Phenol content as correlated to antioxidant activity and gustative characteristics of Tunisian monovarietal virgin olive oils

This article investigated the relationship between the phenol contents, the antioxidant activity and the organoleptic characteristics of the two main Tunisian virgin olive oils (cvv. Chétoui and Chemlali). The Chétoui oils presented the highest values of total phenols, radical scavenging activity and bitter index. Results elaborated statistically put in evidence interesting correlations between the phenol mainly secoiridoid contents with antioxidant activity and the intensity of bitterness of the tested monovarietal virgin olive oils.

**Keywords:** virgin olive oil; Tunisian varieties; phenolic compounds; antioxidant activity; organoleptic characteristics.
INTRODUCTION

The importance accorded to virgin olive oils (VOO) is not due to the presence of fatty acids only, but also to the richness in important functional components, mainly phenols, which act as natural antioxidants and may contribute to the prevention of several human diseases [1]. The most representative phenolic compounds in VOO are simple phenols (phenylethyl alcohol, such as tyrosol and hydroxytyrosol, and other phenolic acids, namely, cinnamic or benzoic acid derivatives), secoiridoids (the aglycons of oleuropein and ligstroside and their respective decarboxylated derivatives), and lignans (\((\alpha\)-pinoresinol and \((\beta\)-1-acetoxypinoresinol) [1].

Phenols are reported to have beneficial health properties and detrimental effect on oil stability. Indeed, these compounds act as primary antioxidants (AH) to inhibit oxidation in virgin olive oil (VOO). They chiefly act as chain breakers by donating radical hydrogen to alkylperoxyl radicals (ROO•) formed during the propagation step of lipid oxidation and subsequently forming a stable radical (A•) through the well-known reaction: ROO• + AH \rightarrow ROOH + A• [2].

The large increase in demand for VOO is due not only to its health virtues but also to its organoleptic properties [3]. In fact, when properly extracted from fresh and good quality fruits, this precious food provides a delicate and unique aroma highly appreciated by consumers [4].

Phenolic compounds contribute to the organoleptic properties of VOO and are commonly described as bitter, pungent and astringent [5]. VOO with a low or moderate level of bitterness are accepted by consumers, but very bitter ones are rejected. The intensity of the bitterness of VOO has been related with the presence of phenolic compounds derived from the hydrolysis of oleuropein, a secoiridoid glucoside characteristic of the Oleaceae [1,6]. These molecules, which are partially soluble in lipids, are conferred to the VOO during its extraction, through an unknown process in which at least two kinds of enzymes, such as glycosidases and esterases, could be involved [7]. To the authors’ knowledge, the bitterness of VOO has been linked generally to the total derivatives of oleuropein [8] or to the total phenol contents [9].

In Tunisia, the second VOO exporter and producer after the European Union, the main variety is Chemlali, which is cultivated in central and southern areas, while, the second cultivar Chétoui is widespread in the north of the country [10]. Thus, the chemical and organoleptic characterization of these two cultivars seems to have a particular economic interest.

Previous works carried out by our research group [11-12] characterized Chemlali and Chétoui VOO based on major and minor compounds as well as on the oxidative stability measured by Rancimat method. Moreover, these articles evaluated the influence of some agronomic factors (i.e. fruit ripeness degree, water supply) on the chemical composition of the two main Tunisian VOO [11-12].

As a continuation of this study, the present investigation was firstly carried out in order to obtain a better understanding of possible relationships between phenol compounds, antioxidant activity and gustative characteristics of the Chemlali and the Chétoui VOO. Moreover this work intended to provide suggestions for agronomic practices, thus leading to the production of Tunisian monovarietal VOO with a moderate level of bitterness and therefore accepted by consumers.

MATERIALS AND METHODS

• Samples
Healthy olive samples were harvested at the same crop season (2005) from the two main Tunisian cultivars (cvv. Chemlali and Chétoui) in their original growing area, in triplicate and at different maturity stages. Oil extraction was performed using an Abencor laboratory oil mill (MC2 Ingenierias y Sitemas, Sevilla, Spain), kneading the olive paste at 28°C.

