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Original Research

Physicochemical Profiling of Olive Oil as Affected by Variety and Maturity: Relevance to Climate Adaptation Strategies

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Abstract

Olive oil quality is shaped by genetic factors, environmental conditions, and the fruit's maturation stage. This study evaluated the chemical composition and quality of virgin olive oils obtained from two principal Tunisian olive varieties: Chemlali Sfax from central Tunisia and Chetoui from the north. The results indicate that olive oil characteristics are impacted by both cultivar and fruit maturity stages. In both varieties, total oil content and fatty acid composition were assessed at different ripening stages, with oil content reaching up to 30% of fresh weight at full ripeness. During maturation, the free acidity increased from 0.2% to 0.4%, and linoleic acid content rose by approximately 15%, especially in both varieties' later ripening stages. The fatty acid profile revealed higher oleic acid levels in Chetoui (up to 68%) compared to Chemlali Sfax (60%). Antioxidant content, such as carotens, decreased from 6.79 to 2.34 for the Chetoui variety and 7.83 to 5.53. The Chemlali Sfax variety and its related parameters, such as phenols and pigments (chlorophyll), tended to decline with fruit ripening, resulting in distinct compositional profiles that can serve as varietal and maturity indicators.

These findings underline the importance of cultivar selection and harvest timing in optimizing olive oil quality and resilience to changing environmental conditions.

Keywords: *Olea europaea* L., fruit ripening, oil content, Chemlali Sfax, Chetoui

Introduction

Olive oil is a fundamental component of the Mediterranean diet [1, 2]. Enhancing olive oil quality deserves special attention in order to guarantee its nutritional and organoleptic properties [3].

Virgin olive oil has a chemical composition that varies based on environmental stressors, such as limited water availability or variations in harvest timing, which play a central role in the biosynthesis of olive oil's key compounds. In addition, variety, geographical production area (altitude, soil composition, and latitude), climate

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conditions, ripening stages, olive tree age, extraction process, and farming practices [4-6]. The effects of harvest timing on the oil yield, quality, stability, and sensorial characteristics are of particular interest to the grower. According to [4], most olive oils produced commercially are of compromised quality due to improper harvest time selection.

During the ripening process, the weight, pulp/stone ratio, color, oil content, chemical composition of the oil, and enzyme activities change considerably in the fruits. All these factors influence the fruit's firmness, ease of the oil, and sensory characteristics [4, 5]. The composition of fatty acids and the levels of polyphenols, tocopherols, sterols, and pigments change with fruit maturation [7, 8]. The magnitude of these changes depends on the cultivar, climate, and growing conditions.

In Tunisia, a southern Mediterranean region, olive growing is one of the main agricultural and agri-food activities, playing a significant socioeconomic role. In fact, Tunisia is the fourth-largest producer of olive oil, with a production of 200 thousand tons for the 2023-2024 season 4. It ranks first in olive oil production and export after the European Union, covering 30% of the total crop area with 1.96 million hectares of olive orchards. Tunisian olive growing is characterized by a rich varietal heritage [5, 9], with olive plantations spread across all agricultural areas from north to south and east to west, constituting one of the important strategic sectors of Tunisia's economy.

The two principal olive varieties, Chemlali Sfax and Chetoui, are responsible for the majority of olive oil production in the region. Chemlali Sfax, predominantly cultivated in central Tunisia, is recognized for its high oil yield and adaptability to varying environmental conditions, making it a staple for large-scale production. It is recognized for its robust resistance to environmental stressors, which is particularly valuable in the face of climate variability and water scarcity challenges. On the other hand, Chetoui, found mainly in the northern regions, is highly prized for its superior sensory qualities, including flavor and antioxidant content. These varieties are known to differ significantly not only in their oil yield but also in the composition of their oil, including the levels of fatty acids, phenolic compounds, and other bioactive substances. Understanding these differences is crucial for optimizing the quality and characteristics of produced olive oil. By choosing these two varieties, we were able to assess the impact of different geographic regions and climatic conditions on the physicochemical characteristics of olive oil, providing a broader perspective on the relationship between genetic factors (variety), environmental influences (geography and climate), and maturity stage.

To explore these differences in greater depth, we conducted an experiment aimed at analyzing how several factors, including the variety, the date of harvest, and the stage of fruit maturation, influence the chemical composition of the oil. By studying these variables, we aim to gain insights into how they impact the quality, nutritional value, and overall characteristics of the olive oil produced from these two important varieties.

Materials and Methods

Plant Material

Olive trees of the two principal Tunisian cultivars, Chetoui and Chemlali Sfax, were studied in two distinct geographical locations in Tunisia. Chetoui trees were cultivated in the Beni Khalled region, located in the Nabeul governorate (36°39' N, 10°36' E), in the north of Tunisia, while Chemlali Sfax trees were grown in the Taous locality of the Sfax governorate (34°56'7"N, 10°36'53"E), in the central region of Tunisia that has a semi-arid climate with higher temperatures and lower rainfall compared to the northern region (Beni Khalled). These two regions differ significantly in terms of climatic conditions, altitude, soil composition, and annual rainfall, all of which influence olive tree growth and oil quality.

– Olive oil samples were collected from mature trees of both cultivars during the fruit maturation process, which spans from early ripening (green stage) to full maturity (black stage). Samples were collected during two consecutive years (2014/2015 and 2015/2016), which allowed for comparison between annual variability and the consistency of cultivar responses to climatic conditions over time.
– The number of samples collected from each cultivar was standardized for years, ensuring statistical significance and replicability. Typically, 3 kg of olive fruits per tree were sampled, with 3 trees selected from each locality per year, resulting in a total of 6 trees per variety per year. This allowed for a comprehensive analysis of the variability within each cultivar, accounting for any environmental or genetic influences on oil quality.

