# Short Note

# Minor compounds and sensory evaluation of Tunisian high-quality olive oil

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This article investigated the minor composition and the sensory evaluation of five high-quality Tunisian virgin olive oil (TVOO) gold awarded in some international olive oil contests. Sterolic and hydrophilic compounds were analysed. Moreover, sensory profiles were carried out by a panel of trained assessors. HPLC–MS analysis showed that samples contain low amounts of simple phenols, appreciable concentrations of secoiridoid derivatives and high bitter index. Moreover, very high sensory evaluation score and overall quality index were registered in tested oils. Results elaborated statically put in evidence interesting correlations between the phenols mainly secoiridoids contents with the intensity of bitterness in high-quality Tunisian olive oils.

**Keywords:** Tunisian virgin olive oil, high-quality, sterols, phenols, sensory profile, correlation.

## 1. INTRODUCTION

According to International Olive Council, the olive oil market is increasing in a global dimension [1]. The growing interest in the dietary consumption of virgin olive oil (VOO) has been attributed to its potential beneficial effect to human health and chiefly the ability to prevent diseases related to oxidative stress such as inflammations, diabetes, coronary diseases, cell ageing and several types of cancers [2]. These biological properties of olive oil are chiefly associated to the presence of minor, unsaponifiable compounds making up to 1-2% of the total content [3]. Sterolic and phenolic compounds are the most bioactive components of the olive oil unsaponifiable fraction. The impact of sterols on human health has already been demonstrated by several researches [4]. Some papers reported that phytosterol may present protection against cancer by different way such as cell division inhibition, tumour cell death stimulation and the alteration of some hormones that are necessary to tumour growth [4]. The phenolic compounds are reported to have a crucial role in the oxidation prevention. In fact, in previous works, we already attributed the oxidative stability of virgin olive oil during the storage to these hydrophilic bioactive compounds [3]. Other authors [5] had reported their importance as antioxidants as well as nutraceutical components. Moreover, these bioactive compounds are responsible for olive oil bitterness and pungency [6].
Tunisia, the smallest African country (163,600 $km^2$), is currently the world's third-largest olive oil exporter and fourth largest producer [1]. Last year, It was the world's third-largest olive oil exporter and fourth largest producer [1]. Even so, the challenge for Tunisia's olive oil industry is not just to augment production but to export branded products having the best commercial quality possible. Henceforth, consumers' increasing need for high sensory olive oil quality has

motivated many prestigious international olive oil competitions (IOOC). Recently, Tunisian producers are gaining recognition by winning awards at important international contests. Many publications reported on the chemical composition and sensory profiles of TVOO [3] but there are no studies on the mentioned parameters in awarded olive oils. This investigation was carried out to obtain a better understanding of possible relationships between some minor compounds and gustative characteristics of five Tunisian VOO. Oils were produced during the crop season 2017/ 2018 from the two main Tunisian cultivars (cvv. Chemlali and Chetoui). NY1 and NY2 oils were awarded in New York; AT: in Athens; JA: in Japan and LO in London. NY1, NY2, and AT are obtained from Chetoui monovarietal olive oils. While JA and LO are blended from Chetoui and Chemlali varieties. The chemical characterisation of such genuine oils is imperative in order to select high-quality VOO for their commercial potential exploit in the future.

## 2. MATERIALS AND METHODS

### 2.1. OLIVE OIL SAMPLES

This investigation was carried out on five high-quality Tunisian olive oils produced during the crop season 2017/ 2018 by the two main Tunisian cultivars (cvv. Chemlali and Chetoui). Tested oils were gold awarded in prestigious international olive oil contests throughout the world. The samples: NY1 and NY2 were awarded by: the New York international olive oil contest; AT: the Athens contest; JA: by the Japan contest and LO, the London comtest. NY1, NY2 and AT are from Chetoui monovarietal olive oils. While, JA and LO are blends from Chetoui and Chemlali varieties. All samples were filtrated and stored at 4°C into dark glass bottles.



