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Research Article

Rheological behaviour, fatty acid composition and pigment content of Moroccan Picholine virgin olive oil during three harvest periods

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Abstract

The physicochemical, rheological, and fatty acid composition of six virgin olive oils extracted from the Picholine variety in the Beni Mellal-Khénifra region (Morocco) were studied and compared over three harvest years: 2018-2019, 2019-2020, and 2020-2021. These results show that all samples are rich in oleic acid, with the lowest amount observed for samples harvested in 2018-2019, followed by samples from 2019-2020 and finally those from the 2020-2021 harvest. It was also shown that all virgin olive oil samples exhibited Newtonian behaviors at shear rates between 60 and 120 s⁻¹ and that the average dynamic viscosity of the 2018-2019 samples was $0.045 \pm 1.14 \cdot 10^{-4}$ Pa.s, below that observed for the 2019-2020 period ($0.063 \pm 4.92 \cdot 10^{-4}$ Pa.s). The latter was in turn lower than the viscosity recorded for the 2020-2021 harvest ($0.071 \pm 1.90 \cdot 10^{-4}$ Pa.s). Analysis of fatty acid composition shows that olive oils with a high oleic acid content have a higher viscosity than those with a low content. Finally, multivariate statistical analyses (Principal Component Analysis and Hierarchical Cluster Analysis) showed good classification between samples according to their physicochemical properties and year of harvest. The olive oils produced in 2018 were significantly different from those harvested in 2020, probably due to their viscosities and fatty acid profiles.

Keywords

chemometrics, fatty acid, rheological behaviors, virgin olive oils, viscosity

Abbreviations

FAC – fatty acid composition; HCA – Hierarchical Cluster Analysis; PCA – Principal Component Analysis; VOO – virgin olive oils

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Introduction

Olive oil is a food staple of Moroccan cuisine and today it is a valuable and recognized ingredient in many other cultures. Olive oil is well known as a vegetable oil that is directly extracted from the fresh fruit of the olive tree *Olea europaea* L., treated mechanically under a controlled temperature regime, and then used in its pure form. This is why olive oil retains its natural properties, similar to those of olive fruit, while preserved in the vacuoles. Olive oil is highly sought after by consumers worldwide due to its abundance of monounsaturated fatty acids and bioactive compounds, as well as its numerous benefits for human health (Lozano-Castellón et al. 2020).

In 2020, world olive oil production was approximately 3.2 million tons, the vast majority of which came from Mediterranean countries (COI 2022). and according to data from the International Olive Council (IOC) for the 2020/2021 crop year, Morocco accounted for about 3% of world olive oil production (COI 2022). "Moroccan Picholine" is the variety most cultivated, it represents more than 90% of the olive trees growing at a national level.

Overall olive oil consumption has grown almost parallel with production over the last three seasons (2019/20, 2020/21, and 2021/22) (International Olive Oil Council - Consumption 2018; International Olive Oil Council - Production 2018). Even in nations without a tradition of cultivating olive oil, this notable rise in olive oil consumption can be attributed to its nutritional value and health benefits, particularly antioxidant, anti-inflammatory, antiviral, anticancer, antitumor, antiviral, and immune-modulating activities (Lozano-Castellón et al. 2020).

The quality and chemical properties of virgin olive oil (VOO) are affected by various factors, such as genotype, soil and climatic conditions, geographic origin, agronomic and irrigation methods, the extraction process, tree age, fruit ripening, and production zone (Miho et al. 2021). In addition, it was demonstrated that storage conditions after processing significantly influence the quality of VOO (Vekiari et al. 2007). Several studies have observed an accumulation of polyunsaturated fatty acids with a decrease in pigments, total phenols, and of oxidative stability during the ripening of the olives (Bouaziz et al. 2004; Gómez-Alonso et al.

2003; Yousfi et al. 2006). Borges et al. showed a decrease in the peroxide value, extinction coefficient at 232 and 270 nm, chlorophyll, carotenoids, and oleic acid contents during ripening (Borges et al. 2017). In the same way, Piscopo et al. observed a lower quality trend in VOO variants resulting from a later ripening state of olives (Piscopo et al. 2018). In addition, knowledge of the physicochemical properties (acid value, peroxide value, fatty acids composition, thermostability, cloud point, thermal value and flash point) and the rheological behavior (viscosity, plasticity, elasticity, pour point) of VOOs are important aspects for the conception an adequate production process, the analysis of the production costs and the final quality evaluation.

Viscosity is a crucial characteristic of VOO and other vegetable oils (Kim et al. 2010). Rheological analysis was used to measure the viscosity of VOO and other olive oil categories (extra virgin olive oil, refined olive oil) to determine if the fluids (olive oils) are Newtonian. The viscosity of liquids depends mostly on the molecular bond forces and their molecular weight (Kim et al. 2010). These rheological behaviors of vegetable oils are influenced by many other factors such as temperature, shear rate, concentration over time, pressure, physicochemical characteristics, fatty acid composition, additives, and catalysts (Wan Nik et al. 2005). The viscosity of a Newtonian fluid stays constant as the shear stress changes. On the other hand, this type of flow is influenced by temperature changes (Santos et al. 2005).

Several research articles and reviews have been published regarding the analytical methods used to identify and characterize VOOs. Nevertheless, the majority of these published techniques have relied on chromatographic techniques, like high-performance liquid chromatography (HPLC) (Palamthodi et al. 2021), gas chromatography, capillary electrophoresis, and polymerase chain reaction (PCR) via DNA fingerprinting. These approaches are expensive, time-consuming, and require skilled operators. Therefore, they are not suitable as a routine screening method or as a fast quantitative analysis at an industrial scale. The aforementioned situation has led analytical chemists to enhance these techniques by increasing their speed, user-friendliness, and compatibility with chemometric treatments.