Oil Extraction

Oil samples were obtained by a cold extraction process using a laboratory mill equipped with a metal crusher, a mixer, and a basket centrifuge. The oil samples were immediately stored in the dark at 0°C until the moment of analysis. The analysis of chlorophylls and carotenes was carried out a few days after the oil extraction. As for the other analyses, no sample was stored longer than 4 months.

Determination of Pomological Parameters: Average Fruit Weight, Moisture, and Fat Content

The average weight of the olives was systematically determined for each olive sample by weighing three samples of 100 fresh fruits. The fat content was evaluated using an Oxford 4000 NMR device (Oxford

Instruments, Oxford, UK) by directly measuring three samples of 50 freshly harvested olives.

The moisture content of the fruit was determined by weighing the same olives after drying in an oven at 105ºC.

Oil Content

According to [6], this parameter is not a criterion for determining oil quality but is helpful in identifying the optimal harvest date. The oil content of an olive cultivar is a key factor in its acceptance by olive growers and processors [7]. Table 2 shows that olive oil yield for the two virgin olive oils (VOO) is important, ranging on average from 41.21 to 57.22 for Chetoui and 29.22 to 48.11 for Chemlali Sfax, respectively. Previous research shows that each olive variety exhibits variation in oil recovery, primarily due to its genetic profile. Additionally, external factors such as climate, temperature, and soil significantly impact oil yield, with temperature being the most influential factor [8]. [9] also observed a positive correlation between variations in oil content and olive size, which is influenced by both external and internal factors. Similarly, [10, 11] indicated that larger olive size, pit weight, and fatty acid composition are directly associated with higher oil content percentages.

Quality Criteria

Estimations of free acidity, peroxide value (IP), and UV spectrophotometric indices (K232 & K270) were evaluated according to the official methods described by the ISO 3960 (2007), the EEC regulation 2568/91, and the International Olive Council [2].

Fatty Acid Composition

In order to determine the fatty acid composition, the methyl esters were extracted from olive oils and analyzed by gas chromatography (GC-MS) after cold saponification by mixing a solution of 0.2 g of oil with 5 ml of hexane with 0.3 ml of 2N methanolic potassium hydroxide. After 3 minutes of agitation and subsequent decantation, the methyl esters' upper layer is collected for analysis. The identification of fatty acids was obtained by comparing their retention times with those of standard compounds, and results were expressed as percent (%) of the relative area.

Pigment Content

Chlorophyll determination was analyzed following the method described by Wolff (1968), based on spectrophotometric quantification by detecting the absorbance at 630, 670, and 710 nm. Virgin olive oil samples were filled directly into 1 cm path-length glass cells (L), and pure carbon tetrachloride was utilized as a control.

The chlorophyll compound was estimated using the following formula:

$$\text{Chlorophyll (mg/kg)} = (A670 - (A630 + A710) / 2) / (0.1086 \times L)$$

The carotenoid fraction was performed from the absorption spectra at 470 nm of 3 g of olive oil dissolved in 25 ml of cyclohexane.

$$\text{Carotene (mg/kg)} = (A470 \times 25 \times 1000) / (E \times 75)$$

With E, the specific extinction is equal to 2000.

Determination of Total Polyphenol Content

Virgin olive oil is the only vegetable oil containing appreciable amounts of natural phenolic substances. The presence of these compounds is often considered a quality criterion. Polyphenols were quantitatively determined using HPLC, according to the method of [2].

2.5 g of olive oil was added to 5 ml of hexane and 5 ml of methanol-water (60/40). The mixture was agitated for 2 min using a vortex mixer and then centrifuged at 3500 rpm for 10 min. After centrifugation, each tube contained two phases: the supernatant, which contains the saponifiable part, and the precipitate, which contains the phenolic compounds. 0.2 ml of the phenolic extract (precipitate), 4.3 ml of distilled water, 1 ml of sodium carbonate (Na_2CO_3), and 4 ml of distilled water were mixed in the flask. Samples were kept away from light for 1 hour until all phenolic compounds were oxidized. The absorbance was measured using a UV/V spectrophotometer at a wavelength of 726 nm.

The following formula determines the polyphenol content:

$$\text{Polyphenols} = (833.32 \times A) + 10.25$$

Statistical Analysis

All parameters were determined in triplicate, and statistical analysis was performed using SPSS software version 20. Analysis of variance (ANOVA) was applied to determine the effects of maturity, variety, and harvest season on the pomological and physicochemical characteristics of olives and oils, respectively. Duncan's test was used to assess significant differences in the aforementioned parameters. Principal component analysis (PCA) was conducted. For all statistical analyses performed, differences were considered significant at $p<0.05$. Multivariate analyses utilized the Pearson correlation approach, and results were presented as heatmaps and PCA biplots performed by XlStat v2017.

Results and Discussion

Pomological Classification of Chetoui and Chemlali Sfax Fruits

Pomological parameters – including fruit weight, oil content, and moisture – were measured at 6 stages of ripening and summarized in Table 1. Significant variations ($p<0.001$) were observed between fruits from adult and young olive trees as ripening progressed. ANOVA revealed that the maturity stage and harvest year primarily influenced fruit weight. Over the two studied seasons, olive weight gradually increased with ripening in both varieties. A significant interaction between agricultural season and variety was also observed, likely due to climatic variations such as temperature and precipitation.

