American Journal of Food Science and Technology, 2018, Vol. 6, No. 6, 228-236
Available online at http://pubs.sciepub.com/ajfst/6/6/1

DOI:10.12691/ajfst-6-6-1

Effects of Geographical Location on Chemical Properties of *Zarazi* Virgin Olive Oil Produced in the South of Tunisia

Sinda El-Gharbi[1,2,*], Meriem Tekaya[1], Alessandra Bendini[3,4], Enrico Valli[3,4], Rosa Palagano[3], Mohamed Hammami[1], Tullia Gallina Toschi[3,4], Beligh Mechri[1]

[1]Laboratoire de Biochimie, USCR Spectrométrie de Masse, LR-NAFS /LR12ES05"Nutrition –Aliments Fonctionnels et Santé Vasculaire», Faculté de Médecine, université de Monastir, 5019 Monastir, Tunisia
[2]Faculty of Science of Gabes, University of Gabes, 6072 Gabes, Tunisia
[3]Department of Agricultural and Food Sciences, Alma Mater Studiorum – University of Bologna, Italy
[4]Inter-Departmental Centre for Agri-Food Industrial Research, University of Bologna, Italy
*Corresponding author: sindagharbi.1@gmail.com

Received August 02, 2018; Revised September 11, 2018; Accepted September 26, 2018

Abstract The effects of geographical location on the chemical composition of olive oil of *Zarazi* cultivar growing in four different regions in the south of Tunisia (Sfax, Tataouin, Matmata and Medenin) were evaluated. Quality indices, oxidative stability, tocopherol content, fatty acid, sterol and phenolic compositions, were analyzed for each oil sample. Significant differences were detected depending on the growing area ($p< 0.05$). In general, the main secoridoids, were present in higher levels in the oil obtained from olives of Sfax region. Statistical analyses showed that acidic and sterolic compositions of olive oil were significantly modified according to the growing regions. The Principal Component Analysis of the different analyzed compounds indicated that the factor "geographical location" significantly affected olive oil quality, and highlighted the high quality of oil samples of Sfax, in terms of their high oxidative stability and their richness in bioactive compounds.

Keywords: *fatty acids, geographical location, olive oil, sterols, phenolic compounds*

Cite This Article: Sinda El-Gharbi, Meriem Tekaya, Alessandra Bendini, Enrico Valli, Rosa Palagano, Mohamed Hammami, Tullia Gallina Toschi, and Beligh Mechri, "Effects of Geographical Location on Chemical Properties of *Zarazi* Virgin Olive Oil Produced in the South of Tunisia." *American Journal of Food Science and Technology*, vol. 6, no. 6 (2018): 228-236. doi: 10.12691/ajfst-6-6-1.

1. Introduction

Virgin olive oil (VOO) is considered as the best oil through its sensory, functional and chemical composition, and make it unique among other vegetable oils because it contains essential nutritional elements (antioxidants, vitamins, etc.) [1]. The factors affecting olive oil chemical composition can be clustered into four groups: environmental (soil, climate), agronomic (irrigation, fertilization), cultivation (harvesting, ripeness), and technological factors (post-harvest storage, extraction system, oil storage conditions). These factors currently affect the physico-chemical quality, the sensory characteristics and the chemical composition of the extracted olive oils [2,3]. Moreover, olive oil quality is linked to the olive cultivar and to the geographical locations of the olive cultivar [4].

Polyphenols, which are important minor compounds and main antioxidants in VOO, are one of the basic parameters to be taken into account in the authentication with respect to geographic region and cultivars [5,6].

The fatty acid composition has a key role in the characterization of olive oil. VOO has high contents of monounsaturated fatty acids, mainly oleic acid which ranges from 56% to 84% [7], thus showing great importance, particularly in relation to its effect on cardiovascular system health [8]. The sterol profile, instead, is widely used for the examination of its authenticity because sterols can be considered as its real fingerprint [1].

Only few authors have focused their attention on the location (geographical area, latitude, altitude) of olive tree crops and how it can influence physical, chemical, sensory and nutritional characteristics of the relative oils.

Tunisian orchards are dominated by the cultivar population *Chemlali* occupying 2/3 of Tunisian olive orchard and providing more than 60% of the national production of the olive oil [9]. Nevertheless, there are other secondary varieties specific of local regions such as *Zarazi* cultivars in the south of Tunisia.

Several studies have been performed to classify virgin olive oils from various geographical areas in Tunisia following their different chemical properties [10]. However, there is no study on the effect of geographic location on the chemical composition of *Zarazi* cultivar, cultivated in the south of Tunisia and appreciated for its high oil content.

In this context, the ultimate objective of this study was to investigate the modification of chemical composition of

Zarazi olive oil obtained from olives of this variety growing in four different regions in the south of Tunisia (Sfax, Matmata, Tataouin and Medenin). The evaluation of the effect of geographic areas on oil quality included the analysis of quality indices and minor compounds (such as phenolic compounds) as well as the profiles of phytosterols and fatty acids. The results were then discussed in order to determine which samples had the best oil quality and nutritional value in terms of richness of bioactive compounds.

2. Material and Methods

2.1. Fruit Samples

The study was performed during the crop season 2014/2015. Olive samples of *Zarazi* cultivar (~3 kg for each sample) were randomly hand-picked from 9 trees at each region in the south of Tunisia: Sfax (34°44′26″ N, 10°45′37″ E), Tataouin (32°55′46″ N, 10°27′06″ E), Medenin (33°21′17″ N, 10°30′19″ E) and Matmata (33°32′40″ N, 9°58′17″ E). All samples showed the same ripening degree (the maturity index was approximately 5) on the basis of the skin color [11].

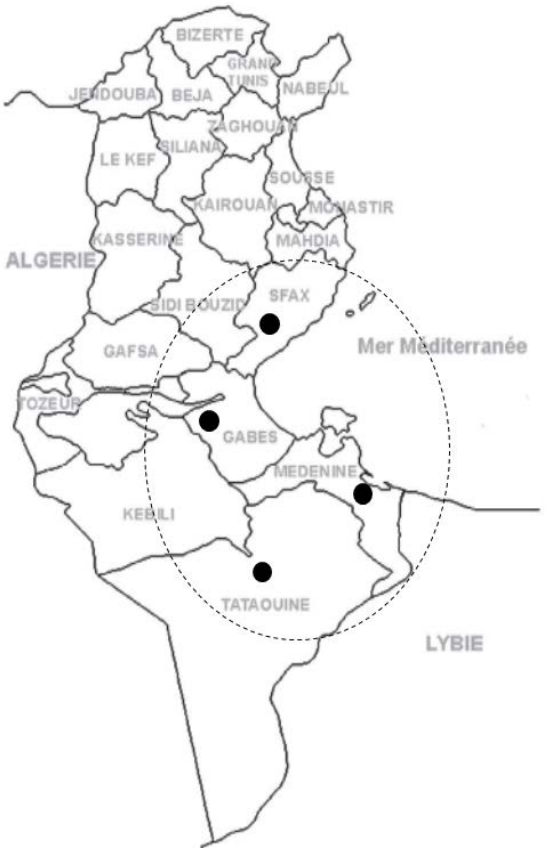

Figure 1. Map for geographical areas where Zarazi olive fruits were collected

2.2. Oil Extraction and Samples

After harvesting, the olive samples were immediately transported to the laboratory where the oil was extracted within 24 h. Only healthy fruits, without any physical damage were processed. Olive oil of four different sites (Sfax, Matmata, Tataouin and Medenin) was extracted using a laboratory oil mill Abencor, which simulates the industrial process of VOO production. Olive oil samples were stored in dark glass bottles at room temperature.