### 2.2. DETERMINATION OF STEROLS, ERYTHRODIOL AND UVAOL CONTENT [7]

The qualitative and quantitative sterol contents of the samples were determined according to the European Official Analysis Methods, described in Annexes V and VI of Regulation EEC/2568/91 of the European Union Commission [7]. The sterols are expressed as mg/kg of total sterols and as percentage of individual sterols. The oil sample was saponified with ethanolic potassium hydroxide solution 2M during 20 min at 30°C approximately. The unsaponifiable fraction, containing the sterols, was removed with diethyl ether. The sterols were separated by chromatography on a silica gel plate. Identification and quantification of the silanised sterol fraction was carried out by capillary gas chromatography with a Hewlett Packard 6890 gas chromatograph equipped with a flame ionisation detector (FID), using a HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm), working as follows: injector 300°C, detector 325°C, oven 260°C, using helium as carrier gas at a flow rate of 1.1 ml/min$^{-1}$. An injection volume of 0.2 µL was used. The injected volume was 0.2 µL, at a flow rate of 1.1 mL/min, using helium as carrier gas. The qualitative analysis of the sterolic and alcoholic fraction was performed after the determination of the retention time of their pure compounds that had been analysed in the same conditions.

### 2.3. EXTRACTION OF THE PHENOLIC FRACTION

The method developed by the International Olive Oil Council was used to isolate the phenolic fraction of olive oils [8]. 4g of the oil sample was added to 2 mL of n-hexane and 4 mL of a methanol/water (60:40, v/v) solution in a 20 mL centrifuge tube. After vigorous mixing, they were centrifuged for 3 min at 1490 × g. The hydroalcoholic phase was collected, and the hexanic phase was re-extracted twice with 4 mL of methanol/water (60:40,v/v) solution each time. Then, the hydroalcoholic fractions were collected, washed with 4 mL of n-hexane and finally concentrated and dried by evaporative centrifuge (Mivac Duo of Genevac Inc., Valley Cottage, NY, USA) in vacuum at 35°C. 100 µL of 3,4-dihydroxyphenylacetic acid solution (0.1 mg mL$^{-1}$) was added as internal standard to 4 g of oil. The phenolic extracts were stored at -20°C until analysis.

### 2.4. CHROMATOGRAPHIC ANALYSIS OF PHENOLS BY HPLC-DAD/MSD

Quali-quantitative phenolic analysis was carried out by HPLC HP 1100 Series instrument equipped with a diode array UV-Vis detector (DAD), mass spectrometer detector (MSD). A column Luna C18 (Phenomenex) of 5 µm particle size and 250 mm, 3.00 mm ID was used. The flow rate of the mobile phase was 0.5 mL min$^{-1}$. The wavelength of DAD was set at 280 nm for simple phenols and secoiridoids. The injection volume was 10 µL. The mobile phase A was water/formic acid (99.5:0.5, v/v) and mobile phase B was acetonitrile.
The mass spectrometer (MS) analyses were carried out using an electrospray (API-ES) interface operating in positive mode using the following conditions: drying gas flow, 9.0 L min$^{-1}$; nebuliser pressure, 50 psi; gas drying temperature, 350°C.

### 2.5. SENSORY ANALYSIS

The sensory characterisation was performed by a fully trained analytical taste panel, composed of fifteen assessors of different nationalities, members of staff of New York olive oil competition. Quantitative descriptive analysis (QDA) was applied in order to identify different sensory profiles between tested VOO, according to International Olive Oil Council [9]. Each taster must identify organoleptic parameters in VOO samples. Samples were analysed using sensory sheets (Bongartz & Oberg., 2011). Each taster smelled and

tasted the oil under consideration in order to access olfactory, gustatory and tactile or kinaesthetic sensations. Ten attributes were evaluated: seven during the olfactory fruity (green/ ripe, grass/leave, tomato, artichoke, almond, apple and banana) phase, and three during the gustatory (bitter, astringent and pungent) phase. Obtained data were used to define the sensory profile for each sample (average values and their standard deviations). The quantitative sensory evaluation (SE) was the final global score attributed to each sample and ranged from 0 - 10.

