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Effect of geographical conditions (altitude and pedology) and age of olive plantations on the typicality of olive oil in Moulay Driss Zarhoun

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Abstract: The exact authentication of virgin olive oil (VOO) origin becomes one of the priorities of the international community, both at the legislative and commercial levels. It also concerns the ultimate destination of this product, at the consumer's level. The uses of geographical indications are considered a reliable way to guarantee the traceability of this product.

The objective of the present work is to study the resultant effect of the geographical area (altitude and pedologic conditions) and age of olive plantations on the typicality of VOO produced in Moulay Driss Zarhoun (MDZ), with the ultimate objective to attribute a protected designation of origin (PDO) to the oil. Previous recommendations of such PDO were based on a set of characteristics (typicality, originality, knowledge of production, fame, and a strong historic anchoring) according to the Moroccan Law (n°25/06). This law, dealing with the recognition and protection of distinctive signs of origin and quality (DSOQ), established the legal framework of protected geographical indications (PGI), PDO, and agricultural labels (AL).

The present research deals with the chemical characterization of VOO from MDZ, mainly its pigment and total phenolic contents, the composition in fatty acids (FA) and triacylglycerols (TAG). Based on chemometric techniques, the interpretation of the results will allow drawing the fingerprints of VOO originating from MDZ. The study was carried out during the 2012/2013 and 2013/2014 crop years and concerned 57 representative olive orchards in the MDZ area. The olives were hand-picked from different trees, at three different classes of altitudes (<300m, 300-600m, 600-900m), at three categories of age (<30 years, 30-50 years, more than 50 years), and in different types of soil. The analysis revealed high contents in total phenols, monounsaturated FA, and triolein. The quantitative differences observed in all analyzed parameters could be attributed in general to altitude and limestone percentage of soil. The impact of age in the determination of typicality of the VOO remains insignificant.

Keywords: Virgin olive oil, typicality, age, altitude, chemical characterization, Moulay Driss Zarhoun, Morocco.

Introduction

VOO plays an important role in the Moroccan agronomy and economy. With an area approximating 1.000.000 ha, the olive sector contributes about 5% in the composition of the Gross Domestic Product (GDP), covers 16% of self-sufficiency in edible vegetable oils, and generates annually more than 15 million work days, the equivalent of 70.000 permanent jobs. The annual VOO production exceeds 95.000 tons, which places Morocco as the world's 6th largest producer of VOO. The olive sector (olive oil + table olives) exports represent 15% of the national food exports; which can be largely increased through an improvement of yields and quality.

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The new Moroccan agricultural strategy (Green Morocco Plan), which covers also the olive oil sector, aims at increasing the yield and quality of fruit production in order to modernize and unlock opportunities in the olive value-chain, particularly for the export market. In this regard, the labeling via DSOQ is one of the measures highlighted in this strategy.

Presently, concerns arise over origin criteria, perceived as an effective tool to protect specific attributes of VOO's. The DSOQ such as PDO are a very interesting approach to promote and safeguard VOO's with high quality standards and distinctive characteristics. The benefits to get out from DSOQ are immense, not only from an economic point of view, but also from the sustainable development of the olive sector. However, for developing countries like Morocco, the DSOQ approaches do not sticks only to these motivations. Indeed, they may constitute a mean for organizing actors into corporations, structuring the actors intervening in the sector, promoting hygiene standards and creating interactive dynamics for developing regions of origin.

With the establishment on 2008 of the Law 25/06, the Moroccan public authorities have accomplished the first step in the qualification procedures of VOO's and other local products. Several VOO's throughout the kingdom were labeled, such as Tyout Chiadma, Ouezzane, Tafersite, Outat El Haj, and Aghmat Aylane.

The chemical composition of VOO's depends on pedo-climatic conditions, agronomic and cultural techniques (cultivar, fertilization, irrigation, pest treatment, time and method of harvesting), postharvest storage, oil extraction systems, and VOO storage (temperature, light) conditions [1,2]. In Morocco, over 90 percent of olive oil is produced from the "Picholine marocaine" cultivar. However, a great difference is shown among the composition of VOO's produced in different regions [3-5].

