Oil quality parameters and quantitative measurement of major secoiridoid derivatives in Neb Jmel olive oil from various Tunisian origins using qNMR

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Abstract

BACKGROUND: Olive oil contains compounds with interesting biological activities which are influenced by the cultivar, the geographic origin and other factors. The aims of this work were to (1) investigate these factors in Neb Jmel olive oil from various Tunisian origins; (2) determine the influence of geographic conditions on phenolic composition of Neb Jmel olive oil and consequently on the antioxidant compounds; and (3) verify whether oils could be discriminated based on geographical origin.

RESULTS: The characterisation of extra-virgin Neb Jmel olive oil produced in its original location has been conducted. Owing to the effect of the genotype and environmental, agronomic and technological factors on the chemical composition of olive oil and its quality, all studied olives were collected at the same season, and their oil obtained under the same processing technique. Many analyses were carried out to characterise the different olive oils: free acidity, peroxide value, fatty acid composition, Rancimat assay, pigments content and phenolic compounds by 1H NMR. A recently developed method for the direct measurement of the oleocanthal and oleacein levels in olive oil by quantitative 1H NMR was applied. The method was applied to the study of four Neb Jmel olive oils samples, and a broad variation of concentrations of all four secoiridoids was recorded. The concentration of each ranged from 55 to 529 mg kg$^{-1}$ and the sum of the four major secoiridoids (known as D3) ranged from 436 to 1063 mg kg$^{-1}$.

CONCLUSION: The quantification of major phenolic compounds of olive oil by NMR indicated that environmental conditions influence the production of qualitative phenolic fractions. All these compounds can be used as base ‘markers’ to characterise and differentiate these olive oil on geographic origin.

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Keywords: Neb Jmel cultivar; oil quality; growing area; qNMR; secoiridoid derivatives

INTRODUCTION

Olive oil is of interest to consumers because of its impressive quality and health beneficial effects. Therefore, consumers notice when special types of olive oil commonly produced from olives from specific varieties cultivated in selected regions (mono-varietal) appear on the market. In Tunisia, olive oil production represents 10% of the total agricultural production. Tunisia ranks as the fourth world producer of 180 000 tons of olive oil (6%), thus making it the fourth world exporter (8.2%) of olive oil after Spain, Italy and Greece, with almost 110 000 tons every year. Tunisian orchards are rich in many cultivars that have been listed in a catalogue comprising 56 different cultivars.1,2 The Tunisian olive-growing areas are found all over the country, in which a broad range of edapho-climatic conditions are found, from lower semi-arid to arid conditions. It is well known that environmental factors, as well as genotype, agronomic and technological factors have a significant impact on the chemical composition of olive oil, and therefore on its quality.3–6

The genetic diversity in the olive sector of Tunisia is very wide; there are many different olive varieties in the various regions of the country. Two cultivars, Chemlali and Chetoui, are the most important from the economic point of view. There are other cultivars that are grown in restricted geographical locations and which have a limited diffusion, such as Neb Jmel.7–9 Virgin olive

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oil (VOO) is unique among other vegetable oils due to its high levels of mono-unsaturated fatty acids (mainly oleic acid) and the presence of minor components, such as phenolic compounds among others. Mono-unsaturated fatty acids have been shown to be predominant in the VOO composition, which is closely associated with its medicinal properties. Nevertheless, the presence of some other bioactive compounds, such as phenolic compounds, tocopherols, pigments and phytosterols, has been proven to be of great importance in this respect. Among these components, phenolic compounds have acquired a good reputation, since they have an important role in the lengthening of the oxidative stability of VOO. They are also partly responsible for certain sensory attributes (they impart bitterness, pungency and astringency to VOO). Moreover, such compounds are secondary metabolites with considerable biological activities, namely anti-cancer, antibacterial, anti-inflammatory and antioxidant effects. Therefore, phenolic compounds are presently among the most studied constituents of VOO. This group of analytes is very heterogeneous, with a broad range of phenolic compounds belonging to diverse chemical classes, mainly to phenolic acids, phenolic alcohols, secoiridoids, lignans, and flavonoids.