Regardless of the maturity stage or year, Duncan's test showed that Chetoui olives had the highest average fruit weight (2.43 g), while Chemlali Sfax olives ranged between 0.98 g and 1.00 g. These findings are consistent with previous studies [4, 12].

Moisture content varied markedly between the two seasons. For Chemlali Sfax, it ranged from 38.95% to 53.67% in 2015/2016, while the 2014/2015 season was marked by even higher moisture, with an average of 54.03%. These differences highlight the strong impact of climatic conditions on olive moisture content [13, 14].

At the beginning of the season (September), the fruits of both varieties were in early ripening stages, characterized by smaller size, lower oil yield, and higher moisture. However, Chetoui olives showed greater fat content and larger size than Chemlali Sfax. By December, mature fruits from both varieties exhibited increased fruit size, higher oil content, and sustained high moisture levels [4].

Principal component analysis (PCA) was conducted on the pomological parameters to identify potential underlying relationship patterns among the variables. As illustrated in Fig. 1, two principal components were extracted, accounting for a total of 92.38% of the variance. After rotation, Component 1 had an eigenvalue of 2.16 and explained 54.22% of the total variance, while Component 2 had an eigenvalue of 1.52, accounting for 38.16%.

The loading plot on the Component 1 vs. Component 2 plane (Fig. 1) shows that moisture is inversely correlated with average fruit weight (FW), fat content on a dry weight basis (F/DW), and fat content on a fresh weight basis (F/FW). These variables all contribute significantly to the variance explained by Component 1. Regarding Component 2, both F/FW and F/DW contribute to its structure, with F/DW showing the strongest influence.

Fig. 1 also highlights that average fruit weight is the variable contributing most strongly to the second principal component. Moreover, fat content increases progressively with fruit maturity, and this accumulation occurs in parallel with the increase in fruit weight. However, high moisture content negatively influences oil yield [14-16]. The same figure suggests that the first principal component primarily reflects oil yield–related variables, whereas the second component is more closely associated with fruit quality traits. Fig. 1 also shows distinctive groups of cultivar/harvest periods.

The comparison between the observation and score plots for the first and second principal components reveals distinct patterns based on the olive variety and harvest period. Samples with positive scores on both components correspond to Chetoui olives harvested between October and December. These samples displayed higher mean values for the variables contributing to both components, notably fruit weight, oil content, and low moisture levels – indicators of advanced maturity and superior fruit quality. In contrast, Chemlali Sfax olives harvested in November and December display positive scores for the first component, reflecting improved oil yield, but negative scores for the second component, suggesting comparatively lower fruit quality parameters such as fruit weight. During the early stages of harvest (from September to mid-October), Chemlali Sfax samples showed negative scores for both components, indicating immature fruits with lower oil content and smaller size. Interestingly, during this same early period, Chetoui olives already exhibit positive scores for the second component, reflecting more favorable fruit quality traits even at earlier stages of maturation.

These findings support the well-established notion that olive oil yield increases with fruit maturity [5, 16, 17]. Therefore, determining the optimal harvest time requires a comprehensive evaluation of key parameters such as average fruit weight, moisture content, and oil content. These factors not only influence oil yield but also have direct economic implications. Given that they are closely linked to the maturity stage as well as climatic and agronomic conditions [3, 18, 19], their progression was carefully monitored across the two studied seasons (2014/2015 and 2015/2016) for both olive varieties.

Evolution of the Physicochemical Characteristics of Virgin Olive oil During Fruit Ripening

The physicochemical quality parameters of Chetoui and Chemlali Sfax olive oils were evaluated at different stages of fruit maturation (Table 2). All samples showed values well within the limits set by the *Regulation (EEC) No. 2568/91* for extra virgin olive oil – namely, free acidity $\leq 0.8\%$, $K270 \leq 0.22$, and $K232 \leq 2.50$ [2]. These results confirm that the oils produced throughout the harvest period maintained excellent physicochemical quality.

Statistically significant differences were observed between the two varieties and across ripening stages for key parameters, particularly free acidity, K232 absorbance, and total phenolic content. Among these, free acidity is a crucial quality indicator. It reflects the

Table 1. Variability of pomological parameters of trees among harvesting periods.

		2014/2015						2015/2016					
	Var	Sept2	Oct1	Oct2	Nov1	Nov2	Dec1	Sept2	Oct1	Oct2	Nov1	Nov2	Dec1
MI	CHT	---	---	2.9±0.7	3.0±0.7	3.2±0.7	3.7±0.4	---	---	2.9±0.7	3.0±0.7	3.2±0.7	3.7±0.4
	CML	1.5±0.2	1.9±0.3	2.0±0.1	2.1±0.3	2.5±0.4	3.1±0.3	1.5±0.3	1.8±0.2	2.0±0.1	2.1±0.2	2.5±0.4	3.0±0.4
FW (g)	CHT	2.00±0.10	2.07±0.09	2.29±0.22	2.98±0.09	3.23±0.11	3.04±0.14	1.81±0.30	2.00±0.17	2.11±0.20	2.29±0.26	2.52±0.33	2.76±0.15
	CML	0.54±0.02	0.65±0.05	0.92±0.07	1.05±0.10	1.19±0.10	0.93±0.08	0.95±0.09	1.07±0.13	1.14±0.09	1.11±0.11	1.15±0.09	1.05±0.11
F/FW (g)	CHT	20.74±0.70	24.73±0.92	24.76±0.80	25.53±1.19	23.84±2.35	25.38±1.43	18.96±0.89	19.86±2.88	23.03±4.07	23.83±4.11	23.71±3.93	23.02±2.16
	CML	13.62±1.39	18.93±1.50	13.75±1.23	12.98±2.03	20.91±4.66	27.79±2.71	23.03±0.66	22.82±0.37	22.95±0.63	27.58±2.17	31.09±1.91	30.58±1.11
F/DW (g)	CHT	41.21±1.53	48.74±1.32	52.02±3.81	52.98±4.58	54.67±6.47	57.22±1.58	40.74±2.49	44.16±2.47	50.17±2.51	50.77±2.69	51.38±2.95	52.12±1.71
	CML	29.22±1.71	35.15±2.46	36.90±3.32	38.88±3.50	48.21±4.63	48.11±2.45	46.02±0.87	48.04±0.67	49.81±0.91	50.51±0.64	50.91±0.72	50.29±0.51
Moisture (%)	CHT	49.39±2.49	52.21±0.49	55.16±1.07	56.25±1.42	58.56±0.60	55.13±0.28	53.61±1.77	54.91±5.16	53.73±6.34	53.71±6.20	53.74±6.21	55.79±3.19
	CML	48.44±0.74	46.40±1.14	58.81±2.60	67.94±5.17	55.96±6.02	46.47±4.85	49.61±1.14	53.76±0.75	52.09±0.96	49.81±0.42	44.64±2.60	38.95±3.21