### 2.6. OVERALL QUALITY INDEX

The overall Quality Index (OQI) introduced by the International Olive Council [10] was used to express EVOO quality numerically. The scale ranges from 0 to 10 and considers 4 quality parameters: the score of sensory evaluation (SE), FFA, $K_{270}$ and PV according to the following equation:

$$OQI = 2.55 + 0.91\ SE - 0.78\ FA - 7.35\ K270 - 0.066\ PV$$

### 2.7. DETERMINATION OF BITTERNESS INDEX

Evaluation of the index of bitterness (IB) in polar extracts was carried out spectrophotometrically at 225 nm [11]. VOO (1g) dissolved in 5 mL n-hexane was extracted with 5 mL MeOH/H2O (60:40, v/v). The mixture was vortexed and centrifuged at 3500 rpm for 10 min. After the removal of the hexane layer, the polar fraction was transferred in a 10 mL volumetric flask and the volume was made up to 10 mL with MeOH/$H_2O$ (60:40, v/v) (stock solution, C0); an aliquot (1.25 mL) was diluted to 5 mL with the same solvent (C1). The absorbance of C1 was recorded at 225 nm by means of a UV-1601 spectrophotometer (Shimadzu Co., Kyoto, Japan). A software package UVPC 3.5 (Shimadzu Scientific Instruments, Inc.) was used for data acquisition and processing.

### 2.8 STATISTICAL ANALYSIS

The results are reported as mean values of at least three repetitions with standard deviations. To verify the association among experimental data, a correlation analysis was performed using SPSS 16.0, (SPSS Inc., 2007). Significant differences among samples were determined by the analysis of variance using Duncan's multiple tests. When probability was greater than 99% ($P < 0.01$) differences were considered as statistically significant.

## 3. RESULTS AND DISCUSSION

### 3.1. STEROLIC COMPOSITION IN HIGH- QUALITY TUNISIAN OLIVE OILS

Table I summarises the sterol contents obtained for the different oils. In all studied oils, total sterol levels were extremely higher than the minimum limit fixed by legislation (1000 mg/kg). Such high sterol content is certainly a good characteristic for olive oils since sterols show great benefits for human health as referred before. As expected for Tunisian oils, the most abundant sterol in olive oil is β-sitosterol (more than 80% of total sterols) [12]. In all cases, they were under the minimum limit established by the EU regulation for virgin olive oil. Oils analysed in this paper showed low campesterol contents under the threshold established by EU Regulations (4%), demonstrating the peculiarity of these TVOO. Stigmasterol is associated to various parameters of the quality of VOO. High levels correlate with high acidity and low organoleptic quality) [12]. All samples contained low levels of this sterol, proving that the oils were produced from healthy fruit) [13]. The other sterolic compounds, such as cholesterol, 24-methylenchlosterol, campestanol, clerosterol, sitostanol, Δ5-24-stigmastadienol, Δ7-Stigmastenol, and Δ7-Avenasterol, were relatively low in the five gold-awarded Tunisian oils. The level of apparent β-sitosterol, expressed as the sum of the contents of β-sitosterol and five other sterols formed by the degradation of the β-sitosterol (sitostanol, Δ5,24-stigmastadienol, clerosterol, Δ5-avenasterol and Δ5,23 stigmastadienol) was also determined. All samples contained more than the established minimum limit of 93%. This is the regulatory minimum limit, indicating that the sum of the remaining sterols does not exceed 7%, thereby confirming the authenticity of the corresponding oils [14]. It is noteworthy that the sterol profile has been proposed as applicable to the characterisation of olive oil and in detecting the presence of some seed oils [14]. The relationships between the sterol composition and gustative characteristics of virgin olive oils were barely studied. Gutierrez et al. [15] suggested that VOO sensorial quality can be revealed by the stigmasterol content and proposed the possibility of determining the oil category by means of its stigmasterol content without the need for an analytical panel to test the sensory quality.

