

Physicochemical characteristics and sensorial profile of two Algerian varietal virgin olive oils (*Chemlal* and *Azeradj*) and their blends

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Olive oils of two most common monovarietal *Chemlal* and *Azeradj* from Bejaia (northern Algeria), were blended at different rates (1/1, 2/1 and 1/2, V/V), in order to examine the influence on quality index, fatty acid composition, minor compounds (α -tocopherol, phenolic compounds, volatile fraction) and organoleptic features.

Results showed a good discrimination between varieties. *Chemlal* olive oil variety contained slightly higher amounts of antioxidants (phenolic compounds and α -tocopherol), while it was higher in palmitic acid and lower in oleic acid compared to *Azeradj* olive oil. *Chemlal* olive oil had less fruity and pungent attributes and very bitter attribute than *Azeradj* which recorded the higher score by the panel.

Blending olive oil contributes to equilibrate the studied parameters particularly the intensity of the positive attributes. Globally, this study suggests that blending olive oil offers possibility to modulate its composition in particular the volatile compounds and consequently its nutritional and sensory quality.

Keywords: olive oil, blends, quality, characterization, fatty acids, volatile compounds, sensory evaluation.

1. INTRODUCTION

The Mediterranean countries supply more than 95% of the world olive oil production which is the major and principal source of fat component for these countries and others. Consumption of olive oil is increasing in non-Mediterranean area due to the growing interest in the Mediterranean diet and its healthful properties, which has been associated with a lower incidence of coronary heart disease and certain cancers [1].

Also, interesting nutritional and sensory properties of olive oil have been known for a long time, due to its use without refining, thus virgin olive oil characteristic aroma, taste, colour, and nutritive properties distinguish it from other edible vegetable oils. The increasing popularity of olive oil has been attributed to its richness in beneficial substances, mainly for its high content of oleic acid, a monounsaturated fatty acid, which range from 56% to 84% of total fatty acids [1], and also, for its minor components, amounting to about 2% of total oil weight, which include more than 230 chemical compounds (e.g. aliphatic and triterpenic alcohols, sterols, hydrocarbons and volatile compounds) [2].

Furthermore, virgin olive oil provides a rich source of natural antioxidants. Among these substances, carotenoids, tocopherols, and phenolic compounds that may act, through different mechanisms, to confer an effective defense system against the free radical attack [3]. The common properties of olive oil were attributed to the phenolic components, mainly to hydroxytyrosol

and oleuropein, potent antioxidants that were confirmed in vitro and in vivo studies [1].

The olive tree has a significant importance for Algeria. It covers over 400.000 ha and localized mainly in Kabylie and in the Oran region. The Bejaia region represents an important part of the national olive orchard with more than 58000 ha and production of 210.000 hL (1/3 of national production) of olive oil during the 2015/16 campaign (Agriculture Chamber, 2016). Varietal structure was characterized by the predominance of the production of oil varieties. Chemlal variety represents over 30% of olive orchard in Algeria. Azeradj variety, particularly very wide spread in the province of Bejaia accompanies populations of Chemlal. Both cultivars are often processed as fruit mixture.

These varieties have already been studied by several authors [4-7]; however, no studies were conducted on their mixtures. The aim of the current study was to evaluate the quality of five extra virgin olive oil samples, through chemical parameters and sensorial analysis and examine the influence of the level of blend on this quality.

2. MATERIAL AND METHODS

2.1. RAW MATERIAL

The material used in this work included two local varieties of olive oil; *Azeradj* (Az) and *Chemlal* (Ch) from Bejaia (northern Algeria). Healthy fruits were harvested by hand from the end of December 2014 to January 2015. The maturity index [8] and fruit weight were determined before oil extraction.

2.2. OIL EXTRACTION AND SAMPLES PREPARATION

Olive oil was obtained using a laboratory oil mill (Levi-Dilon-Lerogsane) with a hammer crusher, thermo-beater (mixer) and a pulp centrifuge. The fruits were crushed with a hammer mill, and then 920 g of olive paste obtained they were mixed for 30 min with 50 ml of warm water (28°C). Then the resulting paste was centrifuged at 3500 rpm for 3 min for oil extraction to recuperate the olive oil. The samples of the two monovarietal *Chemlal* (Ch) and *Azeradj* (Az) olive oil and three blends at different rates (1/1, 2/1 and 1/2, V/V) were used for experimentation and analysis.

2.3. DETERMINATION OF OIL QUALITY PARAMETERS

The determination of the free fatty acids, the peroxide value (PV) and the UV absorption characteristics at 232 nm and 270 nm (K_{232} and K_{270} , respectively) of virgin olive oil were carried out following the analytical methods described in the European Union Commission (EEC/2568/91) [9].

2.4. DETERMINATION OF FATTY ACID COMPOSITION

The fatty acid composition of oils was carried out according to European Union Commission (Regulation EEC/2568/91) [9]. Briefly, the methyl-esters were prepared by vigorous shaking of the oil in hexane (0.2 g in 3 ml) with 0.4 ml of 2 N methanolic potassium hydroxide and analyzed by gas chromatography Dani Master GC equipped with an autosampler and a Flame Ionization Detector (FID).

The operating conditions were set as follow: capillary column Select FAME (100 m × 0.25 mm) (Agilent Technologies, Santa Clara, CA, USA), oven temperature from 140°C (for 5 min) at 240°C with an increase of 4°C/min (maintained for 10 min); detector and injector temperatures were 260°C; carrier gas: helium set at constant flow of 1 ml/min and injection volume 1 µl in split mode (split 1:50). Fatty acids content was expressed as a relative percent of the total area and identified by comparing their retention times with those of standard compounds (Supelco 37 components FAME mix) purchased from Sigma-Aldrich (Milan, Italy).

2.5. PHENOLS CONTENT DETERMINATION

2.5.1. Colorimetric evaluation of total phenols

The extraction and the determination of phenolic compounds were carried out according to the protocol of Favati et al. [10]. Oil sample (1 g) dissolved in hexane (10 ml); then the solution was introduced into a column 1 g of octadecyl C18 previously activated with 6 ml of methanol and 10 ml of hexane. The polar fraction was eluted with 95% methanol. 2 ml phenolic extract 5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent were mixed in a volumetric flask (20 ml). After 3 min, 4 ml of sodium carbonate solution (10%) were added, the content was adjusted to 20 ml with distilled water. After 90 min of incubation in the dark, the mixture was filtered and the absorbance was measured at 765 nm. The total phenols were expressed as mg gallic of acid equivalent per kg of virgin olive oil.