UV-vis spectroscopy is a spectroscopic method that quantifies the total absorption spectrum. The method developed by [Domenici et al. \(2014\)](#) is a relatively new method that measures pigments in extra virgin olive oils from the near UV-Vis absorption spectra, such as β -carotene and lutein among carotenoids, and pheophytin A and pheophytin B among chlorophyll compounds ([Domenici et al. 2014](#); [Lazzerini et al. 2017](#)).

This spectroscopic approach has recently been tested on virgin and extra virgin olive oil samples. It was confirmed by comparing it with the standard HPLC-DAD method ([Lazzerini et al. 2017](#)), confirming its reliability, quality and high traceability. Furthermore, it has been useful to investigate the impact of different crop years on the main pigment content of several extra virgin olive oils. Other methods using near-UV spectra for analyzing olive oils have been developed with stoichiometry approaches. These techniques are very useful for olive oil quality and purity, but their application requires relatively complex data processing and/or appropriate software.

Numerous studies on the rheological properties of VOOs have also been published ([Abedinzadeh et al., 2016](#); [Tudose et al. 2014](#)). These researches have aimed to understand how the composition of vegetable oils, in particular the content of fatty acids, affects their physical parameters and rheological behavior ([Santos et al. 2005](#)).

The main objective of this study is to examine the chemical and physical properties of different VOOs. This includes analyzing their chemical composition, measuring their dynamic viscosity, and determining if there is a relationship between the dynamic viscosity of VOO and their oleic and linoleic acid content. This correlation could help to better understand the physical properties of VOO and predict their behavior in different scenarios and also, to compare results obtained from different sources and harvest years of VOO. This would help identify variations in the chemical and physical characteristics of VOO and determine if these differences have an impact on their overall quality.

Materials and Methods

Sample description. In this study, six VOOs samples were selected and collected in the Beni-Mellal Khénifra region (Morocco) from the

Picholine variety provided by olive growers coming from irrigated orchards. The olives used to produce these samples were harvested in three different seasons, namely 2018, 2019 and 2020. The 2018 samples were harvested between December 10 and 28, the 2019 samples were harvested from December 15 to 28, and the 2020 samples were harvested limited to the period from October 16 to 28. The climatic conditions during all harvest periods are known and can be found on the Internet ([Historique Météo 2009](#)). The local olive growing and harvesting areas are shown in Fig.1. All samples were produced by stainless steel disc mills and then pressed by hydraulic press systems. They were then coded according to their region of origin, namely ATN for "Ait Nouh", SD for "Sidi Amrou", ZC for "Zaouit Chikh", LH for "Lahri", TNT for "Taounate" and KS for "El Kessiba". The samples were stored in black bottles between 12 and 17°C to avoid oxidation, solidification and degradation of olive oil quality, and were analyzed no later than two weeks after sampling. The maturity stage of the olives at harvest was purple (P)/black (B), according to the maturity index defined by Loudiyi et al. ([Loudiyi et al. 1984](#)). All these samples were classified as "Virgin Olive Oils" according to their analytical indices (European regulation CE 1234/2007, annex XVI). All analytical grade chemicals used in this work were purchased from Sigma-Aldrich (France) and used without further purification.

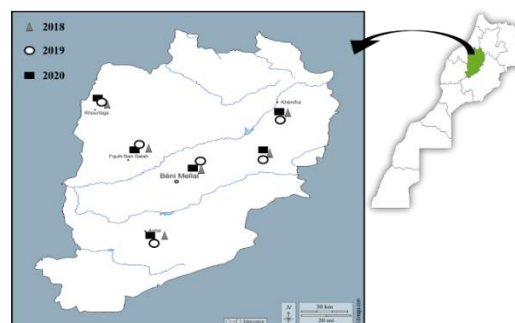


Figure 1. Virgin olive oil samples from different harvest years (2018, 2019 and 2020) produced in the Beni Mellal-Khénifra region (Morocco) from Moroccan Picholine. The place of origin of the olives is indicated for all the samples studied

Physical-chemical characterization

Fatty acid composition. After each sample was prepared according to the standard (EN ISO

5509:2000 standard), fatty acids composition of the virgin olive oils was analysed using an Agilent 7890 series gas chromatograph (Agilent Technologies, Santa Clara, CA), equipped with an autosampler and an HP-88 fused silica capillary column (100 m, 0.25 mm, 0.2 μ m, Agilent Technologies). The temperature of the injector and detector (FID) was set at 250 and 280°C, respectively. The temperature was initially set at 120°C for 1 min, then increased by 10°C per min until it reached 175°C. It was then maintained at this temperature for 10 minutes, followed by a gradual increase of 5°C per minute until it reached 210°C. Finally, it was held at 210°C for 27 min. Helium was employed as the carrier gas. The fatty acids composition was quantified as a percentage of the total peak area of all fatty acids that were identified by comparing their retention times of standard mixtures of fatty acid methyl esters. Three repetitions of each measurement were recorded.

Ultraviolet-visible spectroscopy (UV-vis) analyses. Without dilution or treatment, the virgin olive oil samples were centrifuged at 5,000 rpm for 30 min at room temperature ($\approx$ 25°C), in order to reduce light absorption and scattering caused by suspended particles. Using quartz cells with an optical path of 1 cm, UV-vis absorption spectra of virgin olive oil were measured at room temperature over a wavelength range of 200-720 nm using a UV-vis spectrophotometer (JASCO V-730). The results were then analyzed using an Excel compatible mathematical tool developed by Domenici et al. (Domenici et al. 2014). This approach is described in detail and optimized in previous works (Borello and Domenici 2019; Domenici et al. 2014; Lazzerini and Domenici 2017).

By mathematically analyzing the absorption spectrum of virgin olive oils, we successfully determined the concentrations of the six primary pigments: β -carotene, lutein, pheophytin A, pheophytin B, chlorophyll-a, and chlorophyll-b. This analysis was based on the molar extinction data provided in the original reference (Lazzerini et al. 2017). For this purpose, we used a deconvolution method consisting of separating the experimental spectrum into four orthogonal spectra derived from the original pigment spectra, as described in detail in reference (Borello and Domenici 2019). This procedure allows us to obtain, as output, content of the six pigments, along with other important

parameters like the ratio between the total amount of carotenoids and chlorophyll derivatives, and the percentage of lutein in relation to the carotenoid fraction, which are all significant factors to consider.

