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Running Title: *Cv* Olivastro di Bucchianico characterization

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ABSTRACT

Background

Single cultivar "Olivastro di Bucchianico" extra virgin olive oil is obtained from olives cultivated in a narrow area of the Abruzzo region, Italy. This cultivar is mostly present in the municipality of Bucchianico and in some neighbouring municipalities in the province of Chieti. There is very little research in the literature describing the morphological and chemical characteristics of this cultivar.

Results

A morphological characterization of the plant and the fruit was carried out. In addition, we characterized the chemical, physical-chemical and sensory properties of the extra virgin olive

oil. The following analyses were conducted: free acidity, peroxide value, UV spectrophotometric indices, contents in fatty acid ethyl esters, waxes, tocopherols, fatty acids, triglycerides, sterols, alcohols, phenolic substances, volatile compounds and sensory profile. The analysis of the volatile compounds was performed using a HS-GC-IMS instrument connected to a nitrogen generator for carrier/drift gas production.

Conclusion

The results of the chemical analyses showed good levels of nutraceutical components in the oils, wich were found to be organoleptically well balanced with medium values of fruity, bitter and pungent. The HS-GC-IMS method based on the analysis of 15 volatile molecules might be a useful tool for a chemometric discrimination of the varietal origin for the oils under investigation.

Keywords: *Olea europaea L.*, single cultivar, characterization, sensory profile, volatile compounds, chemometric analysis

INTRODUCTION

The olive tree (*Olea europaea L.*) is the oldest fruit tree species present in the regions surrounding the Mediterranean and, thanks to its nutritional properties and its high economic value, is rapidly expanding into new areas of cultivation.

The high environmental variability of the Italian territory allows the presence of many cultivars of olive trees, whit an estimated number of about 800 cultivars spread throughout the country.[1,2] Most probably, this number is even greater because there are many local varieties about which little information is available. The enhancement and preservation of such a great

germplasm was very important in order to contain genetic erosion and diversify production on the territory with single cultivar Extra Virgin Olive Oils (EVOOs).[3-7]

It has been widely demonstrated that a regular consumption of extra virgin olive oil prevents many diseases, not only due to its fatty acid composition abundant of monounsaturated fatty acids (MUFA), but also to minor components with health-related properties that can be defined as nutraceuticals: phenolic compounds, tocopherols, sterols.[8-11] The concentration of these compounds is variable and strongly influenced by many factors, such as cultivars, pedo-climatic environment, cultivation techniques, time and harvesting system, extraction technology.[12-14] Several studies have been carried out to correlate the chemical composition of olive oil with its geographical origin.[3, 4, 15] At the same time, identifying the olive germplasm is a complex task, given the lack of references and the existence of numerous cases of synonymy and homonymy for the same cultivars. To preserve this genetic diversity within the European Union, EVOOs have been "linked" to their territory of origin through the establishment of standardized protocols of various types, such as PDO (Protected Designations of Origin), PGI (Protected Geographical Indications) and, lastly, TAF (Traditional Agricultural Food Products). In this framework, the production of single EVOO cultivars with relative high consumption and market relevance is crucial for the protection of the typical cultivar of a specific area.

Even if for some traits influenced by external factors, the first and principal step for the study of the genetic diversity within a cultivated plant species was finding morphological descriptors, as they represent the phenological traits normally used in a taxonomic classification.[6,16] The study and recovery of minor cultivars existing in specific cultivation areas is very interesting and could be a resource to expand the range of products offered to consumers. These cultivars are linked to specific environmental conditions, together with the continuous extraction

technology, which has a strong influence on the chemical characteristics, oxidative stability and organoleptic characteristics of extra virgin olive oils.[17,18]

To support the organoleptic determination of virgin olive oils by panel test, it has been recently proposed an interesting analytical approach which is based on the determination of volatile compounds using HS-GC-IMS (Gas Chromatography-Ion Mobility Spectrometry). This method delivers a fingerprint of the aroma which can, then be used to discriminate samples with respect to their quality grade in a relatively simple, fast, and economical way.[19,20]

The objective of this work was to analytically study the native Olivastro di Bucchianico cultivar from a very narrow area of Abruzzo, Italy. Indeed, there is very little research in the literature describing the morphological and chemical characteristics of this cultivar.[2,21,22] Hence, the aim of this work was i) to perform a morphological study of this underexploited local cultivar and a chemical characterization of the produced extra virgin olive oils, ii) to use the HS-GC-IMS technique for varietal traceability.

