

Derivatives of the UV Absorption Spectra, ECN Groups, and Phytosqualene Contents in Virgin Olive Oils from Varieties Grown in the Extreme Conditions of Tataouine Region (Southern Tunisia)

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Abstract: This study was carried out on the analysis of the physicochemical composition of some Tunisian virgin olive oils (VOOs) from varieties grown in the harsh pedoclimatic conditions of the region of Tataouine. According to the UV absorption spectra and the respective derivative ones, all the studied samples belong to the category “extra virgin”. HPLC analysis of triacylglycerols permitted the identification and quantification of four triacylglycerol groups: ECN 44, 46, 48, and 50, which are present at the highest levels in the studied VOOs. Small amounts of some other groups (ECN 38, 40 and 42), were also observed in all samples. The analysis of phytosqualene, and its related alcohol composition, showed that all the studied samples contained levels expected for good quality VOO. The Principal component analysis applied to the analytical data reveal that the great variability in oil composition are influenced exclusively by the genetic factor.

Keywords: ECN groups, Phytosqualene, UV derivative spectrophotometry, Virgin olive oil

1. Introduction

The beneficial effect of the consumption of the virgin olive oil (VOO) on human health is well known and related to the characteristic of the fatty acid composition and the presence of minor components, such as phytosqualene, phytosterols and antioxidant molecules as tocopherols and other phenolic compounds (Owen *et al.*, 2000). Some recent researches have shown the influence of the geographic regions (Ben Temime *et al.*, 2006) and the fruit maturity (Baccouri *et al.*, 2007) on the chemical composition of the VOO, but, to our knowledge, no references can be found regarding the influence of the severe pedoclimatic conditions on the quality of VOO. This study are focused on the characterization of VOOs from varieties grown under the harsh pedoclimatic condition of Tataouine (chalky grounds, very high temperature, low rainfall and the presence of the Sahara which covers more than 70% of the Tataouine region) (Bonvallot, 1986). Many analytical parameters, such as quality parameters, ECN groups, phytosqualene content and related alcohol composition, have been considered in the characterization of the Tataouine varieties.

2. Materials and Methods

2.1. Oil Samples

Four samples of VOOs, grown in the locality of Douirat in the region of Tataouine (southern Tunisia), were analyzed from the following varieties: Chemlali Tataouine, Fakhari Douirat, Zarrazi Douirat, and Dhokar Douirat. Oil extraction was performed using an Abencor laboratory oil mill, kneading the olive paste at 28 °C for 30 min. All samples were stored at -20 °C in darkness until analysis.

2.2. ECN groups Composition

The analysis of triacylglycerols was performed according to the official chromatographic method of the European Economic Community Regulations no. 2568/91 (EEC, 1991). A Hewlett Packard HPLC (HP 1050, Agilent Technology) quaternary pump instrument equipped with a detector of refraction index (IR) model HP 1040 was employed using a Lichrosorb RP18 column (250 × 4.6 mm, 5 µm particle size; Teknocroma, Barcelona, Spain). Settings were: column oven, 45 °C; elution solvent: acetone-acetonitrile

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(60:40, v/v) at a rate of 1.2 mL/min. The injected volume was 10 μ L.

2.3. Quantification of phytosqualene

Phytosqualene determination was accomplished on a reverse phase Nucleosil C18 column (particle size 5 μ m, 125 \times 4.0 mm i.d., Macherey-Nagel, Düren, Germany). The elution solvent was 100% acetonitrile; the flow rate, 1.2 mL/min; and the injection volume, 10 μ L. Detection was achieved at 208 nm.