### 3.2. ERYTHRODIOL AND UVAOL CONTENT IN HIGH-QUALITY TUNISIAN OLIVE OILS

The triterpenic dialcohols (erythrodiol and uvaol), which are also part of the unsaponifiable fraction of the olive oil, are usually analysed together with the sterol fraction. In all cases, the sum of erythrodiol and uvaol in samples was below the established limit of 4.5% for the “extra virgin” olive oil category. These results are consistent with results of other authors on Tunisian varieties [16].

Several authors have described the use of an olive oil's sterol profile to detect possible fraudulent admixtures with lower-value fats. The presence of olive-pomace oil in virgin oil can be detected from the levels of erythrodiol + uvaol [17].

**Table I -** Sterols, phenols and some indexes of quality in TVOO

| | NY1 | NY2 | AT | JA | Lo | Extra virgin olive oil [8] |
|---|---|---|---|---|---|---|
| Cholesterol# | 0.06 ± 0.00[b] | 0.12 ± 0.01[a] | 0.13 ± 0.01[a] | 0.06 ± 0.00[b] | 0.11± 0.01 [a] | ≤ 0.5 |
| Brassicasterol# | 0.01± 0.01 [a] | 0.00± 0.01 [a] | 0.00± 0.01 [a] | 0.00± 0.01 [a] | 0.00± 0.01 [a] | NFL |
| 24-Methylenchlosterol# | 0.10 ± 0.01[b] | 0.10 ± 0.01[b] | 0.22 ± 0.01[a] | 0.10 ± 0.01 [b] | 0.22 ± 0.02[a] | NFL |
| Campesterol# | 3.10 ± 0.41[a] | 2.81 ± 0.25 [b] | 2.66 ± 0.28[b] | 3.16 ± 0.39[a] | 2.68 ± 0.28[b] | NFL |
| Campestanol# | 0.10 ± 0.01[b] | 0.93 ± 0.08[a] | 0.10 ± 0.01[b] | 0.06 ± 0.01 [b] | 0.11 ± 0.01 [b] | ≤ 4 |
| Stigmasterol# | 0.70 ± 0.01 [b] | 0.60 ± 0.01 [b] | 1.16 ± 0.12 [a] | 0.57 ± 0.06 [b] | 1.16 ± 0.12 [a] | NFL |
| Δ 7-Campesterol# | 0.00± 0.01 [a] | 0.01± 0.02 [a] | 0.01± 0.02 [a] | 0.00± 0.01 [a] | 0.01± 0.01 [a] | NFL |
| Δ 5,23 Stigmastadienol# | 0.01± 0.01 [a] | 0.00± 0.01 [a] | 0.01± 0.01 [a] | 0.01± 0.01 [a] | 0.00± 0.01 [a] | NFL |
| Clerosterol# | 0.70 ± 0.06 [b] | 0.30 ± 0.01 [c] | 1.04 ± 0.11 [a] | 0.21 ± 0.03 [c] | 1.00 ± 0.13 [a] | NFL |
| β-Sitosterol# | 89.75 9.64 [b] | 91.32 ± 8.91 [a] | 81.50 ± 9.13 [c] | 92.01 ± 10.45 [a] | 81.54 ± 7.61 [c] | NFL |
| Sitostanol# | 0.44 ± 0.05 [b] | 0.12 ± 0.01 [c] | 0.67 ± 0.08 [a] | 0.14 ± 0.01 [c] | 0.66 ± 0.01 [a] | NFL |
