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Research Article

Effect of Maturity and Environmental Conditions on Chemical Composition of Olive Oils of Introduced Cultivars in Morocco

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This work aims at evidencing the quality and chemical composition of extra virgin olive oils according to stages of maturity and in relation to the geographical location of olives. Three different olive cultivars (Moroccan Picholine, Languedoc Picholine, and Frantoio), grown in two different locations in Morocco (Errachidia and Marrakech), were studied during the two crop years (2016 and 2017) at three stages of maturity (green, purple, and black). This work has been carried out by analyzing several parameters, such as the quality characteristics (acidity and peroxide value), the chemical composition (total phenol content and fatty acid composition) of the oils, and also the fruit characteristics of the olives (maturity index, fruit water content, and oil content). The results obtained in this study indicate that as maturity advanced, there was a slight rise in oil content and acidity, while there was a decrease in fruit water content and peroxide value in both locations during the two crop years. The fatty acid composition of extra virgin olive oil showed a significant increase of linoleic acid and polyunsaturated fatty acids (PUFAs) and a decrease of oleic acid, palmitic acid, monounsaturated fatty acids (MUFAs), and MUFA/PUFA ratio as the maturation process progressed. A significant gradual decrease was noted in total phenol content and bitterness intensity from the green stage to the black. Moreover, olive oil composition differed clearly between the two sites. Therefore, the olive cultivar, harvesting date, and geographic location influence the olive oil characteristics.

1. Introduction

Among the old crops of the Mediterranean basin, the olive seed (*Olea europaea ssp. europaea*) is the most valuable fruit owing to its ecological, economical, and cultural importance. This species includes many cultivars, most derived from empirical selections. A great number of olive cultivars (presumed clones) are grown throughout the world [1]. There are more than 900 million olive trees grown around the world; the Mediterranean basin remains its favorite area with nearly 95% of the world's olive groves [2]. Morocco is among the major olive producing countries, and the olive tree is its main fruit species. It has spread throughout the whole national territory because of its ability to adapt to diverse bioclimatic stages, ranging from mountain areas to arid and Saharan zones [3]. Currently, Morocco is the world's 6th largest olive oil producer behind the European Union, Tunisia, and Turkey [4]. The main cultivated variety in Morocco is the "Moroccan Picholine" locally called "Zeitoun Beldi" covering more than 90% of the Moroccan olive groves [5]. The Moroccan government has made significant efforts to improve the Moroccan olive sector by introducing new varieties that are more productive and better adapted to the climate of the different Moroccan regions. Also, since the adoption of the strategy of the "Green Morocco Plan," new olive tree plantations, as well as the intensification and rehabilitation of national olive orchards, have taken place resulting in a gradual increase of the surface area covered by olive trees. By 2020, this strategy aims at increasing the olive surface to 1 220 000 ha and total olive production to 2 500 000 tonnes, whereas

the production of olive oil will reach more than 330 000 tonnes/year [5].

Extra virgin olive oil is the main source of edible lipids and one of the essential compounds of the Mediterranean diet. In comparison with other vegetable oils, virgin olive oil is precious for its organoleptic and nutritional characteristics and its resistance to oxidation due to its high monounsaturated fatty acid content (MUFAs) and its richness on antioxidants [6]. Olive oil has a relatively variable chemical composition [7]. This variability depends on a set of factors such as agronomic factors (irrigation and fertilization), cultivation practices (harvesting and maturity), technological factors (postharvest storage and extraction system), and geographical production area (altitude, latitude, and edaphological characteristics) [8–10]. Maturity stage represents an important factor related to the quality and chemical composition of olive oil [11]. One of the attributes for evaluating maturity is the skin color. During maturity, olive skin color changes from green at the beginning of the harvest period and then the changes continue and the small reddish-green spots turns from purple to black at the end of the harvest period [12]. The stage of maturity impacts directly the quality of the olive oil since important chemical variations take place during maturation [13]. These changes influenced not only the quality but also the organoleptic and nutritional characteristics and also the oxidative stability of olive oil [14]. In fact, the progress of fruit maturation causes changes in the metabolic processes of certain compounds such as triglycerides, fatty acids, polyphenols, tocopherols, chlorophylls, and carotenoids.

Environmental conditions are critical variables that change the quality and chemical composition of olive oil from one region to another and from one crop season to another [15]. The precipitation and temperature, during olive growth and maturation, present the most significant environmental factors that influence the olive oil composition [16–18]. Recently, several studies investigated the effect of the area of production on the specific characteristics of olive oil [19–22]. According to Arslan et al. [23], the identification of the geographical zone of plantation of the olive is a reliable criterion for the authentication of the quality of the olive oil. Cultivars can respond dissimilarly in different environmental conditions, which lead to oils with different characteristics [24].

Considering all these aspects, the objective of this research work is to evaluate the changes of quality and composition of extra virgin olive oils of the French Languedoc Picholine (LP) and the Italian Frantoio (Fr) cultivars grown in two locations in Morocco (Errachidia and Marrakech) over two crop seasons in comparison with the Moroccan Picholine (MP). This work considered also the influence of maturity and environmental conditions on the quality of olive oils, in order to collect knowledge about the qualitative and quantitative characteristics of the olive oil of these cultivars.

2. Materials and Methods

2.1. Plant Material and Growing Areas. Three cultivars, Languedoc Picholine (LP), Moroccan Picholine (MP), and Frantoio (Fr) grown in two different locations (Errachidia and Marrakech), were selected for this study (Tables 1 and 2). Fruit sampling was carried out at 3 different dates, the first in October when most fruits are green, with scarlet skin and green flesh, the second in November, when the most fruits are purple-green, with white flesh, and the last one in December, when the fruits are almost completely black. After the harvest, the olives were processed into oil within 24 hours according to the Abencor system. Only healthy fruits without any physical damage were extracted. The olives were cleaned of leaves and crushed with hammer mill, and the resulting olive paste was malaxed at 26°C for 30 minutes. The oil was centrifuged at 3500 rpm for 1 min. After filtration, the olive oil samples were transferred into dark glass bottles and stored without headspace prior to analysis.

2.2. Maturity Index. The maturity index of olives was determined from a batch of 100 olive fruits randomly selected according to the method proposed by Uceda and Frias [25]. This method was based on the evaluation of olive skin and flesh colors. Maturity index values ranged from class 0 (100% intense green skin) to class 7 (flesh and skin 100% black). The maturity index is calculated by applying the following formula:

$$\mathrm{MI} = \frac{ax_0 + bx_1 + cx_2 + dx_3 + ex_4 + fx_5 + gx_6 + hx_7}{100}, \quad (1)$$

where a, b, c, d, e, f, g, and h are fruit numbers of each category.

2.3. Oil and Water Contents. The oil content was determined using a Soxhlet apparatus extraction for 6 hours with *n*-hexane. The results are given as percentage of dry weight.

To determine the water content, the olive paste was weighed and transferred to Petri dishes and placed in a 105°C oven for 48 hours until a constant mass was reached. The water content was determined from the difference between the fresh mass and the dry mass and expressed as a percentage of fresh matter.

2.4. Physicochemical Quality Parameters. Free acidity (expressed as % oleic acid) and peroxide value (meq O_2/kg oil) were determined according to the ISO methods: ISO 660 : 2009 (E), 2009, and ISO 3960 : 2007(E), 2007 [26, 27], respectively.

2.5. Total Phenolic Content. Total phenol content was determined according to the Folin–Ciocalteu method. Phenolic extracts were prepared by a triple extraction from a solution of 10 g of olive oil in 20 mL hexane with 30 mL of a methanol-water mixture (80 : 20, v/v). The extract (100 μL) was mixed with 3.9 mL of distilled water and 100 μL of Folin–Ciocalteu reagent and allowed to stand at ambient temperature for 3 min. 1 mL of sodium carbonate solution (20%) was added to the mixture. The tubes were left for 60 min in the dark at room temperature. The absorbance was

Table 1: Characteristics of the two growing sites (Marrakech and Errachidia).