2.3. Quality Indices Determinations

Free acidity (FA, given as % oleic acid), peroxide value (PV, expressed as milliequivalents of active oxygen (meq O_2 kg^{-1} oil) and coefficients of specific extinction at 232 nm and 270 nm were assessed according to analytical methods described respectively in ISO 660:2009, ISO 3960: 2010 and ISO 3656: 2011.

2.4. Carotenoids and Chlorophylls

Carotenoids and chlorophylls (mg kg^{-1} oil) were determined colorimetrically using an UV–Vis 1800 spectrophotometer (Shimadzu Co., Kyoto, Japan), following the method described by Mínguez-Mosquera *et al.* (1991) [12]. The pigment contents are expressed in milligrams of pheophytin *a* and lutein per kilogram of oil, respectively.

2.5. Oxidative Stability

Oxidative stability (expressed as the oxidation induction time (h)), was measured by a Rancimat 743 apparatus (Metrohm Ω, Schweiz AG, Zofingen, Switzerland) according to the procedure described by Tura *et al.* (2007) [13].

2.6. Total Phenol and o-diphenol Contents

Total phenolic and *o*-diphenol amounts in olive oil samples were quantified colorimetrically using an UV–Vis 1800 spectrophotometer (Shimadzu Co., Kyoto, Japan) following the procedure described by Mateos *et al.* (2001) [14]. The results are expressed as mg of gallic acid per kg of oil and the analysis was carried out in triplicate for each sample.

2.7. HPLC Analysis of Phenolic Compounds

The extraction of phenolic compounds was carried out according to IOC/ T.20/Doc No. 29. HPLC analysis was carried out using an Agilent Technologies 1260 series system treated with an automatic injector, a diode array UV-Vis detector (DAD) and a mass spectrometer detector (MSD). A C18 column KINETEX (100 cm x 3.00 mm x 2.6 µm; Phenomenex, Torrence CA, USA) was used, maintained at 40°C during the analyses. The injection volume was 5µl and the flow rate was 0.7 ml min^{-1}. The wavelength was set at 280 nm for phenolic acids, phenyl ethyl alcohols and secoiridoids. Mobile phase was a mixture of water/formic acid (95:5, v/v) (solvent A) and acetonitrile (solvent B). A linear gradient was run from 95% (A) and 5% (B) to 80% (A) and 20% (B) during 3 min, it changed to 60% (A) and 40% (B) in 1 min, after 1 min it changed to 55% (A) and 45% (B), it changed to 40% (A) and 60% (B) after 4 min, and then in 1 min it became 100% (B), it changed to 95% (A) and 5% (B) during 3 min (13 min total time).

The phenolic compounds were determined by comparison of their retention times with and maximum absorbance according to IOC/T.20/Doc No 29, and by interpretation of their mass spectra [15]. The internal standard used was syringic

acid (purchased from Sigma-Aldrich, St. Louis, France), and for the calculation of individual phenols data are expressed as mg of tyrosol per kg of oil, according to IOC [16].

2.8. Determination of Tocopherols

An aliquot of 0.5 g of oil sample was solubilized in 10 ml volumetric flask with isopropanol following the methods described by Anwar *et al.* (2013) [17]. HPLC analysis was performed with an Agilent Technologies 1100 series HPLC apparatus (Paolo Alto, California, USA), comprising a HP pump series 1050 (Darmstadt, Germany) and a DAD detector set at 292 nm was used. The eluting solvents were methanol/water (90:10, v/v) acidified with 0.2% H_3PO_4 (solvent A), and acetonitrile (solvent B). Samples were eluted through a Cosmosil column (π NAP 4.6 mm × 150 mm x 5 µm; Nacalai-Tesque, Kyoto, Japan) according to the following gradient: 100% A maintained for 22 min; 100% B for 13 min; 100% A for 15 min (total run = 50 min). The concentrations of *α*-tocopherol was determined using a calibration curve constructed with different concentrations of *α*-tocopherol (r^2 = 0.998). Results are expressed in µg of *α* tocopherols per g of oil.

2.9. Fatty Acid Composition and Squalene Determination

The fatty acid composition was determined by gas chromatography (GC) after saponification/methylation with methanolic KOH, according to the official method (EEC Reg.2568/91 and following amendment). The identification of fatty acids was performed by the comparison of retention time with standard compounds. Fatty acids percentage was determined by internal standardization without taking into account mass response factor.

2.10. Sterol Composition

The analysis of sterol fractions extracted from olive oils was determined referring to the official method (EEC Reg. 2568/91 and following amendments). Sterol identification was performed by comparing the peak retention times with those of the pure standards of sterols.

2.11. Statistical Analysis

The Statistical Package for Social Sciences (SPSS) program release 16.0 for Windows (SPSS, Chicago, IL, USA) was used for statistical analyses. The results are expressed as mean ± standard deviation (SD) of three measurements for each analytical determination. Significant differences between the values of all parameters were determined at $p < 0.05$ according to the one-way ANOVA: Post Hoc Comparisons (Duncan test). The Principal Component Analysis (PCA) was conducted using XLSTAT 2006 Version 2006.06 (Addinsoft, Paris, France). It was applied to separate oils samples relatives to each growing area according to all parameters investigated.

3. Results and Discussion

3.1. Quality Indices

The basic quality indices of the samples are presented in Table 1. The free acidity of the oils from different regions varied between 0.11 and 0.77 % oleic acid. The peroxide values of the samples were in the range of 10.48 – 18.03 meq O_2kg^{-1}. K_{232} and K_{270} coefficients ranged from 1.79 to 2.10 and from 0.05 to 0.11, respectively. All the oil samples were characterized by quality indices (FA, PV, K_{232} and K_{270}) under the maximum limits for extra virgin olive oil quality grade fixed by the International Olive Council [18]. As reported by Servili and Montedoro, (2002) [19] these analytical parameters are principally affected by factors altering the fruit (e.g., fly attacks, inappropriate harvesting systems, transport and storage of olives) or the oil quality (e.g., the oil extraction procedure and storage conditions). The basic physico-chemical parameters FA, PV, K_{232} and K_{270} were apparently affected by the growing area of olive trees of the same cultivar. Therefore, the significant differences observed between FA, PV, K_{232} and K_{270} of oil samples relative to the different studied regions could not be attributed to the factor 'geographical location' of olive trees, but rather to one of the mentioned preharvest and/or postharvest factors affecting olive oil quality.