| Δ5-Avenasterol# | 3.50 ± 0.41 [b] | 2.54 ± 0.31 [b] | 11.15 ± 1.51 [a] | 2.53 ± 0.35 [b] | 11.13 ± 1.29 [a] | NFL |
| Δ5-24-Stigmastadienol# | 0.60 ± 0.05 [a] | 0.31 ± 0.05 [b] | 0.78 ± 0.09 [a] | 0.45 ± 0.05 [b] | 0.79 ± 0.09 [a] | NFL |
| Δ7-Stigmastenol# | 0.33 ± 0.04 [a] | 0.30 ± 0.04 [a] | 0.17 ± 0.01 [b] | 0.26 ± 0.03 [a] | 0.18 ± 0.01 [b] | ≤ 0.5 |
| Δ7-Avenasterol# | 0.62 ± 0.05 [a] | 0.52 ± 0.04 [b] | 0.42 ± 0.03 [c] | 0.42 ± 0.06 [c] | 0.39 ± 0.04 [c] | NFL |
| Apparent β-Sitosterol# | 94.99 ± 8.67[b] | 94.17 ± 9.44 [c] | 95.14 ± 7.61 [b] | 95.34 ± 10.67 [a] | 95.12 ± 9.88 [b] | ≥ 93 |
| Total sterol§ | 1550 ± 147.32 [c] | 1509 ± 150.87 [e] | 1522 ± 142.23 [d] | 2159.80 ± 214.33 [a] | 1677.30 ± 150.54 [b] | ≥ 1000 |
| Erythrodiol # | 1.54 ± 0.17.62 [b] | 1.18 ± 0.12 [b] | 3.36 ± 0.39 [a] | 1.40 ± 0.15 [b] | 1.41 ± 0.16 [b] | NFL |
| Uvaol # | 0.40 ± 0.02 [b] | 0.98 ± 0.08 [a] | 0.55 ± 0.06 [b] | 0.64 ± 0.08 [b] | 0.25 ± 0.02 [b] | NFL |
| Erythrodiol+Uvaol # | 1.94 ± 0.22 [c] | 2.16 ± 0.31 [b] | 3.91 ± 0.41 [a] | 2.04 ± 0.25 [b] | 1.66 ± 0.17 [c] | NFL |
| SID § | 410.5 ± 42.59[a] | 404.14 ± 42.26[a] | 320.14 ± 33.81[b] | 209.74 ± 24.91[c] | 200.23 ± 22.34[c] | NFL |
| Simple phenols § | 52.14 ± 6.27[b] | 55.89 ± 6.22[b] | 87.70 ± 9.37 [a] | 49.65 ± 5.43 [b] | 18.17 ± 2.11[c] | NFL |
| TP-HPLC § | 504.64 ± 52.34[a] | 482.03 ± 51.84[a] | 429.84 ± 45.62[b] | 281.39 30.28[c] | 250.4 ± 28.91[c] | NFL |
| Bitterness index ($K_{225}$) | 4.02 ± 0.34[a] | 4.86 ± 0.51[a] | 2.13 ± 0.24[b] | 2.78 ± 0.31[b] | 1.12 ± 0.10[c] | NFL |
| SE | 9.20 ± 0.88 [a] | 9.00 ± 0.07 [a] | 8.90 ± 0.91 [a] | 9.30 ± 0.84 [a] | 9.5 ± 0.97[a] | NFL |
| OQI | 8.77 ± 0.87 [a] | 8.68 ± 0.91 [a] | 8.58 ± 0.77 [a] | 9.13 ± 0.95[a] | 9.09 ± 0.98 [a] | NFL |

TVOO: Tunisian virgin olive oil. NY1: TVOO n°1 gold awarded in New York competition NY2: TVOO n°2 gold awarded in New York competition AT: TVOO gold awarded in Athens competition JA: TVOO gold awarded in Japan competition. LO: TVOO gold awarded in London competition. Values are means ± standard deviations of five (n = 5) measurements.