• Apparatus
Phenolic extracts were concentrated and dried by evaporative centrifuge (Mivac Duo of Genevac Inc., Valley Cottage, NY, USA). High-performance liquid chromatography (HPLC) analyses were performed using a HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump delivery system, degasser, and autosampler. Detection was performed with a diode array UV-Vis detector (DAD), mass spectrometer detector (MSD). Spectrophotometric determinations were carried out using an UV-Vis 1601 instrument (Shimadzu Co., Kyoto, Japan), which had a six slots shuttle and a system for temperature control of working conditions.
• Standard, reagents and solvents

The standard for HPLC quantification (3,4-dihydroxyphenylacetic acid) and for the evaluation of antioxidant capacity of phenolic extracts (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Trolox), such as the reagent ABTS [2,2’-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid, diammonium salt] were achieved from Sigma-Aldrich (St. Louis, MO, USA). The standards for the HPLC identification (tyrosol, vanillic and ß-coumaric acids) were from Sigma-Aldrich. Hydroxytyrosol, decarboxymethyl oleuropein aglycon (DAOA) and acetoxypinoresinol were identified by analyzing and comparing their MS spectra with those reported in the literature [13].

• Extraction of the phenolic fraction

A solution of 3,4-dihydroxyphenylacetic acid (0.1 µg ml⁻¹) was used as internal standard. A VOO sample (4 g ± 0.001 g) was added to 1 ml of n-hexane and 2 ml of a methanol/water (60:40, v/v) solution in a 10 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 2 ml of methanol/water (60:40, v/v) solution each time. Finally, the hydroalcoholic fractions were combined, washed with 2 ml of n-hexane to remove the residual oil, then concentrated and dried by evaporative centrifuge in vacuum at 35 °C.

After extraction procedure dry extracts were solvated in 0.5 ml of a methanol/water (50:50, v/v) solution [14]. Extractions were performed in three replicates (n=3). Unless otherwise stated extracts were stored at ?18 °C before use in order to analyze by HPLC-DAD/MSD and by radical scavenging activity.

• Chromatographic analysis of phenols by HPLC-DAD/MSD

A column Luna C18 (Phenomenex) of 5 µm particle size and 250 mm, 3.00 mm ID was used. The mobile phase flow rate was 0.5 ml min⁻¹. The wavelength of DAD was set at 280 nm for phenolic acids, phenyl ethyl alcohols, and secoiridoids. The injection volume was 10 µl. Analyses were carried out at room temperature. The gradient elution was carried out using water/formic acid (99.5:0.5, v/v) as mobile phase A and acetonitrile as mobile phase B of the solvent system [15].

The total run time was 75 min. Phenolic compounds were quantified using a calibration curve made with 3, 4-dihydroxyphenylacetic acid (r²=0.999). The average was calculated by three replications for each sample. The MS analyses were carried out using an electrospray (API-ES) interface operating in positive mode using the following conditions: drying gas flow, 9 l min⁻¹; nebulizer pressure, 50 psi; gas drying temperature, 350 °C. Phenolic compounds were identified comparing retention times (by spiking attempts) and UV and MS spectra of the detected peaks with standards.

• Radical scavenging activity of phenolic extracts by ABTS test

The radical-scavenging capability of phenolic extracts was evaluated by ABTS⁺ radical cation assay according to the Re et al. [16] method with detection at 734 nm. Results were expressed as mmol Trolox/kg of oil using its calibration curve (r² = 0.981).

• Determination of bitterness index

Evaluation of the index of bitterness (IB) in polar extracts was carried out spectrophotometrically at 225 nm according to Gutierrez-Rosales et al. [17].

• Sensory analysis

Sensory analysis was performed by a fully trained analytical taste panel for virgin olive oil of Dipartimento di Scienze degli Alimenti of Università di Bologna (recognized by the Italian Ministry for Agriculture, Food and Forestry Policy - Mipaaf). Quantitative descriptive analysis (QDA) was applied in order to identify different sensory profiles between tested VOO [18]. Each taster was asked to identify gustatory characteristics in VOO samples (specifying the difference in term of major or minor presence of bitter and/or pungent attribute). Each oil sample was analyzed by 10 tasters during three different sessions using the sensory ballot reported in Cerrutani et al. [19]. The sample sets were randomly distributed among the assessors. The test supervisor chose a significance level of 5%.