2.5.2. HPLC analysis of phenolic compounds

The phenolic compounds from olive oil were obtained by the liquid-liquid extraction method of Montedoro et al. [11] with some modifications: 20 g of EVOO were added to 10 ml of methanol/water (80/20, v/v) and homogenized in an Ultra-Turrax model T25 (IKA Labortechnik, Staufen, Germany) at 17800 rpm during 2 min, centrifuged at 2850 rpm for 12 min, and then the supernatant was recuperated. After two repeated extractions, the supernatants were collected and concentrated in a rotavapor at 37°C until it became syrupy. The extract taken in 2 ml of methanol was dried under a nitrogen stream. Prior

to HPLC analysis, the extract was redissolved in 1 ml methanol and filtered through a 0.2 μm PVDF filter. A reversed-phase HPLC was performed using an Agilent Technologies system Mod. 1100 equipped with vacuum degasser, ternary pump, autosampler, thermostated column compartment and detectors (DAD and FLD). AC18 column, Spherisorb ODS-1 (250 \times 4.6 mm, 5 μm particle size) was used with injection sample volume of 20 μl and a flow rate of 1 ml/min. Operating conditions of chromatographic analysis were described previously by Antonini et al. [12]. The mobile phase was composed of 0.2% acetic acid (pH 3.1) in water (solvent A) with methanol (solvent B). The gradient was changed as follows: 95% A/5% B for 2 min, 75% A/25% B in 8 min, 60% A/40% B in 10 min, 50% A/50% B in 16 min, 0% A/100% B in 14 min. This composition was maintained for 10 min and then returned to the initial conditions and equilibration in 13 min; the total running time was 73 min. Secoiridoid derivatives lignans, and phenolic alcohols were detected by using the DAD at wavelength of 278 nm, while the lignans were detected by using the FLD at 280 and 339 nm [13]. The standards were used for quantitative analysis; the results were expressed as mg of phenols/kg of oil.

2.6. α -TOCOPHEROL DETERMINATION

α -Tocopherol was evaluated by high-performance liquid chromatography with direct injection of oil in n-hexane solution as described by Psomiadou & Tsimidou [14] (1998). 1 g of oil was dissolved in 5 ml of n-hexane, filtered with a polyvinylidene difluoride (PVDF) syringe filter (Whatman, Clifton, NJ) and injected into the HPLC system.

For the HPLC analysis of α -Tocopherol the column used was a Li Chrospher-Si 60, 250 mm \times 4 mm with a particle size 5 μm , (Merck, Germany); the injected sample volume was 40 μl . The mobile phase was composed of n-hexane/2-propanol (99.5:0.5 v/v) (A) and n-hexane/2-propanol (70:30 v/v) (B) at a flow rate of 1.2 ml/min. The gradient changed as follows: 100% A for 2 min, to 89% A in 8 min and maintained for 6 min, to 45% A in 2 min, then to 20% A in 2 min, maintained for 6 min, 100% A in 4 min, and maintained for 5 min. The standard (α -Tocopherol) was obtained from Sigma-Aldrich (Milan, Italy). The α -Tocopherol detection was performed using a DAD set at 294 nm. Results were expressed as mg of α -Tocopherol per kg of oil by comparison of the chromatographic area with a standard response curve.

2.7. VOLATILE COMPOUNDS ANALYSIS

2.7.1. Extraction

Volatile compounds were extracted by a headspace-solid phase microextraction (HS-SPME) technique previously described by Selvaggini et al. [15].

Three grams of EVOO were placed in a 10 ml vial and thermostated at 35°C, then the coated SPME fiber (a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) with a length of 1 cm, Stable Flex - Supelco, Inc., Bellefonte, PA, USA) was exposed to the vapor phase for 30 min to adsorb the volatile compounds. Then, the fiber was inserted into the GC injector, set in splitless mode, using a splitless inlet liner of 0.75 mm i.d. for thermal desorption, where it was maintained for 10 min. All the SPME operations were automated by using a Varian CP 8410 Autoinjector (Varian, Walnut Creek, CA, USA).

2.7.2. GC/MS analysis

The volatile compounds were analyzed by GC-mass spectrometry (MS) using the same operating conditions reported by Leone et al. [16]. A Varian 4000 GC/MS equipped with a 1079 Universal Capillary Injector (Varian, Walnut Creek, CA, USA) was employed. The Volatiles compounds were separated using an Agilent J&W fused-silica capillary column (DB-WAXetr, 50 m, 0.32 mm i.d., 1 μm film thickness) (Agilent Technologies, Santa Clara, CA, USA), operating at constant flow with helium at 1.7 ml/min. The GC oven heating program was as follows: at 35°C for 8 min, raised to 45°C at 1.5°C/min, raised to 150°C at 3°C/min, raised to 180°C at 4°C/min and finally increased to 210°C at 3.6°C/min; this final temperature was maintained for 14.5 min. The injector temperature was at 250°C, the temperature of the transfer line was fixed at 170°C. The mass spectrometer was operated in the electron ionization (EI) mode at ionization energy of 70 eV, with scanning in the mass range of m/z 25 - 350 a.m.u. at a scan rate of 0.79 s/scan and a trap set point temperature of 150°C. The GC-MS was operated with the Varian MS Workstation Software, Version 6.6 (Varian, Walnut Creek, CA, USA). The volatile compounds were identified by comparison of their mass spectra and retention times with those of authentic reference compounds. The values of volatile compounds were calculated by reference to a calibration curve for each compound and expressed in micrograms per kilogram of oil.

2.8. SENSORY ANALYSIS

Sensory analysis was carried out by a trained panel of experts (recognized by the International Olive Council), composed of staff members of the APROL (Società Agricola APROL PERUGIA Soc. Coop, Italia), according to current European Union Commission regulations (EEC 1989/2003 modifying the Regulation EEC 2568/91) [17]. For the sensory analysis, each sample of 15 ml, in a normalized cup at 28 \pm 2°C, after removing the cover, was smelled and then tested by the panelist to judge it for its flavor and for its bitterness, pungency and fruitiness, which were positive attributes, and any negative attributes. Re-

sults were expressed as the mean intensity of the sensory perceptions of the tasters.

2.9. STATISTICAL ANALYSIS

All the results are reported as a mean value ($n = 3$). The data from the experiment were subjected analysis of variance (ANOVA), following by the *Newman-Keuls* test using the Statistica 5.5 package (StatSoft'99 edition). The degree of significance of the results was taken to the probability $p < 0.05$.

The figure of sensorial analysis was obtained using Microsoft Excel. Principal Component Analysis (PCA) applied to oil samples according to their values of α -tocopherol, phenolic compounds and fatty acid profiles, volatile compounds, and sensorial evaluation, were carried out using the same software (Statistica 5.5).

3. RESULTS AND DISCUSSION

3.1. OLIVE'S ANALYSIS

The determination of maturity index and the stage of collect of olive fruits was a very important parameter, since it affected the olive oil quality, composition, and stability. The *Azeradj* variety could be classified as a big fruit with 3.47 g and *Chemlal* as a small fruit with 0.98 g. The ripening index values were found equal to 3.12 and 3.76 for *Azeradj* and *Chemlal* varieties, respectively. Significant differences were found between the values at $p < 0.05$.