It is worth mentioning that the dialdehydic form of decarboxymethyl ligstroside aglycone, also known as oleocanthal, is an interesting olive oil phenolic compound because of its health-benefiting properties. Beauchamp et al. have classified oleocanthal as a natural non-steroidal anti-inflammatory drug due to its ibuprofen-like cyclooxygenase (COX-1 and COX-2) inhibiting activity. Its properties are found to be responsible for the therapeutic properties of extra virgin olive oil because inflammation plays a vital role in the development of many chronic diseases, such as cardiovascular disease, and certain kinds of cancers. Furthermore, recent research has proved that oleocanthal is a promising therapeutic agent for the treatment of inflammatory degenerative joint diseases. The dialdehydic form of decarboxymethyl oleuropein aglycone, known as oleacein, has shown similar activities to those of oleocanthal, but the former has displayed notable antibreast cancer properties. Oleacein also has powerful antioxidant activities that are even better than those of hydroxystylosol. The modern finding of the strong anti-inflammatory activity of oleocanthal and its potential health effects that have confirmed the ancient reports, have led us to assume that various types of olive oils could be distinguished on the basis of their content in oleocanthal and related secoiridoids.

To do so, the levels of oleocanthal and its related analogue oleacin in Tunisia olive oils of mono-varietal origin were investigated in relation to the cultivar, geographic origin, and time of harvest. A recent study has shown large differences among oils of different cultivars and origins in Greece. The current work aims to study the effect of geographic origin on the phenolic profile of oils from four accessions belonging to the cultivar ‘Neb Jmel’, and for the first time to apply a recently developed qNMR method for the oil classification of Tunisian oils. The obtained results would contribute to a future traceability of Tunisian virgin olive oils.

**MATERIAL AND METHODS**

**Chemicals and reagents**

Methanol, hexane, acetic acid, n-heptane, acetonitrile and cyclohexane HPLC-grade solvents were purchased from Riedel-deHaen (Buchs SG, Switzerland). Double-distilled water was used as the HPLC mobile phase. Folin-Ciocalteu potassium iodide, reagent was obtained from Fluka (Buchs SG, Switzerland), fatty acid methyl ester (FAME) multi-standards (>99.0%), DCI, and syringaldehyde were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

**Samples**

The samples were obtained from homogenous olive fruits (Olea europaea L.) of Neb Jmel olive cultivar from Kairouan, Mahdia, Sfax and Gabes (Fig. 1). The soil type is sandy loam to loamy sand on a gravel ground. Trees were cultivated under fed rain without fertilisation and without pesticide application. Olive samples were handpicked in triplicate from different trees, during the crop season 2013–2014 (November) to obtain only good quality fresh and healthy fruits. In order to eliminate the influence of the maturation state on olive oil quality, the ripening degree was the same for all the studied olive cultivars (maturation indices were 3.5). Samples were harvested from adult trees (40 years old), separated from each other with a distance of 24 m between two successive trees. The maturity index (MI) was determined by the visual appreciation of colour of olives from three trees (n = 3 × 100), randomly selected, according to the method developed by Hermoso et al., which attributes a maturity index range from 0 (for unripe fruits) to 7 (ripe fruits). Olive fruits, 100 for each sample, were randomly taken, classified into the categories below, and homogenised prior to storage. The categories were 0 for olives with intense green or dark green epidermis; 1 for olives with yellow or yellowish green epidermis; 2 for olives with yellowish epidermis but with reddish spots or areas over less than half of the fruit; 3 for olives with reddish or light violet epidermis over more than half of the fruit; 4 for olives with black epidermis and totally white pulp; 5 for olives with black epidermis and less than 50% purple pulp; 6 for olives with black epidermis and violet (more than 50%) or purple pulp; 7 for olives with black epidermis and totally dark pulp. With a–h being the number of fruits in each category, the MI is: [a*0 + b*1 + c*2 + d*3 + e*4 + f*5 + g*6 + h*7)/100.

