Research Article

Can all the Sardinian varieties support the PDO "Sardegna" virgin olive oil?†

Running Title: Sardinian varieties in PDO Sardegna

Pierfrancesco Deiana[1,4], Mario Santona[1], Sandro Dettori[1], Maria Giovanna Molinu[2], Antonio Dore[2], Nicola Culeddu[3*], Emanuela Azara[3], Eleni Naziri[4], Maria Z. Tsimidou[4]

[1]Department of Agriculture, University of Sassari, Sassari, Italy

[2]Institute of Sciences of Food Production – National Council of Research, Sassari, Italy

[3]Institute of Biological Chemistry – National Council of Research, Sassari, Italy

[4]Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, Thessaloniki (Greece)

***Corresponding author:** Nicola Culeddu, Institute of Biological Chemistry – National Council of Research, Via La Crucca,3 Sassari, 07100, Italy; Email**:** nicla.culeddu@icb.cnr.it

Keywords: PDO Sardegna, olive germplasm, phenolic composition, squalene, sterols

Abbreviations: 3,4-DHPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol; CV, coefficient of variation; EFSA, European Food Safety Authority; EVOO, extra virgin olive oil; FAMEs, fatty acid methyl esters; DAD, diode array detector; MI, maturity index; MUFA, monounsatured fatty acids; p-HPEA-EDA, dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol; PDO, Protected Designation of Origin; PGI, Protected Geographical Indication; PUFA, polyunsaturated fatty acids; VOO, virgin olive oil.

†This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [**10.1002/ejlt.201800135**].

Received: June 5, 2018 / Revised: July 17, 2018 / Accepted: October 1, 2018

Abstract

Protected Designation of Origin (PDO) labels are important tools to promote high quality virgin olive oils (VOO). To better valorize and differentiate among others these labeled products it is necessary to have a deep knowledge of characteristics and features of the monovarietal VOOs that are used. In Sardinia, only one PDO, named 'Sardegna', is registered. Drupes from several local varieties are mixed up before pressing. Four are the principal autochthonous Sardinian varieties: Bosana, Tonda di Cagliari, Tonda di Villacidro, Semidana and respective synonyms. This study examined the chemical and nutritional characteristics of monovarietal VOOs of some of the varieties that comprise the label with the aim to establish the identity and natural variability of the registered product. Results were compared with those from some minor Sardinian and Italian cultivars, all grown under the same agronomic and environmental conditions. Data for the fatty acid and sterol composition together with those for some specific nutrient and non nutrient antioxidants were used to investigate whether the mixing up of many different varieties is favorable in the production of this particular product. PDO varieties achieved broadly minimum quality levels requested by the label regulation and expressed some specific characteristics in accordance to genetic similarities.

Practical applications

Knowledge of chemical composition of VOOs from minor local varieties is of great interest for the promotion of products typical of a specific territory and to improve quality and competitiveness of protected denominations labels. The characterization of VOO admitted to a PDO could help producers to optimize blends obtaining specific nutritional and sensorial features. Moreover, findings are important to highlight some distinctive features among genetic groups that might be useful for further supporting the integrity of the registered product in the near future.

1. Introduction

Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) are quality and geographic labels introduced by the European Union (EU) to promote and protect the quality names of food and wine products. They are connected to a territory and the materials and manufacturing processes inherent to it and promote sustainable agriculture through the production of high added value products[1,2]. According to the Database Of Origin and Registration (DOOR)[3] concerning the olive oil sector, over the past 20 years, EU has registered 124 extra virgin olive oil (EVOO) denominations (109 PDO and 15 PGI). Italy is the country with the highest number, 42 PDO and 4 PGI. The Italian EVOO quality labels usually are referred to a narrow territory and a selected number of olive varieties that thrive under the respective growing conditions. Indeed, most of the 800 autochthonous documented cultivars are well adapted to a specific microclimatic area and are cultivated only in their area of origin[4]. During the last period the Italian strategy on this regard favors the development of PGI labels such as "Sicilia", "Olio di Calabria" and "Marche"[3], under which a larger number of producers, millers and other operators work to achieve a stronger market power and higher visibility in comparison to the potential of having many PDOs within a particular region.