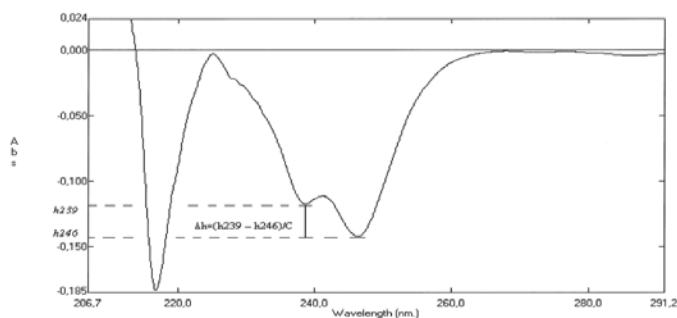


Fig. 1. Example of calculation of the Δh value from the first derivative of the UV spectrum of a VOO sample (Abs, absorbance).

2.4. Determination of Phytosterols.

The content of phytosterols (%) was determined according to procedures described by Sanchez-Casas *et al.* (2004). The apparatus was a Hewlett-Packard instrument model HP 6890 gas chromatograph, equipped with an FID, an HP-5MS capillary column (30 m \times 0.25mm \times 0.25 μ m), and a 6890 Agilent automatic injector. The working conditions of the chromatograph were: injector, 300 $^{\circ}$ C; isothermal column, 260 $^{\circ}$ C; and detector, 325 $^{\circ}$ C.

2.5. Determination of Quality parameters.

Quality of the samples was evaluated based on measurements of acidity, peroxide value and absorbance at 232 and 270 nm (EEC, 1991).

Concerning the first derivatives, the oil solutions (0.5%, wt/vol) were prepared in iso-octane, and the UV spectrum was recorded between 190 and 350 nm. Quantitative data for the first derivatives were expressed as Δh values, calculated as the vertical distance between the two minima at 239 and 246 nm of the first derivative spectrum in the side towards longer wavelengths, divided by the concentration of the sample solution (% wt/vol) (Fig. 1):

$$\Delta h = \frac{|h_{239}| - |h_{246}|}{c}$$

2.6. Statistical analysis

Principal components analysis is performed with XLSTAT software, Version 2007. Significant differences among clones were determined using an analysis of variance, followed by a Duncan's multiple range tests, using SPSS 16.0 for Windows (SPSS Inc., 2007).

3. Results and Discussion

3.1. Free Acidity, Peroxide Index and Specific UV Extinction Coefficient determinations

For all olive oil samples analysed, the values of the physicochemical parameters evaluated (acidity \leq 0.8%, PV \leq 20 mequiv of O₂ kg⁻¹, $E_{1cm}^{1\%}$ 270 \leq 0.22, and $E_{1cm}^{1\%}$ 232 \leq 2.5) (Table 1) fell within the ranges established for the highest quality category of "extra virgin" olive oil, as stated by Regulation EC/1989/2003.

3.2. Derivatives of the UV Absorption Spectra

Concerning the first derivatives, the results of the four samples showed no significant differences ($p < 0.05$) between varieties. All the results showed a Δh which did not exceed 0.6. Based on the work of Grigoriadou and Tsimidou (2006), oils with higher peroxide index and $E_{1cm}^{1\%}$ 232 values had negative Δh values. High positive values are expected for more recent oils whereas high negative values are expected for oils stored for long or under inappropriate conditions. Values around zero are indicative of oils that only marginally fall within the upper commercial category "extra". Our observations showed that all the studied oils belong to the category extra virgin and are in line with those of Grigoriadou and Tsimidou (2006), who showed a good correlation between the $E_{1cm}^{1\%}$ 232 and the Δh ($y = -0.032x + 0.0866$, $R^2 = 0.91$, $p < 0.05$).

Table 1. Means and standard deviations of the quality parameters and some minor compounds evaluated in the studied olive oil samples from the Tataouine varieties.