*** Mean values of the same tree age and the same crop month differ significantly ($p \leq 0.001$) [comparison between harvest dates]. MI: Maturity Index; FW: Fruit weight (g); F/Fruit: Fat content per olive fruit; F/FW: Fat content expressed as percent of fresh weight; F/FD: Fat content expressed as percent of dry weight.

a: Values are means±SD of two successive crop seasons, 2014/2015 and 2015/2016. Oct1 means the first half of the month, and oct2 means the second half of the month.

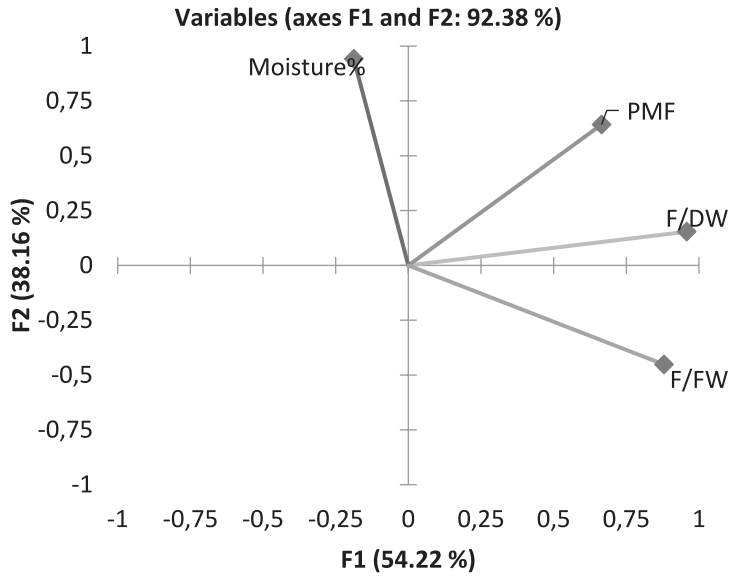

Fig. 1. PCA of the pomological parameters of the Chemlali and Chetoui olive trees during the ripening process for two successive seasons (2014/2015 and 2015/2016).

degree of hydrolytic degradation of triglycerides due to enzymatic or chemical activity, often triggered by poor handling, delays in processing, or advanced ripening [4]. It is also a major criterion in olive oil's commercial classification and nutritional evaluation [2].

According to [19], olives must be harvested manually, processed promptly, and at an appropriate maturity stage to ensure low free acidity. Although the free acidity of Chemlali Sfax oil increased during the later harvest periods, it remained below the 0.8% threshold for extra virgin classification. A similar trend was observed for Chetoui oil. In both cases, acidity levels gradually rose with maturation, in line with previous findings on Turkish olive varieties [20, 21].

This increase in free acidity at advanced ripening stages – particularly in late November and December – is mainly attributed to heightened enzymatic activity, especially from lipolytic enzymes that catalyze the release of free fatty acids [22, 18]. At these stages, free acidity values reached approximately 0.38% for Chetoui and 0.42% for Chemlali Sfax (Table 2). Comparable results were reported by [12], who observed values around 0.62% in February, confirming that delayed harvesting tends to elevate acidity.

Specific Extinction

The specific extinction coefficients K232 and K270 are conventional indicators of olive oil oxidation. K232 reflects the presence of conjugated dienes associated with primary oxidation products, while K270 corresponds to conjugated trienes, indicating secondary oxidation.

K232 values showed highly significant differences depending on the maturation stage, although they were not significantly influenced by harvest year or variety (Table 2). For the Chetoui variety, K232 absorbance ranged from 1.70±0.13 to 2.34±0.17, while Chemlali Sfax varied between 1.77±0.14 and 2.26±0.21 (Table 3). All these values fall within the limits established by the International Olive Council (IOC) for extra virgin olive oil (K232≤2.50), confirming the good oxidative quality of the samples.

Despite the overall compliance with quality standards, K232 values showed significant variation depending on the variety, harvest period, and agricultural year. For example, Chemlali Sfax oils exhibited K232 values ranging from 0.12±0.008 to 0.16±0.03, whereas those of the Chetoui variety ranged from 0.12±0.01 to 0.29±0.03, indicating a broader variation in oxidative stability for this latter variety.