A similar policy probably guided the drawing of the application for the registration of the only virgin olive oil PDO, named "Sardegna"[5], finally approved in 2007. It can be produced along the whole territory and must be characterized at least for the 80% by the four principal autochthonous Sardinian varieties, namely, Bosana, Tonda di Cagliari, Tonda di Villacidro, Semidana and their other names[5]. The remaining 20% may include virgin olive oils (VOOs) obtained from other cultivars grown in the island. Growing areas are concentrated in five main traditional zones, i.e. Sassarese and Alghero, Oristanese and Montiferru, Nuorese and Ogliastra, Medio Campidano and Marmilla, Parteolla and Trexenta[6]. Bandino et al[6], utilizing morphological and structural parameters, described 28 autochthonous varieties. The principal ones are mainly grown in the respective areas of origin and are rarely cultivated out of them. Bosana is the most widespread, including about the 65% of the cultivated trees. Minor ones (e.g. Pizz'e Carroga, Pezz'e Guaddu, Cariasina, Corsicana da Olio and Sivigliana da Olio) are usually present as scattered trees inside secular olive orchards, usually utilized as pollinators. Several studies have been carried out on Sardinian varieties. Studies regarded genetic relationships within Sardinian and between Sardinian and Italian germplasm[7]. Chemical composition and sensory profile have been widely studied for different reasons[8-14]. Moreover, some authors investigated the effect of storage and extraction technology on VOO quality from local cultivars[15,16]. Other studies applied chemometric techniques aimed at discriminating cultivars and/or geographical origin[8,17,18]. The role of microorganisms in Sardinian oleic ecosystems, and in

particular the potential effect of the enzymatic activity of bacteria and yeasts on the sensory and physico-chemical properties of oil, has been recently described[19].

The aim of this work is to describe the principal chemical and nutritional characteristics of the monovarietal VOOs used in the production of the PDO "Sardegna". A comparison with the characteristics of VOOs of some minor Sardinian and Italian varieties obtained from olives grown under the same agronomical conditions in the same experimental olive grove was also carried out. Knowledge of this monovarietal VOOs could provide good guidelines to producers as to how they will provide the optimum blends that can bear the label "Sardegna" and will assist the competitiveness of this important product for the local economy and gastronomy among the Italian and international consumers.

2. Materials and methods

2.1 Experimental design

2.1.1 Area of study and pedoclimatic conditions

The study was carried out at the olive varietal collection field of the Experimental Station "A. Millela" of the University of Sassari, located in San Quirico - Fenosu, Oristano, Sardinia (39°54'12" N, 8°37'19" E), sited at 13 m above sea level. The bioclimate of the area is classified as "*Mediterranean Pluviseasonal-Oceanic; isobioclimate 6: Upper Thermo Mediterranean, Lower Dry, Euoceanic Weak*" [20]. According to the data provided for Oristano by the Department of Meteorology and Climatology Environmental Protection Agency of Sardinia (ARPAS), the annual mean rainfall is 580 mm, mainly concentrated in autumn and winter months; the annual average temperature is 17.1 °C, with an average maximum at 23.9 °C and minimum at 11.3 °C. Winters are mild whereas summers are hot and dry. The intermediate seasons are characterized by a constant high humidity, variability of temperatures and precipitations..

2.1.2 Olive samples

Olive orchard was implanted in 1998 at a space of 6 x 6 m and drip irrigated with ca. 2500 m^3/ha during the period June-October. The collection field was considered appropriate for the present study because it provided genetically certified plant material. The presence in the same grove of almost all the candidate Sardinian varieties for the aim of this study gave the possibility to evaluate the genetic influences on the VOO chemical composition. Varieties in this olive grove were represented by three trees.

Olive samples were collected during 2015 growing season (from 16th November to 2nd December). Olives were mechanically harvested and weighted separately from each tree. Olive fruits from trees that produced at least 17 – 20 kg were processed separately in order to obtain two or three samples

per variety; otherwise the whole olive production of the same variety was utilized to obtain one sample; 31 olive samples coming from 21 varieties were processed (Table 1). The 21 varieties were divided into two groups, in order to simplify the discussion of results:

- Group A was comprised of 14 of the Sardinian varieties that can be used for the production of the PDO "Sardegna": Bosana; Tonda di Cagliari (Nera di Gonnos, Maiorca, Sivigliana da Mensa, Confetto); Tonda di Villacidro (Nera di Oliena, Paschixedda, Terza Grande, Terza Piccola, Corsicana da Mensa); Semidana (Bianca di Villacidro); synonyms (in brackets) have been described from a genetic point of view in 2010[7].
- Group B was comprised of 3 minor Sardinian varieties not included in the 80% of the PDO composition (Corsicana da Olio, Pizz'e Carroga and Sivigliana da Olio), and 4 Italian (Coratina, Frantoio, Itrana and Leccino).