Rheological behaviors. This analysis was carried out to investigate the rheological behavior of VOO using a rotating viscometer (rheolab qc, Anton paar), with a concentric cylinder type CC27 (iso 3219 din 53019) where we put 15 mL of the sample. The analyses are treated by the start RheoPlus software.

The effect of shear rate ranging from 60s⁻¹ to 120s⁻¹ on viscosity was studied using the following equation (Newton's law):

$$\tau = \mu\gamma \quad (1)$$

Where τ is shear stress, Pa, μ dynamic viscosity, Pa.s and γ shear rate, s⁻¹.

Statistical analyses. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using unscrambler version 10.4 software. All tests were performed in three replicates. PCA results were presented in the form of score diagrams. HCA results were presented as dendrograms, using squared Euclidean distances between two samples to determine the similarity between them based on their different attributes (Malomo et al. 2021; Nadif and Govaert 2010; Pages 2004)

Results and Discussion

Fatty acid composition. In vegetable oils, FAC is a critical indicator of their nutritional as well as their oxidative stability. Fatty acid composition of olive oil is very well known to be a source of oleic acid, which is an omega-9 monounsaturated fatty acid. The FAC of virgin olive oils for the three harvest years 2018, 2019, and 2020 were shown in Table 1. For all samples, the fatty acid content was within the standard limits for olive oil established by the International Olive Oil Council (Bajoub et al. 2015; COI 2019), and within the range of published values for Moroccan olive oil (Bajoub et al. 2015; Bouymajane et al. 2020). As can be seen in Table 1. a total of 4 FAs from six samples of virgin olive oil were determined over three years. These are Palmitic (C16:0), stearic (18:0), oleic (C18:1n9), and linoleic (C18:2n6) FAs. There are considerable

variations in the average values of the fatty acid composition of the VOOs according to the samples studied and the harvest years (Table 1.). In all virgin olive oil samples studied, the most abundant fatty

acid is oleic acid, followed by palmitic acid, linoleic acid, and stearic acid, which agrees with other studies like Benito et al. (Benito et al. 2010) and Paciulli et al. (Paciulli et al. 2021).

Table 1. Fatty acids composition of VOO samples from three harvest seasons (2018-2019, 2019-2020, and 2020-2021)

Fatty acids, %	Years	ATN	KS	Samples		TNT	ZC	VOO ^a
				LH	SD			
Palmitic acid (C16:0)	2018-2019	7.80±0.07	9.24±0.13	8.83±0.08	8.72±0.08	9.67±0.16	7.83±0.14	7.5-20.0%
	2019-2020	9.28±0.08	9.55±0.04	9.04±0.18	8.88±0.14	10.08±0.13	8.03±0.11	
	2020-2021	8.13±0.16	9.63±0.01	9.16±0.2	8.09±0.18	9.38±0.06	8.14±0.11	
Stearic acid (18:0)	2018-2019	2.48±0.05	2.09±0.08	2.60±0.06	2.26±0.18	2.23±0.02	2.64±0.08	0.5-5.0%
	2019-2020	3.14±0.03	2.71±0.03	3.13±0.16	3.57±0.03	2.67±0.08	3.62±0.04	
	2020-2021	2.15±0.2	2.2±0.17	3.19±0.04	2.87±0.07	2.24±0.13	2.7±0.35	
Oleic acid (C18:1n9)	2018-2019	79.49±0.06	79.48±0.06	79.48±0.13	80.31±0.03	78.21±0.01	79.34±0.27	55.0-83.0%
	2019-2020	82.03±0.28	81.34±0.10	80.33±0.07	82.72±0.08	80.15±0.08	81.2±0.16	
	2020-2021	81.94±0.03	81.57±0.08	80.56±0.04	82.67±0.07	81.29±0.05	81.62±0.01	
Linoleic acid (C18:2n6)	2018-2019	7.87±0.03	6.81±0.13	6.85±0.21	6.49±0.12	7.51±0.10	7.90±0.01	3.5-21.0%
	2019-2020	7.15±0.21	7.6±0.10	7.12±0.06	8.14±0.04	8.42±0.35	7.93±0.7	
	2020-2021	7.04±0.02	6.35±0.14	6.76±0.03	6.16±0.03	6.8±0.01	7.38±0.11	
Other FA	2018-2019	2.29±0.04	0.19±0.01	2.18±0.15	2.15±0.01	2.34±0.13	2.23±0.16	-
	2019-2020	3.99±0.02	1.17±0.06	2.88±0.05	3.75±0.04	2.67±0.06	3.1±0.04	
	2020-2021	0.74±0.04	0.25±0.08	0.33±0.07	0.3±0.06	0.29±0.07	0.16±0.8	

^aReference value of virgin olive oil established by the International Olive Council (COI/T.15.2013) (IOC (INTERNATIONAL OLIVE COUNCIL) 2019). Each value represents the mean of three determinations (n=3) ± standard deviation.

Indeed, for example, the oleic acid (C18:1) percentages varied between 78.21% (TNT) and 80.31% (SD), and from 80.15% (TNT) to 82.03% (ATN), respectively during the 2018/2019 and 2019/2020 harvest seasons while the percentages of stearic acid (C18:0) vary between 2.09% (KS) and 2.64% (ZC), and from 2.67% (TNT) to 3.62% (ZC), during the 2018/2019 and 2019/2020 harvest seasons, respectively. The other fatty acids have a percentage lower than 4%.

Thus, according to the results obtained during three harvest years, the highest value of oleic acid was found in the virgin olive oils harvested in 2020/2021 (average of 81.81%), followed by the virgin olive oils of the 2019/2020 season (81.29%), knowing that the lowest content of this acid was observed in the virgin olive oils harvested in 2018/2019 (79.38%).

