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Chemical and Sensory Characteristics of Olive Oils Extracted from the Tunisian Olive Varieties

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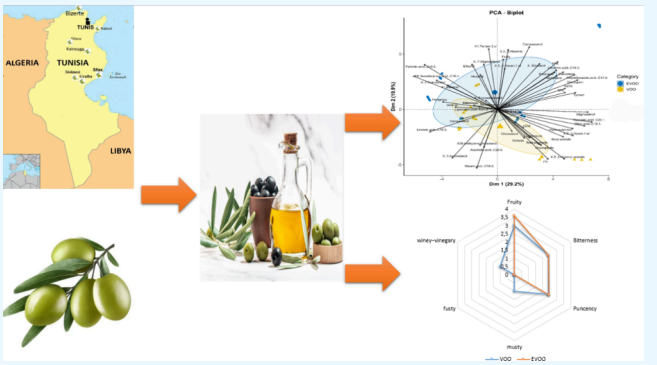
ABSTRACT: The chemical and sensory characteristics of virgin olive oil (VOO) and extra virgin olive oil (EVOO) are assessed in this study, with an emphasis on the relationships between chemical profiles and sensory attributes such as fruitiness, bitterness, pungency, and defect presence. To evaluate their composition and sensory qualities, 18 samples of each type of oil, primarily Chemlali cultivars from various Tunisian regions, were examined. According to chemical analysis, VOO had a richer pigment profile than EVOO because it had a far higher chlorophyll and carotenoid. As for the fatty acid analysis, VOO had a larger percentage of the oleic acid concentration, while EVOO had more palmitic acid. This difference is probably what affects the oils' oxidative stability and sensory qualities. On the other hand, EVOO samples displayed higher concentrations of volatile and phenolic chemicals, which may improve their antioxidant capacity and sensory qualities. While VOO had discernible defects, sensory examination showed that EVOO had a more pronounced fruity profile and was consistently free of sensory defects. While pungency and bitterness were similar for both oil types, EVOO's lack of flaws complies with consumer preferences and premium olive oil quality requirements. EVOO's distinct nutritional profile, characterized by higher oleuropein and TPC levels ($p < 0.05$), increased Δ-7-stigmastenol ($p < 0.001$), and exclusive campestanol, enhances its antioxidant potential and cholesterol-regulating properties. These findings underline the influence of chemical composition on sensory perception in olive oils and highlight cultivar and regional differences. Both oils provide antioxidant and cholesterol-regulating benefits, but their unique chemical and sensory properties determine their suitability for different culinary and medical uses. The correlation between sensory attributes and chemical markers offers information on how to maximize quality of olive oil for desired flavor profiles and nutritional benefits.



1. INTRODUCTION

Virgin olive oil (VOO) and extra virgin olive oil (EVOO) are celebrated worldwide for their exceptional nutrition, rich flavors, and numerous health benefits, making them fundamental components of the Mediterranean diet. These oils are principally produced from the fruit of the *Olea europaea* tree, whose composition and quality are shaped by factors, such as the cultivar, geographical origin, climate, and cultivation practices. Tunisia, a major leader in global olive oil production, is home to about 107 million olive trees spread across 2 million hectares of olive grooves[1] (DGPA, 2023). In the 2021/2022 season, the country's olive oil production reached 240,000 tons, of which nearly 80% was classified as extra virgin[2] (DGPA, 2023). Worldwide, Tunisia ranked as the third largest producer and the second largest exporter of olive oil[3] (COI, 2023). It has also a wide range of native olive cultivars, each contributing to a large array of features, from sensory attributes like fruitiness, bitterness, and pungency to chemical profiles, namely, phenolic compounds, volatile compounds, sterols, and fatty acids.[4,5]

Phenolic compounds are fundamental to the exceptional qualities of olive oil, serving as potent antioxidants that enhance its oxidative stability. These compounds are key contributors to the oil's distinctive bitterness and pungency, which are markers of high-quality extra virgin olive oil.[6]

The phenolic content of olive oil varies widely based on factors such as the olive cultivar, ripeness at harvest, and processing techniques. For example, Tunisian cultivars like *Chemlali* and *Chetoui* yield oils with differing phenolic

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concentrations,[7] which directly affect their sensory attributes. These characteristics are essential not only for shaping consumer preferences but also for determining the commercial classification of olive oils. Positive attributes, such as bitterness and pungency, are highly regarded in sensory evaluations, whereas defects like rancidity or fustiness are deemed undesirable.[8]

Volatile compounds represent a crucial component of olive oil quality, playing a significant role in defining the characteristic aroma of EVOO. These compounds, mainly aldehydes, esters, and alcohols, are products of polyunsaturated fatty acids, and their composition varies depending on the olive cultivar and oil extraction technique used.[9,10] Olive oils from Tunisian indigenous cultivars are characterized by a unique volatile profile that strongly influences sensory attributes such as fruitiness.[5,11] This connection between volatile profiles and sensory qualities is essential for understanding the impact of cultivar-specific traits on olive oil's overall quality.[5,12]

Sterols and fatty acids are also important in determining the composition of olive oil. Sterols are significant in assessing the authenticity and purity of the oil because their composition might indicate whether the oil has been adulterated with other vegetable oils.[13,14] Fatty acids, especially oleic acid, are key components of olive oil and contribute significantly to its health-promoting properties, such as anti-inflammatory and cardioprotective effects.[15,16] The fatty acid profile of olive oil is largely influenced by the olive cultivar, with Tunisian variety frequently exhibiting elevated oleic acid levels. This high oleic acid content is positively associated with enhanced oil stability and health benefits.[17]

Quality parameters, such as free acidity, peroxide value, and UV absorbance, are essential for the classification of olive oil as VOO or EVOO. These parameters indicate the extent of oil degradation and oxidation, which are affected by various factors, including fruit handling, extraction methods, and storage conditions.[18] EVOO is required to meet quality standards that are more stringent than those of VOO, including lower limits on free acidity and peroxide values. These criteria ensure its superior chemical composition and sensory attributes (European Commission, 2022).[19]

The utmost objective of the present research work is to characterize and compare the phenolic, volatile, sterol, and fatty acid profiles of virgin and extra virgin olive oils from well spread Tunisian olive cultivars, namely, *Chemlali*, *Chemchali*, and other regional varieties. Additionally, this study examines the relationship between these chemical compositions of olive oil and sensory attributes, including fruitiness, bitterness, pungency, and defects. By analyzing these correlations, we aim to provide a deeper understanding of how cultivar and processing techniques shape the overall quality of olive oil. This effort seeks to promote Tunisian olive oils in the international market, highlighting their distinctive features and exceptional quality.

