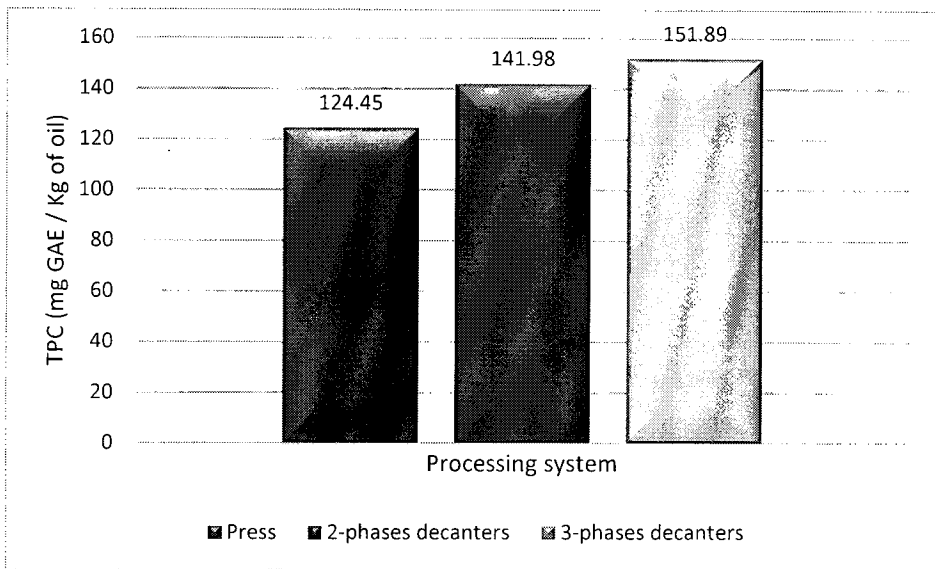

Figure 2.3 Average values of TPC regarding the processing system factors

Regarding the processing system, the three-phase system showed the highest TPC followed by the two-phase and the press system. These results doesn't align with the results obtained in the literature. Khdair et al. (2015), reported a significant differences between the three types of olive mills with the highest level obtained from the two-phase system rather than the three-phase system. Similarly, Gimeno et al., (2002) stated that the TPC is higher in the two-phase system due to the addition of lukewarm water that is used to dilute the olive paste. OO obtained from the three-phase decanter, should have a lower content of phenols because water was used to dilute the olive paste.

Water level has a major negative effect on TPC level, it partially dissolves the phenols and reduces their content (Zamora, 2016). The difference in our results may be due to the oil quality, the two-phase system, the production in mills etc. Aguilera et al., (2015) also reported that the concentration of PC in oil is being decreased during processing of olive fruits with higher water amount.

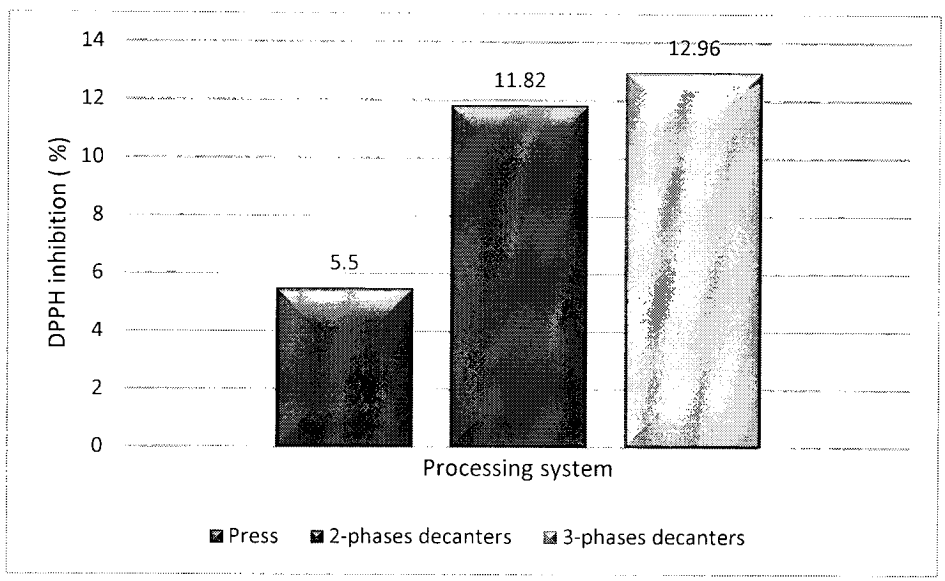

Figure 2.4 Average values of DPPH regarding the processing system factor

The antioxidant capacity is higher in the new processing system in comparison to the traditional one (press). This is logic because the press system is a completely open system, where the paste is exposed to the air oxygen for long time. According to Bendini et al., (2007) in a bulk oil system the hydrophilic antioxidants, such as polar phenols, are oriented in the air-oil interface (a low quantity of air is always trapped in the oil) and become more protective against oxidation than the lipophilic antioxidants, like tocopherols, which remain in solution in the oil. Thus, natural antioxidants exhibit complex properties between air-oil and oil-water interfaces that significantly affect their relative activities. However, it was shown in a study done by Torress et

al., (2006) that the oils from press had higher TPC level than those with the centrifugation. Contradictory results were reported by Salvador et al., (2003), TPC level was greater in the centrifuge-extracted oil than in the press, and lower in the oils extracted using the three-phase system than those from the two-phase system. The respective mean contents for the two-phase, three-phase and press systems were 160, 142 and 100 mg/kg. These differences in natural antioxidant contents affected the oxidative stability. Kalogeropoulos et al., (2014) also reported that over a period of 5 crop years, oils obtained by two-phase showed a higher oxidative stability as compared to those extracted using three-phase and press systems. In addition, Torress et al. (2006) reported that EVOO produced with two-phase system showed highest TPC as compared to those obtained using three-phase system. The differences in TPC and antioxidant capacity may be explained by their water-solubility, the higher water/paste ratios used in three-phase system, the crushing machine, the processing time etc. (Salvador et al., 2003; Torress et al., 2006).