Rheological behaviors. The rheological behavior of virgin olive oils can be used to predict the quality, and the purification of virgin olive oil (VOO), based on their viscosity (Tamborrino et al. 2021). The Fig.2 illustrates that all virgin olive oils (VOOs) show similar flow profiles between 60s⁻¹ and 120s⁻¹

shear rates at 25°C. The obtained results show a linear relationship between the shear stress and the shear rate, which was expressed by Newton's law of viscosity (Equation 1) (Kim et al. 2010; Santos et al. 2005). The viscosities of virgin olive oils at 25°C can be obtained from this curve using the slope of the fits to the experimental shear stress-shear rate data (Table 2). In addition, this variation in viscosity is positively correlated with C18:1 oleic acid composition (i.e., viscosity increases with increasing levels of this type of fatty acid) and negatively correlated with C18:2 linoleic acid composition, respectively (i.e. viscosity decreases with increasing levels of this fatty acid) (Paul et al. 2021). This facilitates the sliding of the molecules one on the other without any chewing and makes the olive oils more fluid (Santos et al. 2005). However, the results obtained justify that virgin olive oils have a Newtonian behavior, which is consistent with the results of other researchers Kim et al. (Kim et al. 2010) and Santos et al. (Santos et al. 2005). Furthermore, the same trend has been observed by various authors using different vegetable oils from different natural sources and employing different

extraction methods, such as the study by Delfan-Hosseini et al. (Delfan-Hosseini et al. 2017), who found that purslane seed oils exhibited Newtonian rheological behavior. And the work of Blayo et al. (Blayo et al. 2001), who showed that the vegetable oils (linseed oil, tung oil, soybean oil, and rapeseed oil) also exhibited Newtonian behavior. Generally speaking, vegetable oils are Newtonian liquids with high viscosity due to their long-chain structure (Chouaibi et al. 2012).

Table 2. Mean viscosity of six VOO samples during three harvest seasons (2018-2019, 2019-2020, and 2020-2021)

Samples	Years	Mean viscosity $\pm$ SD, Pa.s
ATN	2018-2019	$0.045 \pm 1.14 \cdot 10^{-4}$
	2019-2020	$0.063 \pm 4.92 \cdot 10^{-4}$
	2020-2021	$0.071 \pm 1.90 \cdot 10^{-4}$
TNT	2018-2019	$0.050 \pm 1.52 \cdot 10^{-4}$
	2019-2020	$0.086 \pm 3.50 \cdot 10^{-4}$
	2020-2021	$0.074 \pm 2.16 \cdot 10^{-4}$
LH	2018-2019	$0.047 \pm 7.84 \cdot 10^{-4}$
	2019-2020	$0.050 \pm 2.92 \cdot 10^{-4}$
	2020-2021	$0.077 \pm 3.19 \cdot 10^{-4}$
SD	2018-2019	$0.038 \pm 1.54 \cdot 10^{-4}$
	2019-2020	$0.050 \pm 2.61 \cdot 10^{-4}$
	2020-2021	$0.077 \pm 3.54 \cdot 10^{-4}$
KS	2018-2019	$0.046 \pm 5.24 \cdot 10^{-4}$
	2019-2020	$0.069 \pm 2.35 \cdot 10^{-4}$
	2020-2021	$0.068 \pm 2.29 \cdot 10^{-4}$
ZC	2018-2019	$0.050 \pm 1.66 \cdot 10^{-4}$
	2019-2020	$0.043 \pm 1.88 \cdot 10^{-4}$
	2020-2021	$0.075 \pm 2.70 \cdot 10^{-4}$

*Each value represents the mean of three determinations (n=3) $\pm$ standard deviation (SD)

Ultraviolet-visible (UV-vis) analyses. The UV-visible profile of olive oil comes from two different pigment types: chlorophylls and carotenoids. The green color of olive oils is attributed to the presence of chlorophyll pigments, while the yellow color comes from carotenoids. Fig. 3 presents UV-visible spectra of virgin olive oils that are strongly correlated to the profile of these pigments (Lazzerini and Domenici 2017). The presence of conjugated dienes and trienes is indicated by peaks observed at 230 and 270 nm respectively, which are probably due to oxidized forms of phenolic molecules or other compounds such as oxidized forms of fatty acids (Fuentes et al. 2012). In addition, the 300-400 nm range has been associated with various polyphenols (Grazia et al. 2012). The peaks and absorption bands between 390-720 nm

are characteristic of the pigments (chlorophyll and carotenoids) present in virgin olive oil. Lutein, beta-carotene, phytovitin A and phytovitin B are known to show absorption peaks at specific spectral wavelengths: 486, 455 and 432 nm; 490 and 462 nm; 670 and 414 nm; 657 and 437 nm, respectively. These results are in line with previous research of Minguez-Mosquera et al. (Minguez-Mosquera et al. 1990) and Cayuela et al. (Cayuela et al. 2014).

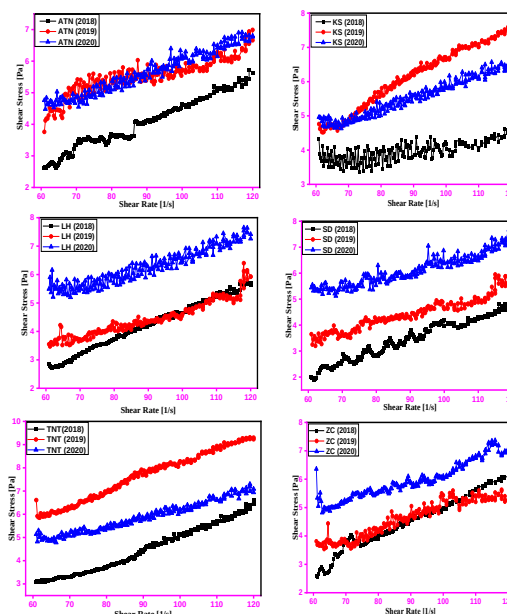


Figure 2. Shear stress versus shear rate of six VOO samples (ATN, KS, LH, SD, TNT, and ZC) at 25°C, during three harvest seasons (2018-2019, 2019-2020, and 2020-2021)