Location	Latitude	Altitude	Longitude	Soil type	Climate
Marrakech	31°38′N	411.6	8°04′W	Silty clay soil	Arid to semiarid
Errachidia	31°55′N	1039	4°25′W	Clayey, silty, and sandy soil	Semidesertic of continental type

Table 2: Temperature and precipitation of Marrakech and Errachidia during 2016 and 2017 crop season.

Region	2016		2017	
	Temperature (°C)	Precipitation (mm)	Temperature (°C)	Precipitation (mm)
Marrakech	24	154	25	216
Errachidia	21	218	23	150

measured at 725 nm. Values are expressed as in mg equivalent of gallic acid per kilogram of oil (mg GAE/kg).

2.6. Bitterness. Bitterness K225 was determined by a solid-phase extraction (SPE) of bitter compounds, using SPE C18 cartridges of 6 mL filled with 500 mg solid phase. For the extraction procedure of the bitter components, a sample of 1.0 g of virgin olive oil was dissolved in *n*-hexane, the cartridge was conditioned by eluting with methanol and *n*-hexane, and the oil was applied to the SPE column. The column was washed with hexane that was run through the cartridge and discarded. The bitter compounds were eluted with methanol/water (50 : 50). The absorbance of the methanolic extract was measured at 225 nm. The intensity of bitterness (IB) was determined according to the method described by Gutiérrez Rosales et al. [28], proposing an equation for the estimation of the bitterness intensity. This equation assumes a linear relationship between the bitterness index (IB) and the K225 ($IB = 13.33 * K225 - 0.837$).

2.7. Fatty Acid Composition. Determination of the qualitative and quantitative composition of fatty acid was performed according to the analytical methods described in the European Commission Regulation [29]. CGC gas liquid chromatography analyses were carried out using a Varian CP 3380 Chromatograph, with a capillary column (CP-Wax 52 CB : $L = 25$ m; $\Phi = 0.25$ mm; $Ft = 0.20\,\mu$m), using a split-splitless injector equipped with CP-8400 autosampler and a FID detector. Chromatographic parameters were as follows: the temperature of the injector was 220°C, the detector temperature was 230°C, and the oven was held at 190°C. The carrier gas was hydrogen. The analysis was repeated three times for each sample.

3. Statistical Analysis

The results were statistically analyzed by the ANOVA test using "XLSTAT Addinsoft TM" software (XLSTAT, 2014). Differences between the average data were compared using least significant difference (LSD), and statistical differences with P values under 0.05 were considered significant.

4. Results and Discussion

4.1. Maturity Index. Olive maturation is a complex process involving several physiological and chemical changes taking place within the fruit [30]. Olive color changes from green to black during this maturation process. The maturity stage presents one of the major important factors affecting olive oil composition [31]. The maturity index, which is widely used for the determination of optimum harvest time, is based on the evaluation of the changes of the olive skin color. The samples of this work were harvested at three different stages of maturity: green, purple, and black. The values of the maturity indices for the two seasons 2016 and 2017 at both locations (Errachidia and Marrakech) are shown in Figures 1 and 2. During the two years of study, at each sampling date, the cultivars showed almost similar maturity indices in the two regions. For the first period of sampling, maturity indices ranged from 1.03 to 1.20 in the Errachidia region and from 1.06 to 1.47 in the Marrakech region, while in the second period of harvest, the maturity indices varied from 2.03 to 2.25 in the Errachidia region and from 2.03 to 2.66 in the Marrakech region. In the last sampling period, the values were found to vary between 3.69 and 4.29 in the Errachidia region and between 3.87 and 4.14 in the Marrakech region. "LP" showed the lowest maturity indices in both seasons and both sites. The "MP" olives originated from the Errachidia region showed the highest value at the first year season and "Fr" at the second one in the Marrakech region. At the last date of sampling (end of December), all the olive cultivars have reached the black stage. The evolution of the maturity index was similar between cultivars during the two studied years in the both sites of our study. According to Gharbi et al. [32], maturity index may depend on the evolution of genetic factor (the cultivar), the cultivation zone, the climatic conditions, and the cultivation practices.

4.2. Oil and Water Contents. The evolution of oil contents expressed as a percent of dry weight was evaluated in Errachidia and Marrakech during the two seasons (Figures 3 and 4). Significant differences were found between the mean values of oil content of the olives during the stages of maturity ($P < 0.05$). Oil content depends on the cultivar, the growing locations, and the maturity stages. Our data collected over two years showed that the evolution of oil content during maturation of olives was similar for the three cultivars, with oil contents increasing as the maturation process progressed. The maximum oil content was reached at the last harvesting period (black olives). This increase during maturation results from a continuing triglyceride-

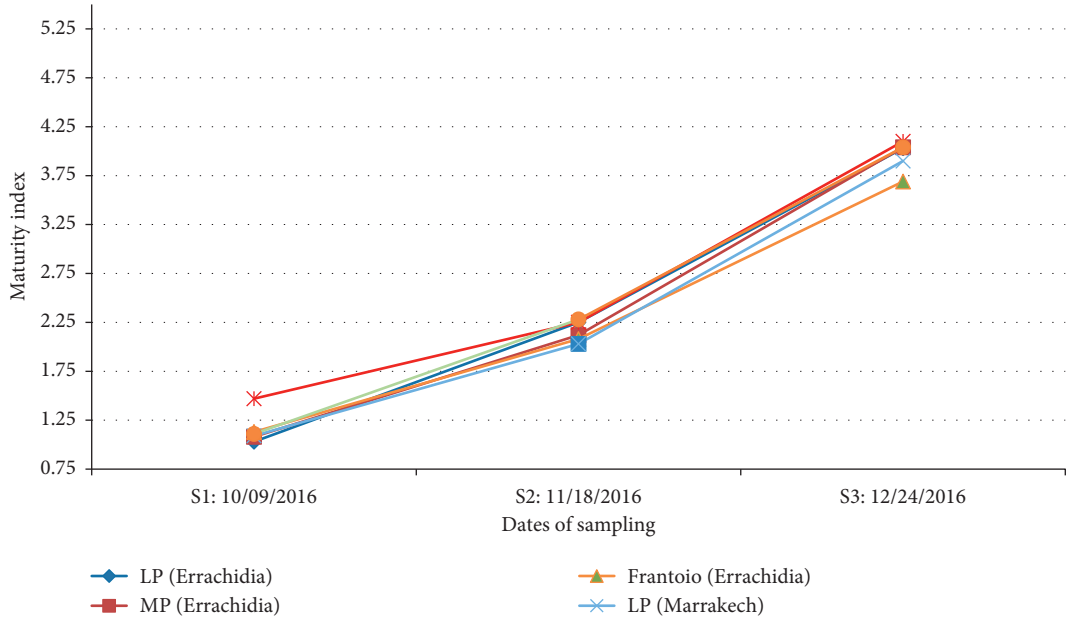

Figure 1: Olive maturity index evolution of the different cultivars grown in Marrakech and Errachidia regions during the 2016 season (LP: Languedoc Picholine; MP: Moroccan Picholine; Fr: Frantoio).

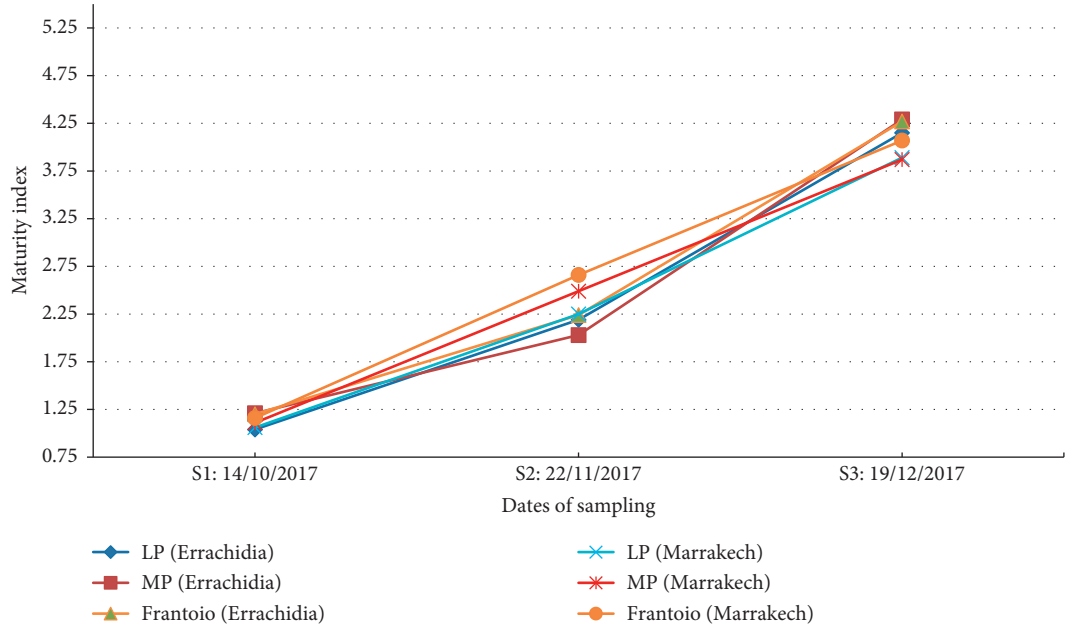

Figure 2: Olive maturity index evolution of the different cultivars grown in Marrakech and Errachidia regions during the 2017 season (LP: Languedoc Picholine; MP: Moroccan Picholine; Fr: Frantoio).