MATERIALS AND METHODS

Morphological description

The Olivastro di Bucchianico olive cultivar can be mostly found in the municipality of Bucchianico and neighbouring municipalities in the province of Chieti. Bucchianico is a small town at an altitude of 330 m, located in the northern part of the provincial territory, close to the seaside resorts of Chieti and Villamagna. Its territory is mainly composed of arable land, but there is also a wide spread presence of olive groves and vineyards. The olive-growing area has a temperate hillside climate with average temperatures around 6 °C in the winter and around 23.5 °C in the summer and relatively abundant rainfall. The Olivastro di Bucchianico

olive trees are characterized by an assurgent bearing, an average foliage and high vigour; the tree is of medium size and the fruiting branches have long internodes. The leaf is of a shiny dark green colour, it is wide and short and has no curvature of the blade. The drupe is ellipsoidal with truncated base and sub-conical apex, of small to medium size ranging from about 1.89 to about 2.31 g, with pulp in percentage varying from 77.4 to 82.7 %. The drupes at the harvest, usually conducted in October, are never completely invaded, with prevalent green and vinous red colours. The drupes show high detachment resistance. The productivity of Olivastro di Bucchianico is medium-high but altering. These cultivars have a good resistance to parasites, other than caries.[22]

Plant material

The olive fruits were handpicked in perfect sanitary conditions at the green stage of ripening in mid and late October and were stored in boxes and bins. The harvest was performed using mechanical facilitators. Seven samples of EVOOs were produced through an extraction system that used two and two half phase centrifugation. The processing temperature was lower than 25°C and the average crushing time lower than 45 minutes. The average oil yield was 15%. Up until the analysis, the oil samples have been preserved at low temperature (15 – 18 °C) in dark-green glass bottles without headspace.

Methods

We performed the following analyses on the samples of single cultivar EVOO "Olivastro di Bucchianico": free acidity, peroxide value, UV spectrophotometric indices, fatty acid ethyl esters (FAEEs), waxes, tocopherols, fatty acid and triglyceride composition, sterol composition and erythrodiol and uvaol content, alcohols content, phenolic substances, volatile compounds, and sensory. Duplicate analyses were performed on each sample.

Analytical parameters

Free acidity

The free fatty acid content was determined as the percentage of oleic acid, according to Annex II of the European Union Commission Regulation EEC/2568/91 and its subsequent amendments.[23]

Peroxide value

The peroxide index was measured as milliequivalents of active oxygen per Kilogram of oil (i.e., meq O_2Kg^{-1}oil), as determined according to Annex III of the European Union Commission Regulation EEC/2568/91 and its subsequent amendments.[23]

UV spectrophotometric indices

The UV absorption characteristics (K_{232}, K_{270}, ΔK) were determined according to Annex IX of EEC /2568/91 and its subsequent amendments. [23]

Fatty acid ethyl esters and waxes content

Fatty acid ethyl esters (FAEEs) and waxes content were determined according to the method described in EEC/2568/91 and its subsequent amendments (Annex XX)[23]. This procedure used the gas chromatography system (HRGC Mega 2 series 8560; Carlo Erba) equipped with a CP-Sil 5CB Low Bleed/MS (Varian, USA) fused silica capillary column (15m x 0.32 mm ID x 0.1 µm film thickness). The oven temperature programme was 80°C for 1 min, then from 80° to 140°C at 20°C min^{-1}, then from 140°C to 340°C at 5°C min^{-1}, then held at 340°C for 20 min. The detector temperature was 350°C. Hydrogen was used as the carrier gas at a column head pressure of 80 kPa. The samples were applied by on-column injection.

Tocopherols

For the HPLC determination of the tocopherols[24], we prepared a solution of 1 g olive oil in 10 mL acetone and used α-Tocopherol (32 mg L^{-1}) as the external standard. The HPLC analysis was conducted using a high-resolution LC 200 liquid chromatograph equipped with a Series

200 UV/Vis detector (Perkin Elmer, Waltham, Massachusetts, USA), a 7725 Rheodyne injector, a 20 µL sample loop, and a Totalchrom workstation for data acquisition (Perkin Elmer, Waltham, Massachusetts, USA). Separation on a Spherisorb ODS2 column (250 x 4.6 mm I.D., 5µm; Waters, Milford, MA, USA) was performed at 25 °C under a constant flow rate of 1 mL min^{-1} with a mobile phase of 0.2% (v/v) H_3PO_4 in water/ methanol/ acetonitrile (2/49/49, v/v/v). The eluted compounds were detected at 292 nm.

Fatty acid composition

Fatty acid composition was determined according to the method described in European Union Commission Regulation EEC/2568/91 and its subsequent amendments (Annex X.B).[23] A gas chromatography system was used (HRGC Mega 2 series 8560; Carlo Erba, Milan, Italy) that was equipped with a SP™ -2380 (Supelco) fused silica capillary column (60 m x 0.32 mm ID x 0.2 µm film thickness). The oven temperature programme was: from 70 to 165°C at 20°C min^{-1}, and hold at 165°C for 23 min, then from 165 to 200°C at 1.5°C min^{-1}, and hold at 200°C for 5 min, then from 200 to 220°C at 2°C min^{-1}, and hold at 220°C for 5 min. The detector temperature was 230°C. Hydrogen was used as the carrier gas at a column head pressure of 60 kPa. The samples (0.4 µL) were injected on-column.

