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Complete List of Authors:	Di Serio, Maria Gabriella; CREA, Research Centre for Engineering and Agro-Food Processing Giansante, Lucia; CREA, Research Centre for Engineering and Agro-Food Processing Del Re, Paolo; CREA, Research Centre for Engineering and Agro-Food Processing Pollastri, Luciano; Regione Abruzzo, Department of Agriculture Panni, Filippo; Università di Bologna, Department of Agricultural and Food Sciences, Alma Mater Studiorum Valli, Enrico; Università di Bologna, Department of Agricultural and Food Sciences and Interdepartmental Centre for Industrial Agro-food Research, Alma Mater Studiorum Di Giacinto, Luciana; CREA, Research Centre for Engineering and agro-food processing
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Analytical study of cultivar "Olivastro di Bucchianico" extra virgin olive oils and its recognition by HS-GC-IMS

Running Title: *Cv* Olivastro di Bucchianico recognition

Authors: Maria Gabriella Di Serio[1], Lucia Giansante[1], Paolo Del Re[1], Luciano Pollastri[2], Filippo Panni[3], Enrico Valli[4], Luciana Di Giacinto*[,1]

[1] CREA Research Centre for Engineering and Agro-Food Processing , I-65012 Cepagatti, PE, Italy

[2] Regione Abruzzo, I-65012 Cepagatti, PE, Italy

[3]Department of Agricultural and Food Sciences, Alma Mater Studiorum—Università di Bologna, 47521 Cesena, Italy

[4] Department of Agricultural and Food Sciences and Interdepartmental Centre for Industrial Agrofood Research, Alma Mater Studiorum—Università di Bologna, 47521 Cesena, Italy

***Correspondence: Dr Luciana Di Giacinto** (CREA - Research Centre for Engineering and Agro-Food Processing (CREA-IT), I-65012 Cepagatti, PE, Italy **Email:** luciana.digiacinto@crea.gov.it)

ABSTRACT

Background

Single olive cultivar "Olivastro di Bucchianico" extra virgin olive oils, obtained from olives cultivated in a restricted area of the Abruzzo region, Italy. Principally is present in the municipality of Bucchianico and in some neighbouring municipalities in the province of Chieti. There are very few research works in 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, extra virgin olive oil was chemical, physical-chemical and sensory characterized. The conducted

analyses were as follows: 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 that resulted 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 plant present in the environment 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, estimated about 800 cultivars throughout in all the national territory.[1,2] Most probably, this number is even greater because there are many local varieties of which few 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 EVOOs.[3-7]

A regular consumption of extra virgin olive oil has been widely demonstrated to prevent many diseases, not only due to its fatty acid composition abundant of monounsaturated fatty acids (MUFA), but also for 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 chemical composition of olive oil with the geographical origin.[3, 4, 15] At the same time, identification of olive germplasm is complicated because no references and numerous cases of synonymy and homonymy for the same cultivars exist. To preserve this genetic diversity within the European Union, EVOOs have been "linked" to their territory of origin through the creation of standardized protocols of various types such as PDO (Protected Designations of Origin), PGI (Protected Geographical Indications) and finally TAF (Traditional Agricultural Food Products). In this framework, the production of single EVOO cultivars, with relative high consumption and market relevance, is very important for the protection of the typical cultivar of a specific area.

Morphological descriptors, even if for some traits influenced by external factors, was the first and principal step for study the genetic diversity within a cultivated plant species and they represent the phenological traits normally used in taxonomic classification.[6,16] The study and recovery of minor cultivars present in particular cultivation areas, is very interesting and could be a resource to expand the offer of products to consumers. The 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]

Recently an interesting analytical approach has been proposed, based on the determination of volatile compounds using HS-GC-IMS (Gas Chromatography-Ion Mobility Spectrometry), to support the organoleptic determination of virgin olive oils by panel test. This method can realize a fingerprint of the aroma for a possible discrimination of the samples with respect to the quality grade in a relatively simple, fast and economical way.[19,20]

This work was made to analytical study native Olivastro di Bucchianico cultivar from a very restricted area of Abruzzo, Italy (Figure 1). There are very few research works in literature describing the morphological and chemical characteristics of this cultivar.[2,21,22] So, the aim of this work was to do i) a morphological study of this underexploited local cultivar and 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 belongs to the municipality of Bucchianico and neighbouring municipalities in the province of Chieti. Bucchianico, with 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 arable land, but with a large presence of olive groves and vineyards. The olive-growing area has a temperate hilly climate with winter temperatures around 6 °C in winter and around 23.5 °C in summer and relatively abundant rainfall. The Olivastro di Bucchianico olive trees have an assurgent bearing, an average

foliage and high vigour; the tree is of medium size, the fruiting branches have long internodes. The leaf is 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 at the mid and late October and stored in boxes and bins. Harvesting was performed with use of mechanical facilitators. Seven samples of EVOOs were produced by an extraction system that used two and two half phase centrifugation. The processing temperature was lower than 25° and the average crushing time lower than 45 minutes. The average oil yield was 15%. The oil samples have been preserved in low temperature (15 – 18 °C) in dark-green glass bottles without headspace before the analysis.