• Statistical analysis

The results reported in this study are the means of at least three repetitions (n=3), unless otherwise stated. To verify the association among experimental data, correlation analysis were performed using SPSS r.11.0.0 statistical software (SPSS Inc., Chicago, IL, USA).
Antioxidant activity of analysed Tunisian VOO

Assays based upon the use of ABTS• radicals are among the most popular spectrophotometric methods for the determination of the antioxidant capacity of food, beverages, and vegetable extracts. They are based on the evaluation of the decrease in absorbance at 734 nm, following the addition of antioxidant extracts. Scavenging activities can be correlated with reference to antioxidants such as Trolox [20]. Several authors have reported the high reproducibility and comparability of this radical scavenging test, due to the same stoichiometry toward Trolox [21].

As shown in Table I, polar extracts from Chétoui VOO showed higher TEAC (Trolox Equivalent Antioxidant Capacity) mean and range values in the ABTS•+ assay, phenol and secoiridoid contents that Chemlali ones.

It is informative to examine the relationship between the antioxidant capacity measured by the ABTS•+ method and the amounts of phenolic compounds in the analyzed Tunisian VOO. Indeed, the antioxidant activity of the phenolic fraction of VOO is mainly due to radical scavenging; this was confirmed by investigating the ability of the polar extracts to scavenge ABTS•+ radicals [21]. This parameter is affected by different factors. It is used to evaluate oil and fat quality as it gives a good estimation of their susceptibility to oxidative degradation. In fact, interesting positive correlations between the phenolic compound contents determined by HPLC and the antioxidant activity were found: TPs-HPLC (total phenols-HPLC) vs ABTS activity, \( r^2 = 0.80, \ p < 0.001 \) (Figure 1, A) and secoiridoid contents (SID) vs ABTS activity \( r^2 = 0.86, \ p < 0.001 \) (Figure 1, B) in the tested VOO.

As already observed in VOO by other authors, secoiridoids are phenolic compounds endowed with high antioxidant activity [1].

Simple phenol levels are low (with respect to the other compounds) and for this reason they are very poorly correlated with the values of the antiradical

**RESULTS AND DISCUSSION**

<table>
<thead>
<tr>
<th>Chemical variable</th>
<th>Chétoui</th>
<th>Chemlali</th>
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<tbody>
<tr>
<td>Secoiridoid derivatives (mg/kg)</td>
<td>339.52 ± 206.92-510.11</td>
<td>35.99 ± 16.47-62.50</td>
</tr>
<tr>
<td>Simple phenols (mg/kg)</td>
<td>49.65 ± 8.86-109.42</td>
<td>12.08 ± 7.84-18.31</td>
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<tr>
<td>Total phenols (mg/kg)</td>
<td>426.43 ± 283.10-567.64</td>
<td>72.35 ± 46.27-112.04</td>
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<tr>
<td>ABTS•+ (mmol/kg)</td>
<td>1.22 ± 0.26-2.33</td>
<td>0.26 ± 0.19-0.36</td>
</tr>
<tr>
<td>Bitterness index ( K_{225} )</td>
<td>4.86 ± 2.01-8.08</td>
<td>0.63 ± 0.21-1.02</td>
</tr>
<tr>
<td>Bitter intensity (by sensory analysis)</td>
<td>3.80 ± 3.00-5.00</td>
<td>1.80 ± 1.00-2.00</td>
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**Table I** - Mean and range values of some chemical and organoleptic parameters of Tunisian virgin olive oil samples of cv. Chétoui and cv. Chemlali

**Figure 1** - Correlations between antioxidant activity with total phenol (TP-HPLC) (A); and with secoiridoid derivatives content (SID) (B) of Tunisian monovarietal VOO.
The correlations recorded could be explained by the antioxidant activity of the phenolic compounds that contribute to the resistance of VOO to oxidation process [1]. The relationship between total phenol contents and antioxidant activity has been widely studied in different foodstuffs: fruit and vegetables [22], wine [23], plants [24], seeds [25] and olive oil [26], showing that where there is a high concentration of total phenol content, the antioxidant activity of that food significantly increases.

