Concentrations of Bioactives and Functional Factors in Destoned Virgin Olive Oil: The Case Study of the Oil from *Olivastra di Seggiano* Cultivar

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Abstract: This work reports on the composition and bionutritional value of destoned (vs. whole) virgin olive oil from *Olivastra di Seggiano* cultivar, one having wild features, which is cultivated in olive areas of Tuscany Region, Italy. Removal of the stone from fruit before processing is an innovative technique that strongly enhanced the already high quality level of this oil variety. There was for this product marked interaction between genetic background and destoning. An in-depth analytical investigation from 2008 to 2010 showed how the innovated olive extraction process led to an improved product standing out for its peculiar features, related mainly to the improved biophenol and volatile composition, as well as to higher concentrations of the lipophilic and vitamin antioxidants (tocopherols and tocotrienols). It had slowly higher levels of oleocanthal (*p-HPEA-EDA*), which was found to be a nutraceutical factor. Its head-space aroma even displayed new volatile phytomolecules and also had higher levels of green volatiles from the LOX-pathway (having as precursors the polyunsaturated fatty acids containing a *cis-cis*-1,4-pentadiene system). Among the other bioactives are to be highlighted the significant levels of *trans*-β-carotene and xanthophylls (lutein, violaxanthin, neoxanthin and other minor carotenoids), whose amount however was not higher with respect to reference oil. Its enhanced bionutritional value was confirmed by the increased intensity of valuable tasting notes, among which stood out the artichoke flavour. Finally it showed higher resistance to autoxidation and longer shelf-life.

Keywords: Destoned olive oil, *Olivastra di Seggiano* variety, hydrophilic and lipophilic antioxidants, aromas, fingerprinting, chemometrics.

INTRODUCTION

Virgin olive oil (VOO), related to its recognized health properties, is being consumed more and more in both producing countries and non-producing ones [1, 2]. Such bionutritional and functional features are related to its contents of bioactive lipophilic and hydrophilic antioxidants as well as to its unique sensory features and to its rather balanced proportions of bioactive fatty acids, such as ω_3 and ω_6 fatty acids [3]. In consequence, it is claimed to be an excellent tool to prevent cardiovascular diseases and the dysmetabolic syndrome [3, 4].

In recent years, we have conducted an investigation targeted to characterize several olive oil kinds (whole; destoned; organic; organic-destoned; POD, protect origin designation; PGI, protect geographical indication; and high quality products), coming from Italian olive growing areas. An analytical database involving the majority of the oil varieties has been developed, from which the analytical data discussed in this paper have been extrapolated [5].

We in this work report on the analytical features of the destoned virgin olive oil from *Olivastra di Seggiano* cultivar, one cultivated in the olive growing areas of the provinces of Grosseto and Siena (Tuscany Region, Italy) and having wild features. It is especially widespread in the origin area of Seggiano Municipality and the other slopes of Mount Amiata, up to an altitude of 400-600 m. In this growing environment the parasite attacks are practically absent or very infrequent.

The above olive cultivar has some synonyms, such as Seggianina, Olivastra di Montalcino and Olivo dell'Amiata. It is self-incompatible and is well pollinated by the cultivars *Frantoio, Moraiolo, Correggiolo, Leccino,* and *Pendolino*. Its ovary abortion reaches a value up to 35% and its vegetation is assurgent. The tree reaches elevated heights. Its fruit is early ripening. Its annual fruit production is not regular. Finally, its oil yield during olive processing is of concern.

Recently, the *Olivastra di Seggiano* oil has obtained a POD trademark from the European Union. A

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protection consortium is operative and a package of production rules has been developed. This oil kind is known to be an excellent product but there are not yet in-depth analytical investigations confirming this popular belief. Therefore, we collected several samples of this crop oil during three consecutive years (from 2008 to 2010) and an analytical characterization of them was carried out.

The purpose of our general study was to characterize the best olive oil varieties from the geographical olive-growing areas of Italy, in order to offer to the Italian and European consumers crop products having both high concentrations of bioactive phytochemicals and high bionutritional value. The specific objective of this special investigation was to elucidate the compositional traits of new POD oil, called *Olivastra di Seggiano* variety, and to evaluate its potential impact on the consumer health. Our attention was focused on the destoned oil kind of this variety, one produced by an innovative processing technique, which is to remove the stone (core) from the olive fruit prior to extraction. This soft technique generates low thermal energy and furthermore removes the oxidoreductase enzymes, plentifully present in the kernel (located inside the stone), thus enhancing the quality level of the resulting product. Comparisons with reference oils (whole oils) were performed.

MATERIALS AND METHODS

Chemicals

Most of the solvents, reagents, and equipments used for the analytical characterization of the oil samples have been given in earlier works [6, 7]. Chemicals were mostly of chromatographic grade and were commercially available from Carlo Erba (Milan, Italy), Fluka (Buchs, Switzerland) and Sigma-Aldrich Chemical (St. Louis, MO, USA).

Taking of Oil Samples

Samples of the studied oil variety were supplied by producers of this product. It was prepared industrially by using the destoning technique. The crushing step was completely eliminated. Kneading was carried out at 28°C and lasted a maximum of 30 min, since there were no emulsions produced by crushing [8]. The malaxed olive paste was then centrifuged by a horizontal two-phase decanter. Olive woody endocarp and the kernel it contains were removed from the fruit before oil extraction by a destoner (exerting bland action). In this way no or low mechanical energy was converted to the thermal one and therefore the oil phase did not undergo thermo-oxidations [5]. The decreased oxidations were also ascribable to the reduced actions of the oxidoreductase enzymes (polyphenoloxidases and peroxidases noticeably), which are plentifully present in the kernel [9]. Reference oils (whole oils) were obtained by implementing substantially the same process steps applied to obtain destoned oils, but a hammer crusher instead of the destoner was used. All oil samples were stored frozen at -20°C before analyzed.