3.2. Chlorophylls and Carotenoids Contents

Chlorophylls and carotenoids are considered as the principal pigments in vegetable oils. As presented in Table 1, chlorophyll and carotenoid concentrations ranged from 0.66 to 0.83 mg kg^{-1} and from 2.05 to 3.53 mg kg^{-1}, respectively. Significant differences were observed between oils of different locations regarding their pigment contents ($p< 0.05$). In fact, olive oils from Tataouin contained the highest amount of carotenoids (3.53 mg kg^{-1}), while oils from Medenin contained the lowest content of pigments (0.66 and 2.05 mg kg^{-1} of chlorophylls and carotenoids respectively).

Table 1. Basic quality characteristics (FA, PV, K_{232} and K_{270}), concentrations of pigments and *α*-tocopherol, and oxidative stability of virgin olive oil samples of *Zarazi* cultivars growing in four locations in the south of Tunisia (Sfax, Tataouin, Medenin, Matmata)

	Sfax	Tataouin	Medenin	Matmata
FA (% oleic acid)	0.11 ± 0.00 ^{d}	0.24 ± 0.03^{c}	0.54 ± 0.03^{b}	0.77 ± 0.01 ^{a}
PV (meq O_2 kg^{-1} oil)	17.69 ± 0.21 ^{a}	18.03 ± 0.33 ^{a}	15.50 ± 0.34 ^{b}	10.48 ± 0.01 ^{c}
K_{232}	1.84 ± 0.02 ^{b}	1.79 ± 0.07 ^{b}	2.10 ± 0.08 ^{a}	2.04 ± 0.11 ^{a}
K_{270}	0.11 ± 0.00 ^{a}	0.10 ± 0.01 ^{ab}	0.10 ± 0.01 ^{b}	0.05 ± 0.00 ^{c}
Chlorophylls (mg kg^{-1})	0.79 ± 0.02 ^{a}	0.83 ± 0.04 ^{a}	0.66 ± 0.04 ^{b}	0.80 ± 0.07 ^{a}
Carotenoids (mg kg^{-1})	2.69 ± 0.02 ^{b}	3.53 ± 0.29 ^{a}	2.05 ± 0.12 ^{c}	2.62 ± 0.10 ^{b}
α-Tocopherol (mg kg^{-1})	183.87 ± 0.17 ^{c}	227.94 ± 6.66 ^{a}	213.68 ± 6.26 ^{b}	181.72 ± 0.00 ^{c}
Oxidative stability (h)	16.21 ± 0.18 ^{a}	2.88 ± 0.11^{c}	3.03 ± 0.09 ^{c}	8.47 ± 0.28 ^{b}

FA: free acidity; PV: peroxide value; K_{232} and K_{270}: spectrophotometric indices.
Each value represents the mean of three determinations (n = 3) ± standard deviation.
$^{a\text{-}c}$Different letters in the same row indicate significantly different values ($p < 0.05$) according to Duncan test.

Geographical origin of *Zarazi* olive trees influenced considerably the pigment content in olive oil. These results are in accordance with those found by Mansouri *et al.* (2013) [20]. These authors observed that, in addition to geographical location and environmental conditions, the cultivar had also a significant effect on pigment content and reported that *Zarazi* cultivar presented the lowest concentration in cholorophylls and carotenoids (0.83 and 3.53 mg/kg, respectively), whereas *Chemlali* cultivar contained the highest amounts of both pigments (13.72 and 6.31 mg/kg, respectively) [21].

3.3. Total Phenol and Ortho-diphenol Contents

Total phenols and *o*-diphenols were quantified in oil samples and the results were represented in Figure 2. Statistical analysis showed significant differences in both total phenol and *o*-diphenol contents between the oil samples from different locations ($p<0.05$). The total phenol content in the olive oils ranged from 602.14 to 707.98 mg gallic acid kg^{-1} oil. Regarding *o*-diphenols, their concentration varied between 213.31 and 626.97 mg kg^{-1}. In fact, the oils from Matmata and Tataouin have the highest amounts of phenols (707.98 and 698.81 $mgkg^{-1}$, respectively. On the other hand, the highest content of *o*-diphenols (626.97 mg kg^{-1}) was registered in the oil from Matmata, than those from other growing areas.

Our results revealed that geographical location of olive trees had a significant effect on total phenol and *o*-diphenol concentrations in the oil, which is in agreement with the findings of Mansour *et al.* (2015) [21]. In the same line with our results, Del Monaco et *al.* (2015) [7] studied 12 olive oil samples from different locations and cultivars in Italy for the characterization of olive oil with respect to varieties and geographical regions, and reported that the total phenol content showed large variation with regard to geographical location.

In addition, several agronomic parameters can modify the phenol contents of olive oil [22], explaining the variation of phenol content in the different oil samples of Zarazi, obtained in some localities in the south of Tunisia. Compared to Chemlali cultivar, Zarazi presents a higher amount of phenolic compounds. In fact, as described in a previous study, [10] have reported that Zarazi variety was characterized by high average content of total phenols (>600 mg/kg), which is in accordance with our results (between 602 mg/kg and 707 mg/kg).

3.4. Quantification of Phenolic Compounds

Table 2 summarizes the information about the phenolic compounds identified in *Zarazi* VOO samples. The main secoiridoids detected in the studied olive oil samples were the dialdehydic form of elenolic acid linked to hydroxytyrosol or tyrosol, respectively named 3,4-DHPEA-EDA (decarboxy methyloleuropein aglycon) and *p*-HPEA-EDA (decarboxy methyl ligstroside aglycon), an isomer of the oleuropein aglycon 3,4-DHPEA-EA.

Statistically significant quantitative variations were recorded in some phenolic compounds following the geographical provenance of the samples. Sfax oil had the highest levels of most secoiridoids particularly 3,4-DHPEA-EDA (116.0 mg kg^{-1}), 3,4-DHPEA-EA (89.0 mg kg^{-1}), and *p*-HPEA-EDA (24.7 mg kg^{-1}), instead of the oil from Medenin that had the highest amount of cinnamic acid (8.5 mg kg^{-1}), but the lowest amount of 3,4-DHPEA (6.0 mg kg^{-1}), than oils from other locations. The 3,4-DHPEA-EDA was detected in higher concentration than the *p*-HPEA-EDA in all samples of olive oils.

The amount of the lignan compound such as acetoxypinoresinol was different in each oil sample; its concentration was much higher in Sfax oil (65.8 mg kg^{-1}). With regard to flavonoid components, the highest levels of luteolin and apigenin were also registered in Sfax oil (24.9 mg kg^{-1} and 11.5 mg kg^{-1}, respectively).