This variability reflects the impact of environmental conditions and knowledge of indigenous people on determining the typicality of VOO. The typicality according to Casabianca et *al*. [6] is a set of properties of similarity and distinction that determine the belonging of a PDO product to one determined category or "Type", making it possible to differentiate, identify and recognize this product as original and unique. The main components used to highlight VOO typicality are fatty acids, polyphenols, sterols, tocopherols, as well as its organoleptic characteristics [4,7-9] .The analysis of TAG in oils from different olive varieties have emphasized their usefulness as discriminating factors between olive varieties, and different regions of [10,11].

The aim of the present work is to study the typicality of VOO in MDZ area, including Volubilis ; the cradle of olive trees in Morocco. This area hosts the oldest vestiges of olive oil production with remains of Roman olive mills dating back to the 3rd century[4,12]. The age of plantations exceeds 50 years for about 2/3 of the olive groves, and the "Picholine marocaine" is the main cultivar. The area is marked by an altitude exceeding 1,180 m, but olive trees are rarely cultivated at altitudes exceeding 900 m. The soils are mostly rich in limestone [13]. The climate falls in the semi-arid Mediterranean, with minimum and maximum monthly mean temperatures lying between 15°C and 30°C. The annual rainfall is around 600 mm.

Criteria investigated for VOO typicality determination include the influence of age, altitude and soil composition. The parameters used to differentiate between VOO are FA, TAG composition, contents of polyphenols and pigments (chlorophylls and carotenoids).

Materials and methods

Sampling

Olive Sampling

The study was carried out during the 2012/2013 and 2013/2014 crop years and concerned 57 representative farms in the MDZ area (Fig. 1). The olives were hand-picked from different trees, at different classes of altitudes (<300m, 300-600m, 600-900m), at three categories of age (<30 years, 30-50 years, more than 50 years), and in different types of soil.

All trees belong to the "Picholine marocaine" cultivar, which constitutes the main olive variety cultivated in this region (representing 98% of the varieties cultivated in the area). The sampled trees were located in traditional cultivation areas, without irrigation or fertilization and with similar cultivation techniques. In order to eliminate the influence of maturation degree on VOO quality, the samples were harvested at a maturation index between 3.5 and 4.0 (Fig. 2), according to the fruit classification based on skin and flesh color [15]. Only fresh, healthy and undamaged drupes were selected.