2. MATERIALS AND METHODS

2.1. Samples. The used samples of monovarietal VOO and EVOO were obtained from olive cultivars of the north and south regions (Tunisia): Chemlali (Sfax), Chemlali (Graïba), Chemlali (Meknesi), Chemlali (Kairouen), Chemcheli (Siliana), Sayali (Nabeul), and Koroneiki (Bizert) (Table 1). Approximately 300 kg of olives from each variety was collected in the last week of October during the harvest season 2022/2023. Their maturity indices range between 1.0 and 1.2 (yellow-green) as reported by IOC (2011).[20] The olives were crushed using a Retsch ZM 200 hammer mill and then gently mixed for 30 min at 25 °C using a Pieralisi Malaxer model X10. The obtained paste was then centrifuged at 3500 rpm for 3 min using a Pieralisi DMF 2000 decanter centrifuge. The extracted oil samples were immediately stored in the dark at 4 °C until analysis.

Table 1. Distribution of Olive Cultivars Used for Oil Samples[a]

origin/variety	VOO (N = 18)	EVOO (N = 18)
Sfax/Chemlali cultivar	6	3
Graïba/Chemlali cultivar	0	3
Meknesi/Chemlali cultivar	3	0
Kairouen/Chemlali cultivar	6	6
Siliana/Chemcheli cultivar	0	3
Nabeul/Sayali cultivar	0	3
Bizerte/Koroneiki cultivar	3	0

[a]VOO: virgin olive oil; EVOO: extra virgin olive oil.

2.2. Quality Parameter Analysis. The measurement of free acidity (FFA), expressed as a percentage of oleic acid in the oil (g/100 g), was realized according to the ISO 660:2020 method.[21] As for the determination of peroxide value (PV) (meq O_2 kg^{-1}), it was carried out following the ISO 3960:2017 method,[22] and UV absorption at 232 and 270 nm (K_{232} and K_{270}) was measured according to the International Olive Council (IOC) standard (IOC, 2019).[23]

2.3. Extraction of Phenolic Compounds (PC). The samples' PC extraction was conducted according to the IOC method (2017),[24] as described by Rodrigues et al.,[25] with minor adjustments. The amounts of 3 g of VOO or EVOO and 250 μL of syringic acid solution (0.15 mg mL^{-1}) prepared in methanol:water (80:20, v v^{-1}) were mixed and shaken in a 12 mL tube. Next, 3 mL of methanol:water solution was supplemented to the solution and vortexed for 30 s. The obtained mixture was then centrifuged at 500 rpm at 4 °C for 5 min. The lower phase was transferred to a separate tube, and the extraction process was repeated twice more. Afterward, the combined methanolic phases were subsequently washed twice with 1.5 hexane to eliminate the oil residues. The final lower phase was collected and employed as the phenolic extract for measuring the total phenolic content and PC.

2.4. Total Chlorophyll and Carotenoid Content Analysis. The total chlorophyll and total carotenoid contents of olive oils were measured by colorimetry at 670 and 470 nm, respectively. Olive oil (7.5 g) was weighed in a 25 mL volumetric flask and mixed with cyclohexane. Specific extinction coefficients were measured using a spectrophotometer (UV-1700, Shimadzu, Japan), and the findings were expressed in mg kg^{-1} of oil.[26]

2.5. Total Phenolic Content (TPC) Analysis. The total phenolic content of the varieties of Tunisian olive oil samples was analyzed using the Folin-Ciocalteu reagent following the earlier established method.[27,28] The amount of 2.5 g of the olive oil sample was weighed, with the successive addition of 5 mL of hexane and then 5 mL of methanol/water (60:40, v/v). The analysis was performed on the methanolic phase. The addition of an aliquot of 0.2 mL from the methanolic phase was first performed to a 10 mL volumetric flask and then to distilled water to reach a volume of 5 mL. A reagent blank using distilled water was also prepared for reference. The quantity of 0.5 mL of the Folin-Ciocalteu reagent was added to

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the mixture, to which 1 mL of sodium carbonate solution (Na_2CO_3) (35%, w/v) was added 3 min later. The obtained mixture was diluted to 10 mL of water. After incubation for 2 h at room temperature, absorbance was measured at 725 nm in 4 mL cuvettes against the reagent blank using a spectrophotometer (UV-1700, Shimadzu, Japan). The total phenol content was expressed in mg of caffeic acid equivalents per kg of VOO or EVOO (mg CAE kg^{-1}).

2.6. Fatty Acid Composition Analysis. The analysis of fatty acid methyl esters (FAME) was conducted using a gas chromatography system (HP 6890, USA) equipped with a flame ionization detector (FID), as recommended by the International Olive Council (IOC, 2015).[29] We weighted 0.1 g of the oil sample. We added 5 mL of *n*-hexane and 1 mL of 0.2 N potassium hydroxide with methanol and mixed vigorously. We performed our analysis with the prepared fatty acid methyl esters using a capillary column (DB-23, 30 m × 0.25 mm, film thickness: 0.250 μm, Agilent J&W GC Columns, USA) with detector and injector temperatures set to 250 °C. The oven temperature program was adjusted with a 2 °C min^{-1} increment from 170 to 210 °C, holding at 210 °C for 10 min. We used the Supelco FAME mix standard to determine the fatty acids of olive oil samples and HP 3365 Chemstation program to evaluate fatty acid peak areas.