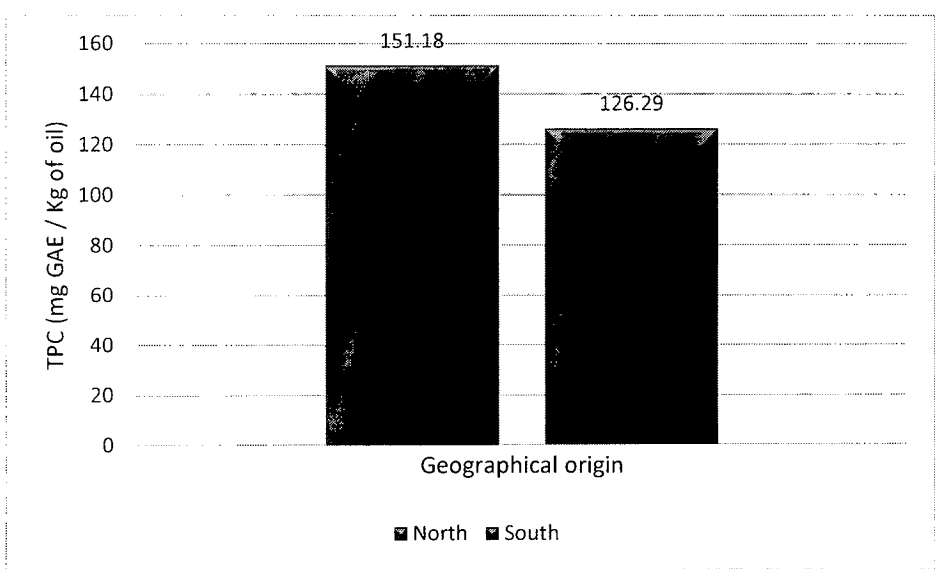

Figure 2.5 Average values of TPC regarding the geographical origin factor

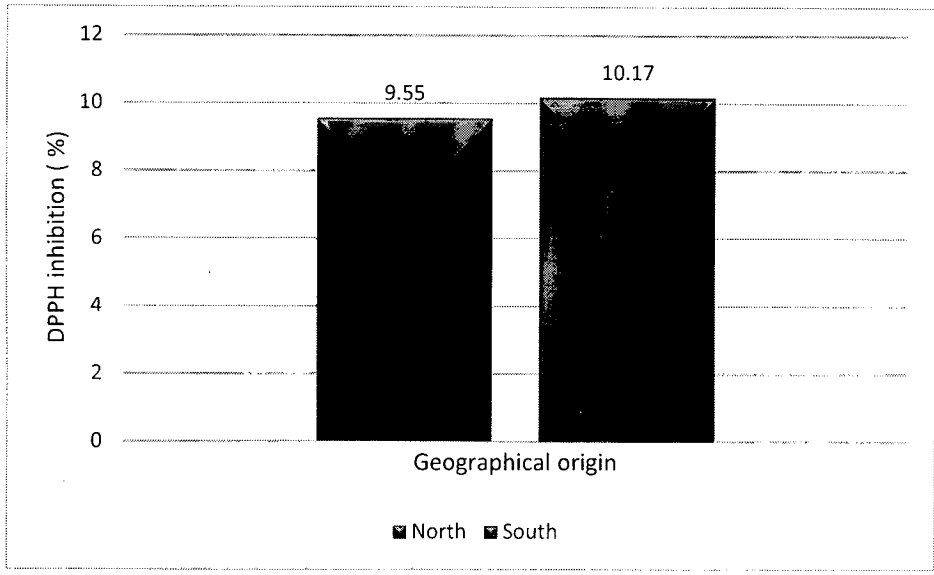

Figure 2.6 Average values of DPPH regarding the geographical origin factor

It is normal to see difference in geographical origin between the two studied regions. However, the results are contradictory between TPC and antioxidant capacity, (figure 2.5 and figure 2.6). The results of the individual phenols obtained by HPLC could serve for interpreting these results. Also, the phenolic content and antioxidant capacity of OO may be influenced by the temperature, altitude, cultivation zone, precipitation and nature of the soil (Aparicio-Ruiz, & Harwood, 2013). The warmer the temperature and the higher the altitude of the orchard, the less the phenolic content level in oil exists (Aparicio-Ruiz, & Harwood, 2013). Kiritsakis (1998) agreed on this point by reporting that the lower the altitude, the higher the PC level. This is mainly due to the fact that lower altitudes have more suitable temperature and sun exposure compared to higher ones. Also, Merchak et al., (2017) confirmed these findings by reporting that the classification of Lebanese OO according to their origin is challenging since the main olive regions including South and North are not clearly separated by their climates. The two regions produce OO and generate 40% of total crop but are 120 km apart of each other and characterized by an annual precipitation of 900 mm

with high rainfall rates in the North than in South. Taking into consideration the geographicall characteristics of the Lebanese territory, the olive regions were designated according to their altitudes and it was reported that both regions showed variations associated with the latitude, altitude and climatic conditions (Merchak et al., 2017).