Analysis of Head-Space Aromatic Volatiles

Pleasant and unpleasant volatiles were stripped from the oil sample with N_2 (1.2 L min^{-1}; 37°C; 2h), trapped on 50 mg of activated charcoal and eluted with 1 mL of diethyl ether. Then they were simultaneously analysed by a dynamic head-space (DHS)-high-resolution gas chromatography (HRGC) method, using Carlo Erba Mega Series 5160 gas chromatograph (Milan, Italy), fitted with a Nordion silica carbowax 20 M capillary column (50 m length; 0.32 mm i.d.; 0.5 µm film thickness) (Helsinki, Finland) and equipped with an on-column injection system, a CO_2 cryogenic accessory (to hold the oven temperature at 25 °C) and a flame ionization detector (FID). The oven temperature programme was as follows: isotherm at 25°C for 7 min, from 25 to 33°C at 0.8°C min^{-1}, from 33 to 80°C at 2.4°C min^{-1}, and from 80 to 155°C at 3.7 °C min^{-1}; final isotherm at 155°C for 20 min. The temperature of the detector was held at 240°C. The carrier gas was H_2 at 30 kPa. The injection volume was 0.5 µL. Quantitation was achieved by peak area integration using a Carlo Erba Mega Series integrator (Milan, Italy). The internal standard was nonan-1-ol (> 99% pure) that was directly added (7-8 mg) to the oil sample (50 g) [10, 11].

Analysis of Lipophilic Antioxidants

The simultaneous extraction of tocopherols and tocotrienols was done according to a procedure developed by us, using for saponification 1 g-sample of olive oil and an alcoholic solution (5 mL of KOH 2N). Such an operation lasts 1 min. Next, a first extraction is carried out in a 100-mL separating funnel, using a mixture (20 mL) of distilled water/diethyl ether (1:3, v/v). Two additional extractions follow, using each time an amount of 10 mL diethyl ether. Subsequently, the supernatant is transferred to another separating funnel and washed with 5 mL distilled water. This operation is repeated until neutrality (checked by phenolphthalein indicator) of the washing water. Finally, the obtained ether extract is transferred to an around-bottom flask

and dried in a rotary evaporator at 30°C. The residue is recovered with 1 mL acetone. The next HPLC analysis [12, 13] was performed according to an analytical method developed by International Olive Oil Council [14]. A 25 cm x 4.6 mm i.d. 5 µm reversed-phase Lichrosorb ODS2 column (Waters Corporation, MA, USA) was used. The mobile phase consisted of an aqueous solution (0.5% H_3PO_4) (solution A) and a methanol/acetonitrile mixture (1:1, v/v) (solution B) added with 4% solution A. A UV detector (wavelength 292 nm) was used.

Analysis of Hydrophilic Antioxidants

Extraction of the simple and hydrolysable biophenolic minor polar (BMP) compounds, such as natural and oxidised derivatives of oleuropein and ligstroside, lignans, flavonoids, phenol acids and phenol alcohols, was done by a methanol/water mixture (80:20, v/v). Their quantification was done by an HPLC apparatus equipped with C_{18} reverse-phase column (4.6 mm × 25 cm; spherisorb 5 µm 100 A° ODS-2, Waters Corporation, MA, USA), with spectrophotometric UV detector at 280 nm and integrator (Perkin Elmer, Beaconsfield, UK). Elution was made by a ternary solvent system including an aqueous solution of H_3PO_4 (0.2%), methanol, and acetonitrile. The internal standard was syringic acid. Results were expressed as mg kg^{-1} tyrosol [15].

Analysis of Chlorophylls, Xanthophylls and Carotenes

Pigments (chlorophylls and chlorophyll derivatives, *trans*-β-carotene, and major xanthophylls) were extracted from the oil samples following the established procedure developed by Mínguez-Mosquera *et al.* [16-18], by using *N,N*-dimethylformamide (DMF) and hexane as solvents. The hexane phase carried over lipids and the carotene fraction, while the DMF phase retained chlorophylls, chlorophyll derivatives and xanthophylls. The obtained chlorophyll and carotene extracts were stored in the dark in a freezer at -40°C, waiting to perform their high-performance liquid chromatography (HPLC) analysis. All extractions were carried out under a green light to prevent pigment alteration.

Major carotenoids, like *trans*-β-carotene, lutein, violaxanthin and neoxanthin, were in addition quantified by thin layer chromatography (TLC) coupled to spectrophotometry. Petroleum ether 65-95°C/acetone/diethylamine (10:4:1, v/v/v) mixture was used as developer. Chlorophyllides and pheophorbides were eluted from the plate with the mixture acetone/pyridine (1:1, v/v). For the HPLC analyses of pigments contained in the oil samples, a Perkin Elmer system Mod. Series 200 (Perkin-Elmer, Beaconsfield, UK) equipped with a YMC 30 analytical column (YMC Europe, Schembek, Germany) with a 5 µm C-30 reversed material (25cm x 4.6 mm i.d.), including a precolumn (YMC 30; S-5 µm, 10 x 4.0 mm i.d.), was used [19, 20].The mobile phase consisted of a mixture of methanol/methyl-tert-butyl ether/water [20]. Quantification of each pigment was carried out using an external standard method. Concerning pyropheophytin *a*, the HPLC method developed by Serani and Piacenti [21] has been applied. A detector UV/VIS (ultraviolet/visible) LC 95 and a column LC-18-DB 3 µm (15 cm x 4.6 mm i.d.) (Perkin-Elmer, Beaconsfield, UK) was used. Based on the concentrations of pheophytin *a* and pyropheophytin *a*, the cold index has been assessed by the formula suggested by Serani and Piacenti [21]: cold index = {[pyropheophytin *a*] - {[pheophytin *a*] x 0.075 + 0.199}} x {[pyropheophytin *a*] / [pheophytin *a*]}. In addition, the colour and chromatic parameters were assessed using the CIE method [10].