forming biosynthesis [33, 34]. A positive correlation ($r^2 = 0.71$) was found between the maturity index and oil content.

In fact, at the first stage of maturity, the average oil content ranged from 22.4% to 36.61% in Errachidia and from 27.0% to 43.3% in Marrakech. In the second stage of maturity, the oil content varied from 26.48% to 50.93% in Errachidia and from 30.6% to 47.7% in Marrakech. In the third stage, the oil content varied from 43.7% to 56.7% in Errachidia and from 38.4% to 53.4% in Marrakech. Furthermore, at the later harvesting date, the Italian cultivar "Fr" and the French "LP" present relatively higher oil contents (>50%) during the two years in both sites compared to the "MP." These differences in the oil content may be attributed to specific climatic conditions in each region [19]. Therefore, it is clear that in addition to the stage of maturity and environmental conditions, the oil content was affected also by the crop year. Salvador et al. [35] reported that the crop season presents an essential factor for the determination of the chemical composition of olive oil.

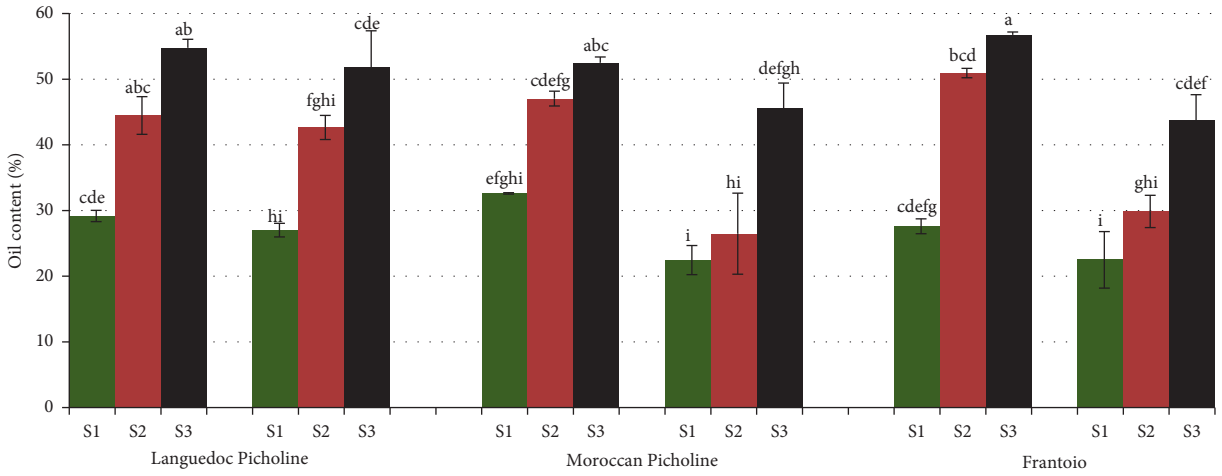

Figure 3: Oil contents (% DW) of different olive cultivars grown in the Errachidia region (East Morocco) at different stages of maturity (S1: green; S2: purple; S3: black). Values are means ± standard deviation (SD) of three replications. Data followed by different letters are significantly different from each other ($P < 0.05$) according to the LSD test.

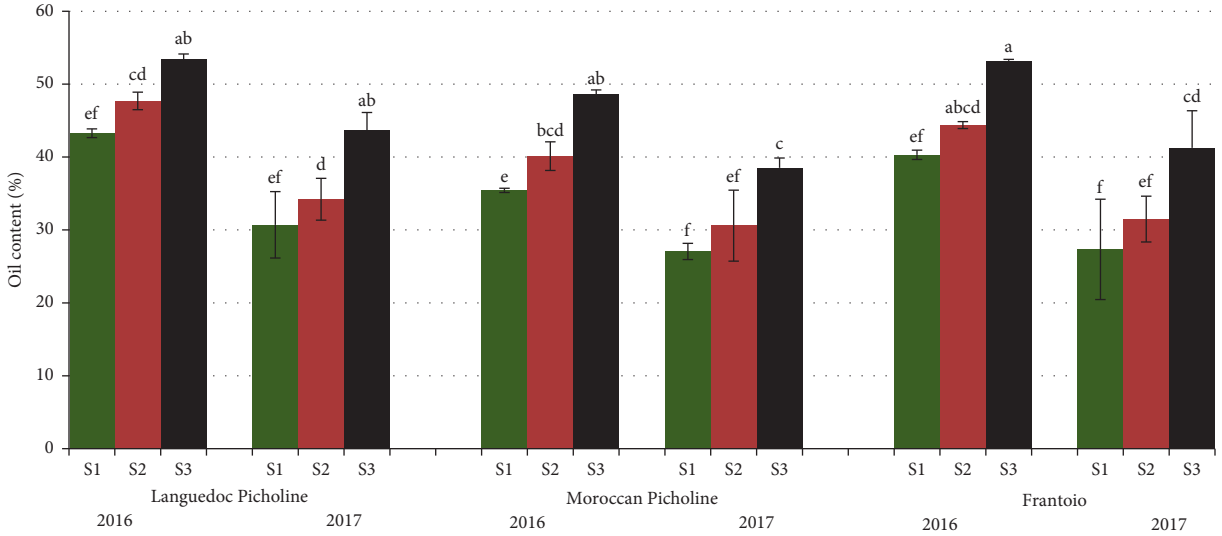

Figure 4: Oil contents (% DW) of different olive cultivars grown in the Marrakech region (Morocco) at different stages of maturity (S1: green; S2: purple; S3: black). Values are means ± standard deviation (SD) of three replications. Data followed by different letters are significantly different from each other ($P < 0.05$) according to the LSD test.

In comparison with the first crop year, the three cultivars showed slightly lower oil contents (% dry matter) at different stages of maturity during the year 2017. The rates of decrease ranged between 7.4% obtained in the case of green olives from the "LP" and 43% of decrease registered for purple olives of the "MP" cultivar grown in Errachidia. Excepting the "LP," the two other cultivars registered the highest decreases during the second stage of maturity (purple olives) in the Errachidia region. At full maturity (black olives), the olive obtained from the cultivars grown in the Marrakech region showed decreases ranging from 18 to 23%, while the decreases were less higher in the Errachidia region (5. 5–13%) for the "LP" and "MP" and almost similar (23%) for the olives from the "Fr" cultivar. Similar results were reported [36, 37]. Alowaiesh et al. [38] stated that the oil content is influenced by the climatic conditions of the region; in fact, low rainfall may cause lower oil content and higher dry matter content in olive fruit. Oil content (% dry weight) is influenced by genetic factor, environmental conditions, and fruit maturity index as reported by Rondanini et al. [39].