For K270, which reflects the formation of secondary oxidation products, all measured values remained within the IOC limits for extra virgin oils. Notably, Chetoui oils consistently exhibited higher K270 values across all harvest stages. Both K232 and K270 values tended to decrease as the harvest advanced, particularly at later stages of fruit maturity. This trend suggests that oxidative degradation was more pronounced in earlier harvests, while later stages yielded oils with greater oxidative stability.

These results are consistent with previous findings reported by [4] and [12], confirming the influence of the maturity stage on olive oil's oxidative indices.

Chlorophylls

The total chlorophyll content is closely linked to olive oil's color and represents an important analytical parameter influencing its sensory quality [23, 24].

The analysis of chlorophyll levels revealed highly significant variations depending on both the olive variety and the fruit ripening stage. Among these factors,

Table 2. Mean values and standard deviations of the physicochemical parameters of Chemlali Sfax and Chetoui oils produced over two consecutive agricultural seasons.

		2012/2013				2013/2014			
		Oct2	Nov1	Nov2	Dec1	Oct2	Nov1	Nov2	Dec1
IM	CHT	2.9±0.7	3.0±0.7	3.2±0.7	3.7±0.4	2.9±0.7	3.0±0.7	3.2±0.7	3.7±0.4
	CML	2.0±0.1	2.1±0.3	2.5±0.4	3.1±0.3	2.0±0.1	2.1±0.2	2.5±0.4	3.0±0.4
Acidity	CHT	0.30±0.02^{ab}	0.26±0.05^{a}	0.33±0.06^{b}	0.35±0.04^{b}	0.32±0.02	0.35±0.01	0.38±0.06	0.36±0.08
	CML	0.34±0.04	0.40±0.02	0.35±0.07	0.32±0.04	0.39±0.11	0.42±0.10	0.41±0.04	0.36±0.07
K232	CHT	2.35±0.18^{c}	2.00±0.25^{b}	2.21±0.10b^{bc}	1.75±0.12^{a}	2.32±0.19	2.20±0.04	1.91±0.14	1.0±0.13
	CML	1.80±0.15	2.26±0.22	2.03±0.10	1.78±0.14	2.17±0.29	2.10±0.16	2.04±0.18	1.85±0.10
K270	CHT	0.29±0.03^{c}	0.21±0.02^{b}	0.25±0.05^{b}	0.14±0.01^{a}	0.26±0.01	0.23±0.01	0.18±0.01	0.12±0.01
	CML	0.13±0.02	0.15±0.01	0.16±0.04	0.14±0.01	0.16±0.02	0.13±0.02	0.13±0.02	0.13±0.01
Chlorophylls	CHT	9.02±1.14	8.56±0.95	5.74±1.16	1.93±0.51	9.77±0.64	8.19±0.79	4.27±0.88	1.52±1.08
	CML	8.28±1.41	4.45±0.42	2.89±1.13	2.17±0.43	7.36±2.81	3.50±0.78	2.13±0.79	1.45±0.55
Carotens	CHT	6.79±1.15	5.81±3.32	3.44±0.75	2.34±0.50	7.25±1.69	5.99±1.25	5.55±1.74	2.29±1.04
	CML	7.83±1.03	8.88±2.44	7.04±0.55	5.53±0.98	9.06±2.97	4.95±1.23	4.54±0.54	2.93±0.63
Polyphenols	CHT	767.51±195.03	662.71±210.69	596.20±196.57	308.83±86.25	876.80±293.46	608.54±146.39	513.33±173.24	509.42±187.67
	CML	690.82±191.05	435.63±157.99	188.58±105.93	204.88±99.31	509.48±187.65	368.33±68.83	381.31±31.74	195.23±46.04

CHT: Chetoui; CML: Chemlali Sfax; IM: maturity index. Oct1 means the first half of the month, and oct2 means the second half of the month.

the maturity stage appeared to be the most influential. In both Chetoui and Chemlali Sfax varieties, chlorophyll content decreased markedly as ripening progressed (Table 2).

These findings align with those of [25], who also examined oil quality in the Chemlali Sfax variety and reported a similar downward trend in chlorophyll content throughout fruit maturation. This decline is commonly attributed to the degradation of chlorophyll pigments during the senescence of the olive fruit, which leads to a loss of green color in the oil.

According to the Duncan test, the crop year also had a statistically significant effect on chlorophyll levels – an observation that contrasts with earlier studies, such as those by [4] and [26], which reported minimal influence of the harvest year on this parameter. Despite these interannual variations, the Chetoui variety consistently displayed the highest chlorophyll concentrations, regardless of the year or harvest period, suggesting a strong varietal influence.

Carotens

Carotenoids, particularly β-carotene, are natural pigments present in virgin olive oil. Their concentration varies depending on several factors, including the olive variety, the degree of fruit maturity, and the harvesting method used [4, 27]. These compounds not only contribute to the oil's characteristic yellow-orange color but also possess antioxidant properties that influence its nutritional and sensory quality. Analysis of variance revealed highly significant differences in carotene content according to both the harvest period and the olive variety (Table 2). As maturation progressed, a marked decrease in carotene levels was observed. For the Chetoui variety, carotene content declined from 6.79 to 2.34 mg/kg, while Chemlali Sfax dropped from 7.83 to 5.53 mg/kg.

Interestingly, the Chemlali Sfax variety consistently exhibited higher carotene concentrations than Chetoui, regardless of the harvest year or stage. This suggests a strong varietal influence on carotenoid accumulation, highlighting Chemlali Sfax as a potentially more carotenoid-rich variety.