Sensory Analysis

An analytical taste panel made up of 12 assessors performed the quantitative descriptive sensory profiling (QDSP), at the oil quality and technology department (OQTD) of our Institute, according to Annex 12 of Reg CEE 2568/91, as modified by Reg CE 702/07 and Reg CE 640/08. Olfactory-gustatory-tactile evaluations were made and the results were collected on a standard profile sheet. In addition to the most remarkable sensory descriptors, shades of each of them were also assessed. Each sensory attribute, including off-flavours, was evaluated on a ten-point scale, with intensities ranging from 0 (no perception) to 10 (extreme). All oil samples were thermostated at 30°C before sensory analysis. By using the statistical programme attached to the above sensory analysis method, the median value for each organoleptic descriptor was evaluated.

Other Analyses

A number of physical and chemical parameters, such as free acidity, peroxide index and spectrophotometric indices, have been determined using the methods described in Reg CEE 2568/91 and subsequent modifications. Methyl and ethyl esters of fatty acids have been quantified according to Reg CE n. 61/11. Other analytical variables, such as carbonyl

index and endurance to autoxidation, have been assessed following the methodologies described in previous works [5, 6].

Statistics

The experimental protocol implemented was based on classification designs including two independent variables, such as harvesting year and processing procedure (destoning vs. crushing). Three replicates for each of the experiments (which lasted three years) were planned. A total of eighteen independent oil samples, that is, nine samples for each processing procedure, was analyzed. Univariate treatments of the data were performed by analysis of variance (ANOVA). When a significant *F* value was found, means were separated using *Tukey's honestly significant difference (HSD) range test.* Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were found to be the better procedures for multivariate treatments of the data. The statistical software packages Statistica® (Release 6.0, Statsoft Inc., Tulsa, OK, USA), Minitab® (Release 15.0, Minitab Inc., State College, PA, USA), SPSS® (Release 12.0, SPSS Inc., Chicago, IL, USA) and Stata® (Release 9.0, Stata Corp., College Station, TX, USA) were used. PCA, also referred to as the eigenanalysis method, is based on the maximum variance criterion and calculates orthogonal linear combinations (principal component scores). HCA produces a hierarchy of partition of objects such that any cluster of a partition is fully included in one of the clusters of the later partitions. Such partitions are best represented by a dendrogram (binary tree) [22-24].

RESULTS AND DISCUSSION

All Tables (**1-5**) give average data (n = 3) for each year and each analytical variable referring to destoned oils only. For both oil kinds (obtained by the two processing procedures) and each analytical variable, they also give the general means (n = 9), such as X (destoned oil) and X' (whole oil).

Green Aromas and Other Head-Space Volatiles

Table **1** shows how the examined oils (destoned products noticeably) were well provided with green volatiles from the lipoxygenase (LOX) pathway, which form either from linoleic acid (LA) or α-linolenic acid (α-LnA) (compounds containing a *cis*-*cis*-1,4-pentadiene system). From the former form essentially the C_6 green volatiles hexanal, hexan-1-ol, and hexyl-acetate, whereas from the latter are prevalently biogenerated the C_6 green volatiles (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, and (*Z*)-3- hexenyl acetate, as well as C_5 compounds, such as pentene dimers, pentenols and carbonyl compounds. These green volatiles are mostly represented by aldehydes, alcohols and esters. In the head-space of the destoned oil kind new volatiles were once again identified, such as α-copaene and α-muurulene, which were absent in the reference product. The higher biogeneration of green volatiles, in the destoned oil, in addition to the genetic background, was also attributable to the low thermal energy developed by destoning, which resulted in higher activity of the hydroperoxide lyase (FAHL), whose inactivation starts at a temperature lower than 15°C [11].

The above LA and α-LnA acids form by triglyceride hydrolysis mediated by acylhydrolase (AH). Next, LOX (whose activity is prevalently genetically determined), transforms LA and α-LnA into their corresponding 9- and 13- hydroperoxides, in a ratio ranging between 65:35 and 65:45, respectively. Only the 13-hydroperoxides from both LnA (13-HPOT) and LA (HPOD) are cleaved by hydroperoxide lyase (FAHL) into C12-oxo-acids, *cis*-3-hexenal and hexanal, since such enzyme has a high substrate specificity. *Cis*-3-hexenal does not accumulate in the oil volatile fraction. Enzymatic transformation of the two aldehydes mediated by isomerases (IR), alcohol dehydrogenases (ADH) and alcohol acetyl transferases (AAT) yields the corresponding C_6 esters and C_6 alcohols. As a result, three branches of volatile C_6 metabolites are biogenerated from LnA, whereas only one forms from LA. An additional branch of short chain volatiles, including oxygenate C_5 compounds, is biosynthesized through another LOX pathway. In this case, 13-HPOT undergoes a β-scission yielding pentene dimers and pentenols through the alkoxyl radical. The subsequent oxidation of pentenols catalysed by an alcohol dehydrogenase yields C_5 carbonyl compounds [25]. The harvesting year (seasonal conditions) also greatly influenced the volatile composition of the oils.

Hydrophilic Simple and Hydrolysable Biofenols

Table **2** shows how the *Olivastra di Seggiano* oil (that destoned noticeably) also had significant contents of hydrophilic antioxidants, such as simple and hydrolysable biophenolics, and of oleocanthal, also called deacetoxyligstrosideaglycon (*p*-HPEA-EDA). The last is characterized by strong bitter taste and has been found to be an aspirin-like compound, exerting anti-inflammatory actions against both COX 1 and COX

Table 1: Composition of the Aromatic Volatile Fraction in Virgin Olive Oil Samples from *Olivastra di Seggiano* Cultivar Collected During Three Consecutive Harvesting Years