Triglyceride composition

Triglyceride composition were executed according to Annex XVIII of Regulation EEC 2568/91 and its subsequent amendments.[23] The HPLC analysis was conducted using a high-resolution SpectraSystem P2000 liquid chromatograph equipped with a Shodex RI SE-61 Refractive Index Detector, and a ERC-3312 Degasser (Thermo Fisher Scientific, Milan, Italy), a 7725 Rheodyne injector, a 10 µL sample loop. Separation on a SuperSphere 100 column (250 x 4.6 mm I.D., 4µm; Waters, Milford, MA, USA) was performed at 25 °C under a constant flow rate of 0.65 mL min^{-1} with a mobile phase of 100% propionitrile.

Sterol composition, erythrodiol and uvaol content and alcohols content

Sterol profile and alcohols content were determined according to the EEC/2568/91 and its subsequent amendments (Annexes V and XIX).[23] The olive oil, with added α-cholestanol and 1-eicosanol as internal standards, was saponified with 2 N potassium hydroxide in ethanolic solution, then the unsaponifiables was extracted with ethyl ether. The fractions were separated from the extract by thin-layer chromatography on a basic gel plate, than recovered from the silica gel and transformed into trimethylsilyl ethers and analyzed by an HRGC 5160 Mega series (Perkin Elmer, Waltham, Massachusetts, USA) equipped with a Zebron Phenomenex ZB-5 capillary column (30 m x 0.32 mm ID x 0.25 µm film thickness). The gas chromatographic conditions for sterols were: column temperature 265°C; hydrogen was used as the carrier gas at a column head pressure of 50 kPa; split ratio 1:50 and substance amount injected into the split system 1 µL; injector and detector temperatures were 280°C and 290°C. The gas chromatographic conditions for alcoholic fractions were: the initial isotherm was set at 180°C for 8 min and then programmed at 5°C min^{-1} to 265°C and a further 15 min at 265°C; the injector and detector temperatures were 280°C and 290°C, respectively.

Phenols composition

The phenolic compounds were extracted from the olive oil employing to the following method: a 500 µL volume of internal standard solution (0.015 mg mL^{-1}) syringic acid in methanol) was added to 2.500 g of oil sample. After removal of the methanol under reduced pressure at < 35 °C, the samples were dissolved in 6 mL hexane and loaded onto solid-phase extraction columns (Discovery DSC-DIOL 500 mg, 3 mL; Supelco, Bellefonte, PA, USA), which were previously conditioned with 6 mL methanol and 6 mL hexane. The samples were then washed with 2 x 3 mL hexane and 4 mL hexane/ethyl acetate (90/10, v/v), and then eluted with 10 mL methanol. After removal of the methanol in a rotary evaporator at a temperature < 35 °C, the

dry residue was taken up with 1 mL methanol/water (1:1, v/v), filtered through a 0.45 µm PVDF membrane, and then injected into the HPLC system. The HPLC analysis of the phenolic extracts was carried out using a high-resolution LC 200 liquid chromatograph, which was equipped with a Series 200 UV/Vis detector (Perkin Elmer, Waltham, Massachusetts, USA), a 7725 Rheodyne injector, and a 20 µL sample loop, using a Totalchrom workstation for data acquisition (Perkin Elmer, Waltham, Massachusetts, USA). Separation on a Spherisorb ODS2 column (250 x 4.6 mm I.D., 5 µm; Waters, Milford, MA, USA) was performed at 25 °C and at a constant flow rate of 1 mL min^{-1}, with mobile phase mixtures composed of A (0.2% [v/v] H_3PO_4 in water), B (methanol) and C (acetonitrile), at the following ternary gradient program (as A/B/C, v/v/v): 0 min, 96/2/2; 40 min, 50/25/25; 45 min, 40/30/30; 60 min, 0/50/50; 70 min, 0/50/50; 72 min, 96/2/2; 82 min, 96/2/2. The eluted compounds were detected at 280 nm. Previously, 20 µL external calibration standard was injected into the HPLC system, to calculate the response factors of syringic acid to tyrosol. Then, a volume of 20 µL per sample was injected. Quantification of the phenolic compounds, expressed as tyrosol as reported in COI/T.20/Doc No 29/2009[25], was performed according to the concentration of the internal standard and on the basis of the response factor of syringic acid to tyrosol.