2.1.3 Maturity Index

Maturity Index (MI) was determined for each olive sample according to the procedure described by the International Olive Council (IOC) [21]. Samples were harvested at an average MI of 3.0 (sd ± 0.7) except for Semidana and Bosana olives that where harvested at different MI: 1.1; 1.4; 3.0; 4.0 Semidana and 2.2; 3.8 Bosana (Table 2).

2.1.4 Oil extraction

Olives (20 – 25 kg), were processed within 18h after harvest using a small scale industrial mill "Sintesi 80" Mori TEM (Tavernelle Val di Pesa, Italy), equipped with a blade crusher, 40 kg vertical malaxator working under reduced pressure and two phase decanter. The extraction was performed maintaining the same parameters for all the samples; environmental air temperature was 20 °C (± 1.5); temperature of olive paste after crushing (3000 rpm) was 24 °C (± 2.5); olive paste was kneaded for 15 min at 25 °C (± 2.5); average decanter temperature was 29 °C (± 2.5) (3500 - 3700 rpm). The oil samples obtained were filtered and stored in 100 mL sealed dark glass bottles without headspace, in the dark at -18 °C until further analysis.

2.2 Standards and solvents

Folin Ciocalteu phenol reagent, hydroxytyrosol (≥98%) and tyrosol (≥98%), oleuropein (≥98%), vanillin (≥99%), vanillic acid (≥97%), caffeic acid (≥98%), p-coumaric acid (≥98%), o-coumaric acid (≥98%), ferulic acid (≥98%), pinoresinol (≥95%), cinnamic acid (≥99%), luteolin (≥98%), apigenin (≥95%), FAMEs mixture and squalene (≥ 98%), were all purchased from Sigma–Aldrich (Milano, Italy and St. Louis, MO, USA). α-tocopherol (α-T) (>96%) and pyrogallol (>98%) were from Fluka Chemie GmbH (Buchs, Switzerland). HPLC grade solvents were used without further purification. 2-Propanol (Chromasolv®), acetone (HPLC 95%) and acetonitrile were all provided from ChemLab (Zedelgen, Belgium). Methanol for HPLC (≥99.9%) and *n*-hexane Chromasolv ®, for HPLC, ≥97.0%

(GC) were purchased from Sigma Co (St. Louis, MO, USA). Ultrapure water (H_2O) was prepared using a Milli-Q system (Millipore Corporation, Billerica, MA, USA).

2.3 Quality parameters, fatty acid methyl esters (FAMEs), sterol composition and triterpene dialcohols analysis

Quality parameters: free acidity (% of oleic acid), peroxide value (meq O_2/kg olive oil), K_{232}, K_{270} and ΔK values, FAMEs, sterolic composition and triterpene dialcohols were determined according to the EU official method for olive oil characteristics and subsequent amendments[22-24].

2.4 Analysis of polar phenolic compounds

2.4.1 Sample preparation

The phenolic extracts were obtained following IOC method[25] with slight modifications. Olive oil sample (4 g) was dissolved in 5 mL of a mixture of methanol/water (80:20, v/v). The mixture was shaken in a mechanical orbital rotating stirrer (30 min) and then centrifuged (5 min, 5000 rpm). The polar extract was removed with a glass pipette. The extraction process was repeated twice and the extracts were combined and filtered through a 0.45 µm PVDV filters. Extracts were stored at -78°C until further analysis.

2.4.2 Total polar phenolic content determination

Total phenolic content was determined on methanolic extracts using the Folin-Ciocalteu assay[26]. Results were expressed as mg of gallic acid equivalents (GAE) per 1 kg of oil by means of a calibration curve of gallic acid (10-40 mg L-1, R^2= 0.996).