Volatiles (mg kg^{-1} 1-nonanol)				*Olivastra di Seggiano* oil	
	2008	2009	2010	X	X'
n-Octane	1.2±0.1a	1.7±0.0b	1.3±0.1a	1.4±0.1A	1,5±0.1A
Acetone	0.8±0.1a	1.3±0.1b	1.2±0.0c	1.1±0.1A	1.3±0.1A
1-Octene	1.7±0.1a	1.6±0.1a	2.2±0.2b	1.8±0.1A	1.8±0.1A
2-Octene	0.1±0.0a	0.1±0.0a	0.1±0.0a	0.1±0.0A	0.1±0.0A
3-Octene	0.3±0.0a	0.1±0.0b	0.3±0.0a	0.2±0.0A	0.1±0.0B
Butyraldehyde	0.2±0.0a	0.3±0.0b	0.1±0.0c	0.2±0.0A	0.3±0.0B
Ethyl acetate	1.1±0.1a	1.1±0.1a	1.3±0.2b	1.1±0.1A	1.3±0.2A
Nonane	2.0±0.0a	2.1±0.1a	2.2±0.2	2.1±0.2A	2.0±0.1A
2-Butanone	7.2±0.4a	13.7±1.0b	9.7±0.7c	10.2±0.8A	9.3±0.7B
2-Methyl-butanal	3.3±2.4a	0.7±0.1b	1.8±0.1c	1.9±0.2A	2.1±0.2A
3- Methyl-butanal	2.1±0.1a	1.6±0.1b	3.0±0.2c	2.2±0.1A	2.5±0.2A
Isopropyl alcohol	0.1±0.0a	0.6±0.0b	0.3±0.0c	0.3±0.0A	0.2±0.0B
Ethanol+2-ethylfuran	2.7±0.2a	3.4±0.2b	3.5±0.2b	3.2±0.2A	3.1±0.2A
Pentene dimer 1	0.2±0.0a	0.7±0.0b	0.3±0.0a	0.4±0.0A	0.2±0.0B
2-Pentanone	36.3±2.5a	17.2±1.1b	12.1±0.9c	21.8±1.5A	10.4±0.8B
Pentenal	0.1±0.0a	0.2±0.0b	0.1±0.0a	0.1±0.0A	0.1±0.0A
Pentene dimer 3	23.0±1.5a	26.0±1.8b	16.6±1.2c	21.9±1.2A	23.8±1.4A
Methyl-2-methyl butyrate	0.9±0.1a	0.3±0.0b	0.1±0.0c	0.4±0.0A	0.5±0.0A
Pentene dimer 9	1.1±0.1a	1.2±0.1b	1.2±0.1c	1.2±0.1A	1.0±0.1A
α-Pinene	23.1±1.5a	25.8±1.8b	16.0±18.0c	21.6±1.4A	20.0±1.3B
2-Butanol	0.6±0.0a	0.5±0.0a	0.7±0.0a	0.6±0.0A	0.6±0.0A
1-Penten-3-one	17.4±1.2a	23.5±1.5b	25.9±1.6c	22.2±1.3A	18.4±1.2B
3-Hexanone	2.1±0.1a	0.5±0.0b	0.5±0.0b	1.0±0.1A	0.8±0.0B
Pentene dimer 8	9.2±0.5a	11.6±0.7b	7.2±0.5c	9.3±0.6A	7.8±0.5B
Pentene dimer 5	15.8±1.0a	18.5±1.3b	10.5±0.6c	14.9±0.9A	12.7±0.8B
Pentene dimer 6	12.0±0.7a	21.1±1.5b	8.7±0.5c	13.9±0.8A	10.5±0.5B
n-Hexanal	16.3±1.1a	31.1±2.2b	21.6±1.5c	23.0±1.2A	19.5±0.9B
β-Pinene	0.1±0.0a	0.7±0.0b	0.1±0.0a	0.3±0.0A	0.1±0.0B
Isobutyl alcohol	4.3±0.3a	0.9±0.1b	0.7±0.0b	2.0±0.2A	2.4±0.2A
3-Pentanol	0.3±0.0a	0.2±0.0b	0.2±0.0b	0.2±0.0A	0,1±0.0B
Isoamyl acetate	0.3±0.0a	4.5±0.3b	0.1±0.0c	1.6±0.1A	1.2±0.1B
Propyl-butyrate	1.9±0.1a	0.4±0.0b	2.8±0.2c	1.7±0.3A	1.2±0.1B
p - xylene	0.2±0.0a	0.1±0.1b	0.1±0.0b	0.1±0.0A	0.1±0.0A
Ethyl pentanoate	0.9±0.1a	0.4±0.0b	0.5±0.0b	0.6±0.1A	0.4±0.0B
1-Penten-3-ol	34.2±2.4a	19.2±1.3b	17.4±1.1c	23.6±1.4A	18.4±1.2B
2-Heptanone	0.3±0.0a	5.6±0.4b	4.1±0.2c	3.3±0.3A	2.6±0.2B
(*Z*)-3-Hexenal	3.6±0.3a	3.8±0.3a	3.7±0.2a	3.7±0.3A	2.2±0.2B
2-Methyl butanol	3.5±0.2a	1.1±0.1b	1.0±0.1b	1.9±0.2A	1.3±0.1B
3-Methyl butanol	4.7±0.3a	2.3±0.2b	2.0±0.1b	3.0±0.3A	2.4±0.1B
(*E*)-2-Hexenal	863.7±60.3a	1152.4±81.5b	873.2±62.1a	963.1±81,3A	658.9±54,5B