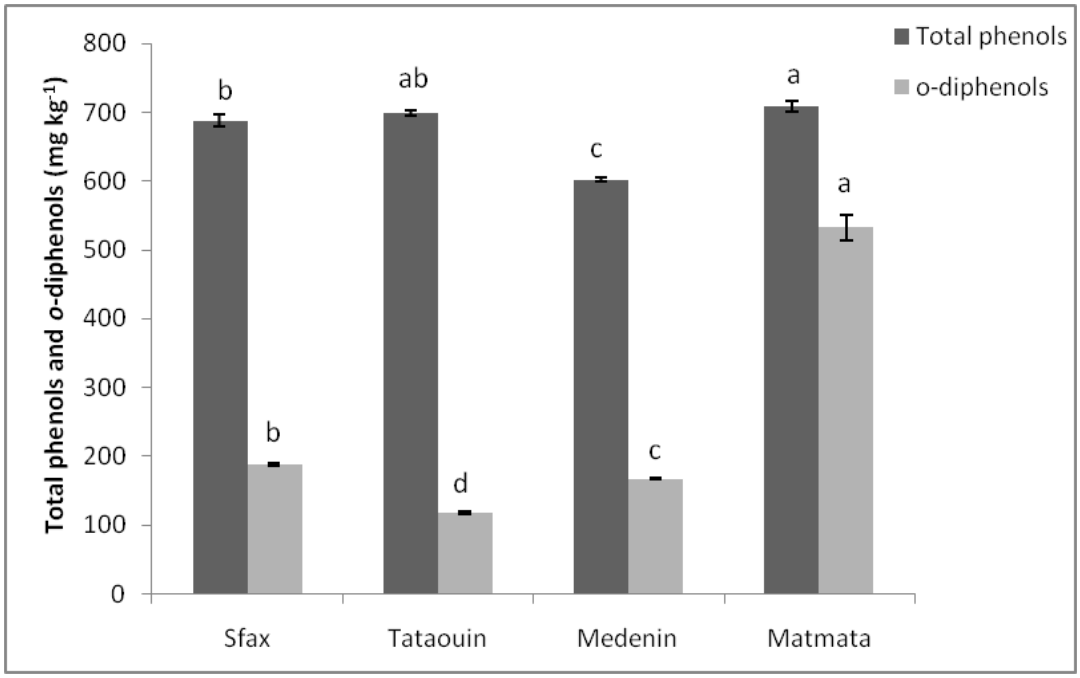

Figure 2. Total phenol and *o*-diphenol contents in olive oil samples from *Zarazi* cultivar growing in four locations in the south of Tunisia. Results are shown as means ± SD (n = 3). [a-b] Different letters indicate significantly different values at $p < 0.05$ according to Duncan test.

It has been observed that 3,4-DHPEA-EDA is the most abundant phenolic compound detected in olive oil extract, which is in accordance with our result [23]. Furthermore, the higher phenolic content in the oil samples from *Zarazi* cultivars was undoubtedly related to the low rainfall in these regions of the south of Tunisia. In previous researches, it was mentioned that water scarcity leads to increased concentration of phenolic compounds in olive oil, because drought generates a stress situation that stimulates phenolic synthesis in olive fruits [2].

Significant differences between geographical areas with regard to the different phenolic compounds have been found in the present work and also in previous studies [23,24]. In fact, according to Bajoub *et al*. (2015) [25] the qualitative and quantitative phenolic composition of VOO were strongly affected by agronomic factors, but also it may be influenced by genetic factor, and environmental conditions related to the geographic regions, especially climate and soil [24]. Our results showed that the concentrations of the main secoiridoids were the most affected depending on the zone of olive tree production. The oil samples relative to the region of Sfax revealed to be very rich in secoiridoids as well as flavonoid compounds, much more than oil samples from other studied regions of the south of Tunisia, which could be a positive feature considering its nutritional implications.

3.5. α-Tocopherol Content

The content of α-tocopherol is shown in Table 1. Olive oil from Tataouin had the higher value (227.94 mg kg^{-1}) of *α*-tocopherol than other sites, whereas the contents of oils from Sfax and Matmata regions were the lowest (183.87 and 181.72 mg kg^{-1}, respectively). Statistical analysis showed a significant difference between the concentrations of *α*-tocopherol in the oil samples from the different geographical areas ($p < 0.05$).

Statistically significant difference shown in *α*-tocopherol among geographic regions can be related to several factors such as climatic conditions, particularly temperature and rainfall. [26] observed that *α*-tocopherol amount in olive oils reached its highest level in the driest years. In this work, *α*-tocopherol was higher in oil samples from regions characterized by a dry climate and an elevated temperature (Medenin and Tataouin). Although *Zarazi* oil of Medenin and Tatouin are not characterized by a high content of total phenols, their richness in *α*-tocopherol may contribute significantly to the enhancement of oil shelf life. This data is in agreement with those found by Laroussi-Mezghani *et al.* (2016) [10].

3.6. Oxidative Stability of Olive Oil

As shown in Table 1, the highest level of oxidative stability was attributed to the oil samples of Sfax (16.21h), while Tataouin oil recorded the lowest value (2.88h). Statistical analysis revealed a significant difference between the oxidative stabilities of oil samples in relation to the geographical area ($p < 0.05$).The different induction times between samples can be explained by the association between fatty acid composition and the contents of the different antioxidants present in these oils [27]. The greatest stability was recorded for olive oil samples from Sfax, followed by those from Matmata. In fact, oil samples from these two regions are characterized by high phenol content and especially *o*-diphenols. This result is in agreement with those of Youssef *et al.* (2011). Some authors have reported that total phenols appear to have great influence on oxidative stability [20], since these compounds are capable to provide an hydrogen atom to the lipid radical established throughout the propagation phase of lipid oxidation [28].

3.7. Fatty Acid Composition and Squalene Content

The main fatty acids, together with squalene, were detected in *Zarazi* olive oil samples obtained from different regions. As shown in Table 3, oleic (C 18:1), palmitic (C 16:0), linoleic (C 18:2) and stearic acids (C 18:0) were the major fatty acids found in all olive oil samples. Generally, fatty acids were within the limits for olive oil from *O.europeae* except for arachidic (C 20:0), gadoleic (C 20:1), behenic (C 22:0) and lignoceric (C 24:0) acids that, in some cases, were slightly above the fixed percentages [18].