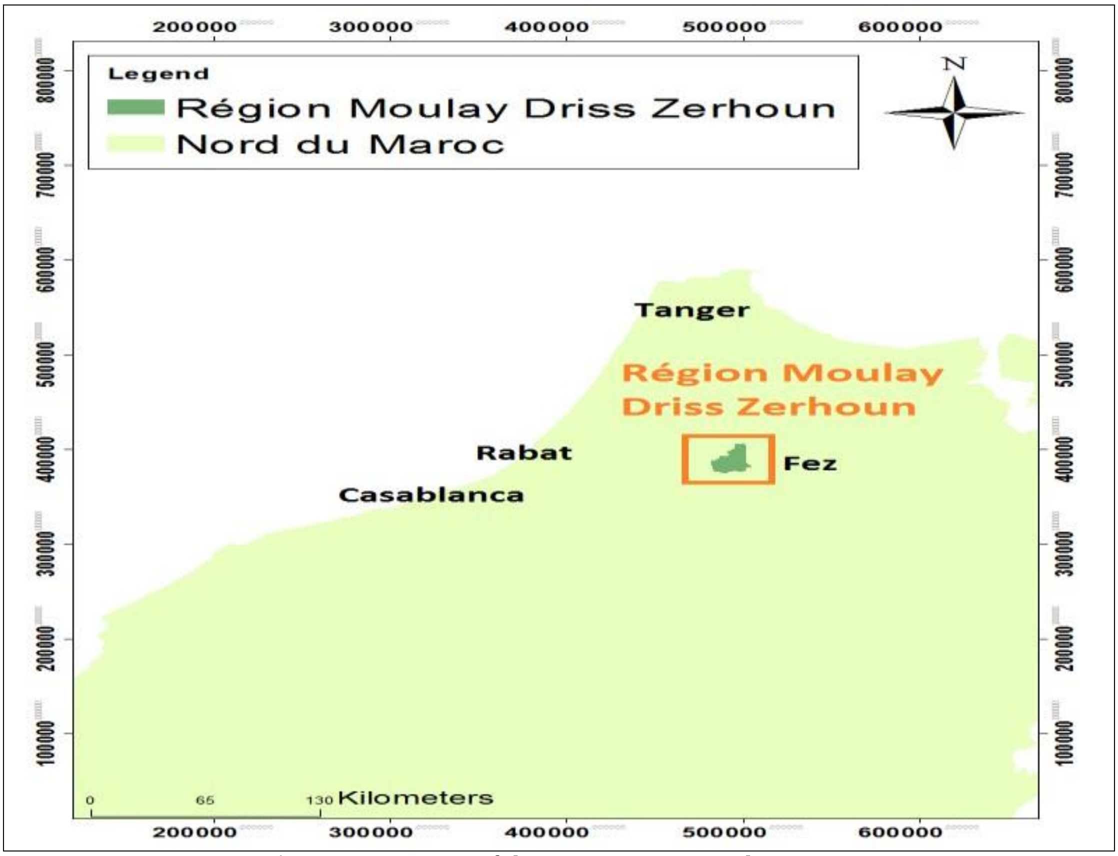

Figure 2. Location of the MDZ area in northern Morocco

Olive sampling was carried out according to the method recommended by the International Olive Council (IOC, 2011) [14].

The crushing method and the olive maturation degree play a crucial role in chemical and sensory attributes of VOO [16]. Therefore, the technique of Abencor was chosen to standardize the technological system of oil extraction.

Figure 2. Color classes and morphometric characteristics of olives (cv; "Picholinemarocaine")

Oil extraction

Extraction of VOO was carried out with the Abencor system, which reproduces the industrial process (at laboratory scale) through three basic elements: hammer mill, paste kneader, and centrifuge. The temperature and time of olive paste malaxing were around 25°C and 30 min, respectively. The VOO's obtained were then decanted, transferred into dark glass bottles and stored at dark with nitrogen gas added at 4°C, until analysis.

Physicochemical quality indices

Free acidity (expressed as % oleic acid), peroxide value (meq O_2/kg oil) and specific extinctions at 232 and 270 nm (K232 and K270) were determined according to the European Commission Regulation EEC 2568/91 [17], as amended. All parameters were determined in triplicate for each sample.

Total phenolic content

Total phenol content was quantified colorimetrically [18]. Phenolic compounds were isolated by a triple extraction from a solution of oil (10 g) in hexane (20 ml) with 30 ml of a methanol-water mixture (60:40, v/v). The Folin-Ciocalteu reagent was added to a suitable aliquot of the combined extracts and the optical density of the solution was measured at 725 nm. Values are expressed as milligrams of caffeic acid per kilogram of oil [19, 20]. A calibration curve was established with caffeic acid, in the same conditions.

Pigment content

Carotenoids and chlorophylls (mg/kg oil) were determined at 470 and 670 nm, respectively, in cyclohexane, using specific extinction values, according to the method of Minguez-Mosquera et *al.* [21].

Fatty acids

Determination of the FA composition was performed via *trans*-esterification into fatty acid methyl esters following the analytical methods described in the European Commission Regulation (EC) no. 2568/91[17], as amended. Gas liquid chromatography (GLC) analysis was carried out with a Varian CP 3380 Chromatograph, equipped with a capillary column (CP-Wax 52 CB: L=25m; Φ= 0.25mm; Ft= 0.20µm), using an injector split-splitless equipped with CP-8400 auto-sampler and a FID detector. The temperatures of the injector, the detector and the oven were held at 220°C, 230°C and 190°C, respectively. The carrier gas was Hydrogen. From these determinations, the percentages of total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids over the total fatty acid content were calculated.