3.3 Correlation between Total Phenolic Content and Antioxidant Capacity of Lebanese OO

Before assessing the relation between TPC and antioxidant capacity, we check the normality of TPC and the antioxidant capacity variables by Kolmogorov-Smirnov test. The first one is normally distributed($D(46) = 0.089; p > 0.05$), the second not($D(46) = 0.187; p < 0.001$). Spearman's rank-order correlation was run to determine the relationship between TPC and the antioxidant capacity measured on OO samples. There was a mid, positive correlation between them, which was statistically highly significant($r_S(44) = 0.489, p < 0.01$).

Logistic Regression

The logistic regression model was statistically significant for the antioxidant capacity, χ^2 (2) = 8.568, $p < 0.05$. The model explained 23.7% (Nagelkerke R^2) of the variance in OO category and correctly classified 71.7% of cases.

Later, a logistic regression was performed to ascertain the effects of TPC and the antioxidant capacity on the OO category. But before running logistic regression 4 major assumptions should be checked. If one of them is violated, the regression couldn't be executed.

Assumption 1: the dependent variable should be measured on a dichotomous scale. This is the case of our dependent variable (OO class).

Assumption 2: they must be one or more independent variables, which can be either continuous or categorical. The two independent variables (TPC and the antioxidant capacity) considered in this study are continuous ones

Assumption 3: Independence of observations: any of our measures is related simultaneously to VOO and EVOO

Assumption 4: There needs to be a linear relationship between any continuous independent variables and the logistic transformation of the dependent variable. In fact, the relationship between the two predictors (TPC and antioxidant capacity) in not a perfect linear one and the correlation between both is not so high. Full model (step 1 model) with predictors (TPC and the antioxidant capacity) were analyzed.

Table 2.4 Omnibus Tests of Model Coefficients

		Chi-square	df	p
Step 1	Step	8.568	2	0.014
	Block	8.568	2	0.014
	Model	8.568	2	0.014

First, it's important to note that the statistics for the Step, Model and Block are the same (8.568) because we have not used stepwise logistic regression. The "p" value is the probability of obtaining this chi-square statistic (8.568) if there is in fact no effect of the independent variables, taken together, on the dependent variable. In this case, the model is statistically significant because the p-value is less than 0.05. As a conclusion, the created model has significantly increased the ability to predict the OO categories of the studied samples.

Table 2.5 Model Summary

Step	-2 Log likelihood	Cox & Snell R^2	Nagelkerke R^2
1	49.518[a]	0.170	0.237

-2 Log Likelihood statistic is 49.518. This statistic measures how poorly the model predicts the OO category. The smaller the statistic the better the model. The explained variation in the dependent variable (OO category) based on the model ranges from 17.0% (Cox & Snell R^2) to 23.7% (Nagelkerke R^2).

The observed values indicate the number of 0's (VOO) and 1's (EVOO) that are observed in the dependent variable (OO categories). The predicted values represent the predicted values of the dependent variable based on the full logistic regression model. This table shows how many cases are correctly predicted (27 cases are observed to be VOO and are correctly predicted to be VOO; 6 cases are observed to be EVOO and are correctly predicted to be EVOO), and how many cases are not correctly predicted (4 cases are observed to be VOO but are predicted to be EVOO; 9 cases are observed to be EVOO but are predicted to be VOO)

Table 2.6 Classification Table for Step 1 (model including the predictors)

			Predicted		
	Observed		OO category		% correct
			VOO	EVOO	
Step 1	OO category	VOO	27	4	87.1
		EVOO	9	6	40.0
	Overall %				71.7

Overall Percentage gives the percent of cases that are correctly predicted by the model (in this case, the full model that we specified above) (Table 2.6). This percentage has increased from 67.4 for the null model (without predictors) to 71.7 for the full model.

The "Variables in the Equation" table (Table 2.7) shows the contribution of each independent variable (TPC and the antioxidant capacity) to the model and its statistical significance. The table values are the ones for the logistic regression equation for predicting the dependent variable (OO category) from the independent variable (TPC and the antioxidant capacity). They are in log-odds units. The prediction equation is:

$$log(p/1-p) = b_0 + b_1 * x_1 + b_2 * x_2$$

Note that "p" is the probability of being in EVOO category.