Only healthy fruits, without any kind of infection or physical damage, were processed. After harvesting, fresh olives (1.5–2.0 kg) were sealed and washed. Neb Jmel olive oils were obtained by extraction with a laboratory mill (a two-phase decanter centrifuge, using an Abencor system). Two hundred and fifty grams of each oil sample were stored in completely full amber glass bottles at 4 °C in the dark without headspace, until analysis.

**Analytical methods**

**Determination of quality indices**

The determination of acidity (A) (given as % of oleic acid), peroxide value (PV) (meqO2 kg−1 of oil), K232 and K270 were carried out following the analytical methods described by the International Olive Council (COI 2013).

**Pigment content**

Chlorophyll and carotenoids were determined colorimetrically as previously described by Minguez-Mosquera et al. The maximum absorption at 670 nm is related to the chlorophyll fraction and that at 470 nm is related to the carotenoids fraction. The applied values of the specific extinction coefficients were E670 = 613 for phoepthyn as a major component in the chlorophyll fraction, and E470 = 2000 for lutein as a major component in the carotenoid fraction. The pigment contents were calculated as follows:

\[
\text{chlorophyll (mg kg}^{-1}) = \left(\frac{A_{670} \times 10^{6}}{613 \times 100 \times d}\right)
\]
carotenoids (mg kg\(^{-1}\)) = \left(\frac{A_{470} \times 10^6}{2000 \times 100 \times d}\right)

where \(A\) is the absorbance and \(d\) is the cell thickness (1 cm).

Fatty acid methyl esters and squalene

Olive oil in \(n\)-heptane (0.12 g per 2 mL) was transmethylated using a cold solution of KOH (2 mol L\(^{-1}\)) (200 \(\mu\)L) according to the European Standard NF EN ISO 5509 Norm.\(^{28}\) Fatty acid methyl esters (FAMES) were analysed according to the European Standard NF EN ISO 5508 Norm.\(^{29}\) The gas chromatographic analysis of FAMES was performed on an Auto System Gas chromatograph equipped with a FID detector (HP 6890 N; Agilent Technologies, J&W Scientific Products, Palo Alto, PA, USA). The column used was a capillary Agilent CP-Sil88 (length 50 m, i.d. 0.25 mm and film thickness 0.20 \(\mu\)m), and the analysis conditions were: the column temperature was first programmed at 165 °C for 25 min, second at a gradient of 5 °C min\(^{-1}\) up to 195 °C, the temperature of injector and detector was set at 250 °C, helium was the carrier gas, with a flow through the column of 1 mL min\(^{-1}\) and 1:100 split ratio, and the injection volume was 1 \(\mu\)L. The identification of fatty acids was performed by comparison of the retention time with those of olive oil fatty acids, whose composition is known. Fatty acid percentages were determined by internal standardisation without taking into account mass response factors.\(^{30}\) The FAMES were identified through a comparison of their retention times versus pure standards analysed under the same conditions. They were quantified according to their percentage area, obtained by the integration of the peaks. The results were expressed as the percentages of individual fatty acids in the lipid fraction. The coefficients of variation were lower than 5% for the most important FAMES and lower than 10% for some minor ones.

Squalene, whose peak is well separated from that of C24:0, was determined along with the fatty acids using an external standard calibration curve, as given in Eqn (1):

\[ A = 248.36C - 0.79 \]  

where \(A\) is the peak area of squalene, and \(C\) is the concentration expressed in mg mL\(^{-1}\) (linearity: 0.030 to 0.60 mg mL\(^{-1}\), \(R^2 = 0.999\)). Final results, calculated on the basis of the analysed oil weight, were expressed in mg kg\(^{-1}\) with a coefficient of variation equal to 1.2% according to the experimental error.