Table 1: continued

Volatiles (mg kg^{-1} 1-nonanol)				*Olivastra di Seggiano* oil	
	2008	**2009**	**2010**	**X**	**X'**
Pentanol	0.1±0.0a	0.2±0.0b	0.3±0.0c	0.2±0.0A	0.1±0.0B
2-Octanone	2.8±0.2a	1.6±0.1b	0.9±0.1c	1.8±0.2A	1.3±0.1B
Hexyl acetate	14.2±1.0a	9.2±0.7b	3.5±0.2b	9.0±0.7A	7.7±0.5B
Octyl aldehyde	1.3±0.1a	17.4±1.2b	10.5±0.6c	9.7±0.5A	7,9±0.4B
(*E*)-2-Penten-1-ol	46.5±3.2a	14.7±0.9b	10.4±0.7c	23.9±1.3A	17.1±1.0B
(*Z*)-3-Hexenyl-acetate	0.1±0.0a	0.3±0.0b	1.0±0.0c	0.5±0.0A	0.2±0.0B
Methyl hexanoate	0.1±0.0a	0.1±0.0a	0.1±0.0a	0.1±0.0A	0.1±0.0A
(*Z*)-2-Penten-1-ol	20.7±1.5a	14.2±0.9b	13.8±1.0b	16.2±1.1A	12.5±0.7B
(*E*)-2-Heptenal	0.7±0.0a	0.2±0.0b	0.2±0.0b	0.4±0.0A	0.4±0.0A
6-Methyl-5-hepten-2-one	0.4±0.0a	0.1±0.0b	0.1±0.0b	0.2±0.0A	0.1±0.0BB
Hexan-1-ol	49.4±0.4a	16.5±1.1b	6.6±0.4c	24.2±1.2A	19.5±1.1B
(*E*)-3-Hexen-1-ol	1.1±0.1a	0.5±0.0b	0.2±0.0c	0.6±0.0A	0.3±0.0B
(*Z*)-3-Hexen-1-ol	38.1±2.6a	41.7±3.0a	39.3±2.6a	39.7±2.7A	28.8±2.0B
Nonanal	0.2±0.0a	0.5±0.0b	0.2±0.0a	0.3±0.0A	0.3±0.0A
(*Z*)-2-Hexen-1-ol	23.1±1.6a	16.1±1.0b	9.6±0.7c	16.3±1.0A	12.8±0.8B
(*E*)-1-Octen-3-ol	0.2±0.0a	5.4±0.3b	3.2±0.2c	2.9±0.2A	2.5±0.1B
1-Heptanol	0.4±0.0a	0.9±0.1b	0.5±0.0a	0.6±0.0A	0.4±0.0B
Acetic acid	0.4±0.4a	2.0±0.1b	1.1±0.1c	1.2±0.1A	2.2±0.2B
α-Copaene	0.2±0.1a	2.6±0.2b	1.2±0.1c	1.4±0.1	n.d.
α-Muurulene	0.3±0.0a	1.4±0.1b	1.1±0.1b	0.9±0.1	n.d.
Propionic acid	0.1±0.1a	1.1±0.1b	4.0±0.3c	1.7±0.1A	2.2±0.2B
Butyric acid	0.6±0.0a	1.0±0.1b	0.7±0.0a	0.8±0.1A	1.1±0.1B

Data are means ± standard deviation (SD) of three independent destoned oil samples. Within each row, values with uncommon lower-case letters (a-c) are statistically different. X=average data (± SD) of three years (n=9) for destoned oils; X'=average data (± SD) of three years for reference whole oils (n=9). Within each row average data with different uppercase letters are statistically different (A-B). For all comparisons the *Tukey's HSD range test*, $p \leq 0.05$, was applied.

2 (cycloxygenases) [26-28]. Four groups of prominent phenol compounds were present in the oils, such as: one including aldehydic and hydroxylic forms of oleuropein aglycon + oxidized aldehydic and hydroxylic forms of oleuropein aglycon; one including the sole compound decarboxymethyl oleuropein aglycon (dialdehydic form); one including decarboxymethyl ligstroside aglycon (oxidized dialdehydic form) + decarboxymethyl ligstroside aglycon (dialdehydic form) + lignans + pinoresinol + acetoxy-pinoresinol; and finally one including the compound ligstroside aglycon (dialdehydic form).

We highlight how these remarkable compounds, having marked bioactivity, have been found to be potent scavengers of the reactive oxygen species (ROS). Moreover, they are largely responsible for the organoleptic and bionutritional properties, and shelf-life of virgin olive oil [5, 6]. Their already marked concentrations in the analyzed oil variety are largely enhanced by destoning that, by removing the stone (known to be one of the hardest woods) from the fruit, causes lowest conversion of mechanical energy to thermal energy. As a result, lowest thermoquinonization of the hydrophlic biophenols is determined.

Lipophilic Antioxidants and Other Quality Parameters

Table **3** shows the significant concentrations of lipophilic antioxidants in the considered oil variety, such as tocopherols and tocotrienols. Both were higher in the destoned oils (obtained by a soft processing procedure). The latter are known to be isoforms of vitamin E and differ from tocopherols for the presence of three double unsaturations in the isoprene chain. They have been found to exert more marked

Table 2: Concentrations of Hydrophylic Biophenols in Virgin Olive Oil Samples from *Olivastra di Seggiano* Cultivar Collected During Three Consecutive Olive Harvesting Years

Polyphenols (mg kg^{-1} tyrosol)			*Olivastra di Seggiano* oil		
	2008	2009	2010	X	X'
Total oleuropein derivatives	87.27±6.50a	144.85±10.28b	152.35±9.90c	128.32±8.31A	109.11±7.52B
Total ligstroside derivatives	82.62±4.32a	121.76±8.65b	92.40±7.67c	98.92±7.4A	83.51±6.6B
Total flavonoids	11.83±0.77a	19.47±1.09b	9.58±0.71c	13.62±1.16A	9.48±0.91B
Total phenol acids	15.61±1.08a	21.29±1.55b	10.04±0.59c	15.46±1.37A	11.28±0.98B
Total phenyl alcohols	4.36±0.27a	6.08±0.46b	4.11±0.28a	4.85±0.32A	3.21±9.21B
Total lignans	12.58±0.69a	12.73±0.94a	24.92±1.52b	16.74±1.28A	13.21±1.11B
Oleocanthal (*p* – HPEA-EDA)	22.10±1.43a	25.30±1.49b	21.60±1.66a	23.0±1.80A	21.4±1.68B

Data are means ± standard deviation (SD) of three independent destoned oil samples. Within each row, values with uncommon lower-case letters (a-c) are statistically different. X=average data (± SD) of three years (*n*=9) for destoned oils ; X'= average data (± SD) of three years for reference whole oils (*n*=9). Within each row average data with different uppercase letters are statistically different (A-B). For all comparisons the *Tukey's HSD range test*, $p \leq 0.05$, was applied.