In addition, the wavelength range between 250 and 350 nm in the UV-Vis spectra of olive oil samples is frequently linked to the absorption of certain compounds, notably polyphenols, tocopherols, and carotenoids (Uncu and Ozen 2019; Vicario et al. 2020). Consequently, the distinctions observed in the peaks between samples from 2020-2021 and those from 2018 and 2019 may result from variations in chemical composition, the concentration of compounds present, or other factors associated with olive growing and processing conditions (Gandul-Rojas et al. 2016; Vicario et al. 2020). For three consecutive years, the pigment content of six virgin olive oils was assessed (Table 3). The results clearly show the variation in these concentrations between different samples.

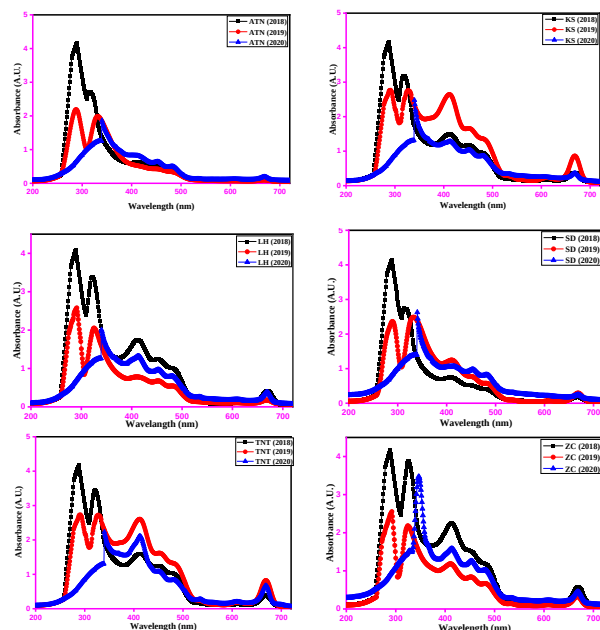


Figure 3. UV-vis absorption spectrum of six VOO samples (ATN, KS, LH, SD, TNT, and ZC), during three harvest seasons (2018-2019, 2019-2020, and 2020-2021)

In general, the highest pigment content was noted for the KS sample produced in 2019-2020 (22.33 mg.kg⁻¹), and the lowest total pigment content was noticed for the ATN sample from the 2019-2020 production with a content of (4.23 mg.kg⁻¹).

The average value of total pigment obtained in this group of VOO samples was approximately 19.88 mg.kg⁻¹, however, as can be seen in the pigment profile presented in Table 3, the total pigment content differences between the three years are sufficiently expressive, with an average of 11.91, 12.28, and 13.71 mg.kg⁻¹ for period 2018-2019, 2019-2020, and 2020-2021 respectively. However, there are several parameters related to this significant variation in pigment content between the three years of olive harvesting, namely environmental and climatic factors, the variety of cultivar (genetic factor), fruit ripeness at harvest time, storage conditions of the olive fruit, the production process and the storage conditions of the olive oils and obviously the production year (Lazzerini et al. 2016).

Statistical analyses. PCA is a statistical technique used for data classification. It is commonly used as the first step in data analysis to determine clusters.

PCA is valuable for processing and interpreting large amounts of quantitative data. Its main function is to extract significant information and create new variables called principal components (PCs). PCs describe the greatest variations between features and can be interpreted independently. This reduces data dimensionality and reveals relationships and deviant characteristics. Hierarchical cluster analysis (HCA) groups samples into clusters by describing a hierarchy. The result is a dendrogram, a graph that shows the arrangement of models as a tree. Dendrograms measure the distance between samples to identify similarity.

Rheological data. The exploitation of rheological data by PCA (Fig.4a) reveals that the two first

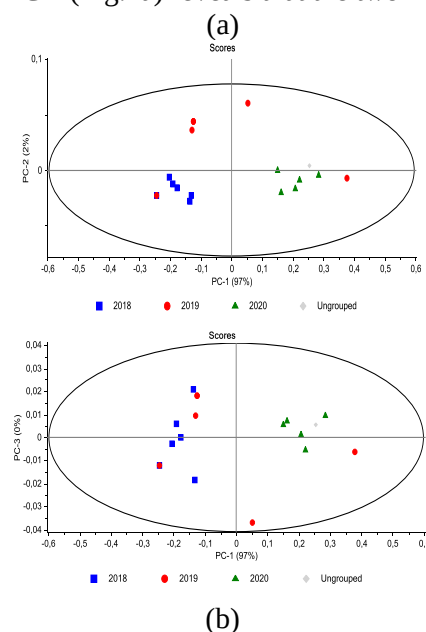


Figure 4. (a) PCA scores plot of rheological data for VOO samples during three harvest years (2018, 2019, and 2020). (b) Dendrogram from an HCA with a single linkage of rheological data for VOO samples during three harvest years using the squared Euclidean distance

Table 3. Pigment content of six VOO samples during three harvest years successive (2018-2019, 2019-2020, and 2020-2021)