	Chemlali Tataouine	Fakhari Douirat	Zarrazi Douirat	Dhokar Douirat
Phytosqualene (mg kg ⁻¹)	2605±4 ^{ax}	6048±6 ^d	3933±3 ^c	2363±2 ^b
Phytosterol (mg kg ⁻¹)	1717±2 ^c	1040±3 ^a	1063±2 ^a	1415±3 ^b
Δ ⁵ Avenasterol (%)	19.54±1.45 ^d	16.14±1.32 ^c	9.55±1.54 ^a	11.83±0.56 ^b
FFA (as OA, g kg ⁻¹)	0.25±0.03 ^a	0.30±0.05 ^b	0.45±0.03 ^{bc}	0.64±0.04 ^c
PI (meq O ₂ kg ⁻¹)	4.00±0.54 ^a	5.00±0.38 ^a	15.46±0.55 ^b	16.03±0.69 ^b
E _{1cm} ^{1%} 270 nm	0.16±0.00 ^a	0.14±0.00 ^a	0.14±0.02 ^a	0.12±0.07 ^b
E _{1cm} ^{1%} 232 nm	2.30±0.03 ^b	1.95±0.02 ^a	1.88±0.06 ^a	2.27±0.07 ^b
E _{1cm} ^{1%} 232 / E _{1cm} ^{1%} 270	14.4 ^a	13.9 ^a	13.4 ^a	18.92 ^b
ΔK x 10 ³	-6.0±0.0 ^a	-2.0±0.0 ^b	7.0±0.1 ^c	-3.0±0.1 ^b
Δh	0.03±0.005 ^a	0.04±0.000 ^a	0.004±0.001 ^a	0.06±0.007 ^a

FFA, free fatty acids; OA, Oleic acid; PI, Peroxide index. $\Delta K = K_{270} - (K_{266} - K_{274})/2$; $\Delta h = (h_{239} - h_{246})/C$ [% , wt/vol]

[‡] Different letters for the same quality parameter indicate significant differences among varieties ($p < 0.05$).

3.3. Phytosqualene content and related alcohol composition

All of the analysed samples contained levels expected for good quality VOOs. The analysis of Dhokar Douirat and Chemlali Tataouine oils showed the lowest phytosqualene content (2363 and 2605 mg/kg oil, respectively), whereas, Zarrazi Douirat and Fakhari Douirat showed an amount of such compound two fold-higher than that of Dhokar Douirat and Chemlali Tataouine VOOs (3933 and 6048 mg kg⁻¹ oil, respectively). Our results confirm that the variability in the phytosqualene content of the studied oils can be attributed to varietal factor.

Table 1 reports the amounts of the precursor of phytosqualene, the phytosterols. Only the total phytosterol and the Δ⁵-avenasterol were presented due to their importance in the authentication and the stability of the VOO during heat treatment. A wide variability of the phytosterols components from one variety to another was observed (Table 1). All of the olive oil samples contained more than 1000 mg/kg, the minimum value established by EU Regulations for the category ‘extra virgin’ olive oil. Moreover, a very high Δ⁵-avenasterol content of the samples was observed, which reached 19.53% of total sterol content in the case of Chemlali Tataouine oil. This amount was almost twice high that found for both Spanish VOOs from the variety ‘Cornicabra’ (Salvador *et al.*, 2001) and Tunisian varieties.

3.4. ECN TAG group compositions

ECN TAG group contents, expressed in percentage of total TAG are shown in **Figure 2**. The predominant ECN TAG groups in all samples are ECN 44, 46, 48, and 50. Together these groups accounted for more than 90 % of the total TAGs in almost all the analyzed VOO samples.

Other minor ECN TAG groups, such as ECN 38, 40 and 42, are also present in almost all samples with an amount less than 2%. The ECN 48 TAG group, was predominant in Chemlali Tataouine, Fakhari Douirat, and Zarrazi Douirat (65 % on average). Its level was higher than that in Dhokar Douirat (38.76%). TAG of ECN 42, 46, and 44 were observed to be highest in Dhokar Douirat (3.17, 37.28, and 16.29%, respectively). Some negligible ECN TAG groups, such as ECN 38, are also detected in the studied VOOs with an amount < 1%. Except in Dhokar Douirat VOO, ECN 40 was not detected in any VOO samples. Chemlali Tataouine, Fakhari Douirat, and Zarrazi Douirat showed small differences in the proportion of ECN 44 and ECN 50 TAG group proportions, with mean values of 4.27 and 7.30%, respectively.