Content of TAG

The determination of TAG's was carried out in a LC Jasco PU-2080 plus intelligent HPLC, equipped with a Jasco CO-2065 plus column, a Jasco RI-930 refractive index detector, and a Jasco AS-2055 auto-sampler. The column used was a Chromsep HPLC Omnispher 5 C18 (250 mm x4.6 mm). The mobile phase used was acetone/acetonitrile (50:50 v/v), with a flow rate of 1.5 ml/min, and an oven temperature of 35°C. The identification of TAG's was similar to that reported by the official method of the European Commission Regulation EEC 2568/91,[17] as amended.

Soil analysis

The physico-chemical analysis of the soils studied included soil texture, pH, organic matter, total limestone, assimilable phosphorus, exchangeable potassium, sodium content, electrical conductivity and soil moisture

Statistical analysis

Statistical data processing was performed using the SPSS software. To study the effect of age and altitude on the different characteristics of the oils, a two way (three age groups and three altitude classes) multivariate analysis of variance (MANOVA) was applied to all characteristics, followed by a two way univariate analysis of variance (ANOVA) for each characteristic taken separately. In case of presence of significant interaction between age and altitude, a three one way analysis of variance (one in each class of altitude) was performed to investigate the effect of age on the characteristic in question. Moreover, in case of existence of significant differences, the analysis of variance was completed by multiple comparisons of means, using the Student-Newman-Keuls test, to identify homogenous groups of means.
Furthermore, we determined the Pearson correlation coefficients to assess the degree of relationship between altitude (taken as quantitative variable) and the different characteristics of the oil.

On the other hand, and to determine the effect of soil parameters on the characteristics of the oils, we opted for the Canonical Analysis (CA). Indeed, CA is a multidimensional descriptive statistical method that aims to explore the relationships that may exist between two groups of quantitative variables observed on the same set of individuals. In our case, the first group of variables concerns the different soil characteristics (e.g., pH, limestone content,..) and the second group relates to the oil characteristics (e.g. phenol and FA contents,..).

Results and discussion

Effect of tree age and altitude

Quality indices

Quality indices (free acidity, peroxide value and UV absorption characteristic, K232, and K270) correspond to the "extra" VOO category, as defined by the Moroccan regulation applying to olive oils and olive-pomace oils.
The high quality of analyzed VOO's may be explained by the healthy and undamaged fruits and the gentle Abencor method used for oil extraction.

The results of statistical analysis showed absence of a significant effect of the growing area conditions on the quality indices.

Pigment contents

The natural pigment contents of VOO's are important quality parameters because they correlate with color and play a key role in the sensorial acceptability among consumers. VOO presents a color range from green-yellow to golden, depending on the variety and the stage of maturity. [22]

Quantitative results of pigment content in MDZ VOO samples are given in Table 1.

The average concentrations of chlorophylls and carotenoids were 5.08 and 1.25 mg kg^{-1}. Oils obtained from olives cultivated at higher altitudes showed higher chlorophyll contents compared with those from lower altitudes (R=0,671, p~000). Chlorophyll average content varied between 3.40 mg/kg for the first class of altitude, 5.34 mg/kg for the second class and 8.76 mg/kg for the last class of altitude. Elevation influenced not only the chlorophyll content but also that of carotenoids which followed the same trend, ranging from 0.93 mg/kg (class<300m) to 2.63mg/kg (class 600-900m). Indeed, the interaction between age of trees and class of altitudes in terms of pigment content was highly significant (p~0.000).