Oxidative stability by the Rancimat method

The oil samples (5.0 g) were heated at 120 °C in a Rancimat equipment (Metrohm Ltd, Herisau, Switzerland), with a continuous air flow of 20 L h\(^{-1}\) passing through the samples. The conductivity

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Figure 1. Descriptions of four Tunisian growing areas for Neb Jmel cultivars.
cells were filled with 60 mL of deionised water. The time needed (hours) for the appearance of a sudden increase in water conductivity, caused by the adsorption of volatiles derived from oil oxidation, was registered as the induction time.

Phenolic compounds

Extraction of phenolic fraction

The phenolic extracts were obtained following the procedure of Chtourou et al., 31 with some modification. The oil sample (4 g) was added to 2 mL of n-hexane and 4 mL of a methanol/water (60:40, v/v) mixture in a 20 mL centrifuge tube. After vigorous mixing, they were centrifuged for 3 min. The hydroalcoholic phase was collected, and the hexane phase was re-extracted twice with 4 mL of the methanol/water (60:40, v/v) solution each time. Finally, the hydroalcoholic fractions were combined, washed with 4 mL of n-hexane to remove the residual oil, then concentrated to a volume of 1 mL by evaporative centrifugation in a vacuum at 35 °C.

Determination of the total phenols and α-diphenols contents

The determination of the total phenolic compounds was performed by means of the Folin–Ciocalteu reagent using the method described by Gargouri et al. 23 The total phenolic content was expressed as milligrams of gallic acid (GA) equivalent per kilogram of oil (y = 0.011x, R² = 0.990). The optical density (OD) was measured at λ = 765 nm, using a spectrophotometer (Shimadzu UV-1800 PC; Shimadzu, Kyoto, Japan).

The concentration of α-diphenolic compounds in the water/methanol extract was determined by the method described by Dridi-Gargouri et al. 33 The total α-diphenolic content was expressed as milligrams of gallic acid (GA) equivalent per kilogram of oil (y = 1.144x, R² = 0.999). The optical density (OD) was measured at λ = 370 nm, using a spectrophotometer (Shimadzu UV-1800 PC).

Olive oil extraction and sample preparation for NMR analysis

Olive oil (5.0 g) was mixed with cyclohexane (20 mL) and acetonitrile (25 mL). The mixture was homogenised using a vortex mixer for 30 s and centrifuged at 6700 g for 5 min. A part of the acetonitrile phase (25 mL) was collected, mixed with 1.0 mL of a syringaldehyde solution (0.5 mg mL⁻¹) in acetonitrile and evaporated under reduced pressure using a rotary evaporator (Buchi, Buchs SG, Switzerland).

NMR spectral analysis

The residue of the above procedure was dissolved in CDCl₃ (750 μL) and an accurately measured volume of the solution (550 μL) was transferred to a 5 mm NMR tube. ¹H NMR spectra were recorded at 600 MHz (Bruker Avance600; Bruker, Karlsruhe, Germany) and 400 MHz (Bruker DRX400). Typically, 50 scans were collected into 32 K data points over a spectral width of 0–16 ppm with a relaxation delay of 1 s and an acquisition time of 1.7 s. Prior to Fourier transformation an exponential weighting factor corresponding to a line broadening of 0.3 Hz was applied. The spectra were phased corrected and integrated automatically using TOPSPIN. Where necessary, accurate integration was performed manually for the peaks of interest. The NMR analysis allows for the quantification of oleocanthal, oleacein, oleuropein aglycon (monoaldehyde form) and ligstroside aglycon (monoaaldehyde form) as previously described. 23, 24

Statistical analysis

The results were expressed as mean ± standard deviation (SD) of three measurements for the analytical determination. Significant differences between the values of all parameters were determined at P < 0.05 according to the one-way ANOVA: Student–Newman–Keuls test, using SPSS Statistics 17.0 for Windows, 2008 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Quality indices