[a,b,c]: Different superscripts for the same quality parameter mean significant differences among samples. #As: percentage of total sterols. §: In mg/kg of oil. Apparent β-Sitosterol:β-Sitosterol+Δ5-Avenasterol+Clerosterol+Sitostanol+Δ5,24-Stigmastadienol + Δ 5,23 stigmastadienol. NFL: non fixed limits by the regulation[8] . SID: secoiridoid derivatives. TP-HPLC: total phenols determined by HPLC. SE: sensory evaluation score. OQI: overall Quality Index

### 3.3. PHENOLIC COMPOUNDS IN HIGH- QUALITY TUNISIAN OLIVE OILS

In olive oil, the most abundant group of phenolic are the secoiridoids (SID) such as aglycons of oleuropein, ligstroside and their respective decarboxylated derivatives, followed by a second group of simple phenols composed by phenylethyl alcohol (tyrosol and hydroxytyrosol) and phenolic acids (cinnamic and benzoic acid derivatives) [3].

HPLC-MS analysis showed that all samples contain low amounts of simple phenols and high concentrations of SID. NY1 had the highest levels of SID 410.5 mg/kg while LO registered the lowest one (200.23 mg/kg of oil). The quantity of simple phenols in tested oils ranged from 18.17 to 87.7 mg/kg of oil in JA and NY2, respectively. Consequently, the highest contents of total phenols determined by HPLC, expressed as mg of 3,4-dihydroxyphenylacetic acid/kg of oil, (TP-HPLC) were observed on NY1 (504.64 mg/kg), whereas LO had the lowest one (250.4 mg/kg) (Tab. I). Previous data reported that the content of total phenol levels varied between 46.27-112.04 mg/kg and 283.10-567.64 mg/kg on Chemlali and Chetoui oils, respectively [3]. The fluctuation in the hydrophilic bioactive compound levels could be attributed to many factors: such as genetic parameters, on environmental and agronomic parameters mainly for the water availability, ripening index) [3].

### 3.4. ORGANOLEPTIC CHARACTERISTICS OF HIGH-QUALITY TUNISIAN OLIVE OILS

Ten sensory attributes were evaluated in this work: seven during the olfactory fruity (green/ ripe, grass/leave, tomato, artichoke, almond, apple and banana) phase, and three during the gustatory (bitter, astringent and pungent) phase. As shown in Table I, oil samples registered a very elevated sensory evaluation score (SE) and overall quality index (OQI). These results were consistent with the existence of an obvious correlation between OQI and SE and highlighted the exceptional organoleptic quality of Tunisian olive oils gold awarded in IOOC. Bitter sensation is a typical attribute of VOO. It is already reported that bitter sensory characteristics are strictly connected by the quali-quantitative phenolic profile of the oils. Moreover, the bitter index (IB) evaluates the intensity of the bitter taste in VOO [6]. Interesting positive correlations between the TP-HPLC contents and the bitter index ($r^2 = 0.7$, $P < 0.001$) were found. In particular, a positive correlation was also recorded between the SID amounts and the bitter index ($r^2 = 0.9$, $P < 0.001$) in the tested oils. The positive correlations between phenol amounts, bitter and pungent intensities were already reported by other authors [5]. Some hydrophilic bioactive compounds mainly elicit the tasting perception of bitterness; however, other phenolic molecules can excite the free endings of the trigeminal nerve located in the palate and