Virgin olive oils	Years	Pigment content (mg.kg ⁻¹)						R ²
		pheophytin-a	pheophytin-b	β-carotene	lutein	chlorophyll-a	chlorophyll-b	
ATN	2018/2019	1.823 ±0.12	0.340±0.08	1.488±0.07	0.190±0.12	0.321±0.08	0.984±0.12	0.962
	2019/2020	1.451 ±0.15	0.207±0.05	1.620±0.12	ND	0.379±0.05	1.127 ±0.15	0.965
	2020/2021	2.615 ±0.17	0.327±0.12	1.135±0.15	0.694±0.16	0.462±0.12	1.356±0.08	0.982
SD	2018/2019	2.978±0.08	0.454±0.07	1.555±0.34	ND	0.422±0.11	0.993±0.21	0.977
	2019/2020	5.433±0.13	0.820±0.06	1.988±0.27	0.548±0.09	0.737±0.23	1.429±0.27	0.968
	2020/2021	6.077±0.05	1.631±0.12	4.698±0.16	ND	0.402±0.31	3.652±0.26	0.978
ZC	2018/2019	8.955±0.2	1.416±0.15	3.024±0.18	1.929±0.11	1.266±0.18	1.384±0.18	0.987
	2019/2020	5.651±0.17	0.784±0.17	2.393±0.24	0.571±0.03	0.555±0.16	1.585±0.12	0.965
	2020/2021	6.718±0.16	0.947±0.19	2.684±0.16	1.165±0.17	0.158±0.21	2.097±0.19	0.978
LH	2018/2019	7.871±0.10	0.855±0.2	3.459±0.06	0.953±0.08	0.977±0.1	1.773±0.09	0.986
	2019/2020	1.662±0.09	0.284±0.11	1.122±0.09	0.992±0.12	0.144±0.05	0.446±0.06	0.968
	2020/2021	6.088±0.19	0.931±0.09	2.767±0.13	0.126±0.06	0.150±0.08	1.929 ±0.17	0.974
TNT	2018/2019	5.810±0.14	0.673±0.06	2.640±0.2	1.698±0.2	0.834±0.13	1.067±0.14	0.972
	2019/2020	11.12±0.17	0.672±0.04	3.329±0.28	1.283±0.31	1.968±0.31	1.516±0.16	0.983
	2020/2021	10.533±0.15	1.446±0.03	2.124±0.19	1.068 ±0.18	0.251±0.15	1.513±0.27	0.984
KS	2018/2019	6.055±0.05	0.853±0.17	3.054±0.17	0.837±0.16	0.820±0.16	1.762±0.07	0.982
	2019/2020	12.135±0.08	0.672±0.08	4.329±0.24	1.283±0.19	1.968±0.06	1.516± 0.16	0.972
	2020/2021	6.918±0.18	1.563±0.16	4.249±0.17	ND	0.414±0.06	3.411±0.19	0.982

ND: not detected. Each value represents the mean of three determinations (n=3) ± standard deviation (SD)

principal components account for 99% of the total variation (0% for PC-3). Observation of the score plot shows a partial grouping of virgin olive oil samples according to their year of collection, accompanied by a few outliers. The PCA shows that there is a strong clustering between the virgin olive oil samples, while some samples collected in 2019 overlap with samples collected in 2018 and 2020. While for the HCA of the rheological data (Fig.4b), we can notice that there is a clustering of the virgin olive oils in two classes, one class (green) that contains the VOOs produced in 2018 and 2019 with a similarity of 95%, and a second class (orange) that contains the virgin olive oils produced in 2020 and two samples of the year 2019 TNT2 and ATN2 with a similarity of 70% and 55% respectively.

UV-Visible data. The PCA analysis of the UV-Visible spectral data (Fig.5a) indicates that the first 3 principal components represent 97% of the total information contained in the spectral database. Observation of the PC1-PC2 score graph shows that there is a clustering of the virgin olive oil samples according to the year of production. The PC1-PC3 graph shows that the virgin olive oils produced in 2018 and 2019 have the same response profile, while the 2020 virgin olive oils have a different profile.

The analysis by HCA (Fig.5b) shows the presence of 2 classes, a class formed by the 2018 and 2019 (orange) virgin olive oils and a class formed by the 2020 virgin olive oils (green).

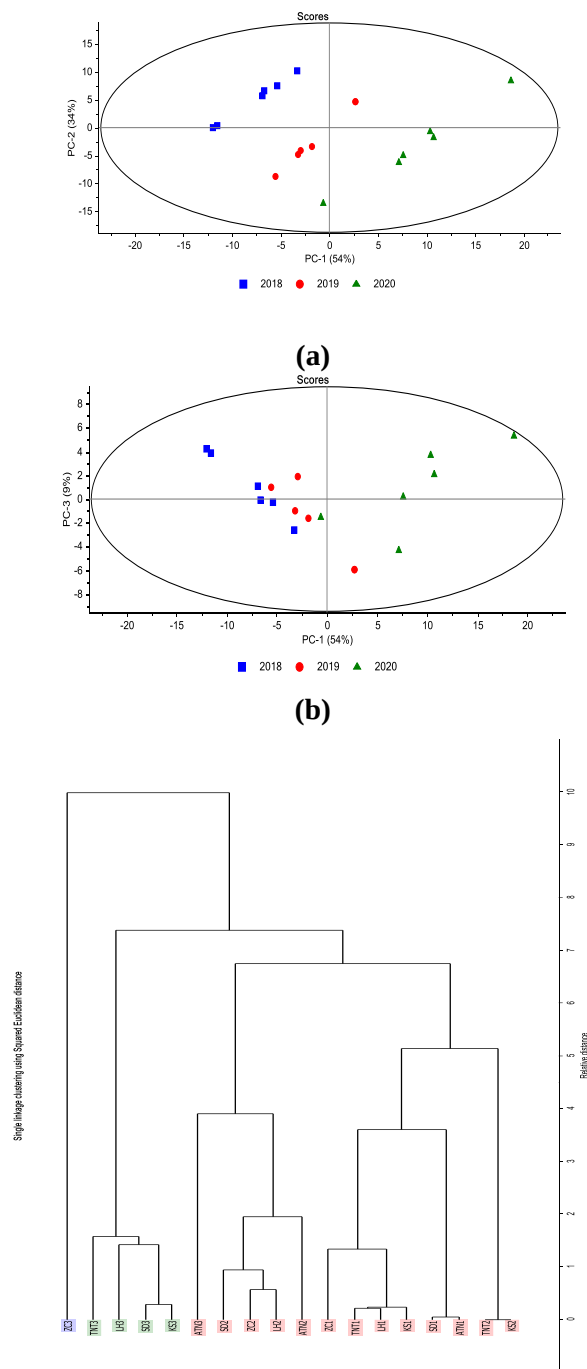


Figure 5. (a) PCA scores plot of UV-Visible data for VOO samples during three harvest years (2018, 2019, and 2020). (b) Dendrogram from an HCA with a single linkage of UV-Visible data for VOO samples during three harvest years using the squared Euclidean distance.