Table 2. Contents of individual phenolic compounds (mg kg^{-1} of oil) determined by HPLC-DAD-MS in the virgin olive oil samples of *Zarazi* variety obtained from four locations (Sfax, Tataouin, Medenin and Matmata)

Phenolic compounds (mgkg^{-1})	Locations			
	Sfax	Tataouin	Medenin	Matmata
3,4-DHPEA	19.0 ± 0.7^{a}	ND	6.0 ± 0.2^{b}	19.8 ± 1.0^{a}
p-HPEA	5.3 ± 0.1^{d}	12.4 ± 0.2^{b}	10.7 ± 0.1^{c}	14.9 ± 0.7^{a}
Cinnamic acid	3.3 ± 0.5^{d}	4.5 ± 0.7^{c}	8.5 ± 0.1^{a}	6.5 ± 0.5^{b}
3,4-DHPEA-EDA	116.0 ± 0.7^{a}	6.5 ± 0.3^{c}	3.8 ± 0.5^{d}	33.2 ± 1.7^{b}
Luteolin	24.9 ± 1.1^{a}	4.2 ± 0.9^{c}	5.7 ± 0.6^{c}	12.2 ± 0.9^{b}
p-HPEA-EDA	24.7 ± 0.6^{a}	10.0 ± 0.2^{d}	11.8 ± 0.5^{c}	15.9 ± 0.9^{b}
Acetoxypinoresinol	65.8 ± 2.1^{a}	18.4 ± 0.4^{c}	18.1 ± 0.6^{c}	26.5 ± 1.7^{b}
Apigenin	11.5 ± 0.8^{a}	3.8 ± 1.6^{c}	2.0 ± 0.6^{c}	6.9 ± 0.4^{b}
3,4-DHPEA-EA	89.0 ± 9.2^{a}	5.3 ± 0.3^{c}	5.4 ± 0.6^{c}	35.3 ± 1.6^{b}
p-HPEA-EA	17.6 ± 2.0^{a}	8.5 ± 0.8^{b}	5.7 ± 1.0^{c}	6.8 ± 0.4^{bc}

p-HPEA-EA: Ligstroside aglycon; 3.4-DHPEA-EA: Oleuropein aglycon; *p*-HPEA-EDA: Dialdehydic form of elenolic acid linked to*p*-HPEA; 3,4-DHPEA-EDA: Dialdehydic form of elenolic acid linked to 3,4-DHPEA; ND: not detected.
Results are expressed as means ± SD of three replicates (n = 3).
[a-c]Different letters in the same row show statistically significant differences ($p < 0.05$) according to Duncan test.

The relative contents of oleic acid varied from 65.25% (Medenin oil) to 74.32% (Matmata oil). Palmitic acid ranged between 8.03% (Matmata sample) and 10.38% (Tataoiun oil) following the geographical location. The highest content of linoleic acid was found in Medenin oil (19.31%). Regarding the ratio of monounsaturated to polyunsaturated fatty acids (MUFA/PUFA), a significant difference between samples ($p< 0.05$) was obvious between regions (Table 3). The highest ratio of MUFA/PUFA was registered in the olive oil samples from Matmata and Sfax regions (5.87% and 5.74%, respectively).

The difference observed between samples of olive oil in relation to the fatty acid composition may be explained by the geographical location, which agree with the findings of Mansour *et al.* (2015) [21]. It is probably attributed to genetic factors and also to environmental conditions during fruit growth and ripening as suggested by Morelló *et al.* (2004) [29]. Piravi-Vanak *et al.* (2012) [30] proved that the fatty acid composition of olive oil is crucially influenced by the variety, latitude, fruit ripening and climatic conditions.

Compared to other researches, Laroussi-Mezghani *et al.* (2016) [10] found that oils of *Zarazi* cultivars obtained from Zarzis region present lipid profiles different from those of Sfax region. These authors found that *Zarazi* oils showed high levels of oleic (C18:1) and stearic (C18:0) acids, which is in accordance with our results. Interestingly, Laroussi-Mezghani *et al.* (2016) [10] observed that oil samples of *Chemlali* cultivars from Sfax region showed high contents of palmitic acid (15 – 20 %), which is in contrast with *Zarazi* oil samples of the present study, characterized by low levels of C16:0 (8.03–10.38%). Compared to *Chemlali* cultivar, that as reported by Chtourou *et al.* (2013) [9] is characterized by a low level of MUFA (~60%), *Zarazi* presents a very interesting fatty acid composition. It is known that the nutritional benefits are mainly attributed to the fatty acid composition, particularly to both great concentration of oleic acid and the ratio saturated / polyunsaturated fatty acids [21].

Moreover, the high stability of *Zarazi* oils from Sfax and Matmata is also probably due to relatively low contents of PUFA and high contents of MUFA. In fact, Aguilera *et al.* (2005) [31] reported that oil samples with great MUFA/PUFA ratio showed higher stability to oxidation. Sfax oils had the highest levels of most secoiridoids which may reinforce their stability to oxidation.

Regarding squalene, it's a triterpenoid hydrocarbon, and the main compound of the unsaponificable fraction of olive oil. Some previous authors suggested that squalene concentration in olive oil is significantly affected by ripeness degree and also by geographical location of olive tree cultivars [32], which confirm the results of our study. Significant differences were observed between the squalene contents of the different olive oil samples relatives to the different studied locations ($p < 0.05$) (Table 3). The highest quantities were detected in the oils of Matmata (3118.4 µg g^{-1}) and Sfax (3092 µg g^{-1}), and the lowest was registered in the oils of Medenin (2006 µg g^{-1}). The obtained results are in accordance with those found previously by Laroussi-Mezghani *et al.* (2016) [10] for *Zarazi* olive oil.

3.8. Effects of geographical location on the phytosterol profile

β-sitosterol, campesterol and Δ-5-avenasterol were the principle sterols present in all oil samples, while stigmasterol, clerosterol and Δ-5,24-stigmastadienol were found as minor sterols (Table 4).

Table 3. Fatty acid composition (%) and squalene content (µg/g) of virgin olive oil samples of Zarazi variety growing in four different geographic areas in the south of Tunisia (Sfax, Tataouin, Medenin, Matmata)