Table 2.7 Variables in the Equation table

							95% C.I.for EXP(B)	
	B	**S.E.**	**Wald**	**df**	**p**	**Exp(B)**	**Lower**	**Upper**
Average TPC	0.015	0.007	4.389	1	0.036	1.015	1.001	1.030
Average DPPH inhibition	0.021	0.034	0.366	1	0.545	1.021	0.955	1.091
Constant	-3.157	1.073	8.660	1	0.003	0.043		

Expressed in terms of the variables used in this research (column B, table 2.7), the logistic regression equation is:

$$log(p/1-p) = -3.157 + 0.015 * Average\ TPC + 0.021 * Average\ DPPH\ inhibition$$

These estimates describe the relationship between the independent variables (TPC and the antioxidant capacity) and the dependent variable, where the dependent variable is on the logit scale. Furthermore, these estimates tell the amount of increase (or decrease, if the sign of the coefficient is negative) in the predicted log odds of EVOO that would be predicted by a 1-unit increase (or decrease) in the predictor, holding all other predictors constant. Accordingly, table 2.7 shows that for every one-unit increase of TPC in reading score (so, for every additional point on the TPC average), it is expected a 0.015 increase in the log-odds of OO category, holding all

other independent variables constant. In addition, for every one-unit increase in the antioxidant capacity score, it is expected a 0.021 increase in the log-odds of OO category, holding all other independent variables constant. On the other hand, Constant is the expected value of the log-odds of OO category when all the predictor variables equal zero. Regarding the S.E. column in table 2.7, it contains the standard errors associated with the coefficients. They are used for testing whether the parameter is significantly different from 0. As for the Wald test (Wald, df and p columns in table 2.7), it is used to determine statistical significance for each of the independent variables. Results show that the TPC ($p = 0.036$) added significantly to the model/prediction, but the antiradical scavenging activity DPPH inhibition (%) ($p = 0.545$) did not. Finally, the numbers in column Exp (B) are the odds ratios for the predictors. They are the exponentiation of the coefficients.

As it was shown in our results that a mid-positive correlation exist between the TPC and antioxidant capacity of Lebanese OO; these findings were consistent with the results found in the literature. Similar to these results, Sevim et al. (2013) reported that the antioxidant capacity of OO is influenced by TPC of oils. The increasing leaf material added to fruits significantly increased the antioxidant capacity level. He also mentioned that the antioxidant capacity level was improved by the addition of phenol-rich olive leaf extract during oil extraction. He reported that the antioxidant capacity of olive leaf extracts depends on the phenolic profile, and that there is a positive correlation between the antioxidant capacity and phenolic content. Another study done Serhan et al., (2016) showed a strong positive correlation between phenols and oxidative stability. In addition, the higher the oleic acid to linoleic acid and MUFA to PUFA ratios, the more stable the oil is because PUFA acids are more prone to oxidation due to their higher number of double bonds. Also a study conducted by Mitsopoulos et al., (2016) about the TPC and the antioxidant

capacity determination for leaves and drupes of the major Greek olive varieties showed a positive correlation (r=0.793) between the antioxidant capacity and TPC. Gorinsteina et al., (2003) also reported a high correlation between TPC and antioxidant capacities measured by four methods including total radical-trapping anti-oxidative potential by ABAP, radical scavenging activity by DPPH, and antioxidant assay by -carotene-linoleate model system and total antioxidant status by ABTS. Furthermore, Šarolić et al., (2014) reported strong correlation between TPC and antioxidant capacity. This study revealed that elevated radical scavenging activity for Leccino OO was correlated to its high TPC. Similarly, Dabbou et al., (2010) reported that the antioxidant capacity of OO was correlated with polar components and the lipid profile which are important for its shelf life.

So, our results indicate the antioxidant capacity may be influenced by factors other than TPC such as the antioxidant compounds, the individual phenols, the hydrogen atom etc. (Šarolić et al., (2014)).

3.4 Lebanese OO grades

Since the TPC level is normally distributed ($D(46) = 0.089; p > 0.05$) (Kolmogorov-Smirnov test) and the equality of variances is assumed ($F = 0.016; p > 0.05$) (Levene test) an independent samples t-test was run to check for significant difference in TPC levels between VOO and EVOO.

The results show a high significant difference between TPC averages in VOO and EVOO ($t(44) = -3.021; p < 0.01$). It was shown that the average TPC for EVOO was higher as compared with those from VOO (figure 2.7). Our results are consistent with a study reported by Kalogeropoulos et al., (2014) that indicated that TPC values for EVOO samples ranged from 65 to 218 mg CAE.kg^{-1} with a mean value of 117 mg CAE.kg^{-1} whereas they ranged between 22

and 187 mg CAE.kg^{-1} for VOO with a mean value of 82 mg CAE.kg^{-1}, this could be due to the TPC that react with lipid radicals to form non-reactive radicals, interrupting the propagation chain. These compounds are able to donate an electron or hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation and stabilize the resulting phenoxyl radical by delocalizing the unpaired electron (Šarolić et al., (2014))