Table 1. Mean values of pigment contents in VOO's extracted from MDZ olives, at different altitudes and categories of age

		Classes of altitude								
		< 300m			300-600m			600-900m		
		categories of age (years)			categories of age (years)			categories of age (years)		
Parameters	Correlation Coefficient with altitude	10-30	30-50	> 50	10-30	30-50	>50	10-30	30-50	>50
Chlorophylls (mg kg−1) (*)	R = 0,671	$3,21^{a}$	$3,05^{a}$	$3,56^{a}$	$5,42^{b}$	$5,31^{b}$	$5,16^{b}$	$8,37^{c}$	$11,86^{d}$	$5,70^{e}$
Carotenoids (mg kg−1) (**)	R= 0,472	$0,76^{a}$	$0,81^{a}$	$1,01^{a}$	$1,27^{b}$	$1,19^{b}$	$1,36^{b}$	$2,23^{c}$	$3,17^{d}$	$1,30^{e}$

(*), (**) In the same class of altitudes, values followed by identical letters indicate that the differences between the means for the 3 categories of age are not significantly different (Student-Newman-Keuls , with p= 0.05).

To determine the nature of this interaction, we studied the effect of different ages in different classes of altitude. Therefore, for the three classes of altitude, chlorophyll content increased independently of age, up to an altitude of 600m. However, for oldest trees, chlorophyll contents stagnated at altitude higher than 600 m, and the same trend was shown for the carotenoid contents. No clear explanation was found for these facts.

Total phenolic content

The determination of total phenol content in VOO is of great nutritional and commercial importance, since these compounds have become the subject of intensive research, due to their biological activities (cancer, atherosclerosis, rheumatoid arthritis), their influence on the organoleptic properties of VOO and their contribution to its oxidative stability [23].

The extra VOO obtained from Picholine olives collected at different altitudes showed quite high total phenols content, exceeding sometimes 850 mg kg^{-1}.

MANOVA analysis showed that there was no significant interaction between age and altitude (p = 0.58) on the phenolic content. The effect of the classes of altitude on phenols was clearly observed, independently of the category of age. For age (Table 2), the Anova showed that there was no significant difference between the different age brackets, but it showed that the rate of polyphenols (Fig. 3) is higher in the oils extracted from trees aged between 30 and 50 years (623.2 mg kg^{-1}, as caffeic acid), younger trees gave the least rich oils in polyphenols (532.8 mg kg^{-1}, as caffeic acid).

Pearson's correlation showed that the total phenol content was positively correlated with the altitude (R = 0,819, p ~ 0.000) ranging from 291,0 mg/kg (for altitude 115m) to 890.1 mg/kg (for altitude 820m). These results are in good agreement with those reported for the "Cornicabra" variety (Salvador et *al*., 2001). Similar mean values were also reported for "Burlat" and "Van" Cherry cultivars [24] and for "Chetoui" cultivar [25]. It is possible that the lower temperatures at higher elevation sites trigger the mechanism of phenols' biosynthesis.

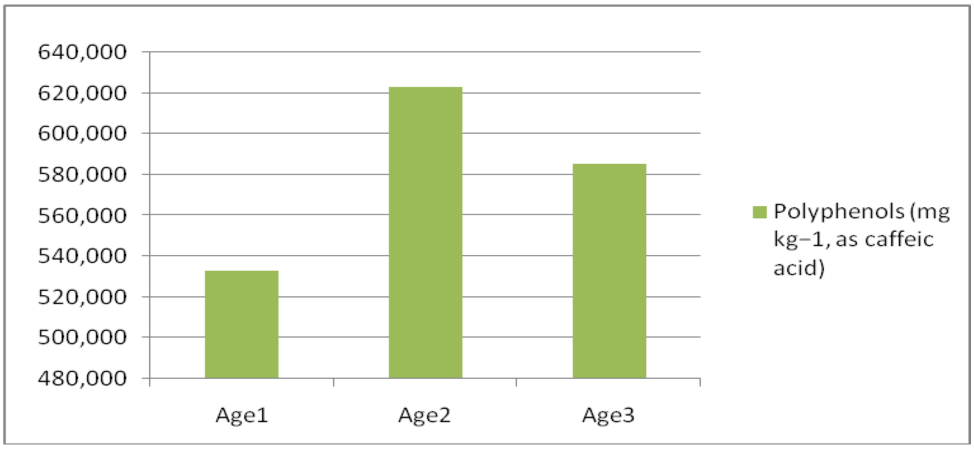

Figure 3. Variation of total phenols' contents (mg kg^{-1}, as caffeic acid) for different tree ages