2.7. Phenolic Compound (PC) Analysis. The phenolic fractions in samples were analyzed using a Waters Alliance e2695 HPLC (Waters, Milford, MA, USA) system equipped with a photodiode array detector (PDA) (Waters 2996, Milford, MA, USA) and an InertSustain C18 column (5 μm, 4.6 × 250 mm, GL Sciences, Tokyo, Japan). The phenolic extract was filtered through a 0.45 μm poly(vinylidene fluoride) (PVDF) syringe filter before injection into the system. The chromatographic separation was performed by using a gradient elution with solvent A (0.1% acetic acid in water) and solvent B (acetonitrile) at a flow rate of 1.0 mL/min. The gradient program was as follows: 0−5 min, 5% B; 5−15 min, 5−25% B; 15−25 min, 25−50% B; 25−30 min, 50−100% B; 30−35 min, 100% B; 35−40 min, back to 5% B. The column temperature was maintained at 30 °C, and the injection volume was 20 μL. Detection was performed at 280 and 320 nm for different phenolic compounds. The HPLC procedure was conducted according to the method described by Veneziani et al.[30] with some adjustments. Each sample was analyzed in triplicate, and the results are expressed as mg kg^{-1}. The quantification was performed using calibration curves obtained from reference standards of the corresponding compounds. The reference standards used for identification and quantification of these phenolic compounds were oleuropein (Sigma-Aldrich, St. Louis, MO, USA), hydroxytyrosol (Cayman Chemical, Ann Arbor, MI, USA), and tyrosol (Sigma-Aldrich, St. Louis, MO, USA).

2.8. Volatile Compound (VC) Analysis. Volatile compounds were extracted and analyzed using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography−mass spectrometry (GC-MS).[31,32] For the extraction, 1.9 g of a virgin olive oil (VOO) sample was weighed and mixed with 0.1 g of a 4-methyl-2-pentanol internal standard (IS) solution (2.5 mg kg^{-1}) in a 20 mL vial. The vial was sealed with a polytetrafluoroethylene septum and agitated for 10 min at 40 °C to allow equilibration of the volatiles in the headspace. After equilibration, the septum was pierced with an SPME needle, and the fiber was exposed to the headspace for 40 min. The SPME fiber (1 cm in length and 50/30 μm film thickness) was purchased from Supelco and contained the Stable Flex stationary phase of divinylbenzene/carboxen/polydimethylsiloxane. The volatiles adsorbed by the fiber were thermally desorbed in the hot injection port of a gas chromatograph coupled to a quadrupole mass spectrometer (Thermo Fisher) for 5 min at 300 °C in splitless mode. Separation of the volatile fractions was achieved using a Thermo Fisher TG-WAXMS capillary column (60 m × 0.25 mm, 0.25 μm coating). Helium was used as the carrier gas, with a flow rate of 1.5 mL min^{-1}. The oven temperature was initially held at 40 °C for 10 min, then increased to 200 °C at 3 °C min^{-1}, and further raised to 250 °C at 150 °C min^{-1}. The ion source and transfer line temperatures were set at 250 and 260 °C, respectively, in the MS quadrupole. Electron impact energy was set to 70 eV, and data were collected in the 40−300 atomic mass unit (AMU) range. Compound identification was performed by comparing the mass spectra with the Wiley 9 MS spectra database (John Wiley & Sons) and verifying retention times with standards of volatile compounds obtained from Sigma-Aldrich. 4-Methyl-2-pentanol (CAS 108-11-2, purity ≥ 98%) was used as the IS. Samples were quantified following the methodologies of Casadei et al. and Aparicio-Ruiz et al.[33,34] Each sample was analyzed in duplicate.

2.9. Tocopherol Analysis. The methods for analyzing the α-tocopherol content were those suggested by Carpenter[35] and IUPAC standard method 2324.[36] A 10 mL flask containing 1 g of olive oil was filled to capacity by using a 1% solution.

Using hexane:2-propanol (99:1) as the mobile phase, the analysis was carried out using high-performance liquid chromatography (HPLC-Agilent 1100, Germany). The flow was 1 mL per minute. The μ-poracil column, which measured 250 mm × 4.6 mm × 5 μm, was purchased from Waters, Ireland. Twenty-five °C was the temperature of the column. The volume of the injection was 20 μL. Utilizing a calibration curve, the amount of α-tocopherol was calculated and expressed as milligrams per kilogram of oil (R^2 = 0.99).

2.10. Sterol Analysis. We used the IOC code COI/T.20/Doc. No 26/Rev. 5 method to determine sterol composition (IOC, 2020).[37] First, we extracted the unsaponifiable parts of the olive oil by using diethyl ether. As an internal standard, 5α-cholestan-3β-ol was used, and then, we used thin-layer chromatography to determine the sterol fraction. Before injection of extract, we silylated with pyridine and BSTFA +TMCS. Capillary column gas chromatography (Agilent Technologies 6850) equipped with a flame ionization detector (FID) with a 30 m × 0.25 mm × 0.25 μm Supelco 24034 column was used for separation sterol peaks. Temperatures of the detector and injector were 290 and 280 °C, respectively. Carrier gas was hydrogen with 0.7/0.8 mL/min. The injection volume of Ume was 1 μL. HP 3365 Chemstation program to evaluate fatty acid peak areas was used.