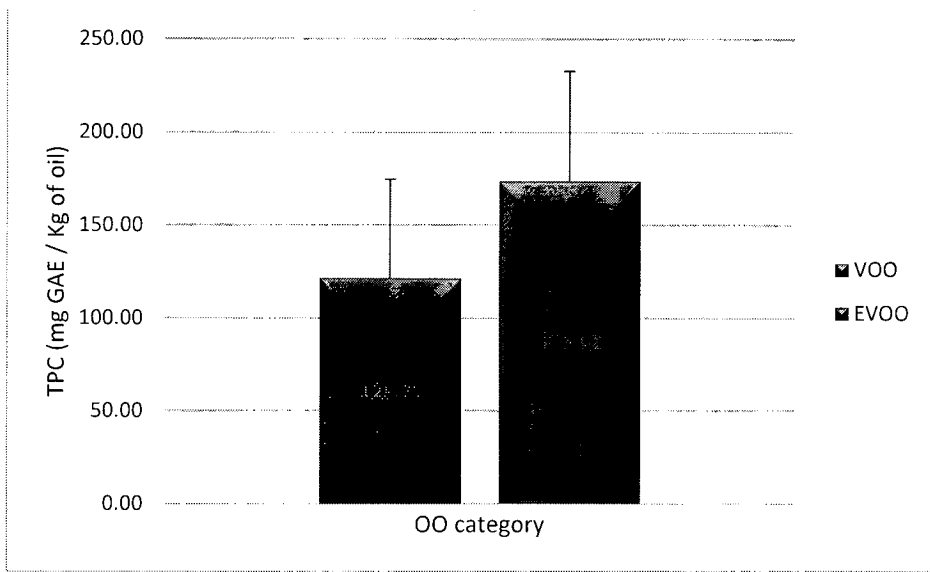

Figure 2.7 TPC averages for VOO and EVOO. The bars represent the standard deviation

Mann-Whitney U test

The antioxidant capacity is not normally distributed($D(46) = 0.187; p < 0.001$). To study the effect of olive oil category on the antioxidant capacity we ran the non-parametric test of Mann-Whitney instead of the independent samples t-test. From the data obtained by running the U test, it can be concluded that the antioxidant capacity in EVOO do not differ significantly from the recorded one in VOO($U = 178.25, p > 0.05$). However, the EVOO can be considered as having the highest antioxidant capacity because it has the highest mean rank (27.10) (figure 2.8). Our

results were consistent with a study done by Silva et al., (2010) which reported that the antioxidant capacity of EVOO was higher than other OO samples and was also positively correlated with the phenolic content of the oil.

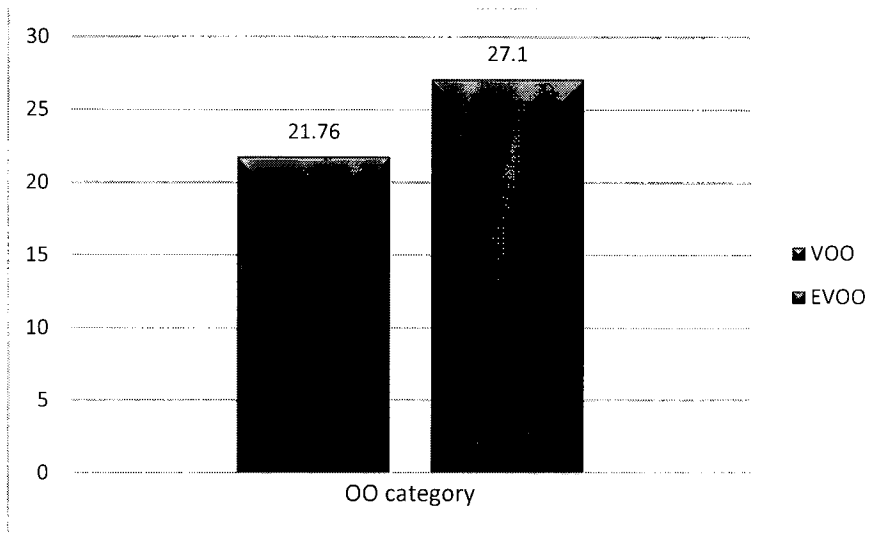

Figure 2.8 DPPH mean rank for VOO and EVOO

4. Limitations

This study was conducted for the purpose of highlighting the effect of geographical areas, harvesting time and processing techniques on the antioxidant capacity and the TPC of OO since no previous studies were performed before. Limitations of this study include the potentially small OO samples gathered that can limit the validity of the results as well as the possibility for generalization. Another limitation is that this study did not assess the individual phenols and their composition but rather provided an evaluation of phenolic compound in general. Also, only three agro-industrial factors were evaluated for TPC and antioxidant capacity levels. Other variables must be taken into consideration to make the study more powerful for later analysis. It was being

reported by the farmers that fruit flies were detected for 2016 crop samples that altered the results. This fly considered a serious pest in the cultivation of olives as it might affect the amount and quality of production in most olive growing areas. The impact of its attacks tend to worsen the quality of the growing areas, with significant variations depending on the variety grown, where it affects olive cultivars and geographical areas.

5. Conclusion

In this study, we have assessed several factors that may have an effect on Lebanese OO quality. It was shown that the geographical origin, the harvesting time and the processing time had no significant effect on OO TPC content and the antioxidant capacity. The results also report a mid, positive correlation between TPC and the antioxidant capacity. In addition, our results show a high significant difference between TPC averages in VOO and EVOO as TPC level was found to be higher for EVOO as compared with those from VOO. The findings of this study provide an overview of several agro-industrial factors that should be taking into consideration while assessing Lebanese OO quality but further studies are needed to confirm our findings and to what extent the OO composition and the agro-industrial factors affect the TPC and the antioxidant capacity of Lebanese OO.