3.2. QUALITY PARAMETERS

The quality indices (free acidity, peroxide value, K_{232} and K_{270}) were used to verify the hydrolytic and oxidative processes that take place in the fruits, and/or during the technological procedures of harvesting, storage prior to olive milling and also during the oil preservation [18]. According to the European Union Commission (Regulation EEC/2568/91) [9], all oils' samples (monovarietal and blends) (Table I), showed very low values of acidity (< 0.8), peroxide index (< 20 meq O₂/kg of oil) and ultraviolet absorptions at 232 and 270 nm (< 2.5 and < 0.22 , respectively), which

were below to the established standards. Therefore, all oils could be classified in the category of extra virgin olive oil.

3.3. FATTY ACID COMPOSITION

The fatty acid composition of the analyzed monovarietal and blend oils was reported in Table II. The main fatty acids were oleic acid (C18:1), palmitic acid (C16:0) and linoleic acid (C18:2). Lower values were detected for other fatty acids (palmitoleic, linolenic, stearic, arachidic and eicosenoic acid). According to European Union Commission Regulation EEC 2568/91 [9], all olive oil samples could be classified as extra virgin category. Monounsaturated oleic acid was the main acid ranging from 58.62% in *Chemlal* variety to 65.90% in *Azeradj* variety. Conversely, a higher content of palmitic acid (19.31%) and linoleic acid (16.77%) was observed in *Chemlal* olive oil than in *Azeradj* olive oil (14.60, and 14.79%, respectively). As reported, the cultivar was the major factor that influenced the fatty acid composition, mainly oleic and linoleic acid contents [19]. The fatty acids of blended olive oil changed almost linearly with the rate of the two monovarietal olive oils. In general, significant differences between all samples ($p < 0.05$) were marked. Our result agrees with the findings of many authors [20, 21].

The percentages of total Saturated Fatty Acids (SFA), Monounsaturated Fatty Acids (MUFA) and Polyunsaturated Fatty Acids (PUFA) of all samples were also calculated (Table II). *Chemlal* olive oil was rich in total SFA and PUFA (21.84% and 17.43%, respectively), effectively due to its higher contents in palmitic and linoleic acids, whilst *Azeradj* olive oil had the lowest SFA and PUFA values (17.60% and 15.36%, respectively) and the higher MUFA values (66.88%) compared to *Chemlal* olive oil (60.73%) due to, essentially, its high content in oleic acid. The blend samples presented average values.

Additionally, to MUFA/PUFA ration, oleic/linoleic acids (O/L) and oleic/palmitic acids (O/P) ratios were also important parameters capable of indicating the quality and stability of olive oil [18]. *Azeradj* registered a high value of O/L and O/P ratios (4.46 and 4.52, respectively) followed by *Az/Ch* (2/1, V/V) blend (4.06 and

Table I - Quality parameters of virgin olive oil samples

Samples	Free Acidity (%)	Peroxide value (meq O ₂ Kg ⁻¹)	K ₂₃₂	K ₂₇₀
Azeradj (Az)	0.24 ± 0.03 ^a	1.83 ± 0.29 ^b	1.30 ± 0.00 ^e	0.18 ± 0.01 ^a
Chemlal (Ch)	0.24 ± 0.03 ^a	1.00 ± 0.00 ^c	1.74 ± 0.01 ^c	0.14 ± 0.00 ^b
Az/Ch (V/V)	0.19 ± 0.03 ^b	3.17 ± 0.29 ^a	1.60 ± 0.00 ^d	0.15 ± 0.01 ^{ab}
Az/Ch (V/2V)	0.19 ± 0.03 ^b	2.67 ± 0.30 ^a	1.84 ± 0.01 ^a	0.14 ± 0.08 ^b
Az/Ch (2V/V)	0.28 ± 0.00 ^a	1.83 ± 0.58 ^b	1.79 ± 0.00 ^b	0.15 ± 0.04 ^{ab}

Mean ± SD ($n = 3$), significant differences in the same row were shown by different letters (a-e) ($p < 0.05$).

3.83; respectively) (Table II). *Chemlal* variety recorded the lowest O/L and O/P ratios (3.50 and 3.04 respectively). Intermediate values were observed with other samples, with significant differences ($p < 0.05$). In summary, the blended oil showed improved fatty acids composition, characterized by the increase of oleic acid and reduction in content of palmitic acid respect of *Chemlal* olive oil.

3.4. PHENOLIC COMPOSITION

It is well known that olive oil possesses a lot of attributes, mainly due to its higher content of phenolic compounds, its nutritional quality, its sensory char-

acteristics [22] and its antioxidant and antimicrobial effects [7].

3.5. PROFILE OF PHENOLIC COMPOUNDS

As shown in Table III, the major phenolic components found in our olive oil samples were similar to those obtained by Reboredo-Rodríguez et al. [21], who affirmed that the phenolic composition of olive oil could be affected by the olive cultivar. *Azeradj* olive oil gave higher significant amounts ($p < 0.05$) of 3,4-DHPEA, *p*-HPEA and 3,4-DHPEA-EDA. However, *Chemlal* olive oil recorded higher concentrations in the following compounds: *p*-HPEA-EDA, (+)-1-Acetoxy-pinoreosinol and (+)-Pinoreosinol and 3,4-DHPEA-EA. Significant differences ($p < 0.05$) were observed among samples. Concerning blend samples, despite slight differences observed between samples ($p < 0.05$), intermediate values for all compounds were found with an influence of the variety (Table III). For all samples (Table III), the total of hydroxytyrosol and its derivatives were higher than other compounds. These results were in accordance with the data published Reboredo-Rodríguez et al. [21]. The presence of hydroxytyrosol in olive oil was advantageous, because it plays an important role in the antioxidant properties [1].