Table 2. Mean values of analyzed parameters in VOO extracted from MDZ olives, at different categories of age

Parameters	Polyphenols(mg kg^{-1}, as caffeic acid)	C16:0	C18:0	C18:1	C18:2	MUFA/PUFA	oleic/linoleic	OOO	LLL	PLL	Total of major TAG (%)
AGE1	532.8	10.2	2.9	73.5	10.6	6.8	7.2	41.5	0.3	0.7	87.8
AGE2	623.2	10.3	3.0	72.9	11	6.5	6.8	42.1	0.2	0.6	88.3
AGE3	585.4	10.3	3.0	73.8	10.7	6.8	7.2	42.4	0.2	0.6	88.4

O= Oleic acid; L= Linoleic acid; P=Palmitic acid

Fatty acids

All the analyzed samples showed FA contents within the ranges required by the European Commission regulation EEC n° 1989/2003.[26] Oleic acid (66.9 to 78.7%) and linoleic acid (7.7 to 16.3%) were quantified as the major FA. Significant differences ($p<0.05$) were found between the levels of the main FA, which were sharply influenced by altitude (Table 3).

Palmitic, stearic and linoleic acids showed a slight decrease with an inconstant variation among altitude locations, whereas the levels of oleic acid decreased gradually, from higher to lower altitudes. On the contrary, palmitic, stearic and linoleic acid levels showed an opposite trend. The MUFA/PUFA and oleic/linoleic ratios increased at the highest elevations (from 5.1 to 8.1) and (from to 5.4 to 8.8), respectively (Table 3). These ratios increases were probably related to the fact that lower temperatures favor oil mono-unsaturation. According to the IOC standard applying to olive oils and pomace-olive oils (IOC, 2015), [27] oleic acid and linoleic acid contents vary between 55-83% and 2.5-21,0%, respectively. There is an inverse proportional relationship between oleic acid and linoleic acid. VOO with high oleic acid will be low in linoleic acid, and vice versa. The reason for this is that climate, altitude and variety all influence the FA profile. In general, cooler, high altitude environments will produce higher oleic acid levels than hot, low altitude environments. Lower temperatures reduce the rate of chemical reactions and microbial activity that may result in the loss of quality of the olive fruits and consequently, the loss in extracted oil quality. Similar results were reported [25,28,29] for different cultivars and different geographical areas.

VOO with high oleic acid is nutritionally preferable and potentially more stable than low oleic olive oil.

From the ANOVA and the measure of Pearson Correlation, a significant correlation (Table 3) was found between altitude and palmitic, stearic, oleic and linoleic acids. Age of olive trees (Table 2) did not affect the composition of VOO in FA, except for some minor FA like palmitoleic acid ($p = 0.02$) and behenic acid ($p = 0.03$).

Table 3. Mean values of analyzed parameters in VOO extracted from MDZ olives, at different altitudes.

Parameters	Altitude 1	Altitude 2	Altitude 3	Correlation coefficient
Polyphenols (mg kg−1, as caffeic acid)	395.5^{a}	571.2^{b}	774.9^{c}	R= 0.819
Palmitic acid	10.7^{a}	10.2^{b}	9.5^{c}	R= - 0.611
Stearic acid	3.4^{a}	2.9^{b}	2.6^{c}	R = - 0.499
Oleic acid	69.9^{a}	73.9^{b}	76.4^{c}	R= 0,765
Linoleic acid	13^{a}	10.5^{b}	8.8^{c}	R= -0,668
MUFA/PUFA	5.1^{a}	6.7^{b}	8.1^{c}	R= 0,737
oleic/linoleic	5.4^{a}	7.1^{b}	8.8^{c}	R= 0,733
Triolein	38.4^{a}	42.1^{b}	45.6^{c}	R = 0.807
Trilinolein	0.4^{a}	0.2^{b}	0.1^{c}	R = -0,654
Palmitodilinolein	0.9^{a}	0.6^{b}	0.4^{c}	R = -0,738
Total of major TAG	86.5^{a}	88.2^{b}	89.7^{c}	R = - 0,750