Table 3: Values of Other Analytical Variables in Virgin Olive Oil Samples from *Olivastra di Seggiano* Cultivar Collected During Three Consecutive Olive Harvesting Years

Analytical variables			*Olivastra di Seggiano* oil		
	2008	2009	2010	X	X'
α-Tocopherol (mg kg^{-1})	185.7±12.3a	204.4±14.5b	147.5±10.2c	179.2±12.5A	158.9±11.2B
γ-Tocopherol (mg kg^{-1})	11.0±0.8a	14.6±0.9b	6.4±0.4c	10.6±0.6A	8.5±0.6B
δ-Tocopherol (mg kg^{-1})	1.1±0.1a	1.6±0.1b	1.5±0.1b	1.4±0.1A	1.1±0.1B
α-Tocotrienol (mg kg^{-1})	2.6±0.2a	2.9±0.2a	3.3±0.2b	2.9±0.2A	2.0±0.1B
γ-Tocotrienol (mg kg^{-1})	1.3±0.1a	2.2±0.2b	1.0±0.1c	1.5±0.1A	1.0±0.1B
Endurance to oxidation – (Swift's test) (h)	17.8±1.2a	15.7±1.1b	16.6±1.0c	16.7±1.3A	14.8±1.0B
ω_6/ω_3 fatty acids	10.8±0.7a	10.2±0.7a	12.1±0.9b	11.0±0.8A	11.2±0.9A
OOL + LnPP (%)	12.0±0.8a	11.8±0.8a	12.0±0.7a	11.9±1.0A	12.1±0.8A
OOO + PoPP (%)	47.2±2.7a	45.8±3.2b	46.1±2.6b	46.4±3.6A	45.8±3.2A
POO (%)	22.6±1.5a	23.4±1.8a	23.7±1.8a	23.2±1.4A	23.4±1.6A
Squalene (%)	0.6±0.0a	1.1±0.1b	0.7±0.0c	0.8±0.1A	0.6±0.0B
Methyl esters (mg kg^{-1})	3.3±0.2a	3.1±0.2a	5.3±0.3b	3.9±0.3A	4.8±0.3B
Ethyl esters (mg kg^{-1})	4.8±0.3a	4.5±0.3a	5.3±0.4b	4.9±0.3A	5.8±0.4B

Data are means ± standard deviation (SD) of three independent destoned oil samples. Within each row, values with uncommon lower-case letters (a-c) are statistically different. X=average data (± SD) of three years (*n*=9) for destoned oils; X'= average data (± SD) of three years for reference whole oils (*n*=9). Within each row average data with different uppercase letters are statistically different (A-B). For all comparisons the *Tukey's HSD range test*, $p \leq 0.05$, was applied.

antioxizing activity compared to the homologous individual tocopherols [29, 30]. In general, the production year significantly affected the contents of these and other functional phytocomponents of the oil. Due to the significant levels of vitaminic and non-vitaminic antioxidants, the oil had a potentially long shelf-life, as suggested also by high values of the endurance to oxidation (Swift's test) parameter.

Table **3** also shows how the oil variety (destoned oil noticeably) had low contents of methyl esters and ethyl esters, indicating again its remarkable quality level, as these compounds are markers of degradation processes. Finally, Table **3** displays a slowly high value of the ω_6/ω_3 fatty acid ratio, one having highest biological meaning, since it affects the balanced formation of the long-chain fatty acids (LCPUFA ω_6 and ω_3), from which the eicosanoid hormones form [5, 6].

Colourings and Chromatic Parameters

Table **4** shows the balanced concentrations of chlorophyll and carotenoid pigments in the analyzed oil variety. Values of the chromatic parameters are

Table 4: Contents of Chlorophylls and Carotenoids (mg kg^{-1}) and Values of Chromatic Parameters (CIE Method) in Virgin Olive Oil Samples from *Olivastra di Seggiano* Cultivar Collected During Three Consecutive Olive Harvesting Years

Pigments and chromatic variables			*Olivastra di Seggiano* oil		
	2008	2009	2010	X	X'
Chlorophyll *a*	1.82±0.09ab	1.75±0.11a	1.94±0.15b	1.83±0.12A	1.91±0.13A
Chlorophyll *b*	0.85±0.06ab	0.79±0.05a	0.98±0.08b	0.87±0.05A	0.91±0,07A
Pheophytin *a*	12.47±1.00a	12.31±0.78a	13.55±1.00b	12.78±1.1A	11.45±0.69B
Pheophytin *b*	0.48±0.03ab	0.37±0.02a	0.53±0.03b	0.46±0.3A	0.47±0.3A
Chlorophyllid *a*	0.18±0.01a	0.16±0.01a	0.25±0.02b	0.20±0.01A	0.17±0.01A
Chlorophyllid *b*	0.13±0.01a	0.11±0.01a	0.17±0.01b	0.14±0.01A	0.16±0.01A
Pheophorbide *a*	0.20±0.01ab	0.18±0.01a	0.22±0.02b	0.20±0.01A	0.26±0.02B
Pheophorbide *b*	0.13±0.01ab	0.11±0.01a	0.14±0.01b	0.13±0.01A	0.13±0.01A
Pyropheophytin *a*	0.49±0.03a	0.47±0.03a	0.50±0.04a	0.49±0.03A	0.53±0.04A
Total chlorophylls	16.75±1.24a	16.25±1.12a	18.28±1.24b	17.09±1.18A	15.99±1.11B
Trans-β - Carotene	2.61±0.14a	2.57±0.17a	2.76±0.17b	2.64±0.17A	2.70±0.17A
Lutein	3.28±0.18ab	3.17±0.22a	3.33±0.25b	3.26±0.20A	3.27±0.24A
Neoxanthin	0.51±0.04ab	0.46±0.03a	0.59±0.04b	0.52±0.04A	0.58±0.04A
Violaxanthin	0.58±0.04a	0.56±0.03a	0.60±0.05a	0.58±0.04A	0,60±0.05A
Rest of carotenoids	0.62±0.04a	0.50±0.04b	0.63±0.04a	0.58±0.04A	0.62±0.04A
Total carotenoids	7.60±0.59a	7.26±0.51a	7.91±0.55a	7.59±0.58A	7.77±0.53B
Chroma (%)	94.2±4.4a	88.1±5.3b	96.5±7.3c	92.9±6.3A	93.1±6.4B
Brightness (%)	86.3±5.7a	87.2±5.0b	84.8±5.0c	60.1±3.9A	58.4±4.0B
Hue (nm)	578±38a	579±39b	578±24c	578±38A	578±37A
Cold index	-0.02±0.00a	-0.02±0.00a	-0.03±0.00b	-0.02±0.00A	-0.03±0.00B