2.11. Sensory Analysis. The sensory analysis was carried out in the laboratory of the National Oil Office (ONH) accredited by ISO 17025:2017 according to "Guidelines for the fulfillment of the requirements of the ISO 17025 standard of sensory testing laboratories with a special reference to virgin olive oil" by the recognition organization: National Accreditation Council (TUNAC). In addition, the competence of the laboratory shall be assessed yearly by the International Olive Council (IOC) in collaboration with an external company.

The determination of the sensory profile was conducted according to the official methods of IOC 2018 and the Guidelines for the accomplishment of requirements of

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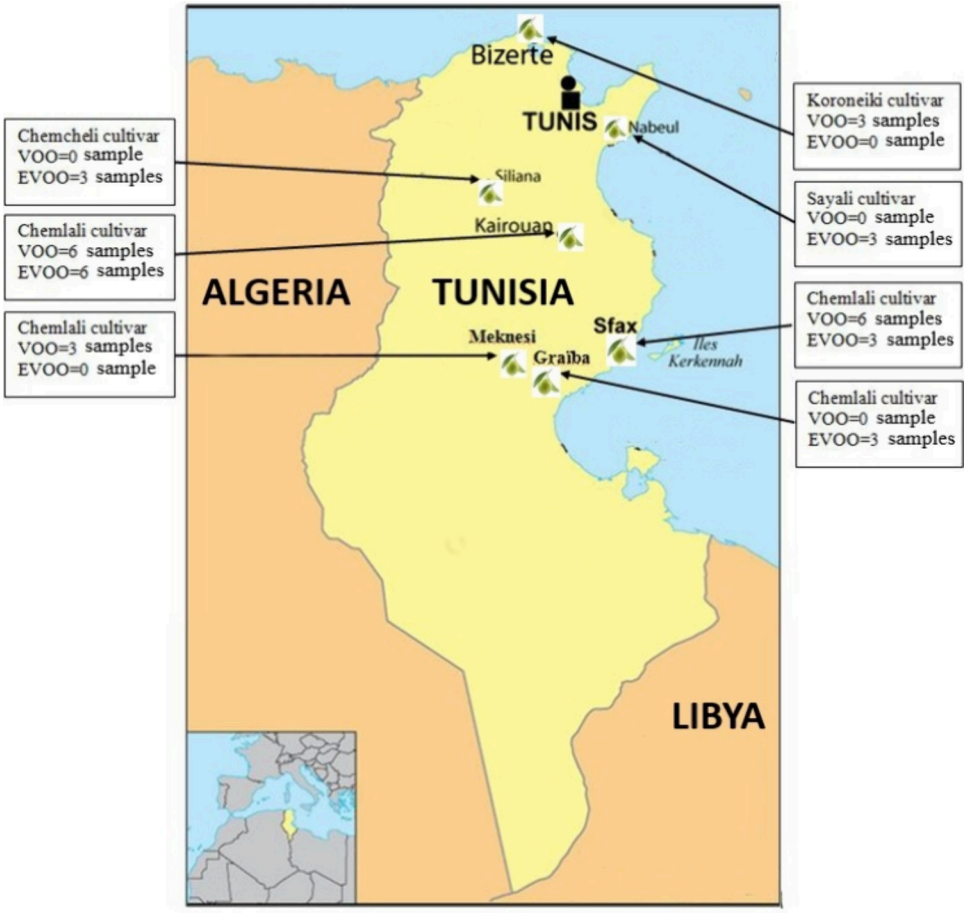

Figure 1. Geographical Distribution of VOO and EVOO olive oil yields in Tunisia.

standard ISO 17025 of sensory testing laboratories with particular reference to virgin olive oil (IOC 2019) by the Tunisian National Office of the oil panel. The latter was composed of eight judges, five of the panelists were males, and three were females aged between 35 and 55 years, who were fully trained in the virgin olive oil assessment. A quantity of 15 mL of each olive oil sample was placed in a tasting glass. The temperature of samples was kept at 28 ± 2 °C. The specific descriptors for a virgin olive oil according to IOC are classified in two sets: three positive attributes were detected, namely, fruity, bitter, pungent, and several negative attributes such as fusty/muddy sediment, musty/humid/earthy, winey-vinegary, acid-sour, rancid, and frostbitten olives (wet wood). Other negative attributes may also have existed, namely, metallic, cucumber, greasy, vegetable water, heated, or burnt. For our case, seven descriptors were detected, namely, fruity, bitter, pungent, rancid, fusty, musty, and winey-vinegary, and were assumed in the samples under study.

2.12. Statistical Analysis. R software (R Foundation for Statistical Computing, Vienna, Austria) version 4.1.2 was used for all statistical analyses. The analysis of the distribution of nonstop variables was conducted by the Shapiro−Wilk test. Variables with Gaussian distribution were expressed as means ± standard deviation (SD) and compared with the Student *t*-test. Variables with non-Gaussian distribution were expressed as the median [25%,75%], and the comparison was completed with the Mann−Whitney *U*-test. Pearson's and Spearman's correlation tests were used to estimate the correlations between continuous variables with Gaussian distribution and non-Gaussian distribution, respectively. For all used tests, statistical significance was defined as $p < 0.05$.

3. RESULTS AND DISCUSSION

Table 1 and Figure 1 exhibit the distribution of olive cultivars used for oil samples across two classes: virgin olive oil (VOO) and extra virgin olive oil (EVOO), with 18 samples in each group. The Chemlali cultivar from diverse regions, including Sfax, Graïba, Meknesi, Kairouen, and Siliana, corresponds to most of the samples. Remarkably, Sfax and Kairouen make significant contributions to both VOO and EVOO samples. In contrast, certain cultivars like the Sayali from Nabeul and Koroneiki from Bizerte appear exclusively in one category, underscoring regional and cultivar diversity in olive oil production.