Values followed by identical letters (for each parameter) are not significantly different (Student-Newman-Keuls , with α= 0.05)

TAG composition

The main TAG peaks in analyzed VOO samples were OOO, POO+SOL, OOL+PLnP+PoOO, POL+SLL+PoOP and SOO (Total 1); these accounted for more than 84% of the total area of peaks in the chromatogram (Fig. 4).

The level of triolein (OOO), was remarkably high, with a mean concentration of 41.6% and a range from 34.3 to 46.7%. A similar triolein content has been reported for the “Picholine marocaine” cultivar [30]. The second peak in order of quantitative importance in oil samples corresponded to the POO+SOL mixture, with an average content of 18.2% and a range from 16.7 to 19.8%. This peak has been reported as the second in importance in oils from different olive varieties [31,32]. The next TAG fraction is OOL+PLnP+PoOO, with a mean content of 17.1%, ranging from 14.3 to 21.1%. The POL+SLL+PoOP, and SOO contents in VOO from MDZ are relatively high (averages of 6.0% and 5.0%, respectively), as compared to the corresponding average contents in other VOO from varieties, such as “Cornicabra” [33]. Similar results for VOO’s from the “Picholine marocaine” cultivar were reported by El Antari et *al*.[3].

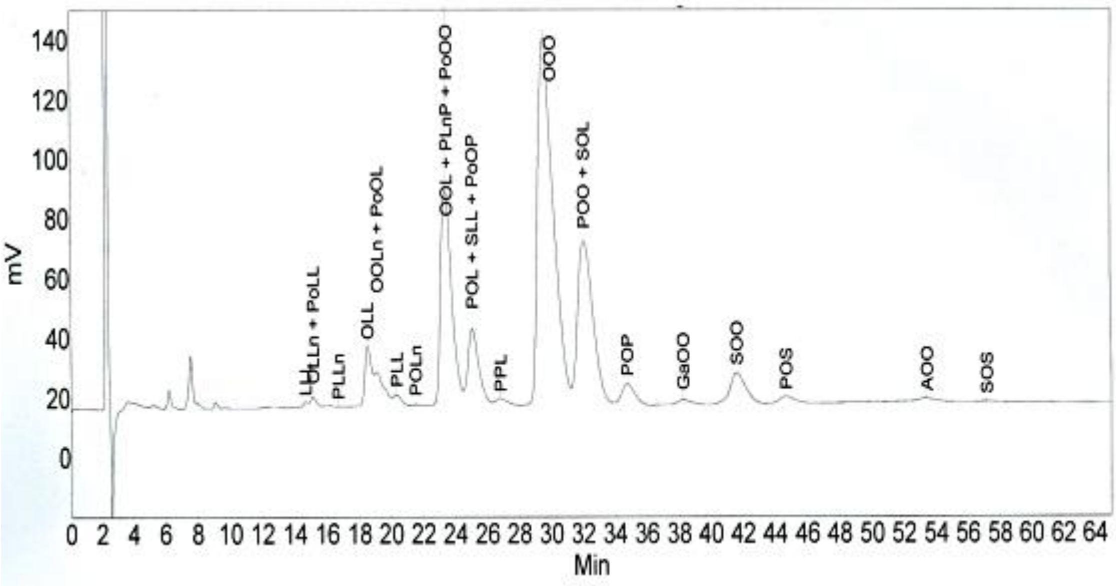

Figure 4. Typical chromatogram of the TAG fraction in VOO

The Pearson's correlation (Table 3) results between altitude and percentage of the triglycerides showed a positive correlation between altitude and triolein (OOO), and between altitude and the amount of the major TAG (OOO, POO+SOL, OOL+PLnP+PoOO, POL+SLL+PoOP). With regard to the FAs in the sn-2 position of the TAGs, oleic acid is predominant. This result can be justified by the 1, 3-random-2-random distribution theory of FA on the glycerol backbone [33].