In all foodstuffs the phenolic compounds practically secoiridoids, are linked to the antioxidant activity, by contributing to its stability and by its capacity to block free radicals. There is a linear correlation between phenol compounds and antioxidant activity, with a decrease in the former implying a fall in the latter.

Organoleptic characteristics of analysed Tunisian VOO

Bitter taste is one of the characteristic attributes of VOO. Its intensity varies greatly and influences consumer attraction and acceptance [17]. Thus, although a low intensity is pleasant, a high one lessens the acceptability to the consumer, causing commercial problem. It is already reported that the bitter index (IB) evaluates the intensity of the bitter taste in VOO [17].

According to Gutiérrez Rosales et al. [17] Chétoui VOO showed very high values corresponding to extremely bitter oils, whereas, Chemlali VOO showed low values corresponding to non-bitter oils. High intensity of bitterness of VOO may not be acceptable to the majority of consumers, despite of their beneficial effect on human health and high resistance to oxidation.

After comparing the IB values with those of TPs-HPLC contents (Table I), it became obvious that the bitter intensity was closely related to the phenol contents in all analyzed VOO. In fact, interesting positive correlations between the TPs-HPLC contents and the bitter index (r² = 0.87, p < 0.001) were found (Figure 2, A). Positive correlations were also recorded between the SID amounts and the bitter index (r² = 0.86, p < 0.001) in all examined samples (Figure 2, B).

Other studies showed that some phenolic compounds mainly elicit the tasting perception of bitterness. Moreover, the intensity of the bitterness highly depends on the genetic and the agronomical factors and is especially abundant in oils obtained from unripe fruits [27].

Considering previous data related to the influence of some agronomic practices on the phenolic contents [11], a postponed harvesting and an irrigation treatment seemed to be adequate in Chétoui olive oil production in order to generate a desirable reduction of the bitterness intensity and, then improve the consumer preference.

It is important to highlight the good correlation between the bitterness index and bitter taste intensity perceived by assessors during quantitative descriptive analysis (QDA) (r² = 0.78, p < 0.001). Indeed, assessors perceived higher bitterness for Chétoui compared to Chemlali samples (Figure 3).

On the other hand, from a sensory point of view, all the examined samples belonged to the extra VOO class, according to EC Regulation no. 796/2002 [28] without any defects (median of the defects was equal to 0) (Figure 3). Furthermore, the qualitative descriptive sensory analysis pointed out that Chétoui samples presented higher fruitiness (through both olfactory and taste analysis) and pungency than Chemlali VOO. Moreover, pleasant secondary flavours of grass, tomato and artichoke were perceived in Chétoui VOO, while, in Chemlali ones the assessors noted especially aromas of flower and almond.

CONCLUSION

In accordance with previous results [1, 8-9, 13], this study pointed out the proportionality of the content on phenolic compounds, practically secoiridoids with the antioxidant activity and the intensity of bitter taste in the main Tunisian VOO. All samples studied were produced with a laboratory scale olive mill; they presented high phenolic content and consequently intense bitter taste. This last consideration is in accordance with previous works [29] that underline a higher extraction of phenolic compounds using low scale mills.

ABBREVIATIONS

ABTS: 2,2'-azinobis(3-ethylbenzothiaziline-6-sulfonate)
TEAC: Trolox Equivalent Antioxidant Capacity
VOO: Virgin Olive Oil
TPs-HPLC: total phenol determined by HPLC
SID: secoiridoid derivatives

REFERENCES
**Figure 2** - Correlations between bitterness index with total phenol (TP-HPLC) (A); and with secoiridoid derivatives content (B) (SID) of Tunisian monovarietal VOO.

**Figure 3** - Sensory profiles of two extra virgin olive oils sample produced from Chétoui (first picking date) and Chemlali (third picking date) by quantitative descriptive analysis (QDA); the intensity of each descriptor is evaluated on a 0-5 points scale; different perception routes: (1) orthonasal, (2) retronasal.


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