	Locations			
	Sfax	Tataouin	Medenin	Matmata
Palmitic (C16:0)	8.61 ± 0.03 ^{c}	10.38 ± 0.24 ^{a}	9.45 ± 0.15 ^{b}	8.03 ± 0.09 ^{d}
Palmitoleic (C16:1)	0.26 ± 0.02 ^{a}	0.27 ± 0.03 ^{a}	0.23 ± 0.04 ^{a}	0.18 ± 0.01 ^{b}
Heptadecanoic acid (C17:0)	ND	0.08 ± 0.02 ^{a}	0.08 ± 0.01 ^{a}	0.06 ± 0.02 ^{a}
Heptadecenoic acid (C17:1)	ND	0.10 ± 0.02 ^{a}	0.11 ± 0.02 ^{a}	0.10 ± 0.00 ^{a}
Stearic (C18:0)	2.84 ± 0.06 ^{a}	2.60 ± 0.05 ^{b}	3.10 ± 0.02 ^{a}	2.90 ± 0.03 ^{a}
Oleic (C18:1)	73.62 ± 0.3 ^{b}	68.35 ± 0.58 ^{c}	65.25 ± 0.22 ^{d}	74.32 ± 0.06 ^{a}
Linoleic (C18:2)	12.22 ± 0.05 ^{c}	15.87 ± 0.13 ^{b}	19.31 ± 0.20 ^{a}	12.30 ± 0.11 ^{c}
Linolenic (C18:3)	0.71 ± 0.05 ^{b}	0.74 ± 0.01 ^{ab}	0.79 ± 0.03 ^{a}	0.50 ± 0.04 ^{c}
Arachidic (C20:0)	0.66 ± 0.04 ^{a}	0.48 ± 0.02 ^{c}	0.62 ± 0.03 ^{ab}	0.53 ± 0.04 ^{b}
Gadoleic acid (C20:1)	0.56 ± 0.00 ^{a}	0.52 ± 0.02 ^{b}	0.53 ± 0.03 ^{ab}	0.51 ± 0.01 ^{b}
Behenic acid (C22:0)	0.26 ± 0.07 ^{a}	0.30 ± 0.03 ^{a}	0.24 ± 0.03 ^{a}	0.26 ± 0.07 ^{a}
Lignoceric acid (C24:0)	0.26 ± 0.07 ^{a}	0.31 ± 0.08^{a}	0.29 ± 0.02 ^{a}	0.31 ± 0.06 ^{a}
SFA	12.63 ± 0.29 ^{c}	14.15 ± 0.40 ^{a}	13.78 ± 0.23 ^{b}	12.09 ± 0.27 ^{c}
MUFA	74.18 ± 0.28 ^{b}	69.24 ± 0.63 ^{c}	66.12 ± 0.22 ^{d}	75.11 ± 0.04 ^{a}
PUFA	12.93 ± 0.05 ^{c}	16.61 ± 0.13 ^{b}	20.10 ± 0.22 ^{a}	12.80 ± 0.13 ^{c}
MUFA/PUFA	5.74 ± 0.04 ^{a}	4.17 ± 0.07 ^{c}	3.29 ± 0.04 ^{d}	5.87 ± 0.06 ^{a}
Squalene (µg/g)	3092 ± 106.21^{a}	2780.8 ± 140.6 ^{b}	2006 ± 99.95^{c}	3118.4 ± 84.06 ^{a}

Each value represents the mean of three determinations (n = 3) ± standard deviation. ND not determined.
$^{a\text{-}c}$Different letters in the same row indicate significantly different values ($p < 0.05$) according to Duncan test.

Table 4. Phytosterol composition (%) of virgin olive oil of Zarazi variety from different locations in Tunisia (Sfax, Tataouin, Medenin and Matmata)

	Locations			
	Sfax	Tataouin	Medenin	Matmata
Campesterol	3.63 ± 0.00 [a]	3.72 ± 0.03 [a]	3.71 ± 0.08 [a]	3.45 ± 0.16 [b]
Stigmasterol	0.62 ± 0.03 [c]	0.96 ± 0.01 [a]	0.92 ± 0.02 [b]	0.65 ± 0.01 [c]
Clerosterol	1.7 ± 0.53 [ab]	1.65 ± 0.54 [ab]	2.29 ± 0.16 [a]	1.18 ± 0.02 [b]
β-sitosterol	83.34 ± 1.16 [ab]	84.55 ± 0.97 [a]	80.19 ± 0.33 [c]	82.33 ± 0.07 [b]
Δ-5-avenasterol	8.90 ± 0.10 [c]	6.84 ± 0.10 [d]	11.43 ± 0.05 [a]	10.71 ± 0.02 [b]
Δ-5,24-stigmastadienol	0.6 ± 0.07 [b]	0.39 ± 0.08 [c]	0.75 ± 0.04 [a]	0.84 ± 0.01 [a]
Apparent β-sitosterol	95.69 ± 0.07 [b]	95.26 ± 0.03 [c]	95.32 ± 0.07 [c]	95.87 ± 0.14 [a]

Apparent β-sitosterol (sum of clerosterol + β-sitosterol + Δ-5-avenasterol + Δ-5, 24-stigmastadienol).
Each value represents the mean of three determinations (n = 3) ± standard deviation.
[a-c]Different letters in the same row indicate significantly different values ($p < 0.05$) according to Duncan test.

As shown in Table 4, individual phytosterol levels changed significantly according to the geographical origin of olive oil samples. In the *Zarazi* cultivar, the highest phytosterol level was *β*-sitosterol, followed by Δ-5-avenasterol, characteristic of the virgin olive oil. These two major sterols were strongly and negatively correlated. Significant differences were observed in *β*-sitosterol and Δ-5-avenasterol contents in olive oil, in relation to the geographical area ($p < 0.01$). The highest level of *β*-sitosterol was observed in Tataouin oil (84.55 %), followed by Sfax (83.34%) and Matmata (82.33%), whereas Medenin oil had the lowest one (80.19 %). *β*-Sitosterol is very interesting from the biological point of view, because it opposes to the intestinal absorption of cholesterol [33]. Regarding Δ-5-avenasterol content, Medenin virgin olive oil showed the highest value (11.43 %), while *Zarazi* virgin olive oil extracted from Tataouin recorded the lowest one (6.84 %).

The analyzed samples contained low levels of stigmastereol and campesterol. In all analyzed oil samples, campesterol level was below the limit threshold fixed by EU Regulations (4%), with a global range between 3.45 % (Matmata) and 3.72 % (Tataouin). There was no statistically significant difference in the campesterol content with regard to the geographical area.

The level of Δ-5,24-stigmastadienol was quite low in all analyzed virgin olive oils and ranged from 0.39 % (Tataouin) to 0.84 % (Matmata). Concerning apparent *β*-sitosterol, it was determined by the sum of the contents of *β*-sitosterol and three other sterols (Δ5, 24-stigmastadienol, clerosterol and Δ-5-avenasterol), and reached more than the established limit of 93%, in the majority of the analyzed oil samples. The oil samples from Matmata were characterized by the highest level of apparent *β*-sitosterol (95.06%). In all samples, the level of stigmasterol was lower than those of campesterol and are in agreement with previous researches [34].

In the present study, the levels of the different phytosterols detected in *Zarazi* oil were similar to those found in the oil samples of *Chemlali* cultivar [9], and similar to some Spanish, Italian and Greek cultivars (respectively, *Arbequina*, *Coratina* and *Koroneiki*) [35].

Many studies revealed that several factors affected sterol content in olive oil such as variety, geographical area, soil type and harvest time [36]. Numerous researchers have mentioned that the geographic location had a significant effect on the amount of sterols that may be linked to various climatic conditions in each growth area, such as rainfall, temperature and humidity [25]. As described by Arafat *et al.* (2016) [36], the low percentage of *β*-sitosterol in the oil may be explained by the low rainfall in the growing area, as for Medenin that is the driest region in the south of Tunisia.

3.9. Principal Component Analysis (PCA)

The data set for the oil samples of the same cultivar in relation to different locations regarding the main studied parameters (total phenols, *o*-diphenols, *α*-tocopherol, some fatty acids, squalene, *β*-sitosterol, Δ-5-avenasterol and oxidative stability) of olive oil was subjected to chemometric analysis by PCA, in an attempt to explore possible underlying pattern of relationships. The selected chemical compounds were considered as the most important parameters to evaluate the nutritional quality of olive oil. The first component explained the 59.52% of the variance, while the second component accounted for 26.95%. The chemometric analysis showed that the four geographical areas were separated into four distinct groups. PCA indicated that clear differentiation exists between oils obtained from these different locations. The variables positively associated with factor 1 were represented mainly by *o*-diphenols, C18:1 and MUFA, oxidative stability, total phenols, squalene and *β*-sitosterol. The oil samples of Matmata were characterized by high contents of *o*-diphenols and high levels of MUFA especially C18:1 (Figure 3). The oil samples of Sfax were characterized by the highest oxidative stability and high contents of total phenols, squalene, *β*-sitosterol, C18:1 and MUFA. All these parameters form two distinct clusters (Figure 3).