Thus, we can conclude that with increasing altitude, monounsaturated FA increase, along with TAG consisting of monounsaturated FA. Conversely, strong negative correlation coefficient ($p< 0.05$) was found between altitude and TAG consisting of palmitic and linoleic acids (PPL) and linoleic acid (LLL). This is related to the FA composition of VOO. Since linoleic and palmitic acid contents decrease with altitude, the TAG comprising such FA will decrease in the same way.

Effect of pedologic conditions:

Fifty seven (57) samples of soil and oils were subjected to canonical correlations analysis to determine the relationship between the variables of the two groups. Soil variables regroup pH, limestone content, moisture, organic matter, available phosphorus, available potassium, nitrogen and electric conductivity. Analytical oil variables regroup phenolic content, FA (C16:0, C18:1, C18:2, C18:3, MUFA/PUFA and oleic/linoleic) and the major TAG (OOO, POO+SOL, OOL+PLnP+PoOO, POL+SLL+PoOP).

Calculation of Wilks' Lambda (F =2.103, $p = 0.016<0.05$) index showed that there is a significant multivariable effect of soil parameters on VOO characteristics at 95% of the confidence level. The main canonical correlations analysis results are reproduced below.

The measure of relationships between the two groups of analyzed variables is determined by calculating a series of pairs of new variables (called canonical variables) which best summarize the main information contained in the original ones. A correlation coefficient is calculated between the two canonical variables in each pair, giving a series of canonical correlation coefficients.

As shown in Table 4, the canonical correlation coefficients are presented in descending order according to their importance in the summarized information.

Table 4. Canonical correlations between VOO characteristics and soil parameters.

Canonical correlation	Value of Correlation coefficient
1	0.87
2	0.75
3	0.59
4	0.53
5	0.41
6	0.33
7	0.29
8	0.24
9	0.08

Since "the smallest" group (soil parameters) includes only 9 variables, thus 9 canonical correlations can be determined. It is to be noted here that the main information is summarized by the two first canonical correlation coefficients. Indeed, there is a strong relation between the two groups of variables, because the first canonical coefficients are very high (0.87 and 0.75).

Furthermore, the results showed that two statistically discriminant functions are formed, the first discriminant function (X) accounts for 52.3% of the total variance. The second (Y) for 22.7% of the total variance. Both discriminants account for 75,0% of the total variance. The X- and Y-loadings indicated that palmitic, linoleic, and linolenic acids could negatively correlate with limestone percentage of soil. On the contrary, oleic acid, MUFA/PUFA and oleic/linoleic levels showed an opposite trend with limestone percentage of soil. Similar results for the influence of the soil parameters on oil composition [18]. The polyphenols and the chlorophyll contents decrease with an increased percent of limestone in the soil. We also noted a negative correlation between the soil organic matter and the linolenic acid content. The other soil parameters did not show a significant effect on the composition of olive oil.

Conclusion

According to the results obtained in this study, the typicality of VOO from MDZ area derives from its chemical composition, especially its high contents in total phenols, pigments, oleic acid, and triolein.
The quantitative differences observed in all analyzed parameters could be attributed in general to altitude and limestone percentage of soil. The impact of age

in the determination of typicality of the VOO remains insignificant.

The current study will contribute to the establishment of a PDO for MDZ VOO, since it includes a comprehensive characterization of the VOO produced in this area and an appropriate determination of its typicality. Organoleptic evaluation of VOO, to be carried later on, might show further significant differences and contributes to a better differentiation of oils in MDZ area.

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