The quality criteria (acidity, peroxide values and UV specific extinction) were determined for Neb Jmel olive oil samples produced in the laboratory scale. The values of these parameters (Table 1) were included in the ranges established for the extra virgin olive oil (EVOO) category (COI 2013). 26

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Free fatty acids (% oleic)</td>
<td>0.55 ± 0.02</td>
<td>0.34 ± 0.01</td>
<td>0.50 ± 0.01</td>
<td>0.18 ± 0.00</td>
</tr>
<tr>
<td>Peroxide value (meq O₂ kg⁻¹)</td>
<td>7.31 ± 0.21</td>
<td>11.25 ± 0.37</td>
<td>8.79 ± 0.12</td>
<td>12.74 ± 0.24</td>
</tr>
<tr>
<td>K₃₂₂</td>
<td>1.78 ± 0.04</td>
<td>1.82 ± 0.02</td>
<td>1.59 ± 0.03</td>
<td>1.66 ± 0.03</td>
</tr>
<tr>
<td>K₇₀</td>
<td>0.17 ± 0.00</td>
<td>0.16 ± 0.00</td>
<td>0.13 ± 0.00</td>
<td>0.15 ± 0.00</td>
</tr>
<tr>
<td>Carotenoids (mg kg⁻¹)</td>
<td>2.85 ± 0.09</td>
<td>3.41 ± 0.10</td>
<td>2.61 ± 0.07</td>
<td>5.83 ± 0.21</td>
</tr>
<tr>
<td>Chlorophylls (mg kg⁻¹)</td>
<td>11.18 ± 0.26</td>
<td>6.23 ± 0.48</td>
<td>9.18 ± 0.10</td>
<td>14.71 ± 0.49</td>
</tr>
<tr>
<td>Oxidative stability (h)</td>
<td>15.14 ± 0.51</td>
<td>12.79 ± 0.25</td>
<td>11.24 ± 0.37</td>
<td>8.34 ± 0.13</td>
</tr>
<tr>
<td>Total phenols (mg kg⁻¹)</td>
<td>1167.03 ± 10.72</td>
<td>984.03 ± 31.94</td>
<td>859.55 ± 35.64</td>
<td>513.57 ± 18.91</td>
</tr>
<tr>
<td>α-Diphenols (mg kg⁻¹)</td>
<td>480.65 ± 7.22</td>
<td>432.41 ± 15.87</td>
<td>401.74 ± 8.53</td>
<td>268.61 ± 5.74</td>
</tr>
<tr>
<td>Squalene (mg kg⁻¹)</td>
<td>3577.63 ± 51.40</td>
<td>3817.83 ± 34.58</td>
<td>3652.46 ± 41.25</td>
<td>3220.82 ± 53.41</td>
</tr>
</tbody>
</table>

Each value represents the mean of three determinations (n = 3) ± standard deviation.

Table 1. Quality indices, total phenols, α-diphenols, oxidative stability and squalene content of virgin olive oils from Neb Jmel cultivars grown in eight different geographical areas.
Table 2. Fatty acid compositions of Neb Jmel oils samples