Table II - Fatty acid composition (%) of virgin olive oil samples

	Azeradj (Az)	Chemlal (Ch)	Az /Ch (1/1)	Az /Ch (1/2)	Az /Ch (2/1)
Myristic acid (C14:0)	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Palmitic acid (C16:0)	14.60 ± 0.02 ^e	19.31 ± 0.05 ^a	17.10 ± 0.02 ^c	17.61 ± 0.04 ^b	16.44 ± 0.07 ^d
Heptadecanoic acid (C17:0)	0.12 ± 0.02 ^b	0.06 ± 0.01 ^d	0.20 ± 0.00 ^a	0.10 ± 0.00 ^c	0.15 ± 0.00 ^a
Stearic acid (C18:0)	2.80 ± 0.05 ^a	2.10 ± 0.00 ^e	2.40 ± 0.00 ^c	2.26 ± 0.00 ^d	2.48 ± 0.00 ^b
Arachidic acid (C20:0)	0.31 ± 0.02 ^c	0.30 ± 0.00 ^{ab}	0.30 ± 0.02 ^a	0.32 ± 0.01 ^b	0.31 ± 0.02 ^{ab}
Behenic acid (C22:0)	≤ 0.01 ^c	0.10 ± 0.00 ^a	0.10 ± 0.00 ^a	0.03 ± 0.01 ^b	0.07 ± 0.01 ^a
Total SFA	17.60 ± 0.38 ^e	21.84 ± 0.04 ^a	20.00 ± 0.03 ^c	20.32 ± 0.01 ^b	19.44 ± 0.04 ^d
Palmitoleic acid (C16:1)	0.65 ± 0.00 ^c	1.83 ± 0.00 ^a	1.26 ± 0.00 ^b	1.93 ± 0.18 ^a	1.13 ± 0.02 ^b
Heptadecenoic acid (C17:1)	0.12 ± 0.03 ^c	0.08 ± 0.00 ^d	0.17 ± 0.00 ^b	0.12 ± 0.01 ^c	0.20 ± 0.00 ^a
Oleic acid (C18:1 ω-9)	65.90 ± 0.08 ^a	58.62 ± 0.03 ^e	62.00 ± 0.02 ^c	60.67 ± 0.15 ^d	63.00 ± 0.05 ^b
11-Eicosenoic acid (C20:1)	0.20 ± 0.01 ^b	0.20 ± 0.00 ^{ab}	0.21 ± 0.00 ^a	0.20 ± 0.00 ^b	0.20 ± 0.01 ^{ab}
Total MUFA	66.84 ± 0.04 ^a	60.73 ± 0.03 ^e	63.61 ± 0.02 ^c	62.91 ± 0.02 ^d	64.44 ± 0.04 ^b
Linoleic acid (C18:2 ω-6)	14.79 ± 0.01 ^e	16.77 ± 0.01 ^a	15.78 ± 0.01 ^c	16.14 ± 0.03 ^b	15.51 ± 0.01 ^d
Linolenic acid (C18:3 ω-3)	0.58 ± 0.00 ^d	0.66 ± 0.00 ^a	0.62 ± 0.01 ^c	0.64 ± 0.03 ^b	0.62 ± 0.01 ^c
Total PUFA	15.36 ± 0.01 ^e	17.43 ± 0.01 ^a	16.40 ± 0.02 ^c	16.78 ± 0.03 ^b	16.12 ± 0.01 ^d
MUFA/ PUFA ratio	4.35 ± 0.00 ^a	3.48 ± 0.00 ^d	3.88 ± 0.00 ^c	3.75 ± 0.01 ^e	4.00 ± 0.00 ^b
O/L ratio	4.46 ± 0.01 ^a	3.50 ± 0.00 ^e	3.93 ± 0.00 ^c	3.76 ± 0.00 ^d	4.06 ± 0.00 ^b
O/P ratio	4.52 ± 0.00 ^a	3.04 ± 0.01 ^c	3.64 ± 0.01	3.44 ± 0.02 ^b	3.83 ± 0.02 ^b

Mean ± SD (n = 3), significant differences in the same row were shown by different letters (a-e) ($p < 0.05$). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. O/L ratio: Oleic acid/ Linoleic acid ratio; O/P ratio: Oleic acid/Palmitic acid ratio.

The total phenols were equal to 779.41 and 533.33 mg/kg by colorimetric determination and equivalent to 526.61 and 492.83 mg/kg by HPLC analysis for *Chemlal* and *Azeradj* olive oils respectively (Table III). Our results were higher than those found by Tura et al. [23] for eighteen Italian varieties of olive oil (55.4 - 615.5 ppm), but close to those found by Ben Temime et al. [24] for *Chetoui* variety from different Tunisian regions (467-748 ppm). These differences could be explained by the effect of numerous factors such as, the cultivar which is an important factor influencing

(+)-Pinoreosinol and 3,4-DHPEA-EA. Significant differences ($p < 0.05$) were observed among samples. Concerning blend samples, despite slight differences observed between samples ($p < 0.05$), intermediate values for all compounds were found with an influence of the variety (Table III). For all samples (Table III), the total of hydroxytyrosol and its derivatives were higher than other compounds. These results were in accordance with the data published Reboredo-Rodríguez et al. [21]. The presence of hydroxytyrosol in olive oil was advantageous, because it plays an important role in the antioxidant properties [1].

The qualitative phenolic composition of olive oil was related to enzymatic activities and fruit composition. As known, during malaxation, many enzymatic activities (β -glucosidase and esterase) cause hydrolysis of secoiridoid glucosides, which are the most abundant phenolic compounds in the olive fruit, to their aglycons derivatives in particular: 3,4-DHPEA-EDA, 3,4-DHPEA-EA, p-HPEA-EDA and p-HPEA-EA that are the result of the hydrolysis of oleuropein, demethyloleuropein and ligstroside.

3.6. α -TOCOPHEROL

Vitamin E, especially α -tocopherol, is an important vegetable oil component contributing to the nutritional value and the antioxidant properties of olive oil [22].

IV. Twenty-four compounds have been characterized in *Azeradj* olive oil and twenty-three in *Chemlal* olive oil and in their blends. These results indicate that there was a quantitative difference between the profiles of the analyzed varieties. The major compounds identified were C6 aldehydes and alcohols. *Azeradj* olive oil variety revealed a higher significant level ($p < 0.05$) of alcohols (91.88% of the whole volatile compounds), which were represented mainly by 1-hexanol (34.46%), (E)-2-hexen-1-ol (33.20%) and (Z)-3-hexen-1-ol (23.50%), followed by lower values of 1-octanol (4.37%), 1-penten-3-ol (1.80%) and (E)-2-penten-1-ol (1.60%). Other alcohols like 1-heptanol and benzyl alcohol were detected in extremely low percentage (0.05-0.4%).

On the other hand, aldehydes were significantly high-

Table III - α -tocopherol and phenolic compounds composition (mg/kg) of virgin olive oil samples evaluated by HPLC