Conclusions

Virgin olive oil samples produced in different regions were compared over three harvest years using several analytical methods (UV-vis, rheological techniques and gas chromatography) and chemometric approaches (PCA, HCA). The results were used to measure the pigment content, fatty acid composition and rheological behavior of the samples. Six different pigments were identified and quantified in virgin olive oil samples from the three harvest years. Four fatty acids, including oleic acid, were identified and their amounts were compared among the three harvest years. The virgin olive oil samples showed Newtonian flow rheological behavior, with higher dynamic viscosity for the samples harvested in 2018 due to a higher amount of oleic fatty acids. Indeed, the variations observed in the total fatty acid composition as well as the physicochemical differences found between the various virgin olive oils studied highlight the influence of the harvest year on the samples analyzed.

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References

- Abedinzadeh S., Torbati M., Azadmard-Damirchi S. Some qualitative and rheological properties of virgin olive oil- apple vinegar salad dressing stabilized with xanthan gum. *Advanced Pharmaceutical Bulletin*, 2016, 6(4): 597-606.
<https://doi.org/10.15171/apb.2016.074>
- Bajoub A., Hurtado-Fernández E., Ajal E.A., Fernández-Gutiérrez A., Carrasco-Pancorbo A., Ouazzani N. Quality and chemical profiles of monovarietal north Moroccan olive oils from “picholine Marocaine” cultivar: Registration database development and geographical discrimination. *Food Chemistry*, 2015, 179(7): 127-136.
<https://doi.org/10.1016/j.foodchem.2015.01.101>

- Benito M., Oria R., Sánchez-Gimeno A.C. Characterization of the olive oil from three potentially interesting varieties from Aragon (Spain). *Food Science and Technology International*, 2010, 16(6): 523-530.
<https://doi.org/10.1177/1082013210367542>
- Blayo A., Gandini A., Le Nest J.F. Chemical and rheological characterizations of some vegetable oils derivatives commonly used in printing inks. *Industrial Crops and Products*, 2001, 14(2): 155-167.
[https://doi.org/10.1016/S0926-6690\(01\)00079-6](https://doi.org/10.1016/S0926-6690(01)00079-6)
- Borello E., Domenici V. Determination of pigments in virgin and extra-virgin olive oils: A comparison between two near UV-vis spectroscopic techniques. *Foods*, 2019, 8(1): 4-8.
<https://doi.org/10.3390/foods8010018>
- Borges T.H., Pereira J.A., Cabrera-Vique C., Lara L., Oliveira A.F., Seiquer I. Characterization of Arbequina virgin olive oils produced in different regions of Brazil and Spain: Physicochemical properties, oxidative stability and fatty acid profile. *Food Chemistry*, 2017, 215(1): 454-462.
<https://doi.org/10.1016/j.foodchem.2016.07.162>
- Bouaziz M., Chamkha M., Sayadi S. Comparative study on phenolic content and antioxidant activity during maturation of the olive cultivar Chemlali from Tunisia. *Journal of Agricultural and Food Chemistry*, 2004, 52(17): 5476-5481.
<https://doi.org/10.1021/jf0497004>
- Bouymajane A., El Majdoub Y.O., Cacciola F., Russo M., Salafia F., Trozzi A., Filali F. R., Dugo P., Mondello, L. Characterization of Phenolic Compounds, Vitamin E and Fatty Acids from Monovarietal Virgin Olive Oils of "Picholine marocaine" Cultivar. *Molecules*, 2020, 25(22):
<https://doi.org/10.3390/MOLECULES25225428>
- Cayuela J.A., Yousfi K., Carmen Martínez M., García J.M. Rapid Determination of Olive Oil Chlorophylls and Carotenoids by Using Visible Spectroscopy. *JAOCs, Journal of the American Oil Chemists' Society*, 2014, 91(10): 1677-1684.
<https://doi.org/10.1007/s11746-014-2515-1>
- Chouaibi M., Mahfoudhi N., Rezig L., Donsi F., Ferrari G., Hamdi S. A comparative study on physicochemical, rheological and surface tension properties of tunisian jujube (*Zizyphus lotus* L.) seed and vegetable oils. *International Journal of Food Engineering*, 2012, 8(2).
<https://doi.org/10.1515/1556-3758.2759>
- Conseil Oléicole International (COI), Coi/T.15/Nc :Norme Commerciale Applicable Aux Huiles D'Olive Et Aux Huiles De Grignons D'Olive, 2019, pp. 1–17. Available at:
<http://www.internationaloliveoil.org/>
- Conseil Oléicole International (COI), production, 2022. Available at: <https://www.internationaloliveoil.org/>
- Delfan-Hosseini S., Nayeibzadeh K., Mirmoghtadaie L., Kavosi M., Hosseini S.M. Effect of extraction process on composition, oxidative stability and rheological properties of purslane seed oil. *Food Chemistry*, 2017, 222(5): 61-66.
<https://doi.org/10.1016/j.foodchem.2016.11.150>
- Domenici V., Ancora D., Cifelli M., Serani A., Veracini C.A., Zandomenighi M. Extraction of pigment information from near-UV vis absorption spectra of extra virgin olive oils. *Journal of Agricultural and Food Chemistry*, 2014, 62(38): 9317-9325.
<https://doi.org/10.1021/jf503818k>
- Fuentes E., Báez M.E., Bravo M., Cid C., Labra F. Determination of Total Phenolic Content in Olive Oil Samples by UV-visible Spectrometry and Multivariate Calibration. *Food Analytical Methods*, 2012, 5(6): 1311-1319.
<https://doi.org/10.1007/s12161-012-9379-5>
- Gandul-Rojas B., Roca M., Gallardo-Guerrero L. Chlorophylls and Carotenoids in Food Products from Olive Tree. In: *Products from Olive Tree*. 2016, pp. 67-98, Print ISBN: 978-953-51-2724-6, eBook ISBN: 978-953-51-4164-8. Available at:
<https://doi.org/10.5772/64688>
- Gómez-Alonso S., Fregapanè G., Desamparados Salvador M., Gordon M.H. Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. *Journal of Agricultural and Food Chemistry*, 2003, 51(3): 667-672.
<https://doi.org/10.1021/jf025932w>
- Grazia A., Ciaccheri L., Azelio A., Cimato A. Optical Absorption Spectroscopy for Quality Assessment of Extra Virgin Olive Oil. In: *Olive Oil - Constituents, Quality, Health Properties and Bioconversions*, 2012, pp. 47-62, Print ISBN: 978-953-307-921-9, eBook ISBN: 978-953-51-4368-0.
<https://doi.org/10.5772/30275>
- Historique Météo, Historique Météo du Maroc, 2009. Available at:
<https://www.historique-meteo.net/afrique/maroc/>
- International Olive Oil Council - Consumption, Huiles D 'Olive - Olive Oils, In International Olive Oil Council (Issue June), 2018. Available at:
<http://www.internationaloliveoil.org>
- International Olive Oil Council – Production, Huiles D 'Olive - Olive Oils, In International Olive Oil Council (Issue June), 2018. Available at:
<http://www.internationaloliveoil.org>
- International Olive Council (IOC), Trade standard applying to olive oils and olive pomace oils, 2019. Available at:
<https://www.internationaloliveoil.org/what-we-do/chemistry-standardisation-unit/standards-and-methods.pdf>