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Kairouan, A.B.M.1</th>
<th>Mahdia, A.B.M.2</th>
<th>Sfax, A.B.M.4</th>
<th>Gabes, A.B.M.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>14.59 ± 0.21^c</td>
<td>14.79 ± 0.30^c</td>
<td>18.49 ± 0.31^b</td>
<td>19.81 ± 0.33^a</td>
</tr>
<tr>
<td>C16:1n-9 + C16:1n-7</td>
<td>1.94 ± 0.03^c</td>
<td>0.99 ± 0.02^d</td>
<td>2.48 ± 0.04^b</td>
<td>3.13 ± 0.05^a</td>
</tr>
<tr>
<td>C16:1n-7</td>
<td>1.85 ± 0.03^c</td>
<td>0.91 ± 0.02^d</td>
<td>2.41 ± 0.04^b</td>
<td>3.05 ± 0.05^a</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.04 ± 0.00^b</td>
<td>0.04 ± 0.00^b</td>
<td>0.04 ± 0.00^b</td>
<td>0.05 ± 0.00^a</td>
</tr>
<tr>
<td>C17:1n-8</td>
<td>0.05 ± 0.00^b</td>
<td>0.06 ± 0.00^b</td>
<td>0.06 ± 0.00^b</td>
<td>0.05 ± 0.00^b</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.50 ± 0.04^c</td>
<td>2.47 ± 0.05^c</td>
<td>3.17 ± 0.05^b</td>
<td>3.93 ± 0.07^c</td>
</tr>
<tr>
<td>C18:1n-9 + C18:1n-7</td>
<td>68.41 ± 0.95^a</td>
<td>66.23 ± 1.32^a</td>
<td>61.37 ± 1.04^b</td>
<td>58.95 ± 0.93^c</td>
</tr>
<tr>
<td>C18:1n-7</td>
<td>2.13 ± 0.03^c</td>
<td>2.07 ± 0.04^c</td>
<td>2.67 ± 0.05^a</td>
<td>2.52 ± 0.04^b</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>10.65 ± 0.15^d</td>
<td>13.62 ± 0.27^a</td>
<td>13.08 ± 0.22^b</td>
<td>12.47 ± 0.21^c</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>0.97 ± 0.01^a</td>
<td>0.95 ± 0.02^a</td>
<td>0.45 ± 0.01^c</td>
<td>0.73 ± 0.01^b</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.41 ± 0.01^b</td>
<td>0.42 ± 0.01^b</td>
<td>0.52 ± 0.01^a</td>
<td>0.53 ± 0.01^a</td>
</tr>
<tr>
<td>C20:1n-9</td>
<td>0.29 ± 0.00^b</td>
<td>0.27 ± 0.01^b</td>
<td>0.15 ± 0.00^c</td>
<td>0.15 ± 0.00^c</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.11 ± 0.00^d</td>
<td>0.12 ± 0.00^b</td>
<td>0.13 ± 0.00^b</td>
<td>0.14 ± 0.00^a</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.04 ± 0.00^b</td>
<td>0.04 ± 0.00^b</td>
<td>0.06 ± 0.00^a</td>
<td>0.06 ± 0.00^a</td>
</tr>
<tr>
<td>C18:1/C18:2</td>
<td>6.42 ± 0.19^a</td>
<td>4.86 ± 0.20^a</td>
<td>4.69 ± 0.16^a</td>
<td>4.73 ± 0.16^a</td>
</tr>
<tr>
<td>ΣSFA</td>
<td>17.69 ± 0.26^c</td>
<td>17.88 ± 0.36^c</td>
<td>22.41 ± 0.38^b</td>
<td>24.52 ± 0.41^a</td>
</tr>
<tr>
<td>ΣMUFA</td>
<td>70.69 ± 1.01^a</td>
<td>67.55 ± 1.35^b</td>
<td>64.06 ± 1.09^c</td>
<td>62.28 ± 1.02^c</td>
</tr>
<tr>
<td>ΣPUFA</td>
<td>11.62 ± 0.16^c</td>
<td>14.57 ± 0.29^a</td>
<td>13.53 ± 0.23^b</td>
<td>13.20 ± 0.22^b</td>
</tr>
<tr>
<td>ΣUFA</td>
<td>82.31 ± 1.17^a</td>
<td>82.12 ± 1.64^a</td>
<td>77.59 ± 1.32^b</td>
<td>75.48 ± 1.12^b</td>
</tr>
<tr>
<td>ΣMUFA/ΣPUFA</td>
<td>6.08 ± 0.18^a</td>
<td>4.64 ± 0.18^a</td>
<td>4.73 ± 0.17^b</td>
<td>4.72 ± 0.14^b</td>
</tr>
</tbody>
</table>

^a–d Different letters in the same line (row) concerning all samples olive oil indicate significantly different values (p < 0.05).
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids.