α -tocopherol	Azerradj (Az)	Chemlal (Ch)	Az /Ch (1/1)	Az /Ch (1/2)	Az /Ch (2/1)
Total phenols (colorimetric method)	309.59 \pm 2.88 ^d 533.33 \pm 13.81 ^c	376.29 \pm 2.69 ^a 779.41 \pm 6.09 ^a	314.73 \pm 2.11 ^c 605.27 \pm 2.61 ^c	321.33 \pm 1.27 ^b 650.20 \pm 8.42 ^b	320.74 \pm 1.72 ^b 552.35 \pm 3.99 ^d
Hydroxytyrosol	19.20 \pm 0.15 ^a	0.63 \pm 0.01 ^e	7.12 \pm 0.03 ^c	4.04 \pm 0.08 ^d	8.92 \pm 0.03 ^b
Tyrosol (p-HPEA)	23.83 \pm 0.17 ^a	1.50 \pm 0.01 ^e	13.37 \pm 0.01 ^c	9.35 \pm 0.16 ^d	19.20 \pm 0.05 ^b
3,4-DHPEA-EDA	194.21 \pm 7.33 ^a	143.61 \pm 0.83 ^b	131.94 \pm 0.01 ^d	130.26 \pm 1.11 ^c	135.00 \pm 0.00 ^c
p-HPEA-EDA	63.18 \pm 0.11 ^e	86.92 \pm 0.52 ^a	73.40 \pm 0.13 ^c	79.16 \pm 0.15 ^b	68.13 \pm 0.10 ^d
(+)-1-Acetoxy-pininosinol	7.38 \pm 0.32 ^e	14.25 \pm 0.64 ^a	9.63 \pm 0.00 ^c	13.28 \pm 0.06 ^b	8.56 \pm 0.02 ^d
(+)-Pininosinol	27.85 \pm 0.14 ^d	40.80 \pm 0.00 ^a	30.15 \pm 0.08 ^c	39.77 \pm 0.06 ^b	26.46 \pm 0.01 ^e
3,4-DHPEA-EA	157.18 \pm 0.68 ^e	238.90 \pm 1.20 ^a	189.30 \pm 0.14 ^c	199.12 \pm 0.60 ^b	183.52 \pm 0.18 ^d
Total phenols HPLC	492.83 \pm 6.00 ^b	526.61 \pm 3.20 ^a	454.91 \pm 0.36 ^d	474.98 \pm 0.36 ^c	449.79 \pm 0.23 ^d

Mean \pm SD (n = 3), significant differences in the same row were shown by different letters (a-e) ($p < 0.05$). 3,4-DHPEA-EDA: dialdehydic form of decarboxymethylenolenic acid linked to hydroxytyrosol; p-HPEA-EDA: dialdehydic form of decarboxymethylenolenic acid linked to tyrosol; 3,4-DHPEA-EA: isomer of oleuropein-aglycone.

Table III contains the values of α -tocopherol in the studied olive oil samples. Higher concentration was detected in *Chemlal* olive oil (376.29 mg/kg) compared to *Azeradj* olive oil with 309.59 mg/kg. This could be the result of the varietal effect, the stage of maturity between *Azeradj* and *Chemlal* olive fruits, as explained by Beltran et al. [22]. For the mixtures samples, statistically no significant differences ($p < 0.05$) between Az/Ch (1/2) and Az/Ch (2V/1V) were shown, where the α -tocopherol contents were 321.33 and 320.74 mg/kg, respectively.

3.7. VOLATILE COMPOUNDS

It is known that oxidation of linoleic and linolenic acid through a series of enzyme genetically determined; the lipoxygenase pathway, are involved in the increase of major group of volatiles compounds of olive oil with good quality. Most of them were formed during the mechanical extraction processes of olive oil [25]. The aromatic composition identified in olive oil samples by HS-SPME-GC/MS is listed in Table

er ($p < 0.05$) in *Chemlal* olive oil accounting for about 68.24% of total volatile compounds, due to the higher amounts of (E)-2-hexenal (72.63%), followed by intermediate levels with (E,E)-2-4-hexadienal (8.10%), hexenal (7.90%) and 2-4-hexadienal (4.78%). Lower levels of aldehydes were detected for other molecules in the range of 0.45 to 1.74%.

As observed by some authors [24, 26], E-2-hexenal was the major volatile detected in olive oil. In our study, in all samples (exempted *Azeradj* olive oil); high amounts of E-2-hexenal was detected within the range of 34.29% and 72.63% (Table IV). This compound is the result of lipoxygenase pathway, which was inversely related to the oxidation degree of olive oil and the maturity of olive fruit [27]. Furthermore, hexenal was found in all samples mainly in the blends. Az/Ch (1/1, V/V) had the highest concentration (37.52%) followed by Az/Ch (2/1, V/V) (36.70%) and Az/Ch (1/2) (28.68%). Hexenal issued during linoleic acid degradation, deriving from either lipoxygenase action or from chemical oxidation, has been related with grassy, green-sweet, and green-apple odors [27].

The esters in olive oil are represented mainly by hexyl-acetate and (Z)-3-hexenyl acetate deriving from the lipoxygenase pathway. The level of esters is usually found to be lower compared to the levels of aldehydes and alcohols [27].

The ratio hexanal/E-2-hexenal was also calculated; all samples showed low values (0.11-1.07). These results agreed with those found by Brkić Bubola *et al.* [28] who indicated that lower hexanal/E-2-hexenal ratio is related to a superior quality and a lower oxidation degree of the oil.

A comparison between our results and those found in some studies was made; differences were observed either in the quantitative and/or qualitative volatile composition of olive oil samples. It could be explained by numerous factors such as: the stage of maturity of the fruit, environmental growth conditions and enzymatic activity [20], harvesting and extraction conditions, which could alter the olive oil volatiles'

profile [29]. In the same context, malaxing conditions may modify the phenol and volatile contents in olive oil and, consequently, its properties [30].

3.8. SENSORIAL EVALUATION

All olive oil samples showed sensory profiles belonging to EVOO category European Union Commission (EEC/2568/91) (Fig. 1) (Table V). The three positive descriptors: fruity, bitter, and pungent vary according to the cultivar. The significant differences were shown between all olive oil samples ($p < 0.05$).

According to Figure 1, *Azeradj* olive oil variety is very appreciated by the panel test (score = 8.12) due to its fruity and pungent attributes with values of 5.64 and 3.42, respectively, followed by *Az/Ch* (2V/1V) blend (score = 7.94) due to, essentially, its bitter and pungent attributes (3.91 and 3.55, respectively). Whereas, *Chemlal* olive oil had less fruity and pungent attributes

Table IV - Concentrations of volatile compounds ($\mu\text{g}/\text{kg}$) identified in virgin olive oil samples