Another interesting parameter is water content, which has a strong influence on oil extraction efficiency. Fruits with higher water content usually produce a poor paste and negatively affect the quality and quantity of the olive oil [40, 41]. Water content during the maturation period is displayed in Figures 5 and 6. Significant differences were observed in the water content between cultivars and stages of maturity ($P < 0.05$). These results demonstrated a decrease in the water content during the maturation period for all the cultivars in both sites. Nevertheless, the rate of decrease in the water content differs between cultivars. In Errachidia, the water content varied from 54.6% to 69.5% at the beginning

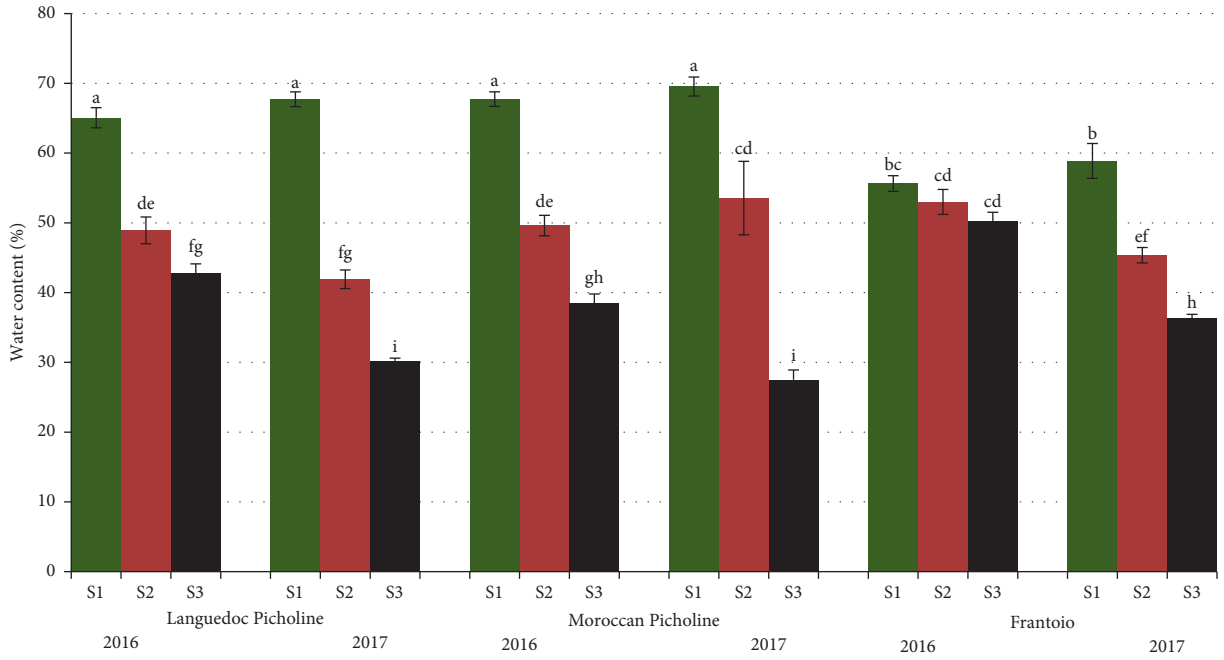

Figure 5: Water contents (% FW) of different olive cultivars grown in the Errachidia region (East Morocco) at different stages of maturity (S1: green; S2: purple; S3: black). Values are means ± standard deviation (SD) of three replications. Data followed by different letters are significantly different from each other ($P < 0.05$) according to LSD test.

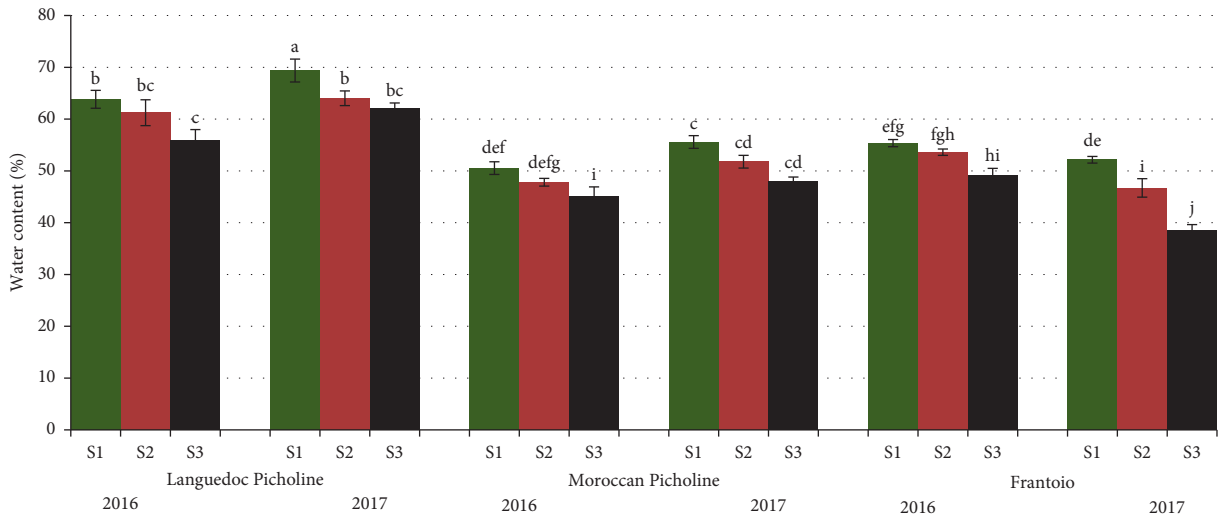

Figure 6: Water contents (% FW) of different olive cultivars grown in Marrakech (Morocco) at different stages of maturity (S1: green; S2: purple; S3: black). Values are means ± standard deviation (SD) of three replications. Data followed by different letters are significantly different from each other ($P < 0.05$) according to LSD test.

of maturity and from 41.9% to 53.8% in the second sampling period and from 27.52% to 50.18% in the last one. In Marrakech, the water content ranged from 54% to 69.4% in the first harvest and from 46.7% to 64.0% in the second harvest and finally from 38.58% to 62.93% in the last one. The water content in olives from Errachidia and Marrakech regions showed significantly different values. This confirms the influence of the environmental conditions (geographical zone and climatic conditions) on the olive water content as reported by Lazzez et al. [42].

Concerning the Errachidia region, the results showed an important decrease in the water content during maturation. Also, at each stage of maturity, this parameter records small variations between the two years of study excepting the last one. During the year 2017, at full maturity, in comparison with the first crop season, the "LP" and the "MP" recorded a water content decrease of 29%, while the "Fr" recorded a water content decrease of 14.42%. According to Al-Maaitah et al. [14], during maturation, cracks on the protective wax around the epicarp or other epidermal openings cause water

loss in the olives. A negative correlation ($r^2 = -0.64$) was found between maturity index and water content.

In the Marrakech region, the results showed a slight decrease in the water content during maturation, as well as low variations at each maturity stage when comparing the two years. In the year 2017, the "LP" and the "MP" recorded a slight water content increase (5–10% depending on the stage of maturity). However, the Italian cultivar "Fr" showed a decrease during the year 2017 during the three stages of maturity. The rates of decrease varied from 5.8% for the green olives and 12.8% for the purple olives and 21.5% at full maturity. According to [43, 44], olive water content is greatly influenced by the precipitation and temperature levels.

4.3. Acidity and Peroxide Value. The results depicted in Table 3 showed the results of acidity and peroxide value of olive oils. The quality parameters of olive oils (acidity and peroxide value) were found to be significantly different during the three stages of maturity ($P < 0.05$). Acidity values range from 0.2 to 0.4% in the first year for all the oils and from 0.2 to 0.7% in the second year. Acidity values seem to be slightly higher during the second year. The pulp cell may be damaged and the intrinsic lipases may have caused some triglyceride hydrolysis. The "Fr" cultivar seems to be the most sensitive to such phenomenon. Acidity levels in the second crop season reached 0.7% for the "Fr" oil obtained in the Marrakech region. However, in all cases, the acidity remained below the limit established by the International Olive Council (IOC) and the European Regulation (0.80%) for oils to be considered as extra virgin. As the maturation advanced, the acidity rises progressively at both sites, and this finding corroborates with the results of other authors [45, 46]. The increase in acidity during maturation can be explained by the increase in lipolytic activity, and also in the advanced stage of maturation, the olives become more sensitive to pathogenic infections and mechanical damage [47].