Volatile compounds: HS-GC-IMS analysis

The analysis of the volatile compounds was performed using a HS-GC-IMS Flavourspec® instrument (G.A.S. Dortmund, Dortmund Germany) connected to a nitrogen generator for carrier/drift gas production (Microprogel, Pordenone, Italy). 2.0 g of each sample were weighted, in a 20 mL headspace glass vial and then hermetically closed with polytetrafluoroethylene septum (PTFE). The sample was incubated at 40 °C for 8 min and 100 µL of the respective headspace was withdrawn using a heated 2.5 mL Hamilton syringe (80 °C) with a 51 mm needle, through an autosampler unit, HT2000H (HTA s.r.l., Brescia, Italy), and

introduced in a splitless heated (80 °C) injector (2 mm ID, 6.5 mm OD × 78.5 mm fused quartz glass). The carrier gas (nitrogen gas with inlet pressure of 4 bar) passed through the GC-IMS injector transferring the sample into the GC column, using a flow ramp set as follows: the flow was initially set at 2 mL min^{-1} (default) for 2 min, then increased to 17 mL min^{-1} for the next 8 min (70% of maximum flow) and maintained at this flow for another 20 min. Finally, the flow was reduced for the next 2 min to the predefined value (2 mL min^{-1}); end of the program was set at 32 min. The analytes were separated in isothermal mode at 40 °C and introduced into the ionization chamber of the IMS where the tritium source (5000 V) ionized compounds eluting from the GC column and the ions reached the drift tube of the IMS through the shutter grid. The drift tube was maintained at a constant temperature of 45 °C. The gas flow rate of nitrogen introduced in the opposite direction of the sample into the IMS (drift gas) was 150 mL min^{-1}.[20]

Sensory profile

The evaluation of the olive oils was performed under the conditions prescribed by the European Union Commission Regulation EEC/2568/91 and its subsequent amendments (Annex XII) by the CREA-Research Centre for Engineering and Agro-Food Processing of Pescara Panel recognized by the International Olive Oil Council (IOC) and the Ministry of Agricultural, Food and Forestry Policies (MiPAAF). Each taster in the panel smelled and tasted the oil under consideration, according to the profile sheet of the Annex XII and to COI/T.20/DOC. 22 – 2005.[26] The following attributes were evaluated: fruity, pungent, bitter, aromatic herbs, tomato, artichoke, almond and grass/leave. Each attribute was evaluated on a scale from 0.0 to 10.0 and statistically processed by calculation of the median. Confidence intervals were constructed considering the attributes with a robust coefficient of variation of 20.0 % or less.

Statistical analyses

For each sample, we obtained from the HS-GC-IMS analysis a heat map (3D chromatogram) from which it was, then, possible to extrapolate a data matrix. Afterwards, by processing this data matrix using the statistical software Unscrambler X, version 10.4, we are able to perform a PCA (Principal Component Analysis).

RESULTS AND DISCUSSION

Following the analyses of the quality indices (Table 1), all samples of "Olivastro di Bucchianico" were classified as EVOOs, according to the EC Regulation (EEC, 1991 and following amendments).

Free acidity of the samples was found in the range from 0.08 to 0.24 % of oleic acids, while peroxide values ranged from 6.2 to 8.8 meq O_2 kg^{-1}. The mean values of the specific extinction parameters K_{232}, K_{270}, and ΔK were respectively 1.66, 0.12 and -0.003; that is, within the EVOO limit according to the EC regulation. The FAEE content of 2.6 mg kg^{-1} was well below the established EU limit for EVOO (35 mg kg^{-1}). These fat-soluble compounds are a valuable indicator of oil quality, as they are generated by improper agronomic and technological practices; in particular, by processes of fermentation and degradation.[27,28] The waxes content was low, with a range from 27 to 40 mg kg^{-1}, a typical feature of extra virgin olive oils. The total tocopherols content was medium-high, around 264 mg kg^{-1} (range 218-337 mg kg^{-1}). The composition and content of these substances depend on several agronomic factors, such as, olive processing conditions and storage quality.[29-32] Extra virgin olive oil is one of the foods richest in vitamin E (tocopherols), with the most representative α-tocopherol, which accounts for about 90% of the total tocopherols with vitaminic and antioxidant actions.

The fatty acid composition presented in Table 2 was also found to fall within the EU Regulation limits for olive oils. Oleic, palmitic, linoleic, and stearic acids were the most abundant

compounds, with mean values of respectively 74.71%, 11.94%, 7.81 %, and 3.02 %.[33] Olive oils are typically characterized by the following values: not less than 73% of oleic acid and not more than 10% of linoleic acid, with an oleic/linoleic ratio > 7. We found high values of monounsaturated fatty acids (Σ MUFAs) in the "Olivastro di Bucchianico" *cultivar,* with a mean of around 75.88%, a maximum value of 78.14%, and a minimum value of 73.82%. It is well known that the oleic acid is extremely important for a healthy and balanced diet, as it induces an increase in oxidative stability and antihypertensive activity, having low-density lipoprotein (LDL) cholesterol–lowering effects.[34] In defining the nutritional properties and varietal characterization of the EVOOs, it is also important to consider the MUFA/SFAs ratio, which for the single cultivar under analysis was found to be at an average level of 4.82.[35] The composition of triglycerides, useful for the characterization of a single cultivar, is reported in Table 3. As it can be seen from this table, the most prevalent compounds were: PLP + OOO + PoPP (42.48%), SOL + POO (23.61%) and OOL + LnPP (12.07%).