	Azerradj (Az)	Chemlal (Ch)	Az /Ch (1/1)	Az /Ch (1/2)	Az /Ch (2/1)
<i>Aldehydes</i>					
(E)-2-Pentenal	3.00 \pm 1.00 ^d	73.50 \pm 3.50 ^a	36.50 \pm 0.50 ^b	36.00 \pm 1.00 ^b	27.00 \pm 0.00 ^c
Hexanal	232.50 \pm 4.50 ^e	300.00 \pm 39.00 ^d	1150.00 \pm 12.00 ^a	839.00 \pm 27.00 ^c	952.00 \pm 6.00 ^b
(E)-2-Hexenal	779.00 \pm 27.00 ^d	2756.50 \pm 147.50 ^a	1191.00 \pm 9.00 ^c	1441.50 \pm 96.50 ^b	889.55 \pm 5.65 ^d
Heptanal	23.50 \pm 13.50 ^d	60.50 \pm 5.50 ^a	40.50 \pm 1.50 ^{bc}	26.00 \pm 1.00 ^{cd}	54.00 \pm 12.00 ^{ab}
(E,E)-2,4-Hexadienal	149.50 \pm 8.50 ^d	307.50 \pm 14.50 ^a	248.00 \pm 4.00 ^b	258.00 \pm 4.00 ^b	200.00 \pm 22.00 ^c
2,4-Hexadienal	77.00 \pm 2.00 ^d	181.50 \pm 12.50 ^c	320.00 \pm 4.00 ^a	293.00 \pm 9.00 ^b	327.50 \pm 3.50 ^a
(E)-2-Octenal	17.50 \pm 3.50 ^c	31.50 \pm 1.50 ^b	19.50 \pm 1.50 ^c	42.00 \pm 2.00 ^a	19.50 \pm 3.50 ^c
Octanal	8.00 \pm 8.00 ^b	17.00 \pm 0.00 ^a	8.50 \pm 1.50 ^b	7.00 \pm 1.00 ^b	9.00 \pm 2.00 ^b
Nonanal	26.92 \pm 5.20 ^{bc}	49.88 \pm 7.87 ^a	17.50 \pm 1.15 ^{cd}	31.06 \pm 6.51 ^b	12.66 \pm 2.74 ^d
(E)-2-Heptenal	0.67 \pm 0.58 ^d	17.50 \pm 0.50 ^c	33.50 \pm 4.50 ^a	28.00 \pm 1.00 ^b	26.50 \pm 0.50 ^b
Total aldehydes	1317.58 \pm 49.70 ^d	3795.37 \pm 231.37 ^a	3065.00 \pm 21.35 ^b	3001.56 \pm 119.01 ^b	2517.71 \pm 13.89 ^c
Hexanal/(E)-2-Hexenal	0.30 \pm 0.00 ^d	0.11 \pm 0.01 ^e	0.97 \pm 0.00 ^b	0.58 \pm 0.02 ^c	1.07 \pm 0.01 ^a
<i>Alcohols</i>					
1-Pentanol	3950 \pm 1.50 ^a	11.00 \pm 2.00 ^d	15.00 \pm 0.00 ^c	10.50 \pm 0.50 ^d	21.50 \pm 0.50 ^b
1-Penten-3-ol	287.50 \pm 31.50 ^a	138.50 \pm 9.50 ^d	253.00 \pm 1.00 ^{ab}	215.50 \pm 13.50 ^{bc}	188.17 \pm 59.07 ^{cd}
(E)-2-Penten-1-ol	255.50 \pm 6.50 ^a	118.50 \pm 10.50 ^e	221.50 \pm 21.50 ^b	173.50 \pm 4.50 ^d	197.50 \pm 14.50 ^c
1-Hexanol	5512.50 \pm 274.50 ^a	113.00 \pm 9.00 ^e	1140.50 \pm 6.50 ^c	671.00 \pm 26.00 ^d	2197.50 \pm 30.50 ^b
(E)-3-Hexen-1-ol	60.50 \pm 4.50 ^a	9.50 \pm 1.50 ^c	10.00 \pm 0.00 ^c	4.50 \pm 0.50 ^d	17.00 \pm 0.00 ^b
(Z)-3-Hexen-1-ol	3758.50 \pm 132.50 ^a	153.00 \pm 9.00 ^e	1189.00 \pm 10.00 ^c	940.67 \pm 59.91 ^d	1725.50 \pm 2.50 ^b
(E)-2-Hexen-1-ol	5310.50 \pm 212.50 ^a	285.33 \pm 20.21 ^e	2358.00 \pm 12.00 ^c	1856.00 \pm 24.00 ^d	2988.00 \pm 27.00 ^b
(Z)-2-Hexen-1-ol	10.50 \pm 1.50 ^a	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b
1-Hexen-3-ol	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
1-Heptanol	13.00 \pm 2.00 ^b	13.50 \pm 2.50 ^b	15.50 \pm 0.50 ^{ab}	12.50 \pm 2.50 ^b	18.00 \pm 1.00 ^a
1-Octanol	698.50 \pm 82.50 ^a	673.00 \pm 52.00 ^a	491.50 \pm 25.50 ^b	364.50 \pm 7.50 ^c	503.50 \pm 56.50 ^b
Benzyl alcohol	8.00 \pm 0.00 ^b	8.50 \pm 5.50 ^b	29.50 \pm 1.50 ^a	33.50 \pm 2.50 ^a	31.00 \pm 1.00 ^a
Phenylethyl alcohol	40.50 \pm 4.50 ^d	180.00 \pm 3.00 ^a	60.50 \pm 1.50 ^c	65.50 \pm 2.50 ^b	57.00 \pm 0.00 ^c
Total alcohols	15995.00 \pm 576.00 ^a	1703.83 \pm 124.53 ^e	5784.00 \pm 23.00 ^c	4347.67 \pm 97.85 ^d	7944.67 \pm 57.95 ^b
<i>Esters</i>					
Hexyl acetate	62.50 \pm 13.50 ^d	61.00 \pm 11.00 ^d	79.70 \pm 2.00 ^b	63.40 \pm 1.00 ^c	113.95 \pm 36.50 ^a
(Z)-3-Hexenyl acetate	33.50 \pm 9.50 ^d	1.50 \pm 0.50 ^e	47.22 \pm 8.69 ^b	38.65 \pm 2.50 ^c	65.65 \pm 28.50 ^a
Total esters	96.00 \pm 4.00 ^c	62.50 \pm 10.50 ^d	126.92 \pm 1.03 ^b	102.05 \pm 0.35 ^c	179.60 \pm 6.50 ^a

Mean \pm SD (n = 3), significant differences in the same row were shown by different letters (a-e) ($p < 0.05$).

with very bitter attribute. Additionally, sensory profiles of all blends olive oils were similar with *Chemlal* olive oil for the bitter attribute, and with *Azeradj* olive oil for the pungent attribute, varying only slightly in intensities. The median of the defects was zero for all olive oil samples, meaning that testers did not perceive any specific off-flavour or taste, and which confirm, again, that our oils could be classed as extra virgin category. Phenolic components could affect most of the taste and flavor attribute mainly bitter taste, pungency, and astringency sensations. Higher intensities for these descriptors were perceived in the VOO which has higher levels of 3,4-DHPEA oleuropein derivatives and p-HPEA ligstroside derivatives [31]. Moreover, hydroxytyrosol and its derivatives influenced on sensory evaluation more than tyrosol and its derivatives, while secoiridoids derivatives of hydroxytyrosol are the main contributors to olive oil bitterness [18]. Our results confirmed that olive oil's characteristic aroma was associated with those substances (Table III). Cecchi & Alfei [26] reported that, the strongest correlation between sensory attributes and volatile molecular markers was found, including mainly alcohols, aldehydes, ketones, hydrocarbons, esters, furans and acids. Several of which were produced from linoleic and linolenic acids through the lipoxygenase pathways, and from fatty acid or amino acid metabolism [30]. In our oils (Table IV), only aldehydes, alcohols and esters were identified, where [E]-2-hexenal (most abundant among C₆ aldehydes) provides the typical "green and bitter note" of olive oil [27]. As expected by Reboredo-Rodríguez et al. [18], the fatty acid composition influenced the organoleptic characteristics of the oils, mainly MUFA contents and MUFA/PUFA ratio which were correlated with a bitter taste, whereas, high content of SFA lead to an in-

crease of viscosity and persistence on the mucous of the oral cavity, producing an effect known as a fatty sensation.