Polyphenols

The polyphenol content of olive oil is known to vary significantly depending on the cultivar, agronomic practices, ripening stage, and storage conditions [28, 29]. In our study, the total phenolic content (Table 2) showed clear variations according to both the harvest period and the olive variety. Oils derived from the Chetoui variety consistently exhibited higher levels of phenolic compounds than those from Chemlali Sfax. The highest concentration was recorded at the early ripening stage for Chetoui, reaching approximately 876.8 ppm. Throughout the entire maturation process, Chetoui oils remained richer in total phenols (ranging from 308.83 to 876.8 ppm), confirming the strong varietal influence on this parameter.

For both varieties, polyphenol content generally increased from early October to mid-November, followed by a gradual decline at more advanced ripening stages. This trend is consistent with observations reported by [4] and [12]. In contrast to Chetoui, Chemlali Sfax oils showed significantly lower phenolic content, reinforcing the importance of optimal harvest timing to maximize polyphenol levels in this variety.

To further explore the relationships between quality attributes and maturity, a Principal Component Analysis (PCA) was conducted using a dataset that included quality indices (K232, K270), total phenols, chlorophylls, carotenes, and fatty acid profiles across different harvest stages. The PCA revealed four components, with the first two (F1 and F2) explaining 81.04% of the total variance. The loading vectors are illustrated in Fig. 2.

The maturity stage of the fruit, an environmental stressor, is a critical factor influencing the quality of olive oil. Olives harvested at early ripening stages, characterized by higher levels of green pigments (chlorophyll), tend to produce oil with higher acidity and greater antioxidant properties, such as increased polyphenol content. As the fruit ripens, the oil content increases, but the phenolic compounds tend to decrease. Hence, harvesting timing is a key factor in determining oil quality, with early harvesting yielding oil with higher stability and nutritional benefits [25, 30].

Component 1 (F1), accounting for 52.31% of the variance, was primarily associated with olive oil quality parameters such as K232, K270, chlorophylls, carotenoids, polyphenols, and peroxidase activity. Component 2 (F2), which explained 28.73% of the variance, was mainly linked to ripening indicators, including the maturity index and free acidity. The rotated component matrix (Table 3) confirmed the strong contributions of these variables to their respective components.

The oxidative stability of olive oil is closely linked to its fatty acid composition, particularly the ratio of monounsaturated to polyunsaturated fatty acids. Chetoui olive oil, characterized by high oleic acid content and low linoleic acid levels, generally displays greater oxidation resistance than Chemlali Sfax oil, which contains a higher proportion of polyunsaturated fatty acids. These compositional differences are further influenced by the fruit maturity stage and environmental conditions across harvest years [13, 25].

PCA score plots revealed interesting distribution patterns across harvest dates. In the early harvest (October), Chemlali Sfax samples showed a positive score on F1 and a negative score on F2, whereas Chetoui samples were positively associated with both components. As ripening progressed (November), Chemlali Sfax oils shifted toward negative scores on both axes, reflecting a decline in both quality and ripeness-related attributes. In contrast, Chetoui samples retained a positive association with F1, highlighting the

Table 3. Mean values and standard deviations of fatty acids from oils of two oil-producing varieties during the olive ripening process.

Acides gras (%)	CHT					CML				
	Oct-01	Oct-02	Nov-01	Nov-02	Dec-01	Oct-01	Oct-02	Nov-01	Nov-02	Dec-01
C16:0	12.18±0.32^{a}	11.72±0.61^{a}	11.72±0.85^{a}	11.28±1.09^{a}	10.75±0.85^{a}	19.07±0.15^{a}	18.63±0.15^{ab}	18.47±0.41^{ab}	18.89±0.87^{b}	17.87±0.31^{b}
C16:1	0.32±0.01^{a}	0.35±0.06^{a}	0.32±0.02^{a}	0.37±0.11^{a}	0.32±0.01^{a}	1.36±0.48^{a}	1.93±0.15^{b}	2.18±0.08^{b}	2.40±0.17^{b}	2.27±0.21^{b}
C17:0	0.04±0.01^{a}	0.04±0.01^{a}	0.04±0.00^{a}	0.04±0.01^{a}	0.05±0.00^{a}	0.04±0.01^{a}	0.03±0.00^{a}	0.04±0.00^{a}	0.04±0.00^{a}	0.04±0.01^{a}
C18:0	2.78±0.10^{a}	2.66±0.25^{a}	2.52±0.04^{a}	2.52±0.09^{a}	2.69±0.22^{a}	2.30±0.17^{a}	2.25±0.15^{ab}	2.11±0.19^{ab}	2.06±0.14^{ab}	1.93±0.15^{b}
C18:1	71.84±0.21^{a}	70.39±0.74^{ab}	68.51±1.21^{bc}	66.89±0.51^{cd}	65.95±2.35^{d}	63.27±1.57^{a}	66.90±7.30^{a}	61.47±0.55^{a}	60.23±0.74^{a}	60.93±0.57^{a}
C18:2	11.97±0.77^{a}	13.39±0.80^{a}	16.04±0.38^{b}	17.08±0.80^{b}	18.93±1.11^{c}	11.63±0.55^{a}	13.56±1.35^{ab}	13.77±1.39^{ab}	14.60±1.42^{b}	15.03±1.01^{b}
C18:3	0.53±0.02^{a}	0.51±0.01^{a}	0.58±0.05^{a}	0.57±0.03^{a}	0.57±0.05^{a}	0.76±0.05^{a}	0.72±0.01^{ab}	0.65±0.02^{b}	0.60±0.03^{c}	0.57±0.03^{c}
C20:0	0.41±0.03^{a}	0.42±0.03^{a}	0.43±0.03^{a}	0.43±0.03^{a}	0.45±0.02^{a}	0.43±0.03^{a}	0.44±0.04^{ab}	0.44±0.00^{b}	0.41±0.02^{b}	0.37±0.02^{b}
C20:1	0.40±0.02^{a}	0.45±0.02^{b}	0.47±0.02^{b}	0.44±0.01^{b}	0.45±0.01^{b}	0.38±0.07^{a}	0.34±0.05^{ab}	0.27±0.02^{bc}	0.24±0.01^{cd}	0.18±0.02^{d}

CHT: Chetoui; CML: Chemlali Sfax; C16:0, palmitic acid; C16:1, palmitoleic acid; 17:0, margaric acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid; C20:1, gondoic acid.
Oct1 means the first half of the month, and oct2 means the second half of the month.

variety's ability to maintain higher quality markers even at advanced stages of ripening.