Methods

Free acidity, peroxide value, UV spectrophotometric indices, content in fatty acid ethyl esters (FAEEs), waxes, tocopherols, alcohols, phenolic substances, and volatile compounds , fatty acid and triglyceride compositions, sterol composition and content, and sensory profile were performed on single cultivar EVOO “Olivastro di Bucchianico” samples have undergone analyses. Duplicate analyses were performed on each sample.

Analytical parameters

Free acidity (Annex II), peroxide value (Annex III), ultraviolet spectrophotometric indices (Annex IX), ethyl esters and waxes contents (Annex XX), fatty-acid composition (Annex X.B), triglyceride composition (Annex XVIII), sterol composition and content (Annex V), and alcohols content (Annex XIX). were performed according to the official methods of the European Union Commission Regulation EEC/2568/91 and its subsequent modifications.[23]

Tocopherols

For the HPLC determination of the tocopherols[24], a solution of 1 g olive oil in 10 mL acetone was prepared. α-Tocopherol (0.32 µg/10 µL) was used 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 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.

Phenols composition

The phenolic compounds were extracted from the olive oil according to the following method: a 500 µL volume of internal standard solution (0.015 mg/mL 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 at a constant flow rate of 1 mL/min, 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 each 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, from which 100 µL of the respective headspace was withdrawn using a 2.5 mL

Hamilton syringe with a 51 mm needle, through an autosampler unit, HT2000H (HTA s.r.l., Brescia, Italy), and introduced in a splitless heated injector (2 mm ID, 6.5 mm OD × 78.5 mm fused quartz glass). From here, the analytes of the headspace are subjected to a double sequential separation: GC, passed into a low polar column FS-SE-54-CB-0.5, 30 m, 0.32 mm ID, film thickness 0.5 µm (94% methyl-5% phenyl-1% vinylsilicone); IMS (equipped with a tritium ionizing radioactive source at 5000 V), passed into a 9.8 cm long drift tube (Gesellschaft für Analytische Sensorsysteme mbH, G.A.S.; Dortmund, Germany).[20]

Sensorial profile

The evaluation of olive oils was performed under the conditions described in European Union Commission Regulation EEC/2568/91 and its subsequent modifications (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 of the panel has 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 attributes evaluated were 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 median; the confidence intervals was used considering the attributes with a robust coefficient of variation of 20.0 % or less.

Statistical analyses

A heat map (3D chromatogram) was obtained from each sample, from which it was possible to extrapolate a data matrix. Subsequently, processing this data matrix using statistical software Unscrambler X, version 10.4, it was possible to perform a PCA (Principal Component Analysis).

RESULTS AND DISCUSSION

The analyses for quality indices (Table 1) allowed for all samples of "Olivastro di Bucchianico" to be classified as EVOOs according to EC Regulation (EEC, 1991 and following amendments). Free acidity of the samples was in the range from 0.08 to 0.24 % of oleic acids, peroxide values between 6.2 to 8.8 meqO_2/kg. Mean values of the specific extinction parameters K_{232}, K_{270} and ΔK were respectively 1.66, 0.12 and -0.003, so within the EVOO limit according to EC regulation. The FAEE content of 2.6 mg/kg is well below the established EU limit for EVOO (35 mg/kg). These fat-soluble compounds are a valuable indicator of oil quality resulting from improper agronomic and technological practices, in particular from fermentation and degradation processes.[27,28] The waxes content was low, with a range from 27 to 40 mg/kg, typical of extra virgin olive oils. Total tocopherols content was medium-high, around 264 mg/kg (range 218-337 mg/kg). Composition and content of these substances depend e.g. on several agronomic factors, olive processing conditions and the storage.[29-32] Extra virgin olive oil is one of the foods richest in vitamin E (tocopherols) and the most representative is 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 within EU Regulation limits for olive oils. Oleic, palmitic and linoleic and stearic acids were the most abundant with mean values respectively of 74.71%, 11.94%, 7.81 % and 3.02 %.[33] These values are typical of olive oils: oleic acid not less than 73% and linoleic acid not more than 10% with an oleic/linoleic ratio > 7. Monounsaturated fatty acids values (Σ MUFAs) in "Olivastro di Bucchianico" *cv* was high, mean around 75.88% with the maximum value 78.14% and minimum value 73.82%. The oleic acid is extremely important for a healthy and balance diet, leading to an increase in

oxidative stability, antihypertensive activity, and low-density lipoprotein (LDL) cholesterol–lowering effects.[34] In defining the nutritional properties and varietal characterization of the EVOOs, it is also important MUFA/SFAs ratio, that in the single cultivar studied is average of 4.82.[35] The composition of triglycerides, useful for the characterization of single cultivar, was reported in Table 3. As can be seen from this table the most represented compounds are: PLP + OOO + PoPP (42.48%), SOL + POO (23.61%) and OOL + LnPP (12.07%).