3.9. GENERAL STATEMENTS

The Principal Component Analysis (PCA) (Fig. 2) applied to the olive oil samples regarding their interactions with all determination parameters showed that, two groups could be distinguished, which were in two opposite areas. The first group was composed by *Chemlal* olive oil variety and *Chemlal/Azeradj* (2V/V) blend, while the second group, consisting of three other samples; *Azeradj* olive oil variety, *Chemlal/Azeradj* (V/V) and *Chemlal/Azeradj* (V/2V), blends. These groups were separated according to their different physicochemical composition and property, except for some common points (Fig. 2). *Chemlal* and *Chemlal/Azeradj* (2V/V) samples revealed higher content in antioxidants and aldehydes molecules (heptanal, (E)-2-octenal, octanal, (E)-2-heptenal, etc.), with bitter and pungent attributes. Whereas *Azeradj* with other blend samples were richer, mainly, in unsaturated fatty acids and alcohol components (1-pentanol, (1-hexanol, (Z)-2-hexen-1-ol, 1-octanol, etc.) with a fruity attribute. All these results, lead us to confirm that the variety as well as the mixing rate between oils monovarieties influenced quality, composition, and properties of oil samples.

4. CONCLUSION

According to the obtained results both of monovarieties and blends of the studied olive oils could be categorized as extra virgin olive oils. *Azeradj* olive oil

Table V - Organoleptic profile of virgin olive oils samples

	Azerradj (Az)	Chemlal (Ch)	Az /Ch (1/1)	Az /Ch (1/2)	Az /Ch (2/1)
<i>Negative attributes</i>					
Fusty	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Musty	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Vinegar	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Metallic	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Rancid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Otherdefectattributes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>Positive attributes</i>					
Fruity	(5.64 ± 0.62a)A	(2.57 ± 0.59d)B	(3.65 ± 0.56c)A	(4.85 ± 0.68b)A	(3.74 ± 0.30c)A
Bitter	(3.47 ± 0.82b)B	(4.12 ± 0.47a)A	(3.52 ± 0.38ab)A	(3.89 ± 0.71ab)B	(3.91 ± 0.57ab)A
Pungent	(3.42 ± 0.89a)B	(2.50 ± 0.84b)B	(3.42 ± 0.34a)A	(4.11 ± 0.75a)B	(3.55 ± 0.82a)A
Score	8.12 ± 0.23 ^a	7.37 ± 0.23 ^c	7.81 ± 0.26 ^b	7.69 ± 0.37 ^b	7.94 ± 0.18 ^{ab}

Mean ± SD, significant differences in the same row were shown by different letters (a-c) for horizontal comparison and (A-B) for vertical comparison (p < 0.05).

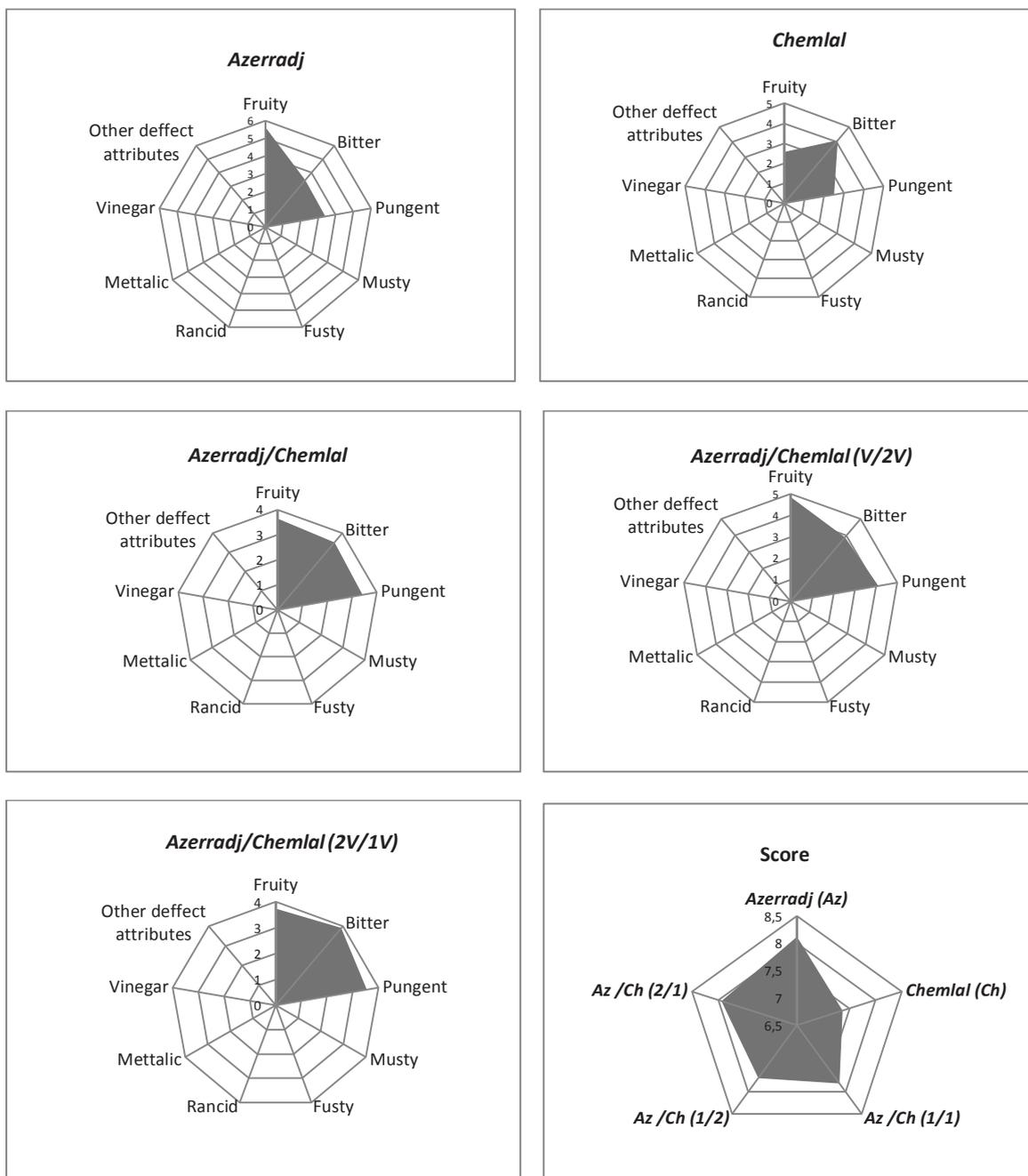


Figure 1 - Sensorial wheels of monovarieties and binary blend virgin olive oil samples

variety was very appreciated by the test panel due to its fruity and pungent attributes, followed by *Az/Ch* (2V/1V). Whereas, *Chemlal* olive oil had less fruity and pungent attributes with very bitter aspect. Moreover, sensory profiles of all blends were like *Chemlal* olive oil for the bitter attribute, and to *Azeradj* olive oil for the pungent and fruity attribute.