Acidic Composition

Fatty acids are widely recognized as key parameters for characterizing and classifying olive oils [19].

Their composition plays a crucial role in determining olive oil's nutritional and organoleptic quality. Several factors influence the fatty acid profile, including olive variety, maturity stage, and environmental conditions such as climate [4, 27, 28, 31]. Some authors have even used fatty acid profiles to classify olive oils by geographical origin [32], while others report only minor variations in the main fatty acid – oleic acid (C18:1) – within the same variety grown in different regions.

Our study of the fatty acid composition of extra virgin olive oils from Chemlali Sfax and Chetoui varieties confirms their compliance with International Olive Oil Council (IOOC) standards. The oils contain several fatty acids, including myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), margaric (C17:0), margaroleic (C17:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), and gadoleic acid (C20:1).

This composition is marked by the dominance of oleic acid, followed by palmitic, stearic, and linoleic acids. Minor components such as palmitoleic, linolenic, arachidic, and gadoleic acids were present in low concentrations, while myristic, margaric, and margaroleic acids remained below 0.2% in all samples.

We observed notable trends during olive ripening: linoleic acid content increased, palmitic acid decreased, and oleic acid remained relatively stable. These variations likely reflect genetic differences between cultivars and the influence of environmental and developmental factors [5, 14, 25, 33, 34]. Some authors have used this profile as a classification parameter for olive oils according to their origins [30, 32, 35-38]; others note rather minor variations in primary fatty acid levels (C18:1). In the same olive variety, even if grown in different locations.

The increase in linoleic acid may be attributed to the action of the enzyme oleate desaturase, which catalyzes the conversion of oleic acid (C18:1) into linoleic acid (C18:2) during fruit maturation.

Table 3 shows that young fruits (drupes) have relatively low levels of oleic and linoleic acids, around 20% in both varieties. As maturation progresses, oleic acid becomes the major fatty acid in the mesocarp, particularly in the Chetoui variety, which exhibits the highest oleic acid content. In contrast, Chemlali Sfax shows lower levels. Palmitic acid decreases from 12.18% to 10.75% in Chetoui and 19.07% to 17.87% in Chemlali Sfax, confirming the maturation-related shift in fatty acid profiles. Similarly, the increase in linoleic acid during ripening supports findings from earlier studies [38].

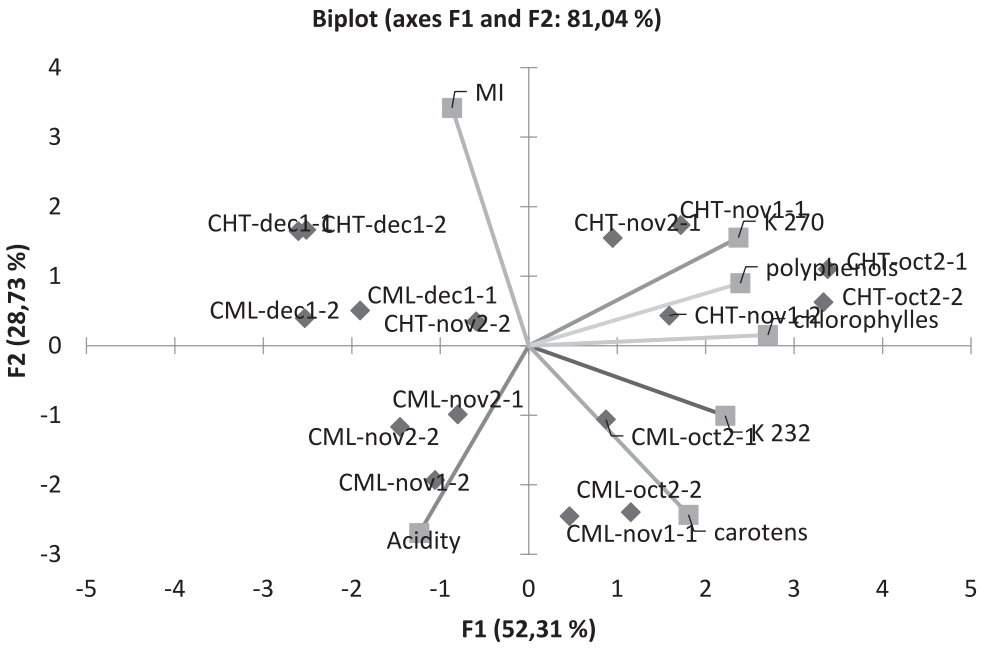

Fig. 2. The principal components analysis (PCA) was applied to the dataset of the analytical parameters of oils of two varieties of olive trees, Chemlali and Chetoui, during the maturation process for two successive seasons (2014/2015 and 2015/2016). CML: Chemlali, CHT: Chetoui

The high oleic acid content can be explained by the desaturation mechanism, which involves the conversion of stearic acid into oleic acid by the enzyme stearoyl-ACP desaturase. This enzymatic reaction introduces a double bond into the saturated fatty acid chain, forming the monounsaturated oleic acid. In addition, the desaturation of oleic acid to linoleic acid is catalyzed by the enzyme FAD2 (oleate desaturase), which adds a second double bond to the fatty acid chain, leading to the production of the polyunsaturated linoleic acid.