Data are means ± standard deviation (SD) of three independent destoned oil samples. Within each row, values with uncommon lower-case letters (a-c) are statistically different. X=average data (± SD) of three years (*n*=9) for destoned oils; X'= average data (± SD) of three years for reference whole oils (*n*=9). Within each row average data with different uppercase letters are statistically different (A-B). For all comparisons the *Tukey's HSD range test,* $p \leq 0.05$, was applied.

consistent with them. Hue values suggested how this product was characterized by a yellow colour, which prevailed over green. The olive processing technique based on destoning caused lower thermal destruction of the pigments, even though the destoned oil had lower concentrations of these substances (compared to the reference oil). This because destoner exerts, with respect to metal crusher, lower effects on the fruit hypodermis tissue, were both chloroplasts and chromoplasts are essentially located. However, in general, no substantial differences between the two oil kinds were found for individual pigment contents, but these differences were rather significant for the total pigment value. The pigment profile also was affected by the harvesting year.

The green colouring fraction included either chlorophyll *a*, chlorophyll *b*, or chlorophyll derivatives (chlorophyllides *a* and *b*, pheophytins *a* and *b*, pheophorbides *a* and *b*, pyropheophytins *a* and *b*, and pyropheophorbides *a* and *b*). Chlorophyllides are chlorophylls that have lost the phytol moiety by means of chlorophyllase enzyme action. This reaction is the first chlorophyll catabolism step. Pheophytins are Mg-free chlorophyll derivatives. Pheophorbides are chlorophylls that have lost both the central magnesium atom and the phytol moiety and can originate from either pheophytins or chlorophyllides. Finally, pyropheophytins and pyropheophorbides are pheophytins or pheophorbides that have lost the ester group $–CO_2-CH_3$ [20].

Pheophytin *a*, as expected, was by far the prominent green native colouring, whereas lutein, violaxanthin and neoxanthin were the major xanthophyll compounds occurring in the oil variety. Appreciable levels of *tran*-β-carotene, a provitaminic antioxidant, were detected. The cold index, one proposed to discover fraudulent addition of deodorized oil to virgin olive oil [21], showed a negative value for

Table 5: Median Values of Organoleptic Attributes in Virgin Olive Oil Samples from *Olivastra di Seggiano* Cultivar Collected During Three Consecutive Olive Harvesting Years

Sensorial descriptors			*Olivastra di Seggiano* oil		
	2008	2009	2010	X	X'
Fruitness	8.5±0.3a	8.7±0.4a	8.6±0.4a	8.6±0.4A	7.0±0.2B
Bitter	6.8±0.2a	6.6±0.2a	6.6±0.2a	6.6±0.2A	7.6±0.3B
Spicy	6.1±0.2a	5.9±0.2ab	6.2±0.3b	6.1±0.3A	6.8±0.3B
Artichoke	5.6±0.1a	5.4±0.1a	6.2±0.2b	5.7±0.2A	5.0±0.1B
Almond	3.3±0.1a	2.8±0.1b	2.7±0.1b	2.9±0.1A	2.3±0.1B
Banana	2.5±0.1a	2.3±0.1a	2.5±0.1a	2.4±0.1A	1.8±0.1B
Mint	1.9±0.1a	1.6±0.0b	1.6±0.0b	1.7±0.0A	1.3±0.0B
Sage	1.8±0.0a	1.7±0.0b	1.4±0.0c	1.6±0.0A	1.3±0.0B
Citron	1.5±0.0	1.6±0.0a	1.6±0.0a	1.6±0,0A	1.2±0.0B

Data are means ± standard deviation (SD) of three independent destoned oil samples. Within each row, values with uncommon lower-case letters (a-c) are statistically different. X=average data (± SD) of three years (n=9) for destoned oils; X'= average data (± SD) of three years for reference whole oils (n=9). Within each row average data with different uppercase letters are statistically different (A-B). For all comparisons the *Tukey's HSD range test*, $p \leq 0.05$, was applied.

the investigated oil variety, thus confirming its authenticity and genuineness.

Sensory Features and Other Analytical Traits

Finally, Table **5** gives the organoleptic profile of the product, indicating how its peculiarity is to be attributed mainly to its sensory characteristics and noticeably to the remarkable artichoke taste. Minor pleasant sensations were perceived by assessors, such as those reminiscent of almond, banana, mint, sage and citron. Its fruitiness flavour was quite marked, whereas the bitter and spicy ones were normal. In the destoned oil kind these sensorial features were somewhat more pronounced compared to the reference oil kind.

The values of other analytical parameters related to quality, such as free acidity, peroxide index, UV (ultraviolet) spectrophotometric indices, carbonyl index, and others, were all very low in the analyzed oil kinds (data not shown). Triacylglycerols and fatty acids had the typical profile of the high quality olive oils.