6. References

Adnan I. Khdair, Salam Ayoub and Ghaida Abu-Rumman, 2015. Effect of Pressing Techniques on Olive Oil Quality. *American Journal of Food Technology, 10: 176-183.*

Aguilera, M. P., Jimenez, A., Sanchez□Villasclaras, S., Uceda, M., & Beltran, G. (2015). Modulation of bitterness and pungency in virgin olive oil from unripe "Picual" fruits. *European Journal of Lipid Science and Technology, 117*(9), 1463-1472. doi:10.1002/ejlt.201400432

Alarcón Flores, M. I., Romero-González, R., Garrido Frenich, A., & Martínez Vidal, J. L. (2012). Analysis of phenolic compounds in olive oil by solid-phase extraction and ultra high performance liquid chromatography-tandem mass spectrometry. *Food Chemistry, 134*(4), 2465-2472. doi:10.1016/j.foodchem.2012.04.058

Aparicio-Ruiz, R., and Harwood, J. (2013). Handbook of olive oil : Analysis and properties (2;2nd;2nd 2013; ed.). Boston, MA: Springer. doi:10.1007/978-1-4614-7777-8

Baiano, A., Terracone, C., Gambacorta, G., and Notte, E. L. (2009). Changes in quality indices, phenolic content and antioxidant activity of flavored olive oils during storage. Journal of the American Oil Chemists' Society, 86(11), 1083-1092. doi:10.1007/s11746-009-1446-8

Baiano, A., Terracone, C., Viggiani, I., Nobile, M. A., & Del. (2013). Effects of cultivars and location on quality, phenolic content and antioxidant activity of extra-virgin olive oils. *JAOCS, Journal of the American Oil Chemists' Society, 90*(1), 103-111. doi:http://dx.doi.org/10.1007/s11746-012-2141-8

Balasundram, N., Sundram, K., and Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chemistry, 99(1), 191-203. doi:10.1016/j.foodchem.2005.07.042

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A. M., Segura-Carretero, A., Fernández-Gutiérrez, A., & Lercker, G. (2007). Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade Alessandra. Molecules, 12(8), 1679-1719.

Boskou, D. (2006;2015;)., L., Luque de Castro, María Dolores, and Rallo, L. (2012). Olive oil: Chemistry and technology (2nd;2; ed.). US: Elsevier.

Chalak, L., Haouane, H., Essalouh, L., Santoni, S., Besnard, G., & Khadari, B. (2015). Extent of the genetic diversity in Lebanese olive (Olea europaea L.) trees: a mixture of an ancient germplasm with recently introduced varieties. *Genetic Resources and Crop Evolution, 62*(4), 621-633.

Chehade, A., Bitar, A. E., Kadri, A., Choueiri, E., Nabbout, R., Youssef, H., ... & Trani, A. (2016). In situ evaluation of the fruit and oil characteristics of the main Lebanese olive germplasm. *Journal of the Science of Food and Agriculture*, *96*(7), 2532-2538.

Dabbou, S., Gharbi, I., Dabbou, S., Brahmi, F., Nakbi, A., and Hammami, M. (2011). Impact of packaging material and storage time on olive oil quality. African Journal of Biotechnology, 10(74), 16937.

De Fernandez, María de los Angeles, SotoVargas, V. C., & Silva, M. F. (2014). Phenolic compounds and antioxidant capacity of monovarietal olive oils produced in Argentina. Journal of the American Oil Chemists' Society, 91(12), 2021-2033. doi:10.1007/s11746-014-2558-

Del Monaco, G., Officioso, A., D'Angelo, S., La Cara, F., Ionata, E., Marcolongo, L., Morana, A. (2015). Characterization of extra virgin olive oils produced with typical Italian varieties by their phenolic profile. *Food Chemistry, 184*, 220-228. doi:10.1016/j.foodchem.2015.03.071

Delisi, R., Saiano, F., Pagliaro, M., & Ciriminna, R. (2016). Quick assessment of the economic value of olive mill waste water. *Chemistry Central Journal, 10*(1), 1-5. doi:http://dx.doi.org/10.1186/s13065-016-0207-7

El Riachy, M., Priego□Capote, F., LeónVirgin olive oil phenolic profile and variability in progenies from olive crosses. Journal of the Science of Food and Agriculture, 92(12), 2524-2533. doi:10.1002/jsfa.5662

El Riachy, M., Priego-Capote, F., Rallo, L., Luque-de Castro, M. D., & Leon, L. (2013). Phenolic composition of virgin olive oils in cultivars for narrow hedgerow olive orchards. *European Journal of Lipid Science and Technology, 115*(7), 800. 10.1002/ejlt.201300001

Favati, F., Caporale, G., and Bertuccioli, M., (1994). Rapid determination of phenol content in extra virgin olive oil. Grasas y Aceites,1994;45(1-2)68-70.

Ferhat, R., Lekbir, A., Ouadah, H., Kahoul, M. A., Khlalfa, L., Laroui, S., & Alloui-Lombarkia, O. (2017).Effect of extraction solvent on total phenolic content, total flavonoid content, and antioxidant activities of Algerian pomace olive oil. *International Food Research Journal*, *24*(6), 2295-2303.

Galvano, F., La Fauci, L., Graziani, G., Ferracane, R., Masella, R., Di Giacomo, C., ... & Galvano, G. (2007). Phenolic compounds and antioxidant activity of Italian extra virgin olive oil Monti Iblei. *Journal of medicinal food*, *10*(4), 650-656.