Peroxide value was used as an indicator of olive oil primary oxidation. The changes of peroxide value during maturation were similar for the three cultivars: a decrease was observed as the maturation process progressed (Table 3). All olive oils presented peroxide values which do not exceed the maximum permitted limit for their classification as extra virgin olive oils (<20 mEq O2/kg). The Italian "Fr" is characterized by the highest peroxide values in the both locations at different stages of maturity, which do not exceed the prescribed limit (<20 mEq O2/kg). The interaction between olive cultivar and harvesting date significantly influenced the peroxide value. At full maturity, the olive oils showed low peroxide values. These observations are in agreement with those of Matos et al. [48] and Salvador et al. [35]. Baccouri et al. [8] attributed theses low peroxide values to a decrease in the activity of the lipoxygenase at full maturity of the olives.

4.4. Total Phenolic Content and Oil Bitterness Evaluation. Phenolic compounds are an important factor that influences the chemical composition and sensory quality of olive oil. These compounds are responsible for the sharp and bitter taste of the oil and improve also their resistance to oxidation [49]. A series of enzymatic and chemical reactions take place during the maturation of the fruit, resulting in the production of free phenols and inducing the variations of several phenolic compounds. These changes have an influence on the chemical quality, the oxidation stability, the sensory properties as well as the nutritional value of the olive oil [50]. Figures 7 and 8 show the total phenol content (mg gallic acid/kg oil) found in both sites and both years tested. The phenol content shows significant differences according to the cultivar and the stages of maturity ($P < 0.05$). Total phenol content in oils (obtained by colorimetric determination) ranged between 272 and 615 mg GAE/kg in the first stage of maturity in Errachidia and between 323 and 805 mg GAE/kg in Marrakech. For the purple stage, the phenol content varied between 194 and 446.54 mg GAE/kg in Errachidia and between 256 and 540 mg GAE/kg in Marrakech. In the last stage, phenol content was found to vary between 135 and 423 mg GAE/kg in Errachidia and between 112 and 509 mg GAE/kg in Marrakech. These results are in agreement with those of some authors demonstrating that the total phenol content in olive oil varied from 50 to 1000 mg/kg, depending on the geographical area, the stage of maturity, and the oil extraction process [51, 52]. Our data also showed that the phenol content varied according to the crop season. Rainfall distribution and amount affect the phenolic content of olive oils [15].

The effect of the maturity stage on phenol content was clearly observed. The studied oils had high levels of phenol content at early stages of maturity, and then the phenol content decreased as the fruit matured. For all cultivars, there was a decrease in phenol content during maturation in both sites. These results are consistent with those reported by other authors [53–55]. The percentage of decrease differs between cultivars at the three stages of maturity. This decrease in phenol content was probably due to polyphenoloxidase activity and by the presence of exit holes which exposed the olive pulp to environmental factors [56]. In fact, to obtain olive oil with high phenol content, harvest at the first stages of maturity is recommended and necessary. The reduction in total phenol content during maturation showed a negative correlation ($r^2 > -0.52$).

The comparison of the obtained phenolic content for "LP" and "FR" cultivars grown in Morocco to those of their origin sites demonstrated that "Fr" grown in Morocco showed a phenol content within the limit determined by Beltrán et al. [57]. This Italian cultivar when cultivated in two different regions in Italy (Marche and Toscana) gave significantly different phenol levels (450.68 and 173.0 ppm, respectively) [58]. Compared with the results of Madeo et al. [24], the "LP" at the first crop season had low levels of phenol content in both sites, but, during the second season, this cultivar had a higher phenol content exceeding the results reported in the literature. The difference in phenol content of "LP" between the two years of our study may be due to differences in climatic conditions (especially precipitations and temperature). These results are in line with some authors who reported variations of the phenolic contents in olive oil

Table 3: Acidity and peroxide value of olive oils from three cultivars grown in Errachidia and Marrakech (Morocco) at different harvesting dates.

	Quality parameters	Crop seasons	Olive cultivars								
			Languedoc Picholine 1	Moroccan Picholine 1	Frantoio 1	Languedoc Picholine 2	Moroccan Picholine 2	Frantoio 2	Languedoc Picholine 3	Moroccan Picholine 3	Frantoio 3
Errachidia	Acidity	2016	0.23 ± 0.01^{hi}	0.26 ± 0.01^{gh}	0.29 ± 0.01^{fg}	0.27 ± 0.01^{gh}	0.27 ± 0.01^{gh}	0.35 ± 0.01^{def}	0.36 ± 0.01^{de}	0.34 ± 0.00^{def}	0.38 ± 0.00^{cd}
		2017	0.35 ± 0.04^{def}	0.20 ± 0.02^{i}	0.33 ± 0.01^{ef}	0.47 ± 0.02^{b}	0.30 ± 0.03^{fg}	0.42 ± 0.02^{bc}	0.55 ± 0.01^{a}	0.34 ± 0.00^{def}	0.45 ± 0.00^{b}
	Peroxide value	2016	10.55 ± 0.05^{e}	11.44 ± 0.05^{d}	11.97 ± 0.05^{c}	6.77 ± 0.05^{j}	7.43 ± 0.04^{h}	8.76 ± 0.04^{f}	5.60 ± 0.20^{k}	5.24 ± 0.07^{k}	10.27 ± 0.12^{h}
		2017	8.99 ± 0.19^{f}	12.88 ± 0.09^{b}	15.47 ± 0.11^{a}	7.48 ± 0.24^{ij}	7.02 ± 0.10^{g}	11.86 ± 0.10^{c}	5.30 ± 0.10^{k}	7.30 ± 0.09^{hi}	8.10 ± 0.10^{g}
Marrakech	Acidity	2016	0.27 ± 0.01^{ij}	0.26 ± 0.01^{jk}	0.27 ± 0.01^{jk}	0.30 ± 0.01^{hij}	0.34 ± 0.00^{fgh}	0.36 ± 0.00^{ef}	0.32 ± 0.01^{fgh}	0.35 ± 0.01^{efg}	0.38 ± 0.00^{e}
		2017	0.20 ± 0.02^{l}	0.22 ± 0.00^{kl}	0.56 ± 0.00^{c}	0.47 ± 0.02^{d}	0.31 ± 0.02^{ghi}	0.63 ± 0.00^{b}	0.65 ± 0.02^{b}	0.39 ± 0.01^{e}	0.71 ± 0.03^{a}
	Peroxide value	2016	10.59 ± 0.14^{ef}	9.96 ± 0.52^{f}	15.37 ± 0.32^{a}	8.05 ± 0.35^{ghi}	8.56 ± 0.16^{gh}	12.52 ± 0.50^{c}	7.91 ± 0.13^{hij}	7.39 ± 0.11^{ij}	8.51 ± 0.12^{c}
		2017	11.93 ± 0.12^{cd}	13.35 ± 0.10^{b}	11.49 ± 0.10^{d}	7.48 ± 0.11^{ij}	8.73 ± 0.06^{g}	11.27 ± 0.10^{de}	6.30 ± 0.10^{k}	3.49 ± 0.10^{l}	7.28 ± 0.10^{j}

Values are means ± standard deviation (SD) of three replications. Data followed by different letters are significantly different from each other ($P < 0.05$) according to LSD test.