3.1. Quality Parameters. Table 2 compares the legal quality parameters and pigment contents between virgin olive oil (VOO) and extra virgin olive oil (EVOO), each of which contains 18 samples. For free fatty acids (FFA), no substantial difference is observed between VOO (0.30%) and EVOO (0.29%; $p = 0.214$). Likewise, there is no significant difference in the peroxide value (PV), K_{232}, and K_{270} values between the two oil types. However, the chlorophyll content is particularly higher in VOO (3.45 mg/kg) compared to that in EVOO (2.88 mg/kg) ($p = 0.027$). Carotenoid levels are particularly higher in VOO (2.53 mg/kg) than in EVOO (1.76 mg/kg) ($p = 0.004$), leading to a substantially higher total pigment content in VOO (5.97 mg/kg) compared to EVOO (4.36 mg/kg) ($p = 0.006$). The tocopherol content is comparable across

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Table 2. Quality Parameters and Pigment Contents of VOO and EVOO[a]

	VOO (N = 18)	EVOO (N = 18)	P-value
FFA (% oleic acid)	0.30 (0.24−0.31)	0.29 (0.25−0.36)	0.214
PV (meq O_2/kg)	9.70 (7.19−13.39)	7.82 (7.10−8.92)	0.171
K_{232}	2.13 (1.91−2.32)	1.99 (1.86−2.08)	0.181
K_{270}	0.11 (0.09−0.14)	0.10 (0.09−0.13)	0.767
chlorophylls (mg/kg)	3.45 (2.39−4.68)	2.88 (1.83−3.16)	0.027
carotenoids (mg/kg)	2.53 (1.95−2.65)	1.76 (1.31−2.36)	0.004
total pigments (mg/kg)	5.97 ± 1.62	4.36 ± 1.41	0.006
tocopherol	348.09 (332.10−358.77)	329.66 (322.93−428.26)	0.481
fruity	3.00 (2.80−3.00)	3.60 (3.20−3.80)	<0.001
bitternes	2.40 (2.30−2.60)	2.40 (2.10−2.70)	0.999
pungency	2.40 (2.40−2.50)	2.45 (2.20−3.00)	0.617
defects	1.50 (1.00−2.00)	0 (0−0)	<0.001

[a]VOO: virgin olive oil; EVOO: extra virgin olive oil; FFA: free fatty acids; PV: peroxide value; significant differences (p < 0.05).

both oil types, showing no significant difference. Overall, the table emphasizes the sensory and pigment differences between VOO and EVOO, with VOO exhibiting higher pigment content levels but more defects than EVOO.

3.2. Sensory Attributes. Table 2 highlights sensory parameters indicating that EVOO is significantly fruitier (p < 0.001), with bitterness and pungency at comparable levels. The key difference lies in the absence of defects in EVOO, whereas VOO reveals significant defects (p < 0.001) (Figure 2).

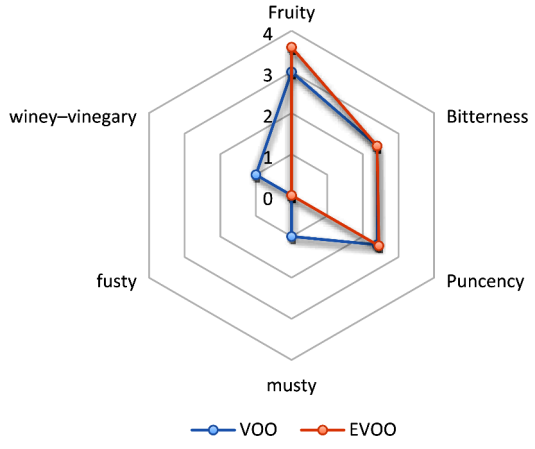

Figure 2. Sensory wheels of VOO and EVOO.

3.3. Fatty Acid Profile. Table 3 provides the fatty acid profiles of VOO and EVOO, based on the analysis of 18 samples of each, comparing their respective fatty acid compositions. Indeed, palmitic acid (C16:0) is substantially higher in EVOO (17.76%) compared to VOO (16.41%) (p = 0.004). This aligns with studies confirming that the palmitic acid content can vary, depending on environmental conditions and cultivar characteristics. Oleic acid (C18:1), a key monounsaturated fatty acid (MUFA) known for its health benefits, reveals a significantly higher concentration in VOO (62.49%) than EVOO (60.14%) (p = 0.046). This variation could affect the oxidative stability and flavor of the oils.

Table 3. Fatty Acid Profile (%) of VOO and EVOO[a]

fatty acid (%)	VOO (N = 18)	EVOO (N = 18)	P-value
miristic acid (C14:0)	0.01 (0.01−0.01)	0.01 (0.01−0.01)	0.999
palmitic acid (C16:0)	16.41 (14.00−17.37)	17.76 (16.77−19.25)	0.004
palmitoleic (C16:1)	1.97 (1.33−2.27)	1.88 (1.50−2.29)	0.568
heptadecanoic acid (C17:0)	0.04 (0.04−0.05)	0.04 (0.03−0.04)	0.122
stearic acid (C18:0)	2.80 (2.42−2.86)	2.97 (2.58−3.10)	0.074
oleid acid (C18:1)	62.49 (58.85−64.55)	60.14 (56.23−63.77)	0.046
linoleic acid (C18:2)	15.59 (15.47−16.60)	15.72 (14.62−17.45)	0.776
linolenic acid (C18:3)	0.72 (0.69−0.73)	0.68 (0.65−0.76)	0.567
arachidic acid (C20:0)	0.45 (0.42−0.46)	0.47 (0.44−0.49)	0.025
gadoleic acid (C20:1)	0.18 (0.18−0.29)	0.21 (0.16−0.26)	0.231
behenic acid (C22:0)	0.09 (0.09−0.09)	0.09 (0.09−0.10)	<0.001
lignoceric acid (C24:0)	0.01 (0.009−0.03)	0.01 (0.008−0.01)	0.105
SFAs	19.61 (17.47−20.39)	21.47 (19.55−22.81)	0.007
PUFAs	16.32 (16.19−17.32)	16.46 (15.19−18.10)	0.776
MUFAs	64.66 (61.13−66.34)	62.11 (58.95−65.53)	0.046
MUFAs/PUFAs	3.97 (3.52−4.17)	3.83 (3.25−4.21)	0.924

[a]VOO: virgin olive oil; EVOO: extra virgin olive oil; significant differences (p < 0.05).