García, A., Rodríguez-Juan, E., Rodríguez-Gutiérrez, G., Rios, J. J., & Fernández-Bolaños, J. (2016). Extraction of phenolic compounds from virgin olive oil by deep eutectic solvents (DESs). *Food Chemistry, 197*(Pt A), 554-561. doi:10.1016/j.foodchem.2015.10.131

Gargouri, B., Zribi, A., and Bouaziz, M. (2015). Effect of containers on the quality of chemlali olive oil during storage. Journal of Food Science and Technology, 52(4), 1948-1959. doi:10.1007/s13197-014-1273-2

Genovese, A., Caporaso, N., Villani, V., Paduano, A., and Sacchi, R. (2015). Olive oil phenolic compounds affect the release of aroma compounds. Food Chemistry, 181, 284-294. doi:10.1016/j.foodchem.2015.02.097

Gimeno, E., Castellote, A. I., Lamuela-Raventós, R. M., De la Torre, M. C., & López-Sabater, M. C. (2002). The effects of harvest and extraction methods on the antioxidant content (phenolics, α-tocopherol, and β-carotene) in virgin olive oil. *Food Chemistry*, *78*(2), 207-211.

Gómez-Caravaca, A. M., Maggio, R. M., and Cerretani, L. (2016). Chemometric applications to assess quality and critical parameters of virgin and extra-virgin olive oil. A review. *Analytica Chimica Acta,* 913, 1-21. doi:10.1016/j.aca.2016.01.025

Gómez-Rico, A., Fregapane, G., & Salvador, M. D. (2008). Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding virgin olive oils. *Food Research International, 41*(4), 433-440. doi:10.1016/j.foodres.2008.02.003

Gorinstein, S., Martin-Belloso, O., Katrich, E., Lojek, A., Číž, M., Gligelmo-Miguel, N., . . . Trakhtenberg, S. (2003). Comparison of the contents of the main biochemical compounds and the antioxidant activity of some spanish olive oils as determined by four different radical scavenging tests. *The Journal of Nutritional Biochemistry, 14*(3), 154-159. doi:10.1016/S0955-2863(02)00278-4

Goulas, V., Orphanides, A., Pelava, E., and Gekas, V. (2015). Impact of thermal processing methods on polyphenols and antioxidant activity of olive oil polar fraction: Stability of olive oil phenolics during heating. *Journal of Food Processing and Preservation,* 39(6), 1919-1924. doi:10.1111/jfpp.12430

Gouvinhas, I., de Almeida, José M M M, Carvalho, T., Machado, N., and Barros, Ana I R N A. (2015). Discrimination and characterisation of extra virgin olive oils from three cultivars in different maturation stages using fourier transform infrared spectroscopy in tandem with chemometrics. *Food Chemistry,* 174, 226-232. doi:10.1016/j.foodchem.2014.11.037

Heffernan, N., Smyth, T. J., FitzGerald, R. J., Soler□Vila, A., & Brunton, N. (2014). Antioxidant activity and phenolic content of pressurised liquid and solid–liquid extracts from four irish origin macroalgae. *International Journal of Food Science & Technology, 49*(7), 1765-1772. doi:10.1111/ijfs.12512

http://www.thegreekoliveestate.com/healthy-evoo/

International Olive Council. (2016). Trade Standard Applying to Olive Oils and Olive PomaceOils

Jimenez, B., Callejón, R., Sánchez□Ortiz, A., Ortega, E., Lorenzo, M. L., and Rivas, A. (2014). Agronomic parameters, quality indices, and sensory attributes of virgin olive oils from Hojiblanca and Picudo varieties from three successive crop years. European Journal of Lipid Science and Technology, 116(12), 1647-1653. doi:10.1002/ejlt.201400043

Kalogeropoulos, N., Kaliora, A. C., Artemiou, A., & Giogios, I. (2014). Composition, volatile profiles and functional properties of virgin olive oils produced by two-phase vs three-phase centrifugal decanters. *LWT-Food Science and Technology*, *58*(1), 272-279.

Karakaya, S. (2004). Bioavailability of phenolic compounds. *Critical Reviews in Food Science and Nutrition, 44*(6), 453-464. 10.1080/10408690490886683

Kiritsakis, A. K. (1998). Flavor components of olive oil—A review. *Journal of the American Oil Chemists' Society, 75*(6), 673-681. doi:10.1007/s11746-998-0205-6

Kotkin, C., and Newspapers, M. (2008, Feb 27). Beyond EVOO: Types of olive oil. Star–NewsRetrievedfrom http://search.proquest.com/docview/285614419?accountid=28281

Laincer, F., Laribi, R., Tamendjari, A., Arrar, L., Rovellini, P., & Venturini, S. (2014). Olive oils from Algeria: Phenolic compounds, antioxidant and antibacterial activities. *Grasas y Aceites*, *65*(1), 001.