The balance between these fatty acids depends on both genetic and environmental factors, such as the cultivar, climate, and growing conditions. For example, olives grown in warmer, arid regions tend to produce oils with higher oleic acid content, which contributes positively to both nutritional value and shelf life.

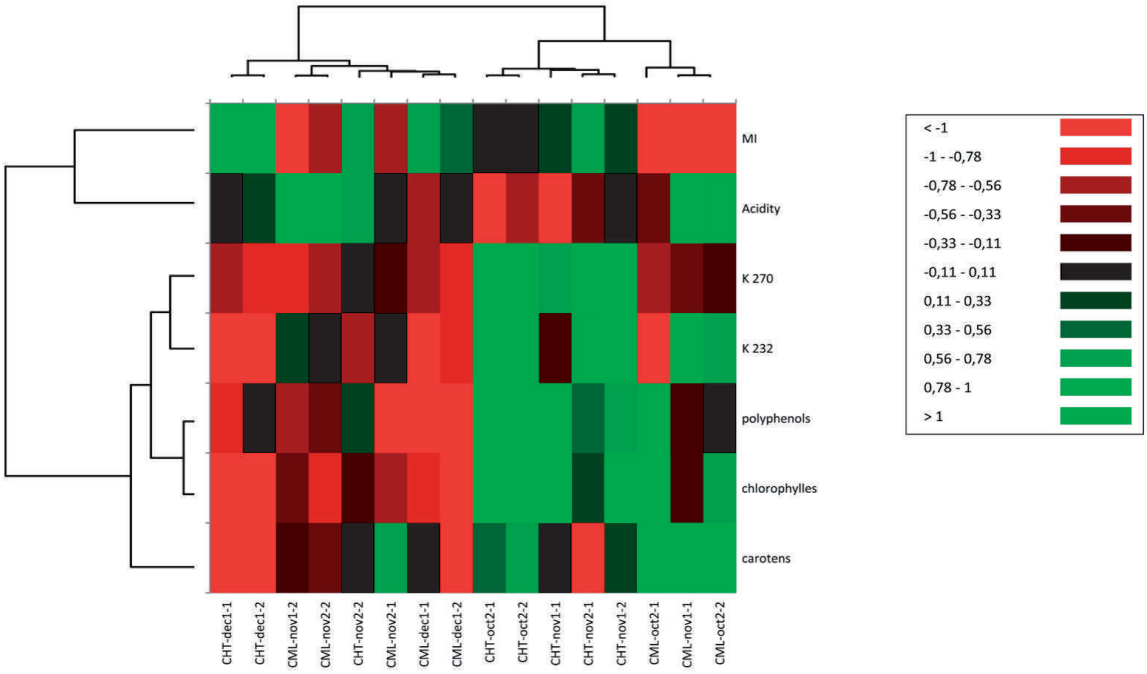

Fig. 3. Heatmap and hierarchical clustering for maturity index, acidity, K232, K270, polyphenols, chlorophylls, and carotens.

The linoleic acid content can also be linked to the oxidative stability of virgin olive oils. Although linoleic acid is an essential fatty acid with health benefits, it is more susceptible to oxidation due to its multiple double bonds. In contrast, oleic acid enhances oxidative stability thanks to its single double bond, making it less prone to degradation. Therefore, a higher oleic/linoleic acid ratio is often desirable in olive oils to ensure better resistance to oxidation, improved flavor preservation, and longer storage time [38, 39].

The heatmaps obtained in Fig. 3 permit us to discriminate two groups; the first one corresponds to CT-oct2, CT-nov1, CT-nov2, CM-oct2, and CM-nov1 that correlate with most of the parameters, such as carotens, chlorophylls, polyphenols, K232, and K272. The second group, composed of CT-nov2, CT-dec1, CM-nov2, and CM-dec1, correlates with a high maturity index and acidity.

As olives ripen, oleic acid tends to decrease while linoleic acid increases, leading to reduced oxidative stability, particularly in Chemlali oils harvested at later stages [40]. Additionally, climatic factors such as temperature and rainfall impact fatty acid biosynthesis, with warmer years typically favoring higher linoleic acid levels. Chetoui, grown in cooler northern regions, tends to maintain a more stable composition and oil quality across seasons, while Chemlali, adapted to southern arid zones, shows greater variability and reduced oxidative stability. Therefore, early harvesting is recommended for Chemlali to ensure better oil preservation.

Conclusions

This study highlights the significant influence of both the olive variety and maturity stage on the pomological traits of the fruit and the physicochemical properties of virgin olive oil. Our results show that while olive oils produced at early ripening stages exhibit similar quality parameters across varieties, differences become more pronounced as maturation progresses. At later stages, young fruits tend to offer higher oil yields and improved fruit quality, whereas oil extracted from mature fruits displays superior overall quality in terms of composition and stability.

The comparative analysis of pomological parameters, quality indices, and fatty acid profiles between the Chemlali Sfax and Chetoui cultivars revealed both qualitative and quantitative variations throughout the ripening process. Principal Component Analysis (PCA) proved valuable in identifying the optimal harvest period for each variety, supporting more informed decision-making for olive oil production.

Nonetheless, further research is needed to validate these findings and to deepen the understanding of how varietal and maturity differences affect olive oil quality, particularly under varying climatic and agronomic conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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