Pigment content
The olive oil colour is directly associated with the chlorophyll and carotenoid contents, and it has been proposed as a characterising factor and as a quality index linked to the oil extraction method and to the olive cultivar. In addition, the colour is the first attribute of virgin olive oil evaluated by consumers. For the studied oils, while carotenones were found at average concentrations between 2.61 and 5.83 mg kg⁻¹, the average concentration of chlorophylls varied between 6.23 and 14.71 mg kg⁻¹ according to the growing area. This result is in good agreement with those previously reported by other authors.

Fatty acid composition
The average fatty acid compositions are reported in Table 2. Along with squalene, 14 fatty acids were detected in the Neb Jmel olive oils. Oleic (18:1n-9), palmitic (16:0), linoleic (18:2n-6), and stearic acids (18:0) are the main fatty acids found in all olive oils, accompanied by a further 10 fatty acids present in lower percentages. The mono-unsaturated isomers of fatty acids having 16 carbon atoms (hypogaeic acid: 16:1n-9 and palmitoleic acid: 16:1n-7) and 18 carbon atoms (oleic acid: 18:1n-9 and z-vaccenic acid) are considered separately contrary to the commercial standards (trade standard) of the IOC. The determination of minor fatty acids is very important for the accurate knowledge of the exact lipid profiles during the authentication of the varietal origin of olive oils. It is to be noted that diverse fatty acid compositions were shown in the Neb Jmel oils of different regions. Oleic acid has always been the most plentiful compound, with a variable content between 57.43% and 66.28% of the total fatty acids. Palmitic acid content varied between 14.59% and 19.81%, according to the geographical area. Neb Jmel olive oils also contained low amounts of linolenic (C18:3n-3), arachidic (C20:0), and palmitoleic acids (C16:1n-7) (Table 2). Linoleic acid was the dominant poly-unsaturated fatty acid, ranging from 10.65% to 13.62%, whereas linolenic acid (C18:3n-3) ranged from 0.45% to 0.97%. With respect to the other Tunisian olive cultivars, the variations in oleic and linoleic acid contents observed in olive oil samples obtained from Neb Jmel cultivar are probably related to cultivar–environmental interaction during the development and maturity of the fruits. The different altitudes (Fig. 1) of the growing areas are likely to elucidate the differences observed for the fatty acids composition. This agrees well with the results described by other authors for oils from olives growing at different altitudes. Apart from altitude and temperature, other climatic variables such as the soil characteristics...
of the olive grove zones and the salinity may have an impact the chemical composition of the resulting EVOO.

**Squalene contents**

Squalene is the major olive oil hydrocarbon. Indeed, this terpenoid makes up more than 90% of the hydrocarbons fraction.\(^{37}\) The squalene content in Neb Jmel olive oils varied between 3220.82 mg kg\(^{-1}\) and 3817.83 mg kg\(^{-1}\). It is to be noted that there were differences according to the regions where the samples were collected. Neb Jmel olive oil from Mahdia presents the highest content of squalene reaching 3817.83 mg kg\(^{-1}\) of oil, which is similar to the data previously reported in previous research.\(^{38,39}\)

**Total phenols and \(\omega\)-diphenols**

Phenols are important antioxidants that protect biological systems against oxygen radicals. Besides, phenolic substances do not only affect VOO stability but also contribute to oil flavour, particularly the typical bitter taste of olive oil. The Kairouan region revealed higher levels of these compounds (Table 1). The effects of the environment and pedo-climatic conditions on these compounds were clearly observed on the basis of the different behaviours of each growing area. The samples of Neb Jmel olive oil obtained from the fruits of trees cultivated in Kairouan were found to have a higher content in total phenols reaching 1167 mg kg\(^{-1}\) (Table 1). The \(\omega\)-diphenol content in the olive oil samples was found to vary broadly according to the olive tree cultivation area. The Neb Jmel olive oil samples obtained from the fruits of olive trees cultivated in Kairouan were found to have a higher content in \(\omega\)-diphenols, about 480 mg kg\(^{-1}\) (Table 3). So, significant differences between geographical area were observed with regard to total phenols and \(\omega\)-diphenols. The contents in total phenols and \(\omega\)-diphenols in the olive oils of Neb Jmel cultivar varied according to the region, which confirms the results of other authors\(^{32,35,38}\) for the oil of Chemlali, Oueslati and Neb Jmel.