The sterols composition of "Olivastro di Bucchianico" is shown in Table 4. The most prevalent sterols were: β-sitosterol (85.8%), Δ-5-avenasterol (6.5%) and campesterol (3.0%). The value of apparent β-sitosterol, that is the sum of β-sitosterol and its four adjacent phytosterols (clerosterol, sitostanol, Δ-5-avenasterol and Δ-5,24-stigmastadienol), was 94.6% higher than the EC established legal lower bounds. High levels of apparent β-sitosterol have a positive effect on reducing total plasma cholesterol and LDL cholesterol.[36,37] Also the total sterols (1274 mg kg^{-1}) were higher than the lower limit established by the EC legislation (1000 mg kg^{-1}). Sterols and triglycerides can mark the difference among various EVOOs.[38,39] We have determined (Table 5) the presence of both diterpenic alcohols (with 20 carbon atoms) and tripterpenic alcohols (with 30 carbon atoms) in the unsaponifiable fraction of olive oil, which reaches up to 25-30%. Among the ones in the first category, the most represented variety was

the phytol (318.4 mg kg^{-1}), partly coming from the degradation of the chlorophyll. The composition of the triterpenic fraction seems to be specific to the botanical family from which the oil is derived.[40] It is mainly composed of cycloartenol (380.4 mg kg^{-1}) and 24-methylen-cycloartanol (317.9 mg kg^{-1}) followed by β-amyrin + butyrrospermol (90.2 mg kg^{-1}) and α-amyrin (1.2 mg kg^{-1}). The alcoholic composition also includes citrostadienol (218.8 mg kg^{-1}), a 4-methylsterol, and total aliphatic alcohols whose content (140 mg kg^{-1}) is below the limit laid down in the EU standard for EVOOs. In "Olivastro di Bucchianico" the most abundant phenols (Table 6) are 3,4 DHPEA-EDA (76.3 mg kg^{-1}), p-HPEA-EDA (65.3 mg kg^{-1}), pinoresinol and 1-acetoxypinoresinol (58.9 mg kg^{-1}), 3,4-DHPEA-EA,H (37.8 mg kg^{-1}). Many studies report how derivatives of oleuropein and ligstroside, 3,4-DHPEA-EA and p-HPEA-EDA show antioxidant and health properties.[41,42] Moreover, secoiridoids and lignans have effect on the bitter and pungent attributes in EVOOs.[43-45] The total phenols content was medium (306 mg kg^{-1}).[46,47] In Figure 1 it is reported the sensory profile of "Olivastro di Bucchianico" was reported. The profile was characterized by medium perceptions of fruity, with well-balanced notes of bitter and pungent. Medium notes of grass/leave and almond were present with mean values of respectively 2.2 and 3.0, while low notes of artichoke, aromatic herbs and tomato were found whit mean values of respectively 2.2, 0.6 and 0.6.

In order to verify the discriminatory potential of the HS-GC-IMS method on EVOOs oils on the basis of the cultivars they belong to, Olivastro di Bucchianico samples were analyzed together with other single cultivar oils from Central and Southern Italy. In particular, 10 EVOOs derived from Canino, Ogliarola, Coratina, Moraiolo and Peranzana cultivars (2 samples from each cultivar) were subjected to the same HS-GC-IMS analysis as Olivastro. From the heat maps obtained, we took into consideration only the 15 volatile compounds selected within the European H2020 project OLEUM and commented in Valli et al., 2020. Their respective signals,

present in the form of a monomer and/or a dimer in the chromatogram, were highlighted using VOCal software (Gesellschaft für Analytische Sensorsysteme mbH, G.A.S.; Dortmund, Germany). Using a specific function of this software, it was possible to export these results as a data matrix (Table 7 and Table 8), which was, then, used for the construction of a PCA. Figure 3 shows the scores plot obtained from the PCA (explained variance PC1 and PC2: 70%). Although more samples would be required for a more robust level of discrimination, the PCA showed that the 7 Olivastro di Bucchianico EVOOs turned out to be separated from the other 10 analyzed samples, showing a promising discrimination of this cultivar based on its volatile profile from the other varieties.

CONCLUSIONS

The analytical study conducted in this paper on cv. Olivastro di Bucchianico EVOOs, which are obtained from olives cultivated in a small area of the Abruzzo region (Italy), highlighted its interesting compositional characteristics. The samples showed an average medium-high content of total tocopherols (264 mg kg^{-1}), high monounsaturated fatty acids values of around 75.88%, and a good oleic/linoleic ratio (9.57). The average total phenols content was medium, at 306 mg kg^{-1}. The sensory profile of "Olivastro di Bucchianico" was characterized by medium perceptions of fruity, with well-balanced notes of bitter and pungent, accentuated hints of grass/leave and almond with mean values of respectively 2.2 and 3.0, and low intensity notes of artichoke, aromatic herbs and tomato. The research has also encompassed the analysis of volatile compounds using the HS-GC-IMS, in order to verify the discriminatory potential of this method in distinguishing different types of cultivar. In particular, Olivastro di Bucchianico cv EVOOs samples were analyzed in conjunction with other 10 single cultivars oils derived from

Canino, Ogliarola, Coratina, Moraiolo and Peranzana cv. The chemometric analysis of the data makes it possible to separate the Olivastro di Bucchianico cv EVOOs from the others, thus resulting as a promising approach for their discrimination, even if more samples would be required to confirm it. This preliminary study puts in evidence the compositional characteristics of the single cultivar EVOOs under analysis, in order to propose a characterization study to the competent institutions. This contribution can also be valuable for the purpose of defining the specifications of PDOs, PGIs, PATs.