Azeradj olive oil was characterized by a high content of alcohols followed by *Az/ch* (2V/V) blend. However, aldehydes were significantly higher in *Chemlal* olive oil, followed by *Az/Ch* (V/2V) and other blend samples. Lower values of the Hexenal/E-2-Hexenal ration

were found in our samples, which mean that, these oils had a high quality and a low oxidation degree. Globally, this study suggests that blending olive oil offers possibility to modulate its composition, in particular the volatile compounds and consequently its nutritional and sensory quality.

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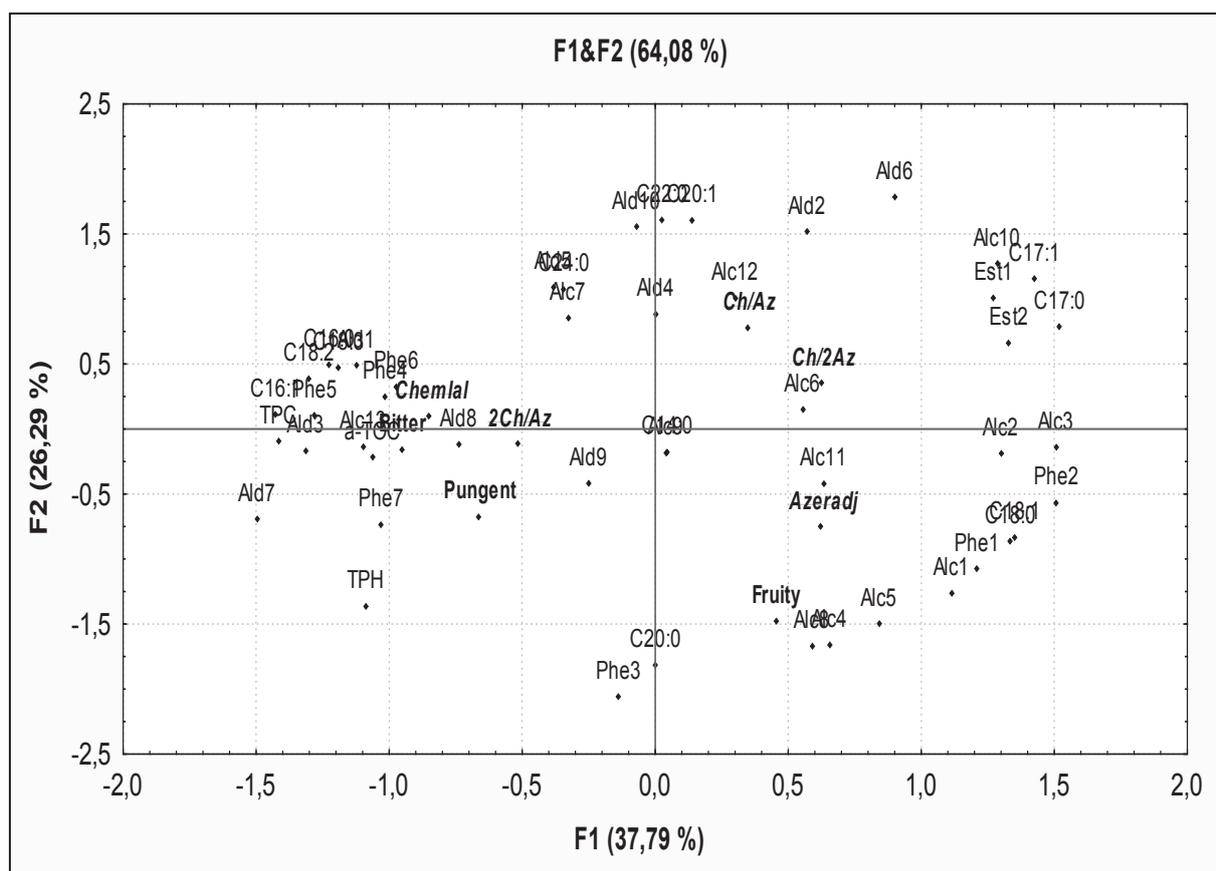


Figure 2 - Principal Component Analysis applied to all samples and to average data of α -tocopherol, fatty acids composition, polyphenolic, volatile and organoleptic profiles.

Ch: Chemlal; Az: Azeradj. a-TOC: α -tocopherol. C16:0 (Palmitic acid); C17:0 (Heptadecanoic acid); C18:0 (Stearic acid); C20:0 (Arachidic acid); C22:0 (Behenic acid); C16:1 (Palmitoleic acid); C17:1 (Heptadecenoic acid); C18:1 (ω -9Oleic acid); C20:1 (Eicosenoic acid); C18:2 ω -6 (Linoleic acid); C18:3 ω -3 (Linolenic acid). Phe 1: Hydroxytyrosol (3,4-DHPEA); Phe 2: Tyrosol (*p*-HPEA); Phe 3: 3,4-DHPEA-EDA; Phe 4: *p*-HPEA-EDA; Phe5: (+)-1-Acetoxy-pinoreosinol; Phe6: (+)-Pinoreosinol; Phe7: 3,4-DHPEA-EA. Alc1:1-Pentanol; Alc2:1-Penten-3-ol; Alc3: (*E*)-2-Penten-1-ol; Alc4: 1-Hexanol; Alc5: (*E*)-3-Hexen-1-ol; Alc6: (*Z*)-3-Hexen-1-ol; Alc7: (*E*)-2-Hexen-1-ol; Alc8: (*Z*)-2-Hexen-1-ol; Alc9: 1-Hexen-3-ol; Alc10: 1-Heptanol; Alc11: 1-Octanol; Alc12: Benzyl alcohol; Alc13: Phenylethyl alcohol. Ald1: (*E*)-2-Pentalen; Ald2: Hexanal; Ald3: (*E*)-2-Hexenal; Ald4: Heptanal; Ald5: (*E,E*)-2,4-Hexadienal; Ald6: 2,4-Hexadienal (*i*); Ald7: (*E*)-2-Octenal; Ald8: Octanal; Ald9: Nonanal; Ald10: (*E*)-2-Heptenal. Est1: Hexylacetate; Est2: (*Z*)-3-Hexenyl acetate. Myristic acid (C14:0).

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