Tataouin location formed another group represented by the variables negatively associated with factor 1 of PCA and included C16:0, C18:3 and *α*-tocopherol. Finally, Medenin oil samples formed the fourth cluster characterized by the highest level of polyunsaturated fatty acid (C18:2).

PCA showed discrimination between the oil samples relatives to the four studied regions and clearly showed the high quality and nutritional value of *Zarazi* oil of Sfax comparing to the other regions, due to its higher stability to oxidation and its richness in bioactive compounds such as MUFA (particularly C18:1), *β*-sitosterol, phenolic compounds and squalene.

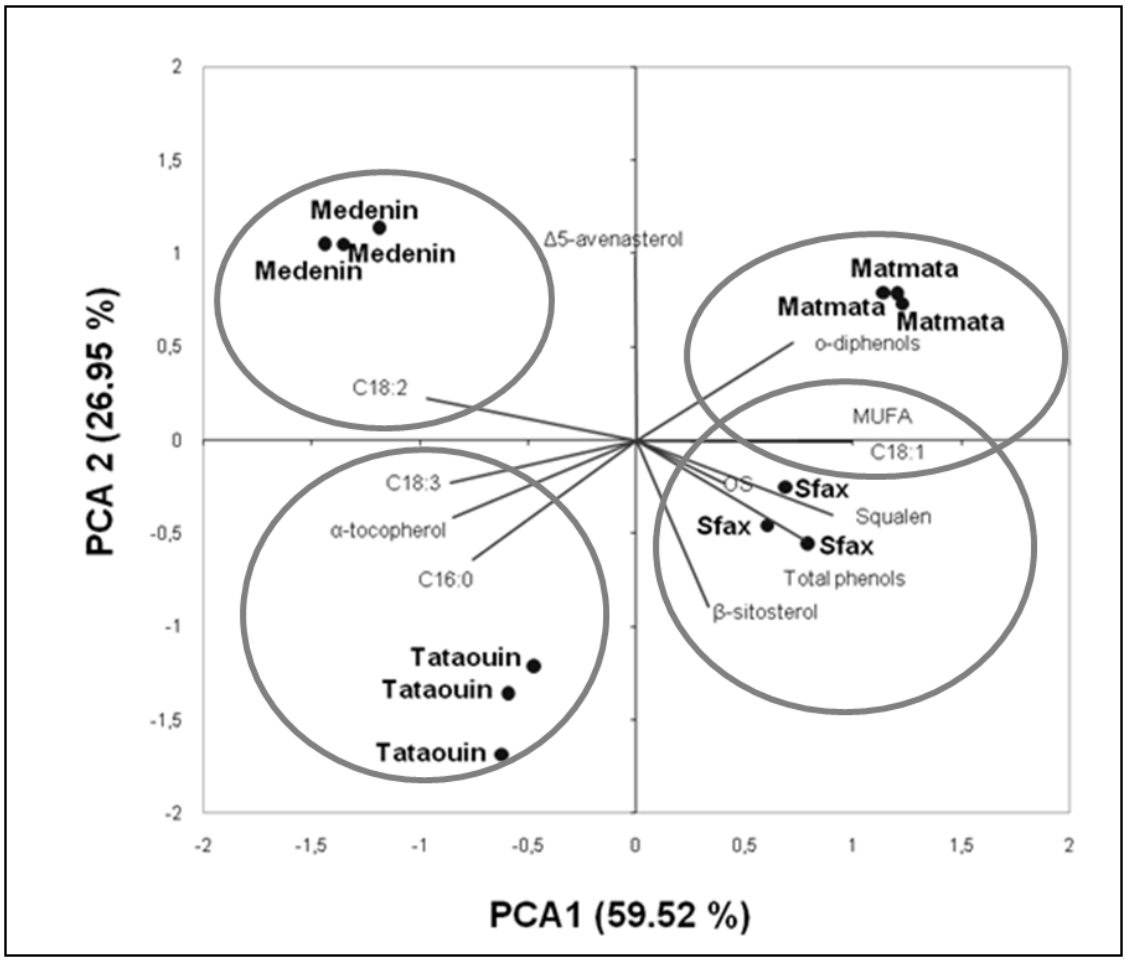

Figure 3. Principal Component Analysis. Projection on the factorial plane of oil samples obtained from different geographical regions (•) and different analyzed parameters (variables)

4. Conclusion

The present study has shown that, the most of analytical parameters, particularly phenolic compounds and the profiles of fatty acids and phytosterols showed consistent variability among growing area. *Zarazi*, a Tunisian cultivar, when grown under the same arid conditions (South of Tunisia), produced oils with different qualities. The oil samples from the region of Sfax, followed by those from Matmata, have the best quality and stability to oxidative degradation and, also, high nutritional value in terms of their richness in bioactive compounds. The geographic area appears to have a significant effect on the chemical composition of olive oil. This behavior can be explained by the variability in soil characteristics and climatic conditions (rainfall, temperature and humidity) between the studied regions. The findings of this study revealed that cultivar and geographical area conditions play a major role in the determination of oil quality characteristics. Oils from an identical variety of olive tree (*Zarazi* variety) growing in southern Tunisia have very different qualities depending on the growing region of cultivars.

Acknowledgments

This research was supported by the Ministry of Higher Education and Scientific Research in Tunisia. We would like to thank all the members of LR-NAFS /LR12ES05 laboratory «Nutrition-Functional Food and Vascular Health» and also the members of Department of Agricultural and Food Sciences, Alma Mater Studiorum – University of Bologna, Italy for their contribution to this research.

Conflict of Interests

The authors have not declared any conflict of interests.

References

[1] Bozdogan Konuskan D, Mungan B (2016) Effects of Variety, Maturation and Growing Region on Chemical Properties, Fatty Acid and Sterol Compositions of Virgin Olive Oils. JAOCS, Journal of the American Oil Chemists' Society 93:1499-1508.

[2] Arslan D, Karabekir Y, Schreiner M (2013) Variations of phenolic compounds, fatty acids and some qualitative characteristics of Sariulak olive oil as induced by growing area. Food Research International 54:1897-1906.

[3] Noorali M, Barzegar M, Sahari MA (2014) Sterol and Fatty Acid Compositions of Olive Oil as an Indicator of Cultivar and Growing Area. Journal of the American Oil Chemists' Society 91:1571-1581.