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FIGURE LEGENDS

Figure 1 Sensory profile of the EVOOs from Olivastro di Bucchianico cv

Figure 2 Scores plot obtained from the PCA

APPENDIX: ABBREVIATIONS USED

EVOOs, Extra Virgin Olive Oils**, FAEE,** Fatty Acid Ethyl Esters**, SFAs,** Saturated Fatty Acids**, MUFAs,** Monounsaturated Fatty Acids**, PUFAs,** Polyunsaturated Fatty Acids, **PCA,** Principal Component Analysis.

Table 1. Qualitative characteristics of the EVOOs from Olivastro di Bucchianico cv.

Determinations:	Mean	Max	Min	S. D.
Free acidity (% oleic acid)	0.15	0.24	0.08	0.06
Peroxide value ($meqO_2\ kg^{-1}$)	7.5	8.8	6.2	1.0
K_{232}	1.66	1.98	1.44	0.17
K_{270}	0.12	0.20	0.07	0.043
ΔK	-0.003	-0.002	-0.004	0.001
FAEE ($mg\ kg^{-1}$)	2.6	4.3	0.1	1.7
Waxes ($mg\ kg^{-1}$)	33	40	27	5
Tocopherols ($mg\ kg^{-1}$)	264	337	218	47

S.D.: standard deviation

Table 2. Fatty acid composition of the EVOOs from Olivastro di Bucchianico cv.

Fatty acid composition (%):	Mean	Max	Min	S.D.
Myristic acid (C14:0)	0.01	0.01	0.00	0.004
Palmitic acid (C16:0)	11.94	14.14	10.89	1.30
Palmitoleic acid (C16:1)	0.71	1.00	0.52	0.19
Heptadecanoic acid (C17:0)	0.12	0.15	0.08	0.03
Heptadecenoic acid (C17:1)	0.18	0.26	0.12	0.04
Stearic acid (C18:0)	3.02	3.17	2.82	0.14
Oleic acid (C18:1)	74.71	77.12	72.46	1.66
Linoleic acid (C18:2)	7.81	8.32	6.63	0.60
Arachic acid (C20:0)	0.46	0.54	0.42	0.04
Linolenic acid (C18:3)	0.58	0.64	0.53	0.04
Eicosenoic acid (C20:1)	0.27	0.31	0.24	0.03
Behenic acid (C22:0)	0.11	0.19	0.06	0.04
Lignoceric acid (C24:0)	0.08	0.15	0.04	0.04
Oleic/linoleic	9.57	10.93	9.27	0.99
∑ SFAs	15.74	17.69	14.69	1.11
∑ MUFAs	75.88	78.14	73.82	1.51
∑ PUFAs	8.39	8.93	7.17	0.62
MUFAs/SFAs	4.82	5.03	4.42	0.42

SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; S.D.: standard deviation

Table 3. Triglycerides composition of the EVOOs from Olivastro di Bucchianico cv.

Triglycerides composition (%):	Mean	Max	Min	S.D.
LLL	0.08	0.17	0.03	0.06
OLLn+PoLL	0.14	0.27	0.08	0.07
PLLn	0.06	0.07	0.02	0.02
OLL	1.50	1.65	1.12	0.19
OOLn+PoOL	1.18	1.33	1.08	0.09
PLL+PoPoO	0.49	0.63	0.28	0.12
POLn+PPoPo+PPoL	0.61	0.75	0.52	0.07
OOL+LnPP	12.07	13.00	11.14	0.64
PoOO	1.10	1.65	0.69	0.39
SLL+PLO	5.61	6.45	4.49	0.71
PoOP+SPoL+SOLn+SPoPo	0.70	0.98	0.53	0.18
PLP+OOO+PoPP	42.48	46.38	39.35	2.77
SOL+POO	23.61	25.33	22.31	1.23
POP	3.88	5.01	3.29	0.67
SOO	5.21	5.72	4.76	0.43
POS+SLS	1.28	1.52	1.18	0.11

P: palmitic; Po: palmitoleic; S: stearic; O: oleic; L: linoleic acids; Ln: linolenic acid.
S.D.: standard deviation

Table 4. Sterol composition and erythrodiol and uvaol content of the EVOOs from Olivastro di Bucchianico cv.