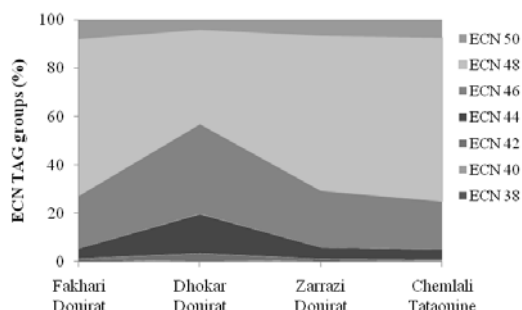


Fig. 2. ECN TAG group compositions in the studied olive oil sample from the Tataouine varieties. With ECN, equivalent carbon number; ECN=CN-2DB, where CN is the number of carbon atoms in all acyls, and DB is the number of double bonds.

3.5. Chemometrics.

In order to study how the studied parameters are useful in chemometric analysis to discriminate between varieties, a principal component analysis (PCA) was performed. The first and the second principal components were sufficient to display the data structure, since they explained 74.70% of the total variance. By examining the scores-plot (Fig. 3) in the area defined by the first and the second principal components, the samples were separated into four groups based on the studied parameters. Group I, which is located on the left side of the scores-plot, is composed of Dhokar Douirat. Group II, which is located in the symmetrical position of the scores-plot, consists of Zarrazi Douirat. Group III, is located at the top of the scores-plot, and is formed by Chemlali Tataouine. Group IV is located on the right side of the scores-plot and includes Fakhari Douirat. Since the samples were well described by the scores-plot, the loading plot (Fig. 3) was analysed in order to show which variables influenced the group separation. Group I was characterised by the highest content of ECN 40, 42, 44, and 46. Group II was characterised by a high level of phytosqualene, acidity and ΔK . Group III was characterised by the highest level of phytosterol. Finally, group IV was characterised by the lowest ECN 38, 48, and 50.

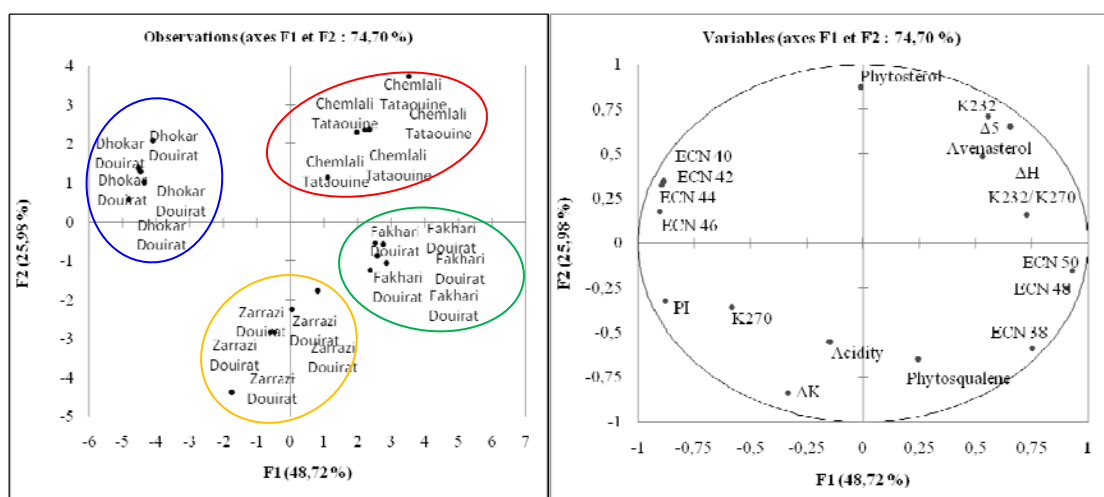


Fig. 3. Loading plot (Projection of the variables) and Scores-plot (Projection of the samples studied) by dimensions F1 and F2 from principal component analysis (PCA) applied to all the studied parameters.

4. Conclusions

All the obtained results showed that the great variability in oil composition among the studied varieties are influenced exclusively by the genetic factor.

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