Locatelli, M., Gindro, R., Travaglia, F., Coïsson, J., Rinaldi, M., & Arlorio, M. (2009). Study of the DPPH -scavenging activity: Development of a free software for the correct interpretation of data. *Food Chemistry, 114*(3), 889-897. doi:10.1016/j.foodchem.2008.10.035

Merchak, N., El Bacha, E., Bou Khouzam, R., Rizk, T., Akoka, S., and Bejjani, J. (2017). Geoclimatic, morphological, and temporal effects on Lebanese olive oils composition and classification: A 1H NMR metabolomic study. Food Chemistry, 217, 379-388. doi:10.1016/j.foodchem.2016.08.110

Minioti, K. S., and Georgiou, C. A. (2010). Comparison of different tests used in mapping the Greek virgin olive oil production for the determination of its total antioxidant capacity. Grasas y Aceites, 61(1), 45-51. doi:10.3989/gya.010508

Mitsopoulos,G.,Papageorgiou,V.,Komaitis,M. and Hagidimitriou,M.(2016). Total phenolic content and antioxidant activity in leaves and drupes of ten olive varieties. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 44(1), 155.

Nakbi, A., Issaoui, M. and Dabbou, S. (2010). Evaluation of antioxidant activities of phenolic compounds from two extra virgin olive oils. *Journal of Food Composition,* (7), 711-715.

Rashed, M. M. A., Tong, Q., Abdelhai, M. H., Gasmalla, M. A. A., Ndayishimiye, J. B., Chen, L., & Ren, F. (2016). Effect of ultrasonic treatment on total phenolic extraction from *Lavandula Pubescens* and its application in palm olein oil industry. *Ultrasonics Sonochemistry, 29*, 39-47. doi:10.1016/j.ultsonch.2015.07.014

Salvador, M. D., Aranda, F., Gómez-Alonso, S., & Fregapane, G. (2003). Influence of extraction system, production year and area on cornicabra virgin olive oil: A study of five crop seasons. *Food Chemistry, 80*(3), 359-366. doi:10.1016/S0308-8146(02)00273-X

Šarolić, M., Gugić, M., Tuberoso, C. I. G., Jerković, I., Šuste, M., Marijanović, Z., & Kuś, P. M. (2014). Volatile profile, phytochemicals and antioxidant activity of virgin olive oils from Croatian autochthonous varieties Mašnjača and Krvavica in comparison with Italian Variety Leccino. *Molecules*, *19*(1), 881-895.

Serhan, M. HoudaYounes and Jessica Chami (2016) Physicochemical Changes in BaladiOlive Oil as a Function of Production Area and Extraction System in North Lebanon. *J Food Technol Nutr Sci*, *1*(003).

Servili, M., Esposto, S., Fabiani, R., Urbani, S., Taticchi, A., Mariucci, F., Montedoro, G. F. (2009). Phenolic compounds in olive oil: Antioxidant, health and organoleptic activities according to their chemical structure. Inflammopharmacology, 17(2), 76-84. doi:10.1007/s10787-008-8014-y

Sevim, D., Tuncay, O., and Koseoglu, O. (2013). The effect of olive leaf addition on antioxidant content and antioxidant activity of "memecik" olive oils at two maturity stages.

JAOCS, Journal of the American Oil Chemists' Society, 90(9), 1359-1369. doi:http://dx.doi.org/10.1007/s11746-013-2282-4

Silva, L., Pinto, J., Carrola, J., & Paiva-Martins, F. (2010). Oxidative stability of olive oil after food processing and comparison with other vegetable oils. *Food Chemistry, 121*(4), 1177-1187.p

Talhaoui, N., Gómez-Caravaca, A. M., León, L., De la Rosa, R., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2016). From olive fruits to olive oil: Phenolic compound transfer in six different olive cultivars grown under the same agronomical conditions. *International journal of molecular sciences, 17*(3), 337.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., and Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis, 19(6), 669-675. doi:10.1016/j.jfca.2006.01.003

Torres, M. M., & Maestri, D. M. (2006). The effects of genotype and extraction methods on chemical composition of virgin olive oils from Traslasierra Valley (Córdoba, Argentina). *Food Chemistry, 96*(4), 507-511.

Veneziani, G., Esposto, S., Taticchi, A., Urbani, S., Selvaggini, R., Di Maio, I., Servili, M. (2017). Cooling treatment of olive paste during the oil processing: Impact on the yield and extra virgin olive oil quality. Food Chemistry, 221, 107-113. doi:10.1016/j.foodchem.2016.10.067

Vossen, P. (2007). Olive oil: history, production, and characteristics of the world's classic oils. *HortScience, 42*(5), 1093-1100.

Xiang, C., Xu, Z., Liu, J., Li, T., Yang, Z., & Ding, C. (2017). Quality, composition, and antioxidant activity of virgin olive oil from introduced varieties at Liangshan. *LWT-Food Science and Technology, 78*, 226-234.

Yorulmaz, A., Erinc, H., & Tekin, A. (2013). Changes in olive and olive oil characteristics during maturation. *Journal of the American Oil Chemists' Society, 90*(5), 647-658.

Zadeh, A. N., Tayefe, M., & Shariffar, A. (2008). Influence of extraction method and production area on antioxidant content (phenolic compounds and tocopherol) in virgin olive oil from Iran, Greece and Italy. *Asian Journal of Chemistry, 20*(7), 5229-5232. Retrieved from https://search.proquest.com/docview/1512875748?accountid=28281

Zamora, R. (2016). Olive and olive oil bioactive constituents.– D. Boskou, editor.–AOCS press, urbana, IL, USA. 2015.– XXIII+397 pages.—ISBN 978-16306741-2. Grasas y Aceites, 67(2), e140"