Determinations:	Mean	Max	Min	S.D.
Sterol composition (%):				
Colesterol	0.2	0.4	0.1	0.1
Campesterol	3.0	3.2	2.9	0.1
Campestanol	0.0	0.1	0.0	0.0
Stigmasterol	0.7	0.8	0.6	0.1
Δ-7-Campesterol	0.1	0.1	0.0	0.0
Δ-5.23-Stigmastadienol	0.0	0.1	0.0	0.0
Clerosterol	0.6	0.8	0.4	0.2
β-Sitosterol	85.8	88.5	80.3	3.0
Sitostanol	1.0	1.1	0.7	0.1
Δ-5-Avenasterol	6.5	11.5	3.7	2.9
Δ-5.24-Stigmastadienol	0.8	1.0	0.6	0.2
Δ-7-Stigmastenol	0.5	0.8	0.3	0.2
Δ-7-Avenasterol	0.8	1.1	0.7	0.1
Apparent β-Sitosterol	94.6	95.0	94.3	0.2
Total sterols (mg kg^{-1})	1274	1409	1099	128
Erythrodiol +Uvaol (%)	1.48	1.98	1.01	0.35

S.D.: standard deviation

Table 5. Alcoholic content of EVOOs from Olivastro di Bucchianico cv.

Determinations:	**Mean**	**Max**	**Min**	**S.D.**
Alcohols content (mg kg^{-1}):				
Phytol	318.4	418.8	205.3	85.5
Geranylgeraniol	14.5	19.6	10.4	2.9
1-Docosanol (C22)	5.2	6.9	3.9	1.2
1-Tetracosanol (C24)	22.1	27.8	16.1	5.1
1-Hexacosanol (C26)	66.7	84.3	50.1	16.0
1-Octacosanol (C28)	28.1	41.4	20.1	8.4
β-Amyrin + Butyrospermol	90.2	127.3	55.2	29.9
α-Amyrin	1.2	3.5	0.1	1.2
Cycloartenol	380.4	516.2	200.5	128.3
24-Methylen-cycloartanol	317.9	420.9	168.8	114.6
Citrostadienol	218.8	289.9	145.4	55.8
Total aliphatic alcohols (mg kg^{-1})	140	187	94	39

S.D.: standard deviation

Table 6. Phenols content of EVOOs from Olivastro di Bucchianico cv.

Phenols content (mg kg^{-1} of tyrosol):	**Mean**	**Max**	**Min**	**S.D.**
3,4-DHPEA	1.0	1.9	0.3	0.6
p-HPEA	2.4	5.5	0.0	2.4
Vanillic acid	1.2	3.2	0.4	1.2
Vanillin	2.0	2.7	1.0	0.6
p-Coumaric acid	1.7	1.9	1.5	0.2
Hydroxytyrosyl acetate	0.4	0.7	0.0	0.3
Ferulic acid	3.0	8.4	0.6	3.2
o-Coumaric acid	0.1	0.2	0.0	0.1
3,4-DHPEA-EDA ox	0.0	0.0	0.0	0.0
3,4-DHPEA-EDA	76.3	203.2	24.8	74.2
3,4-DHPEA-EA	23.8	46.9	6.8	17.2
Tyrosyl acetate	0.6	1.0	0.3	0.3
p-HPEA-EDA ox	15.2	25.2	6.5	7.6
p-HPEA-EDA	65.3	130.1	37.8	37.6
Pinoresinol, 1-acetoxypinoresinol	58.9	73.3	52.3	9.5
Cinnamic acid	3.5	5.1	1.8	1.4
p-HPEA EA	0.8	1.5	0.0	0.7
3,4-DHPEA-EDA, -EA, -H ox	0.8	1.2	0.5	0.3
Luteolin	3.1	4.9	2.4	1.0
3,4-DHPEA-EA, -H	37.8	73.7	19.2	21.1
p-HPEA-EA, -H ox	5.8	9.4	2.8	2.4
Apigenin	1.0	1.4	0.2	0.5
Methyl-luteolin	8.2	14.5	5.1	3.7
p-HPEA-EA, -H	4.1	7.6	1.9	2.6
Total phenols	306	535	182	135

S.D.: standard deviation

Table 7. Data matrix used to build the PCA. The values (mV) represents the intensity of the dimer signals of each volatile compounds present in the heat maps for each samples.