Sterols composition of "Olivastro di Bucchianico" was shown in Table 4. The most representative sterols have been β-sitosterol (85.8%), Δ-5-avenasterol (6.5%) and campesterol (3.0%). The value of apparent β-sitosterol, the sum of β-sitosterol and four adjacent phytosterols (clerosterol, sitostanol, Δ-5-avenasterol and Δ-5,24-stigmastadienol), was 94.6% superior at EC legal minimal limits established. High levels of apparent β-sitosterol have a positive effect on reducing total plasma cholesterol and LDL cholesterol.[36,37] Total sterols (1274 mg/kg) were superior to the lower limit established by EC legislation (1000 mg/kg). Sterols and triglycerides can characterize the different EVOOs.[38,39] In the unsaponifiable fraction of olive oil, the presence of both diterpenic alcohols (with 20 carbon atoms) and triterpenic alcohols (with 30 carbon atoms) has been determined (Table 5), which make up to 25-30%. Among the first ones the most represented is the Phytol (318.4 mg/kg), partly coming from the degradation of 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) and 24-Methylen-cycloartanol (317.9 mg/kg) followed by β-amyrin + butyrrospermol (90.2 mg/kg) and α-amyrin (1.2 mg/kg). The alcoholic composition also includes citrostadienol (218.8 mg/kg), a 4-methylsterol, and total aliphatic alcohols whose content (140 mg/kg) 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), p-HPEA-EDA (65.3 mg/kg), pinoresinol and 1-acetoxypinoresinol (58.9 mg/kg), 3,4-DHPEA-EA,H (37.8 mg/kg). 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).[46,47] In Figure 2 sensory profile of "Olivastro di Bucchianico" was reported. It 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 value respectively 2.2 and 3.0 and low notes of artichoke, aromatic herbs and tomato with mean value 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 of the Canino, Ogliarola, Coratina, Moraiolo and Peranzana cultivars (2 samples of each cultivar) were subjected to HS-GC-IMS analysis at the same time as Olivastro. From the heat maps obtained, only 15 volatile compounds, selected within the European H2020 project OLEUM and commented on Valli et al., 2020, were considered. Their respective signals present in the form of a monomer and/or 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 the results as data matrix (Table 7 and Table 8) which was used for the construction of a PCA. Figure 3 shows the scores plot obtained from the PCA (explained variance PC1 and PC2: 70%). The 7 Olivastro di Bucchianico EVOOs resulted separated from the other 10 analyzed

samples, showing a promising discrimination of this cultivar based on the volatile profile with respect to the others.

CONCLUSIONS

The herein performed analytical study conducted on cv. Olivastro di Bucchianico EVOOs, obtained from olives cultivated in restricted area of the Abruzzo region (Italy) highlighted interesting compositional characteristics. Samples showed an average medium-high content of total tocopherols (264 mg/kg), high monounsaturated fatty acids values, around 75.88% and a good oleic/linoleic ratio (9.57). The average total phenols content was medium, 306 mg/kg. 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 value respectively, 2.2 and 3.0 and low intensity notes of artichoke, aromatic herbs and tomato. The research was extended to the analysis of volatile compounds by HS-GC-IMS for verifying the discriminatory potential of the method according to the cultivar. In particular, Olivastro di Bucchianico cv EVOOs samples were analyzed as well as other 10 single cultivars of the Canino, Ogliarola, Coratina, Moraiolo and Peranzana cv. The chemometric analysis of the data allowed to distinguish the Olivastro di Bucchianico cv EVOOs from the others. This preliminary study put in evidence the compositional characteristics of the studied single cultivar EVOOs, in order to propose a characterization study to the competent institutions. This contribution can also be evaluated for the purpose of defining the specifications PDOs, PGIs, PATs.

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FIGURE LEGENDS

Figure 1 Olivastro di Bucchianico cultivation area in the Abruzzo region (Italy)

Figure 2 Sensory profile of the EVOOs from Olivastro di Bucchianico cv

Figure 3 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 ($mEqO_2/kg$)	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)	2.6	4.3	0.1	1.7
Waxes (mg/kg)	33	40	27	5
Tocopherols (mg/kg)	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; 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)	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):				
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)	140	187	94	39

S.D.: standard deviation

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

Phenols content (mg/kg 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