[4] Kiralan M, Ozkan G, Koyluoglu F, et al (2012) Effect of cultivation area and climatic conditions on volatiles of virgin olive oil. European Journal of Lipid Science and Technology 114: 552-557.

[5] Manai-Djebali H, Krichène D, Ouni Y, et al (2012) Chemical profiles of five minor olive oil varieties grown in central Tunisia. Journal of Food Composition and Analysis 27:109-119.

[6] Kesen S, Kelebek H, Selli S (2014) LC--ESI--MS Characterization of Phenolic Profiles Turkish Olive Oils as Influenced by Geographic Origin and Harvest Year. Journal of the American Oil Chemists' Society 91: 385-394.

[7] Del Monaco G, Officioso A, D'Angelo S, et al (2015) Characterization of extra virgin olive oils produced with typical Italian varieties by their phenolic profile. Food Chemistry 184: 220-228.

[8] Rotondi A, Magli M (2004) Ripening of olives var. Correggiolo: Modification of oxidative stability of oils during fruit ripening and oil storage. Journal of Food, Agriculture & Environment 2: 193-199.

[9] Chtourou M, Gargouri B, Jaber H, et al (2013) Comparative study of olive oil quality from Chemlali Sfax versus Arbequina cultivated in Tunisia. European Journal of Lipid Science and Technology 115:631-640.

[10] Laroussi-Mezghani S, Yveline LD, Molinet J, et al (2016) Biodiversity of Tunisian virgin olive oils: varietal origin classification according to their minor compounds. European Food Research and Technology 242:1087-1099.

[11] Boskou D, Blekas G, Tsimidou M (2006) Chapter 4: Olive Oil Composition. In: Olive Oil Chemistry, Properties, Health Effects. pp 41-72.

[12] Isabel Mínguez-Mosquera M, Gandul-Rojas B, Montaño-Asquerino A, Garrido-Fernández J (1991) Dertermination of chlorophylls and carotenoids by high-performance liquid chromatography during olive lactic fermentation. Journal of Chromatography A 585: 259-266.

[13] Tura D, Gigliotti C, Pedò S, et al (2007) Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (Olea Europea L.) and correlations with oxidative stability. Scientia Horticulturae 112: 108-119.

[14] Mateos R, Espartero JL, Trujillo M, et al (2001) Determination of Phenols, Flavones, and Lignans in Virgin Olive Oils by Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode Array Ultraviolet Detection. Journal of Agricultural and Food Chemistry 49:2185-2192.

[15] Lerma-Garcia MJ, Simó-Alfonso EF, Chiavaro E, et al (2009) Study of chemical changes produced in virgin olive oils with different phenolic contents during an accelerated storage treatment. Journal of Agricultural and Food Chemistry 57:7834-7840.

[16] International Olive Council (2009) Determination of Biophenols in Olive Oils By Hplc. International Olive Council 29:1-8.

[17] Anwar P, Bendini A, Gulfraz M, et al (2013) Characterization of olive oils obtained from wild olive trees (Olea ferruginea Royle) in Pakistan. Food Research International 54:1965-1971.

[18] International Olive Oil Council (2015) Trade Standard Applying to Olive Oil and Olive-Pomace Oil. 1-17.

[19] Servili M, Montedoro G (2002) Contribution of phenolic compounds to virgin olive oil quality. European Journal of Lipid Science and Technology 104:602-613.

[20] Mansouri F, Ben Moumen A, Lopez G, et al (2013) Preliminary Characterization of monovarietal virgin olive oils produced in eastern area of Morocco. In: InsideFood Symposium. International Closing Symposium of EU FP7 project: Integrated sensing and imaging devices for designing, monitoring and controlling microstructure of foods. pp 1-6.

[21] Mansour A Ben, Gargouri B, Flamini G, Bouaziz M (2015) Effect of Agricultural Sites on Differentiation between Chemlali and Neb Jmel Olive Oils. J Oleo Sci 392:381-392.

[22] Servili M, Selvaggini R, Esposto S, et al (2004) Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that affect their occurrence in the oil. Journal of Chromatography A 1054:113-127.

[23] Ouni Y, Taamalli A, Gómez-Caravaca AM, et al (2011) Characterisation and quantification of phenolic compounds of extra-virgin olive oils according to their geographical origin by a rapid and resolutive LC-ESI-TOF MS method. Food Chemistry 127: 1263-1267.

[24] Criado MN, Morelló JR, Motilva MJ, Romero MP (2004) Effect of growing area on pigment and phenolic fractions of virgin olive oils of the arbequina variety in Spain. Journal of the American Oil Chemists' Society 81:633.

[25] Bajoub A, Carrasco-Pancorbo A, Ajal EA, et al (2015) Potential of LC-MS phenolic profiling combined with multivariate analysis as an approach for the determination of the geographical origin of north Moroccan virgin olive oils. Food Chemistry 166:292-300.

[26] Beltrán G, Aguilera MP, Rio C Del, et al (2005) Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. Food Chemistry 89: 207-215.

[27] Hrncirik K, Fritsche S (2005) Relation between the endogenous antioxidant system and the quality of extra virgin olive oil under accelerated storage conditions. Journal of Agricultural and Food Chemistry 53:2103-2110.

[28] Shahidi F, Janitha PK, Wanasundara PD (1992) Phenolic Antioxidants. Critical Reviews in Food Science and Nutrition 32: 67-103.

[29] Morelló JR, Motilva MJ, Tovar MJ, Romero MP (2004) Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chemistry 85:357-364.

[30] Piravi-Vanak Z, Ghasemi JB, Ghavami M, et al (2012) The Influence of Growing Region on Fatty Acids and Sterol Composition of Iranian Olive Oils by Unsupervised Clustering Methods. Journal of the American Oil Chemists' Society 89: 371-378.

[31] Aguilera MP, Beltrán G, Ortega D, et al (2005) Characterisation of virgin olive oil of Italian olive cultivars: "Frantoio" and "Leccino", grown in Andalusia. Food Chemistry 89: 387-391.

[32] Manzi P, Panüli G, Esti M, et al (1998) Natural Antioxidants in the Unsaponiüable Fraction of Virgin Olive Oils From Diþerent Cultivars. J Sci Food Agric 77:115-120.

[33] Plat J, Mensink RP (2005) Plant stanol and sterol esters in the control of blood cholesterol levels: Mechanism and safety aspects. American Journal of Cardiology.

[34] Fernández-Cuesta A, León L, Velasco L, De la Rosa R (2013) Changes in squalene and sterols associated with olive maturation. Food Research International 54: 1885-1889.

[35] Pardo JE, Cuesta MA, Gómez R, et al (2012) Quality of virgin olive oil from the Designation of Origin (D.O.) "Aceite Campo de Montiel" (Ciudad Real, Spain) from two crop seasons. Rivista Italiana delle Sostanze Grasse 89: 103-111.

[36] Arafat SM, Basuny, Amany M.M. Elsayed ME, Soliman HM (2016) Effect of pedological, cultivar and climatic condition on sterols and quality indices of olive oil. Scientia Agriculturae 13: 23-29.