Samples	Ethyl acetate	Ethyl propanoate	3-methyl-1-butanol	Propanoic acid	(*E*,*E*)-2,4-hexadienal	(*E*)-2-heptenal	6-methyl-5-hepten-2-one	Ethanol	Acetic acid	Hexanal	(*E*)-2-hexenal	1-hexanol	1-octen-3-ol	(*Z*)-3-hexenyl acetate	Nonanal
C1	203.7	331.4	229.2	397.7	377.4	396.3	419.4	548.9	333.2	800.5	845.0	395.1	399.8	401.0	376.4
C2	336.1	325.9	229.2	567.7	384.0	396.3	433.4	431.6	345.6	924.1	1000.0	389.1	392.2	406.7	376.4
CR1	493.4	314.8	284.5	549.8	357.5	354.9	440.4	1000.0	388.8	951.1	871.1	353.2	376.8	344.5	339.4
CR2	434.8	602.0	221.3	447.0	350.9	343.1	454.4	852.5	444.3	1000.0	869.2	347.2	369.1	344.5	333.2
M1	527.7	314.8	245.0	676.8	364.1	354.9	447.4	681.8	499.8	595.9	801.4	353.2	384.5	344.5	345.6
M2	450.7	309.3	237.1	570.8	364.1	354.9	433.4	612.8	351.7	577.9	717.3	347.2	376.8	350.2	345.6
O1	455.9	336.9	272.7	784.9	377.4	396.3	419.4	647.5	432.0	888.0	818.5	395.1	376.8	406.7	376.4
O2	462.3	325.9	260.8	568.7	384.0	396.3	433.4	653.0	413.5	898.3	820.9	395.1	384.5	395.4	376.4
OL1	809.5	331.4	229.2	745.0	377.4	408.2	412.4	281.7	407.3	508.4	801.0	389.1	376.8	395.4	388.8
OL2	1000.0	458.4	347.8	1000.0	523.0	573.8	496.3	673.1	549.2	279.3	942.0	568.7	446.0	593.1	555.4
OL3	768.9	314.8	715.3	978.0	364.1	349.0	398.5	592.3	450.5	360.4	355.5	359.2	384.5	355.8	357.9
OL4	779.9	314.8	446.6	929.7	364.1	354.9	405.5	615.2	456.6	818.5	550.2	359.2	392.2	350.2	357.9
OL5	777.3	309.3	656.0	967.5	364.1	360.8	405.5	468.1	382.6	388.7	385.1	353.2	376.8	355.8	345.6
OL6	806.3	314.8	407.0	827.9	364.1	354.9	426.4	719.3	555.4	598.5	734.4	359.2	392.2	350.2	345.6
OL7	816.4	314.8	565.1	995.8	357.5	360.8	426.4	195.0	357.9	455.6	639.0	359.2	384.5	350.2	351.7
P1	683.9	353.5	237.1	369.4	397.2	408.2	433.4	443.4	382.6	741.3	945.1	395.1	384.5	406.7	388.8
P2	333.0	336.9	233.2	244.5	377.4	402.2	405.5	256.1	382.6	854.6	423.3	401.1	399.8	418.0	382.6

Table 8. Data matrix used to build the PCA. The values (mV) represents the intensity of the monomer signals of each volatile compounds present in the heat maps for each samples.

Samples	(*E*)-2-heptenal_M	(*E*,*E*)-2,4-hexadienal_M	Ethyl acetate_M	Ethyl propanoate_M	3-methyl-1-butanol_M	(*Z*)-3-hexenyl acetate_M	Hexanal_M	(*E*)-2-hexenal_M	1-hexanol_M	Acetic acid_M
C1	382.2	387.9	374.4	351.4	146.4	368.7	977.9	878.3	280.7	263.9
C2	374.9	395.4	421.7	375.6	191.8	368.7	985.7	879.1	280.7	265.2
CR1	360.2	402.8	615.4	393.8	501.0	354.5	975.7	864.2	285.6	353.6
CR2	367.5	387.9	343.8	351.4	278.4	354.5	1000.0	865.0	280.7	399.7
M1	367.5	387.9	494.9	339.3	387.6	368.7	847.7	861.1	270.9	389.2
M2	374.9	380.5	410.6	351.4	346.4	368.7	801.3	862.6	270.9	331.1
O1	382.2	387.9	863.8	454.4	443.3	375.8	986.8	873.6	290.6	827.2
O2	389.6	395.4	684.0	393.8	362.9	368.7	968.0	871.3	315.2	1000.0
OL1	382.2	402.8	532.9	351.4	177.3	375.8	835.5	876.8	334.9	476.3
OL2	470.4	462.5	918.4	563.5	459.8	489.2	681.0	1000.0	645.2	773.1
OL3	374.9	395.4	528.3	393.8	983.5	382.8	716.3	821.0	295.5	497.4
OL4	374.9	402.8	832.3	490.8	732.0	368.7	933.8	859.5	310.3	547.5
OL5	374.9	395.4	628.4	399.9	1000.0	375.8	732.9	830.5	354.6	558.0
OL6	374.9	402.8	790.5	339.3	688.7	368.7	802.4	859.5	330.0	753.3
OL7	374.9	380.5	1000.0	357.5	913.4	368.7	742.8	863.4	270.9	409.0
P1	389.6	402.8	532.0	363.5	146.4	382.8	854.3	873.6	275.8	325.9
P2	374.9	402.8	420.8	357.5	127.8	368.7	875.3	861.1	265.9	298.2

M: monomer