With respect to the stearic acid (C18:0) and linoleic acid (C18:2) levels, they are similar across both oil types with no significant differences. Interestingly, the concentration of arachidic acid (C20:0) is much higher in VOO compared to EVOO (p = 0.025), even though the impact of this on quality remains insufficiently explored. Lignoceric acid (C24:0), known for its role in enhancing the oil stability, exhibits no major differences between the two groups.

Regarding fatty acid categories, saturated fatty acids (SFAs) are significantly more abundant in EVOO (21.47%) compared to VOO (19.61%) (p = 0.007), while polyunsaturated fatty acids (PUFAs) show no significant variation. The ratio of MUFAs to PUFAs is similar between the two oils (p = 0.924), suggesting that both oils maintain a healthy balance of fatty acids, which is critical for cardiovascular health. Nevertheless, the overall MUFA content of VOO (64.66%) is much higher than that of EVOO (62.11%; p = 0.046), thus reinforcing its potential for boosting heart health.

Therefore, it can be concluded that while both oils exhibit advantageous fatty acid profiles, VOO has a higher oleic acid and MUFA content, which is likely to enhance health benefits, whereas EVOO contains higher palmitic acid and SFAs. These differences could be accredited to factors such as cultivar variety, geographic origin, and extraction methods, aligning with recent research on olive oil quality variability.

3.4. Individual Major Phenolic Compounds and Total Phenolic Content (TPC). Table 4 displays a comparison of the levels of different phenolic compounds in VOO and EVOO. The findings suggest that EVOO generally contains higher concentrations of phenolic compounds compared to VOO, essentially for oleuropein and total phenolic contents (TPC). These variations were found to be statistically significant (p < 0.05), indicating that EVOO may offer greater antioxidant benefits thanks to its more elevated levels of these health-promoting compounds. Nonetheless, the hydroxytyr-

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Table 4. Phenolic Compounds (mg kg^{-1}) in VOO and EVOO[a]

phenolic compound	VOO (N = 18)	EVOO (N = 18)	P-value
hydroxytyrosol (mg/kg)	4.23 (1.36−36.40)	12.52 (4.45−15.19)	0.506
tyrosol (mg/kg)	9.34 (5.80−25.09)	17.94 (11.55−26.33)	0.058
oleuropein (mg/kg)	49.57 (24.38−280.29)	235.46 (62.71−284.32)	0.046
TPC (mg/kg)	208.36 (165.89- 234.78)	306.00 (196.95−397.50)	0.014

[a]VOO: virgin olive oil; EVOO: extra virgin olive oil; TPC: total phenolic content; significant differences ($p < 0.05$).

osol levels did not vary substantially between the two types of olive oil.

3.5. Volatile Compounds. A detailed comparison of the volatile compound compositions in VOO and EVOO is listed in Table 5. While some volatile compounds display comparable concentrations in both types of oil, numerous considerable differences arise. Interestingly, and as indicated by the p-values less than 0.05, EVOO was proven to contain significantly higher levels of (*E*)-hex-3-enal, 1-penten-3-ol, and ethanol compared to VOO. These findings suggest that EVOO has a different volatile profile, potentially contributing to its unique flavor and aroma characteristics. The elevated concentrations of some volatile compounds in EVOO may also be associated with particular health benefits pertaining to this type of olive oil. Nevertheless, additional research is needed to thoroughly clarify the functional importance of these compositional differences.

Table 5. Composition of Volatile Compounds (mg kg^{-1}) in VOO and EVOO[a]

volatile compound	VOO (N = 18)	EVOO (N = 18)	P-value
(*E*)-hex-3-enal	0.038 (0.030−0.045)	0.046 (0.032−0.054)	0.021
(*E*)-2-hexen-1-ol	0.30 (0.26−0.41)	0.37 (0−0.64)	0.774
(*Z*)-3-hexen-1-al	0.28 (0.19−0.44)	0.25 (0.13−0.42)	0.162
octanal	0 (0−0.73)	0 (0−0)	0.287
hexyl acetate	0.69 (0.69−0.74)	0.69 (0.68−0.72)	0.464
1-penten-3-ol	0.45 ± 0.13	0.55 ± 0.08	0.011
hexanal	0.39 (0.34−0.84)	0.52 (0.38−0.63)	0.623
ethanol	2.61 (1.19−3.18)	9.77 (2.70−15.00)	0.010
1-hexanol	1.62 (1.51−2.66)	1.59 (1.50−2.54)	0.692
(*Z*)-3-hexenyl acetate	0.88 (0.86−1.24)	0.87 (0.84−1.02)	0.105
(*E*)-2-hexenal	3.73 (2.29−6.29)	4.24 (2.67−5.38)	0.776

[a]VOO: virgin olive oil; EVOO: extra virgin olive oil; significant differences ($p < 0.05$).

3.6. Sterol Compounds. Table 6 exhibits a thorough analysis of the sterol compound composition in VOO and EVOO. It is worth mentioning that substantial differences emerge for specific compounds. However, some sterols exhibit similar concentrations in both oil types. Interestingly, EVOO contains significantly higher levels of Δ-7-stigmastenol relative to VOO, confirmed by a p-value of less than 0.001. Additionally, campestanol is solely identified in EVOO. These findings substantiate the EVOO's distinctive sterol profile, likely to contribute to its unique nutritional characteristics and potential health benefits. The elevated concentrations of certain sterols in EVOO could be linked to particular biological activities, such as antioxidant features and cholesterol regulation. However, further research is required to thoroughly understand the functional significance of these compositional differences.