**Oil stability**

Oxidation stability is an important property characterising the quality of olive oil, which is affected by lipid composition and diverse antioxidant compounds, whose levels may be influenced by cultivar, year and place of production.\(^4\) The oxidative stability of the extra-virgin olive oils was measured as the induction time determined using the Rancimat method. The highest oxidative stability was presented by Kairouan Neb Jmel oil with a mean value of 15.14 h. This characteristic may be partially attributed to the highest MUFA/PUFA ratio (6.08 ± 0.18) and the highest content in phenolic compounds (1167.03 ± 10.72 mg kg\(^{-1}\)). Since the other three regions have almost the same value of the ratio (MUFA/PUFA), the difference at the oxidative stability may be explained by the little difference from the levels of the content in phenolic compound. Such result is in accordance with the data previously reported by other authors.\(^{35,38,40}\)

**NMR analysis**

The NMR analysis allows for the quantification of oleocanthal, oleacein, oleuropein aglycon (monoa ldehyde form) and ligstroside aglycon (monoa ldehyde form) as previously described.\(^{23,24}\) The oleocanthal and oleacein content was high, at least in three out of four cases, when compared with the values obtained in a previous study of Greek and Californian oils.\(^{24}\) It is very interesting to note that the total phenol value as measured by the Folin

![Image](image-url)
method was not able to predict the oil with the highest concentration in oleocanthal. Moreover, the o-diphenol measurement allows for the prediction of the oil with the highest concentration in oleacein. Those observations demonstrate the usefulness of the NMR method for the exact determination of the key phenolics of olive oil in comparison with the photometric methods that give only a rough estimation. However, the photometric methods were able to predict the ranking of the four oils only when all measured phenolics were quantitated as in the case of the D3 index.

The most significant observation of this study is that the edaphoclimatological conditions play a very important role in the phenolic profile of the olive oil. Although all samples came from the same cultivar, harvested at the same maturity and processed under the same conditions, the phenolic profile was quite different as demonstrated by the NMR spectra of the aldehydic region (Fig. 2) especially between olive oil obtained from fruit harvested from Mahdia (A.B.M.2) and olive oil obtained from fruit harvested from Gabes (A.B.M.3). Most probably, the maturity index as estimated mainly by the colour of the fruit is not always well correlated with the phenolic content of the corresponding oil. For example, oil obtained from Gabes (A.B.M.3) presented the lowest phenolic content. In previous studies, it was shown that the oleocanthal and oleacein concentration but presented the lowest phenolic content. In previous studies, it was shown that the oleocanthal and oleacein levels were diminishing in relation to the maturity and the harvest time. Herein we show that even with the same maturity, the phenolic profile can show big differences demonstrating that other parameters can also play a significant role.

CONCLUSION

According to the obtained results, we can say that the production area has significantly influenced the phenolic composition of the ‘Neb Jmel’ olive oil cultivar being oils produced from ‘Kairouan’, ‘Mahdia’ and ‘Sfax’, the richest in polyphenols. The NMR method for the quantification of the major olive oil phenolics is a very useful tool for the study of olive oil and the monitoring of the delicate role of the cultivation conditions on the phenolic profile.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Higher Education and Scientific Research of Tunisia (Contract programme LR14ES08), for their support of this research work. They also wish to extend their thanks to Mrs Leila Mahfoudhi, an English teacher at the Sfax Faculty of Science, for having proofread this paper.

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