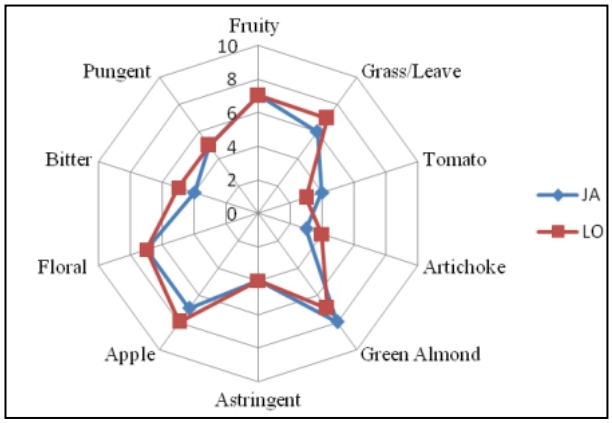


*Fig. 1.a*

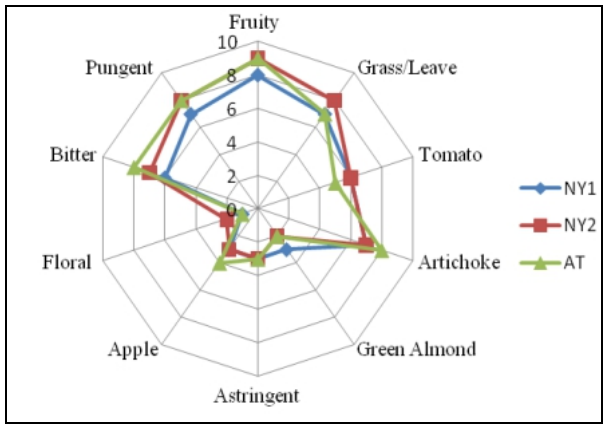


*Fig. 1.b*

**Figure 1 –** *Fig. 1.a:* Sensory profiles of NY1; NY 2 and AT oils by quantitative descriptive analysis.
*Fig. 1.b :* Sensory profiles of JA and LO oils by quantitative descriptive analysis.
The intensity of each descriptor is evaluated on a 0-10 points scale.

also in the gustative buds increasing to some chemesthetic sensations such as pungency, astringency and metallic attribute. Moreover, the strength of bitterness and pungency is chiefly attributed to the genomic factor and the harvest period [3]. The Quantitative Descriptive Analysis (QDA) carried out by Rotondi et al. [18] confirmed that the decrease in bitterness was related to a reduction in the total phenols and especially the SID levels.

In terms of sensory features, NY1, NY2 and AT had very similar gustative profiles (Fig. 1.a). While LO and JA profiles were practically equal (Fig. 1.b). The Quantitative Descriptive Analysis pointed out that NY1, NY2, and AT presented higher fruitiness and pungency than LO and JA oils. Moreover, pleasant secondary flavours of grass, tomato and artichoke were perceived in NY1, NY2 and AT (Chetoui monovarietal olive oils). Whereas, in LO and JA (blends of Chetoui and Chemlali olive oils), the assessors especially noted aromas of flower and green almond. Tunisian olive oil is

appreciated worldwide as a high-quality oil with its own personality. In particular, the Chetoui variety that has won in all international contests studied in this work. The Chétoui variety is characterised by a deep green colour and a mouth-watering aroma. Green, fresh and specific flavour of tomato and artichoke, it has that distinct peppery taste of high-quality oil. Hence, the Chétoui variety is particularly appreciated by the trained judges of the studied contests. While the delicate fruity and floral notes of Chemlali, the main Tunisian cultivar, appeal to many palates and are the ideal variety for blending, the livelier and more pungent Chetoui variety is what drew the attention when tasted.

## 4. CONCLUSION

This is the first chemical and sensory evaluation of the TVOO gold-awarded in international contests. It is interesting to mention that Chetoui and Chemali, the two main Tunisian varieties, have particular gustative proprieties. Furthermore, the current study demonstrated the relationship between the chemical composition, practically hydrophilic bioactive compounds, and the gustative proprieties appreciated by tasters. With the obtained results, it is possible to conclude that the gustative proprieties and, consequently, the commercial potential of the Tunisian olive oil are greatly attributed to the chemical composition of this genuine product.



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