Results of Multivariate Analyses

Only two plots, one generated by the software STATA and another by the software SPSS, have been

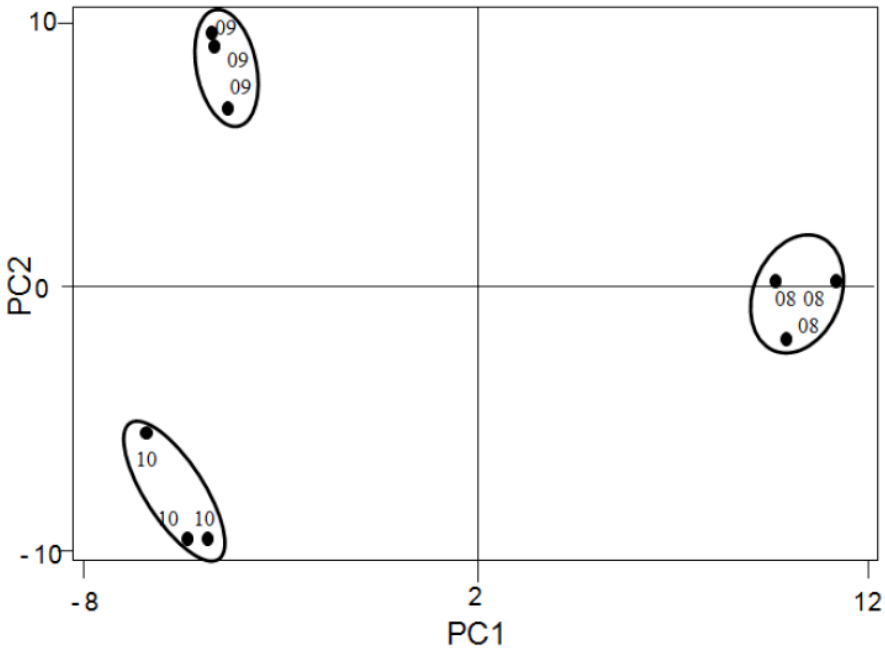

Figure 1: Score plot by dimension 1 and 2 from PCA obtained by processing the phenol fraction dataset of destoned *Olivastra di Seggiano* olive oil samples collected during three consecutive harvesting years. Statistical software used: STATA®.

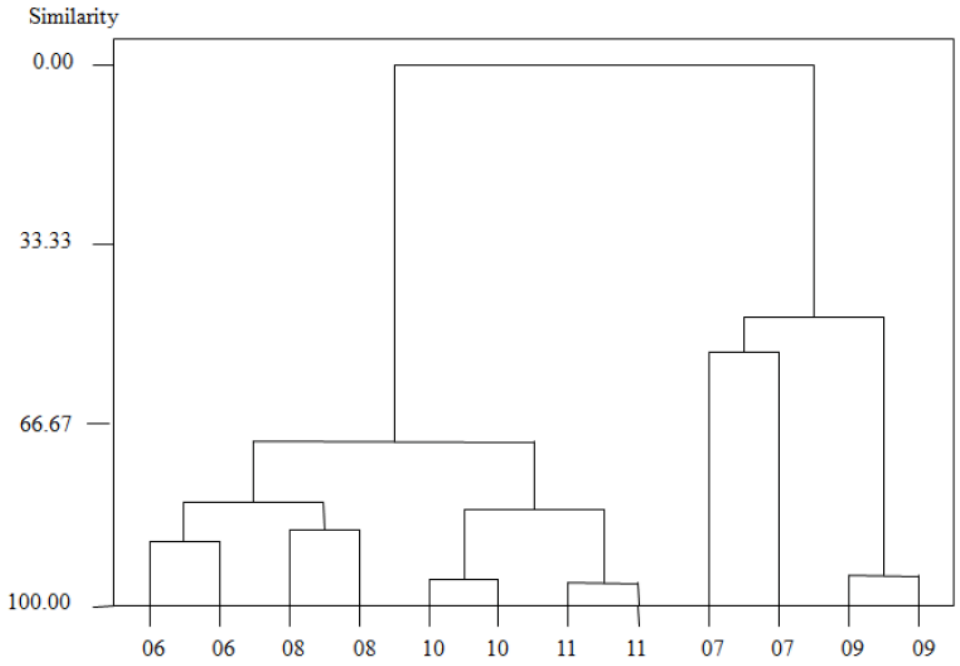

Figure 2: Dendrogram obtained by processing the volatile fraction dataset of destoned *Olivastra di Seggiano* olive oil samples collected during three consecutive harvesting years. Statistical software used: SPSS®.

included in this work. The PCA score plot (Figure **1**) shows how this multivariate method, when applied to the phenol fraction dataset of the destoned oil, was effective in discriminating the oil samples by harvesting year. In fact, along the first dimension (accounting for 71.5% of the total variance) were discriminated those collected in 2008 (negative half, second and third quadrants), as well as those collected in 2010 (positive half, fourth quadrant), whereas along the second dimension, accounting for 16.5% of total variance, were discriminated those collected in 2009 (positive half, first quadrant). The dendrogram in Figure **2** (obtained by processing the volatile fraction dataset) shows how the HCA multivariate method was likewise effective in grouping the destoned oil samples by harvesting year.

CONCLUSION

The excellent analytical composition of the *Olivastra di Seggiano* oil was attributable to its genetic store. However, the destoning technique, applied prior to olive processing, significantly enhanced its quality and typicalness characteristics. This innovative technique could actually help to enhance either naturalness or functionalization degree of other olive oil varieties as well. On the other hand, many other olive production areas, having features similar to those referring to the studied oil variety, are found in Italy and other European olive producing countries. Hence, their excellent products, further improved by applying destoning, will have a great impact on the public health. Moreover, they will play a role in increasing both competitiveness and economic sustainability of the olive production chain.

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