- Kim J., Kim D.N., Lee S.H., Yoo S.H., Lee S. Correlation of fatty acid composition of vegetable oils with rheological behaviour and oil uptake. *Food Chemistry*, 2010, 118(2): 398-402. <https://doi.org/10.1016/j.foodchem.2009.05.011>
- Lazzerini C., Cifelli M., Domenici V. Pigments in extra virgin olive oils produced in different mediterranean countries in 2014: Near UV-vis spectroscopy versus HPLC-DAD. *LWT - Food Science and Technology*, 2017, 84(10): 586-594. <https://doi.org/10.1016/j.lwt.2017.06.025>
- Lazzerini C., Cifelli M., Lazzerini C., Cifelli M., Domenici V. Pigments - Virgin Olive Pigments in in Extra Extra- Virgin Olive Oil : Oil : Authenticity Authenticity and and Quality Quality. In: *Products from Olive Tree*, 2016, pp. 99-114, Print ISBN: 978-953-51-2724-6, eBook ISBN: 978-953-51-4164-8. <https://doi.org/10.5772/64736>
- Lazzerini C., Domenici V. Pigments in extra-virgin olive oils produced in tuscany (Italy) in different years. *Foods*, 2017, 6(4): 1-11. <https://doi.org/10.3390/foods6040025>
- Loudiyi W.D., Chmitah M., Loussert R., Mahhou A., Boulouha B. Morphologic and physiologic characters of olive clones from Picholine Marroqui variety. *Olivae*, 1984, 3(4): 26-31.
- Lozano-Castellón J., López-Yerena A., Rinaldi de Alvarenga J.F., Romero del Castillo-Alba J., Vallverdú-Queralt A., Escribano-Ferrer E., Lamuela-Raventós R.M. Health-promoting properties of oleocanthal and oleacein: Two secoiridoids from extra-virgin olive oil. *Critical Reviews in Food Science and Nutrition*, 2020, 60(15): 2532-2548. <https://doi.org/10.1080/10408398.2019.1650715>
- Malomo A.A., Olawoye B., Olaniran A.F., Olaniyi I., Adedayo A., Adeniran H.A., Abiose S.H. Evaluation of sugar and free amino acid during fermentation of ogi from maize, acha and sorghum. *Food Science and Applied Biotechnology*, 2021, 4(2): 111-118. <https://doi.org/10.30721/fsab2021.v4.i2.136>
- Miho H., Moral J., Barranco D., Ledesma-Escobar C.A., Priego-Capote F., Díez C.M. Influence of genetic and interannual factors on the phenolic profiles of virgin olive oils. *Food Chemistry*, 2021, 342(4): 128357. <https://doi.org/10.1016/j.foodchem.2020.128357>
- Minguez-Mosquera M.I., Gandul-Rojas B., Garrido-Fernandez J., Gallardo-Guerrero L. Pigments present in virgin olive oil. *Journal of the American Oil Chemists' Society*, 1990, 67(3): 192-196. <https://doi.org/10.1007/BF02539624>
- Nadif M., Govaert G. Cluster Analysis. *Data Analysis*, 2010(1): 215-255. <https://doi.org/10.1002/9780470611777.ch7>
- Paciulli M., Difonzo G., Conte P., Flammini F., Piscopo A., Chiavaro E. Physical and thermal evaluation of olive oils from minor Italian cultivars. *Foods*, 2021, 10(5) :1004. <https://doi.org/10.3390/foods10051004>
- Pages J. Analyse factorielle de données mixtes. *Revue de Statistique Appliquée*, 2004, 52(4): 93-111. Availalbe at: http://archive.numdam.org/article/RSA_2004_52_4_93_0.pdf
- Palamthodi S., Shimpi S., Tungare K. Polyphenols profiles and antioxidant activities of extracts from Capsicum chinense in vitro plants and callus cultures. *Food Science and Applied Biotechnology*, 2019, 2(1): 30-37. <https://doi.org/10.30721/fsab2019.v2.i1.56>