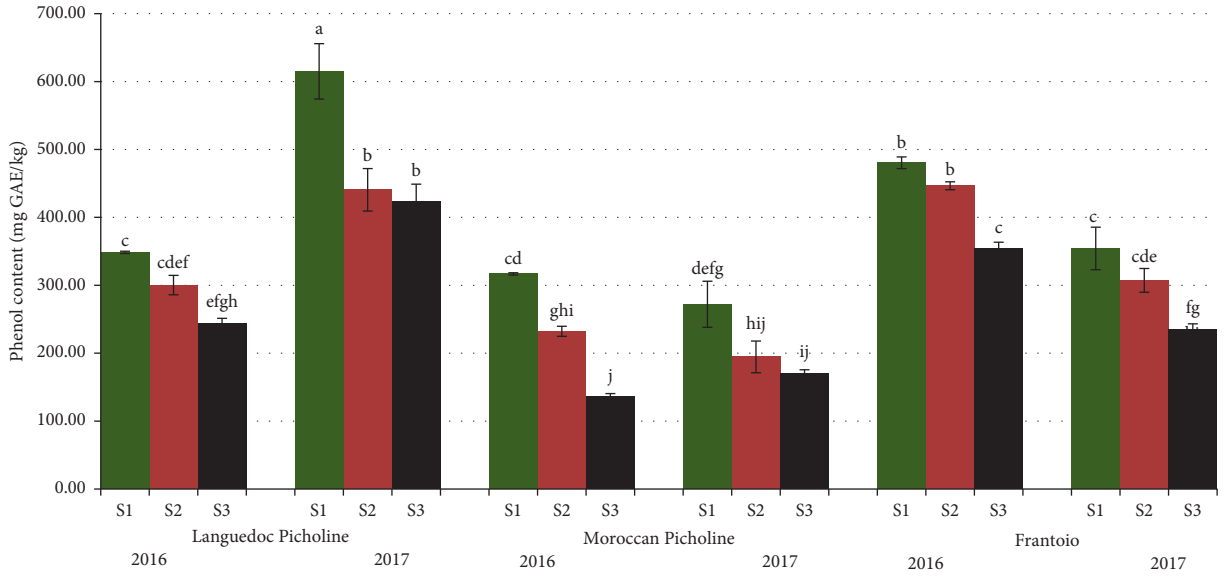

Figure 7: Phenol content in virgin olive oils of three cultivars grown in Errachidia (East Morocco) at different stages of maturity (S1: green; S2: purple; S3: black). Values are means ± standard deviation (SD) of three replications. Data followed by different letters are significantly different from each other ($P < 0.05$) according to LSD test.

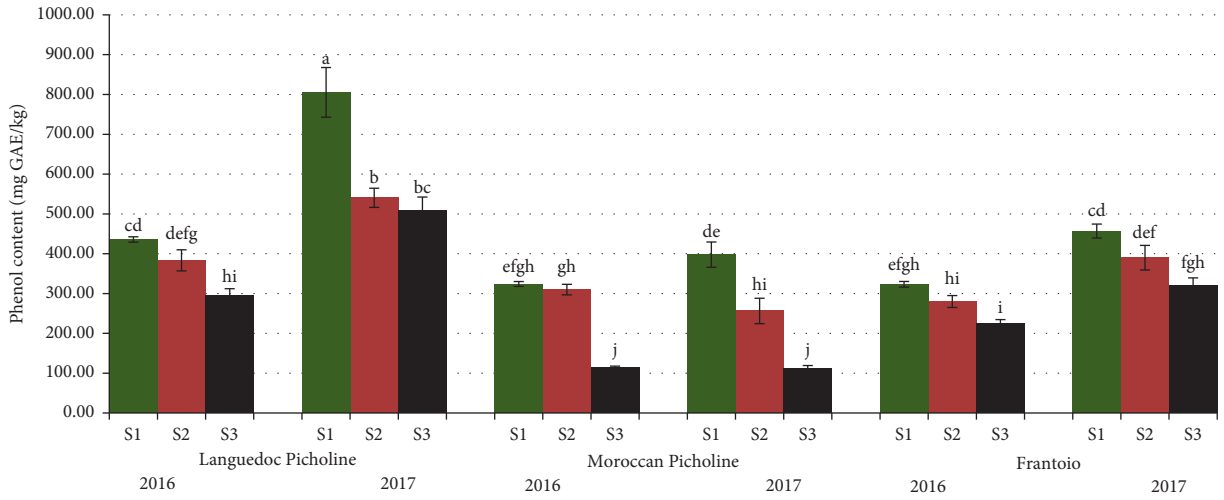

Figure 8: Phenol content in virgin olive oils of three cultivars grown in Marrakech (Morocco) at different stages of maturity (S1: green; S2: purple; S3: black). Values are means ± standard deviation (SD) of three replications. Data followed by different letters are significantly different from each other ($P < 0.05$) according to LSD test.

depending on complex interactions between the olive cultivar, the degree of fruit maturation, and the geographical location [59–63].

Bitterness presents a positive sensory attribute of olive oil that enhances the overall flavor related to unripe olive fruit, and is often positively related to the presence of phenolic compounds [64]. Bitterness can be estimated by the measurement of the specific absorbance at 225 nm (K225). The bitterness index (K225) assesses the intensity of the bitter taste of virgin olive oil. Bitterness intensity results are reported in Table 4. Statistical differences ($P < 0.05$) were found between cultivars and stages of maturity in terms of bitterness index. Bitterness intensities decrease as the maturation of olives advances. Jiménez et al. [65] and Franco et al. [66] reported that olive maturation has an impact on olive oil bitterness. The oils obtained from green olives were excessively bitter than oils of purple and black olives. According to Gutiérrez Rosales et al. [28], the most intense bitterness (>5) indicated a very bitter olive oil, in fact despite the beneficial effects of bitterness on human health and their high resistance to oxidation, but the high intensity of bitterness of virgin olive oils may not be acceptable to the majority of consumers.

Our results showed a great variability in this measured parameter, which can be extremely variable (high or low), between cultivars due to the genetic effect and growing region. In the Marrakech region, "Fr" oils obtained at full maturity were found to have higher bitterness scores.

Table 4: Bitterness intensities of the virgin olive oils obtained from three cultivars grown in Errachidia and Marrakech at different stages of maturity.

Location	Cultivars	Bitterness intensity	
		2016	2017
Errachidia	Languedoc Picholine 1	4.48 ± 0.01^{d}	3.21 ± 0.00^{f}
	Languedoc Picholine 2	2.12 ± 0.51^{ij}	2.93 ± 0.02^{fg}
	Languedoc Picholine 3	1.30 ± 0.03^{l}	1.78 ± 0.00^{k}
	Moroccan Picholine 1	4.73 ± 0.01^{d}	9.94 ± 0.02^{a}
	Moroccan Picholine 2	3.92 ± 0.01^{e}	5.17 ± 0.07^{bc}
	Moroccan Picholine 3	2.64 ± 0.00^{gh}	1.73 ± 0.00^{k}
	Frantoio 1	5.79 ± 0.01^{b}	2.37 ± 0.01^{hi}
	Frantoio 2	3.32 ± 0.00^{f}	1.55 ± 0.01^{kl}
	Frantoio 3	1.46 ± 0.01^{kl}	1.24 ± 0.00^{l}
Marrakech	Languedoc Picholine 1	3.83 ± 0.00^{b}	2.43 ± 0.00^{e}
	Languedoc Picholine 2	1.82 ± 0.00^{g}	1.69 ± 0.19^{h}
	Languedoc Picholine 3	1.19 ± 0.00^{i}	1.64 ± 0.00^{h}
	Moroccan Picholine 1	2.37 ± 0.00^{ef}	5.13 ± 0.03^{a}
	Moroccan Picholine 2	1.94 ± 0.12^{g}	2.51 ± 0.00^{e}
	Moroccan Picholine 3	1.94 ± 0.00^{g}	1.66 ± 0.00^{h}
	Frantoio 1	3.70 ± 0.00^{b}	3.32 ± 0.00^{c}
	Frantoio 2	2.87 ± 0.00^{d}	2.84 ± 0.00^{d}
	Frantoio 3	2.73 ± 0.07^{d}	2.22 ± 0.00^{f}

Values are means ± standard deviation (SD) of three replications. Data followed by different letters are significantly different from each other ($P < 0.05$) according to LSD test.

Significant effects of the cultivar on bitterness were confirmed by Rotondi et al. [67]. Bitterness intensities were found to be different from one year to another and depending on growth site. It is clearly suggested that the weather conditions prevailing each year had an influence on the intensity of bitterness, which differs according to the crop seasons and geographical sites. As reported by Gawel and Rogers [68] and Škevin et al. [69], the bitterness in olive oils depends on a series of factors, such as olive cultivar, fruit maturity, climatic conditions, geographic zone, and the process of extraction of oil. A positive correlation ($r^2 = 0.78$) was found between phenol content and bitterness intensity.