Table 6. Composition of Sterol Compounds (%) in VOO and EVOO[a]

sterol compound (%)	VOO (N = 18)	EVOO (N = 18)	P-value
cholesterol	0.13 ± 0.07	0.08 ± 0.02	0.049
24-methylene-cholesterol	0.21 (0.17−0.25)	0.19 (0.08−0.21)	0.124
campesterol	3.15 (2.97−3.22)	3.15 (3.08−3.31)	0.547
campestanol	0 (0−0)	0 (0−0.05)	0.008
stigmasterol	0.50 (0.41−0.85)	0.51 (0.43−0.59)	0.862
Δ-7-compesterol	0 (0−0)	0 (0−0)	0.999
Δ-5,23-stigmastadienol	0 (0−0)	0 (0−0)	0.999
clerosterol	0.95 (0.91−0.97)	1.00 (0.95−1.03)	0.020
β-sitosterol	80.80 (79.95−82.40)	81.58 (79.38−86.85)	0.486
sitostanol	0.42 (0.40−0.43)	0.44 (0.39−0.51)	0.418
Δ-5-avenasterol	12.07 (10.65−12.53)	10.96 (5.76−13.26)	0.527
Δ-5,24-stigmastadienol	0.58 ± 0.12	0.65 ± 0.15	0.113
Δ-7-stigmastenol	0.34 ± 0.06	0.51 ± 0.11	<0.001
Δ-7-avenasterol	0.73 ± 0.12	0.80 ± 0.19	0.419

[a]VOO: virgin olive oil; EVOO: extra virgin olive oil; significant differences ($p < 0.05$).

3.7. Principal Component Analysis (PCA). Figure 3 reveals a principal component analysis (PCA) in the form of a biplot, comparing sensory variables (fruity, bitterness, pungency, and defects) and biological parameters between two categories of olive oil: VOO and EVOO. Blue points represent EVOO samples, while yellow triangles represent VOO samples. The first two principal components account for approximately 49.1% of the total variance, indicating that while they capture a substantial proportion of the data set's variability, additional components may be required to fully describe the underlying structure of the data. This level of explained variance suggests that the analyzed variables contribute meaningfully to the differentiation between VOO and EVOO but also highlights the complexity of the data set, which may be influenced by other unaccounted factors. It is important to clarify that PCA does not directly compute correlations between variables but rather groups samples based on similarities in the intensity of the analyzed parameters. To provide a clearer representation of the relationships among variables, we included a correlation circle plot (Figure 4), which illustrates the contribution and associations of each parameter with the principal components. This visualization allows for a better understanding of how variables are interrelated within the PCA framework. The correlation circle confirms the trends observed in the biplot while ensuring a more rigorous interpretation of the PCA results.

The variable fruity is closely linked to volatile compounds such as 1-penten-3-ol and ethanol, and other phenolic compounds like tyrosol. This correlation is more prominent in EVOO samples (blue points aligned with the fruity arrow), indicating that extra virgin olive oils tend to have a more noticeable fruity character thanks to the existence of these compounds. Nonetheless, VOO samples (yellow triangles) seem to be less connected to these compounds, asserting a less fruity sensory profile compared to that of EVOO.

VOOs embodied by the yellow triangles are likely to be more associated with exclusive phenolic compounds, such as hydroxytyrosol, tyrosol, oleuropein, and TPC, located in the

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ACS Omega 2025, 10, 16579−16588

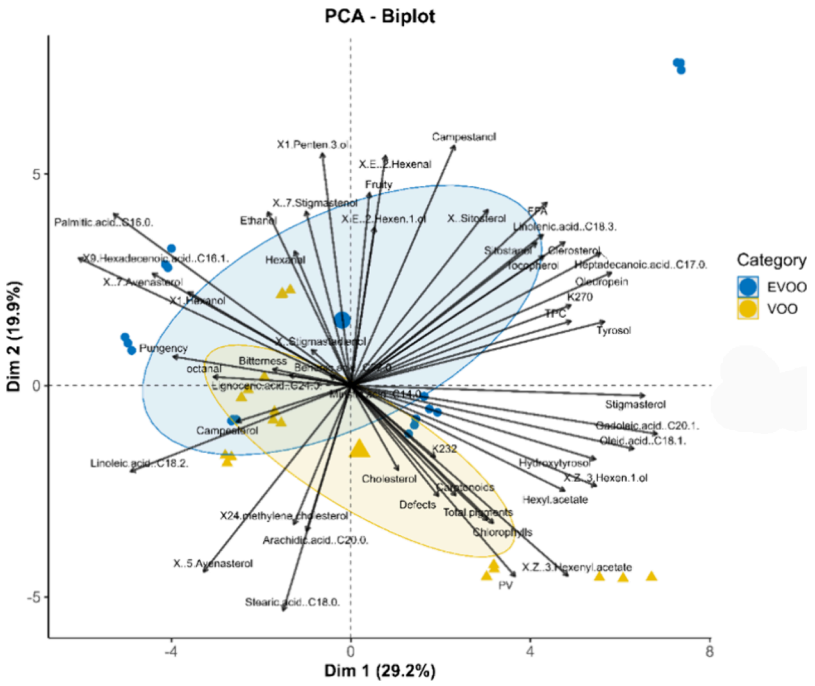

Figure 3. Bioplot of study.