4.5. Fatty Acid Composition. The fatty acid methyl esters (FAME) composition of the oils from the analyzed cultivars is shown in Tables 5 and 6. As shown, the fatty acid composition of the studied oils is variable, depending on the stage of maturity, the crop season, and the place of cultivation. The cultivars showed similar trends of fatty acids levels in both regions during the maturation process. There were wide variations and significant ($P < 0.05$) differences among the fatty acid profiles of the studied oils during the different stages of maturity.

The oleic acid is known to greatly contribute to the oil stability and quality of olive oil. It is the principal monounsaturated fatty acid in all the samples and is present in higher concentrations (>55%) of the total fatty acid. Oleic acid (C18 : 1) was influenced by the maturity stage and the geographic zone. This major monounsaturated fatty acid (MUFA) decreased as the maturity of olives advanced. The highest average oleic acid values were found for MP during the two seasons and in both locations. The linoleic acid presents the highest level among the polyunsaturated fatty acid in olive oils. This fatty acid is more sensitive to oxidation than monounsaturated fatty acids, the percentage of linoleic acid increases progressively during maturation from the green stage to the black stage, and the highest percentage of linoleic acid was observed at the last maturity stage. Oleic and linoleic acids showed the opposite trend during maturation. In fact, an inverse relationship has been found between oleic acid and linoleic acid and this can be explained by the activity of the oleate desaturase enzyme which converts oleic acid to linoleic acid [33]. This interconversion of oleic and linoleic acid is accelerated by water stress [70]. A negative correlation ($r^2 = -0.86$) was confirmed between oleic acid and linoleic acid. Furthermore, it can be noted that the palmitic acid represents the major saturated fatty acid in olive oil, slightly decreased as the fruit ripened. Sakouhi et al. [71] also had found higher amounts of palmitic acid at the green stage compared to the purple and black stages. The palmitic acid level decreases during maturation possibly as a result of a dilution effect [72]. The fatty acid composition was significantly affected by growing site and the crop season. The highest percentages of oleic acid and palmitic acid were found in Errachidia, while Marrakech registered the highest percentage in linoleic acid. The variation of this composition may be due to the impact of environmental conditions (including altitude, precipitations, temperature, soil characteristics, and salinity). This result is in accordance with the finding of Servili et al. [73], demonstrating that the fatty acid composition is related to the geographic zone, the climatic conditions (characteristics of the olive grove zones), and the salinity of soil. According to Hlima et al. [74], the differences observed between selected regions for the fatty acid composition can be explained by the difference in altitude and temperature between the chosen regions. Previous studies have concluded that water absorption and temperature are the main factors that affect the rate and metabolism of fatty acids in olives [75]. Anastasopoulos et al. [47] also reported that the fatty acid composition varied depending on the maturation of fruit and crop year.

In comparison with oils obtained from these European cultivars when they are growing in original growing area in France and Italy, "LP" and "Fr" cultivated in Morocco (Errachidia and Marrakech), produced olive oil with lower amount of oleic acid [76, 77]. The difference detected between samples of olive oil in relation to the fatty acid composition is probably due to the interaction of cultivars and climatic conditions during fruit growth and maturity [54, 78].

The SFAs (saturated fatty acids), MUFAs (monounsaturated fatty acids), and PUFAs (polyunsaturated fatty acids) percentages and the MUFA/PUFA ratio were also calculated. A clear trend on the decrease of the total monounsaturated fatty acids and on the increase of total polyunsaturated is noticed as the olive maturation advances. Changes observed, from first to last sampling, in MUFAs and PUFAs was also confirmed by a high negative correlation ($r^2 = -0.99$). Saturated fatty acids showed a similar behavior to that described for palmitic acid. The ratio MUFA/PUFA ratio is important due to the effects on the

Table 5: Fatty acid composition (%) of the virgin olive oils of three cultivars grown in Errachidia (East Morocco) during the two crop seasons (2016 and 2017).

	Cultivars	C16 : 0	C18 : 1	C18 : 2	ΣSFA	ΣPUFA	ΣMUFA	MUFA/PUFA
2016	Languedoc Picholine 1	16.34 ± 0.04^{c}	66.81 ± 0.07^{h}	11.49 ± 0.03^{ij}	16.43 ± 0.05^{d}	7.14 ± 0.08^{n}	66.99 ± 0.08^{hi}	9.38 ± 0.10^{b}
	Languedoc Picholine 2	13.49 ± 0.07^{h}	66.68 ± 0.14^{h}	14.46 ± 0.00^{d}	13.53 ± 0.07^{fg}	15.43 ± 0.02^{d}	66.85 ± 0.14^{hi}	4.33 ± 0.00^{kl}
	Languedoc Picholine 3	13.29 ± 0.06^{h}	66.51 ± 0.10^{h}	14.68 ± 0.03^{c}	13.36 ± 0.06^{fg}	15.62 ± 0.04^{c}	66.70 ± 0.30^{h}	4.27 ± 0.02^{l}
	Moroccan Picholine 1	12.40 ± 0.09^{i}	75.56 ± 0.14^{a}	9.46 ± 0.02^{n}	12.46 ± 0.09^{h}	9.55 ± 0.03^{l}	75.72 ± 0.18^{b}	7.96 ± 0.04^{d}
	Moroccan Picholine 2	9.97 ± 0.00^{j}	74.14 ± 0.14^{c}	9.49 ± 0.02^{n}	10.01 ± 0.00^{i}	10.56 ± 0.01^{k}	74.37 ± 0.16^{bc}	7.04 ± 0.01^{e}
	Moroccan Picholine 3	9.46 ± 0.05^{k}	72.24 ± 0.23^{d}	11.37 ± 0.03^{j}	9.51 ± 0.04^{i}	12.22 ± 0.02^{h}	72.50 ± 0.20^{d}	5.93 ± 0.02^{h}
	Frantoio 1	18.36 ± 0.11^{a}	65.78 ± 0.05^{i}	12.52 ± 0.02^{g}	18.53 ± 0.10^{a}	6.14 ± 0.02^{o}	66.09 ± 0.04^{ij}	10.82 ± 0.02^{a}
	Frantoio 2	16.47 ± 0.04^{c}	63.52 ± 0.32^{k}	12.30 ± 0.00^{h}	16.57 ± 0.04^{d}	13.32 ± 0.01^{g}	63.69 ± 0.31^{k}	4.78 ± 0.02^{i}
	Frantoio 3	16.33 ± 0.03^{c}	63.34 ± 0.30^{k}	13.54 ± 0.01^{e}	16.38 ± 0.02^{d}	14.54 ± 0.04^{f}	63.45 ± 0.24^{k}	4.36 ± 0.03^{k}
2017	Languedoc Picholine 1	17.27 ± 0.15^{b}	64.65 ± 0.36^{j}	13.34 ± 0.01^{f}	19.06 ± 0.18^{a}	14.52 ± 0.02^{f}	66.18 ± 0.27^{j}	4.56 ± 0.01^{j}
	Languedoc Picholine 2	15.33 ± 0.05^{e}	60.78 ± 0.16^{l}	19.89 ± 0.04^{b}	16.76 ± 0.20^{cd}	21.01 ± 0.03^{b}	61.79 ± 0.18^{l}	2.94 ± 0.01^{m}
	Languedoc Picholine 3	14.00 ± 0.18^{g}	60.23 ± 0.01^{l}	20.51 ± 0.00^{a}	16.63 ± 0.13^{d}	21.55 ± 0.12^{a}	61.28 ± 0.05^{l}	2.84 ± 0.02^{m}
	Moroccan Picholine 1	13.48 ± 0.00^{h}	74.82 ± 0.07^{b}	10.44 ± 0.02^{l}	14.75 ± 0.41^{e}	12.09 ± 0.02^{h}	76.00 ± 0.11^{a}	5.98 ± 0.02^{h}
	Moroccan Picholine 2	12.45 ± 0.01^{i}	70.93 ± 0.09^{e}	13.31 ± 0.01^{f}	13.82 ± 0.38^{f}	14.82 ± 0.05^{e}	72.00 ± 0.06^{g}	4.86 ± 0.01^{i}
	Moroccan Picholine 3	9.59 ± 0.01^{k}	69.38 ± 0.20^{g}	16.38 ± 0.03^{l}	12.68 ± 0.01^{gh}	17.22 ± 0.04^{j}	70.03 ± 0.06^{e}	4.40 ± 0.03^{f}
	Frantoio 1	15.76 ± 0.01^{d}	72.41 ± 0.02^{d}	7.09 ± 0.04^{o}	17.64 ± 0.53^{b}	7.93 ± 0.06^{m}	73.59 ± 0.07^{c}	9.28 ± 0.07^{c}
	Frantoio 2	15.48 ± 0.11^{e}	70.23 ± 0.22^{f}	9.73 ± 0.06^{m}	17.34 ± 0.27^{bc}	10.53 ± 0.10^{k}	71.80 ± 0.19^{ef}	6.82 ± 0.04^{f}
	Frantoio 3	14.85 ± 0.10^{f}	69.59 ± 0.20^{g}	10.61 ± 0.04^{k}	16.80 ± 0.05^{cd}	11.47 ± 0.03^{i}	71.25 ± 0.23^{f}	6.21 ± 0.00^{g}

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid. *Each value is an average of three determinations, and values in the same row with different letters show statistically significant differences ($P < 0.05$).