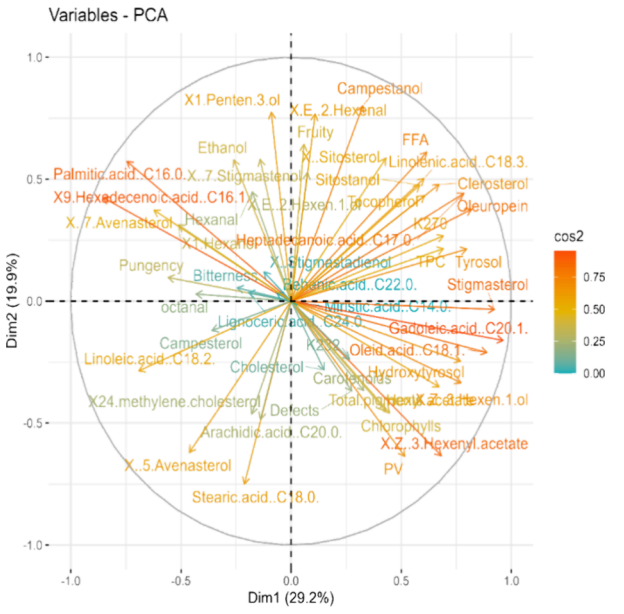

Figure 4. Circle of correlation (Cos2).

same direction in the biplot. This indicates that EVOOs are recognized by a more extreme sensory profile, while VOOs are characterized more by a lower concentration of phenolic compounds. Defects are located in a direction opposite fruitiness, proving a negative impact on the observed quality of the oils.

In the analysis of olive oils, phenolic compounds like hydroxytyrosol, tyrosol, and oleuropein play a pivotal role in identifying quality and bioactive traits.[38] These compounds are recognized for their antioxidant, anti-inflammatory effects, and cardiovascular health benefits.[7] In parallel, sensory attributes such as bitterness, pungency, and fruitiness are directly influenced by these same phenolic compounds, contributing to the complexity and intensity of flavors, essentially in EVOO.[39]

Research has recently shown that EVOO often has higher levels of phenolic compounds thanks to production methods that better conserve these molecules.[40,41] These compounds are also responsible for specific sensory characteristics. For example, oleuropein and hydroxytyrosol are robustly associated with bitterness and pungency. However, virgin olive oils (VOO) typically contain lower phenolic concentrations, which attenuate their sensory intensity and oxidative stability.[42] Hence, the importance of these molecules in perceived quality and the differentiation between EVOO and VOO is highlighted by the relationship between sensory analysis and phenolic compounds.[6,43]

On the other hand, bitterness is connected to compounds like octanal, ethanol, campesterol, β-sitosterol, and linoleic acid. Both VOO and EVOO samples reveal correlations with bitterness, although VOO points are more dispersed, advocating greater variability in bitterness among these oils. Although both categories are associated with bitterness, EVOO (blue points) demonstrates a more stable correlation with bitterness-related compounds compared to VOO.

Pungency is correlated with compounds such as ethanol and 1-hexanol, which are volatile compounds known to contribute to a pungent sensation. EVOO samples display a stronger correlation with pungency than VOO samples, suggesting that extra virgin olive oils tend to be spicier. As with bitterness, EVOO displays a more stable correlation with pungency-related compounds.

Defects are associated with compounds, such as oleic acid and palmitic acid. VOO samples are more strongly related to these compounds and the defect variable, which accords well with the fact that VOO can contain sensory defects that are less present or absent in EVOO. The yellow points (VOO) are closer to the defect arrow, confirming a stronger correlation with sensory defects in this category, while EVOO, which must adhere to firmer quality standards, is less correlated with

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defects. So, stricter quality standard is less associated with defects.

Fruity, bitterness, and pungency are more strongly interrelated with compounds present in EVOO, reflecting the superior quality of these oils, which are characterized by more prominent sensory profiles. On the other hand, defects are more closely associated with VOO samples, indicating lower quality compared to EVOO. This analysis underlines the qualitative differences between extra virgin and virgin olive oils, with EVOO generally being associated with positive sensory features and fewer defects, while VOO elucidates a stronger association with sensory defects.

This principal component analysis (PCA) highlights key sensory and chemical differences between VOO and EVOO, underscoring the higher quality of EVOO. The strong correlation between fruity, bitterness, and pungency with volatile and phenolic compounds in EVOO aligns with recent studies that accentuate the importance of these compounds in defining the sensory attributes of high-quality olive oils.[44] In particular, compounds like 1-penten-3-ol and tyrosol, strongly linked to EVOO's fruity profile, are increasingly renowned as markers of superior quality.[45]

Furthermore, the stability of bitterness and pungency in EVOO, as visually associated with bioactive compounds like campesterol and β-sitosterol in the PCA representation, aligns with the findings of Kottaridi et al.[46] Their study demonstrated that these compounds not only enhance sensory attributes but also contribute to the health benefits of EVOO. The presence of these compounds in higher concentrations in EVOO versus VOO suggests a more robust oxidative stability and health-promoting potential.[47]

On the other hand, the stronger association of VOO with sensory defects, which is linked to higher concentrations of fatty acids like oleic acid, supports the findings of Morales et al.,[48] who noted that such defects often emanate from inadequate production processes. Overall, this analysis confirms that EVOO consistently outperforms VOO in both sensory quality and chemical composition, thus reinforcing its status as a premium product.[44]

4. CONCLUSIONS

This study underscores the superior quality of EVOO compared to VOO, as evidenced by its stronger graphical associations with positive sensory attributes, such as fruity, bitterness, and pungency. Moreover, its close association with beneficial volatile and phenolic compounds further reinforces its exceptional composition and health-promoting properties. In contrast, VOO exhibits a stronger link to sensory defects, highlighting the distinct qualitative differences between these two olive oil categories. These findings provide valuable insights into the factors influencing olive oil quality and may support efforts to optimize the production and classification standards.

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Notes

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