Table 6: Fatty acid composition (%) of the virgin olive oils of three cultivars grown in Marrakech (Morocco) during the two crop seasons (2016 and 2017).

	Cultivars	C16 : 0	C18 : 1	C18 : 2	ΣSFA	ΣPUFA	ΣMUFA	MUFA/PUFA
2016	Languedoc Picholine 1	15.37 ± 0.07^{c}	62.04 ± 0.11^{h}	17.65 ± 0.05^{f}	15.40 ± 0.07^{e}	18.96 ± 0.01^{f}	62.24 ± 0.10^{e}	3.28 ± 0.00^{h}
	Languedoc Picholine 2	15.24 ± 0.04^{c}	58.57 ± 0.14^{g}	21.83 ± 0.04^{c}	15.30 ± 0.03^{e}	23.19 ± 0.02^{c}	58.71 ± 0.11^{g}	2.53 ± 0.00^{l}
	Languedoc Picholine 3	14.67 ± 0.08^{d}	56.34 ± 0.06^{k}	24.21 ± 0.02^{b}	14.73 ± 0.07^{f}	25.71 ± 0.04^{b}	56.50 ± 0.04^{h}	2.20 ± 0.00^{m}
	Moroccan Picholine 1	11.69 ± 0.01^{g}	72.50 ± 0.26^{a}	11.27 ± 0.04^{m}	11.72 ± 0.01^{i}	12.55 ± 0.03^{n}	72.60 ± 0.23^{a}	5.78 ± 0.01^{b}
	Moroccan Picholine 2	11.43 ± 0.02^{h}	69.74 ± 0.18^{b}	13.84 ± 0.02^{j}	11.49 ± 0.01^{i}	15.21 ± 0.05^{k}	69.92 ± 0.16^{b}	4.60 ± 0.00^{e}
	Moroccan Picholine 3	10.92 ± 0.03^{j}	65.31 ± 0.01^{f}	18.21 ± 0.00^{e}	11.00 ± 0.04^{j}	19.65 ± 0.13^{e}	65.53 ± 0.03^{d}	3.34 ± 0.02^{gh}
	Frantoio 1	16.25 ± 0.03^{a}	65.15 ± 0.73^{f}	16.55 ± 0.03^{h}	16.28 ± 0.04^{d}	17.40 ± 0.02^{f}	65.25 ± 0.15^{i}	3.75 ± 0.01^{i}
	Frantoio 2	16.21 ± 0.09^{ab}	60.10 ± 0.13^{i}	17.03 ± 0.07^{g}	16.26 ± 0.08^{d}	17.90 ± 0.09^{h}	60.24 ± 0.11^{f}	3.37 ± 0.01
	Frantoio 3	11.40 ± 0.03^{h}	55.08 ± 0.15^{l}	18.09 ± 0.01^{e}	11.42 ± 0.03^{i}	18.99 ± 0.01^{f}	55.15 ± 0.14^{i}	2.90 ± 0.01^{i}
2017	Languedoc Picholine 1	14.85 ± 0.07^{d}	63.88 ± 0.14^{g}	16.10 ± 0.14^{i}	17.01 ± 0.05^{c}	17.13 ± 0.06^{j}	64.64 ± 0.45^{d}	3.77 ± 0.01^{f}
	Languedoc Picholine 2	14.27 ± 0.10^{e}	60.63 ± 0.04^{i}	20.68 ± 0.02^{d}	16.28 ± 0.05^{d}	21.72 ± 0.02^{d}	61.31 ± 0.21^{e}	2.83 ± 0.01^{j}
	Languedoc Picholine 3	13.23 ± 0.03^{f}	55.81 ± 0.39^{kl}	25.56 ± 0.09^{a}	14.88 ± 0.32^{e}	26.65 ± 0.09^{a}	56.45 ± 0.24^{h}	2.13 ± 0.00^{m}
	Moroccan Picholine 1	11.71 ± 0.03^{g}	71.11 ± 0.05^{b}	12.43 ± 0.05^{l}	13.66 ± 0.06^{g}	13.48 ± 0.05^{m}	71.93 ± 0.20^{a}	5.36 ± 0.00^{c}
	Moroccan Picholine 2	11.31 ± 0.21^{hi}	66.76 ± 0.72^{e}	16.48 ± 0.22^{h}	13.47 ± 0.17^{h}	17.66 ± 0.13^{i}	67.55 ± 0.73^{c}	3.83 ± 0.01^{f}
	Moroccan Picholine 3	11.07 ± 0.11^{ij}	61.02 ± 0.06^{hi}	21.98 ± 0.02^{c}	13.36 ± 0.10^{gh}	23.18 ± 0.08^{c}	61.73 ± 0.02^{e}	2.67 ± 0.01^{k}
	Frantoio 1	15.24 ± 0.08^{c}	68.37 ± 0.10^{d}	10.02 ± 0.11^{n}	17.05 ± 0.03^{b}	10.76 ± 0.05^{o}	69.81 ± 0.88^{b}	6.54 ± 0.05^{a}
	Frantoio 2	15.97 ± 0.04^{b}	66.03 ± 0.20^{ef}	13.53 ± 0.08^{k}	17.69 ± 0.03^{b}	14.18 ± 0.12^{l}	67.66 ± 0.23^{c}	4.77 ± 0.06^{d}
	Frantoio 3	16.45 ± 0.08^{a}	59.98 ± 0.48^{i}	17.65 ± 0.01^{f}	19.14 ± 0.13^{a}	$18.53 \pm 0.0.08^{g}$	61.69 ± 0.32^{e}	3.35 ± 0.03^{gh}

SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid. *Each value is an average of three determinations, and values in the same row with different letters show statistically significant differences ($P < 0.05$).

nutritional properties and oxidative stability of olive oils. The MUFA/PUFA ratio was higher at the green stage and decreased during the olive maturation. This ratio (MUFA/PUFA) was higher in the oils obtained from Errachidia in both crop seasons.

5. Conclusion

The present research work provides information about the maturation patterns of three cultivars (Moroccan Picholine, Languedoc Picholine, and Frantoio) grown in two locations in Morocco (Errachidia and Marrakech) and how they are influenced by environmental conditions during the two crop seasons. According to the results, progress in maturity was accompanied by a slight rise in oil content and acidity, while water content and peroxide value decrease. As the maturation process progressed, a series of changes occur, including modifications in the fatty acid profile (oleic and palmitic acids decreased as ripening progressed, while the linoleic acid increased, also the ratio of MUFA/PUFA decreased during ripening). Significant decrease was noted in phenol content and bitterness intensity. It was demonstrated that the crop season is also an essential factor for the determination of the chemical composition of olive oil.

Languedoc Picholine and Frantoio when grown in Morocco produced oils with some differences (phenol content and fatty acid composition) compared to those obtained in their traditional growing area. Nevertheless, this research evidenced as well that the environmental conditions influence significantly the chemical composition of olive oil. The findings of this research have shown also that the stage of maturity and geographical conditions have a major role in the determination of the characteristics of olive oil.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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