2.4.3 RP-HPLC-DAD and LC-MS analysis of the polar phenolic compounds

Analysis of phenolic compounds was performed on an Agilent 1100 LC System (Agilent Technologies, Palo Alto, CA, USA) consisted of a quaternary pump (G1311A), degasser, column thermostate, auto-sampler (G1313A), diode array detector (G1315 B, DAD) and a Luna C18 column (250 x 4.6 mm, 5 µm) from Phenomenex (Torrance, CA, USA) with a security guard cartridge (4 × 2 mm). The flow rate was set at 1 ml/min and the column temperature at 30 °C. Elution was carried out with a ternary mobile phase of solvent A (water and 0.1% trifluoracetic acid), solvent B (methanol) and solvent C (acetonitrile). The following gradient program was performed: initial percentage eluent composition was 96:2:2 (A:B:C) v/v/v; 50:25:25 from 0 to 40 min; 40:30:30 from 40 to 45 min; 0:50:50 from 45 to 60 min. This composition was maintained for 10 min, then returned to initial conditions and left to equilibrate for 12 min. Total run time was 82 min. The injection volume was 20 µL. Phenolic compounds were detected at 240, 280 and 320 nm. Identification of polar phenolic compounds was made by the means of standards. When standards were not commercially available (e.g. secoiridoids and acetoxypinoresinol), identification was made by LC-MS analysis performed as follows. An Agilent Technologies (Palo Alto, CA, USA) 1200 series LC equipped with

a Q-Exactive Orbitrap (Thermo Fisher Scientific, Bremen, Germany) mass spectrometer was used for LC MS analysis. Chromatographic separation was achieved with a Gemini C18 column (100 mm × 4.6 mm, 3 µm, 110 Å, (Phenomenex, Torrance, CA, USA) using a mobile phase consisting of 0.2% acetic acid in water (A) and acetonitrile (B) at a flow rate of 500 µL/min. Phenolic compounds separation was obtained using the following linear gradient: A/B (v/v): 0 min 90/10, 0.1-20 min 70/30, and 20.1-40 min 50/50, 40.1-50 min 30/70 and 50.1-60 min 30/70. Mass detection was carried out after electrospray ionization in both Positive and Negative scan ion mode (HESI+ and HESI-). The source voltage was 3.5 kV and 3.2 kV, respectively, for negative and positive ion mode. Nitrogen was used as the sheath and aux gas, with flow rates of 30 and 5 arbitrary units, respectively. The aux gas heater was set at 280 ºC, and the capillary temperature was 300 ºC. HRMS mode operations in Full Scan was: resolution (FWHM) 70000; AGC target, 10^6; injection time: 250 ms; scan range, 130–1000 m/z. Parallel-reaction-monitoring (PRM) and Target selected ion monitoring data dependent (tSIM-dd MS/MS) were also tested as MS/MS mode of acquisition: Data-dependent scanning was carried out without the use of a parent ion list. Operation parameters were as follows: (FWHM) resolution 70000 for precursor ions and 35000 for product ions; AGC target, 10^6 (precursor ions), $2{\cdot}10^5$ (product ions); injection time, 250 ms (precursor ions), 120 ms (product ions). An external calibration for mass accuracy was carried out the day before the analysis according to the manufacturer's guidelines. Data were analyzed using XCalibur software v. 3.0.63 (Thermo Fisher Scientific). Quantification of polar phenols was performed by HPLC-DAD using the calibration curves of a mixture of 13 standards (1.5, 3, 4.5, 6, 7.5 mg/kg). Secoiridoids - dialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), dialdehydic form of decarboxymethyl elenolic acid linked to tyrosol (p-HPEA-EDA), oleuropein and ligstroside aglycon, dialdehydic forms of oleuropein and ligstroside aglycon, and respective isomers - and elenolic acid were quantified using the calibration curve of oleuropein, while acetoxypinoresinol was quantified using the calibration curve of pinoresinol as also described by Bajoub et al.[27]. Results were expressed as mg of phenols per kg of oil. Repeatability of the method was found satisfactory for all the identified compounds (CV = 1.42 – 9.14%, n = 5). Samples were analyzed in triplicate.

2.5 HPLC analysis of tocopherols

HPLC analysis of tocopherols was performed on a system consisted of a pump, model P4000 (Thermo Separation Products, San Jose, CA), a Midas autosampler (Spark, Emmen, The Netherlands) and a UV 6000 LP DAD (Thermo Separation Products) in series with an SSI 502 fluorescence detector (FLD) (Scientific Systems Inc., State College, PA, USA). The data were processed with the aid of Chrom Quest software (version 3.0, Thermo Separation Products). Determination of α-T was performed following the method proposed by Psomiadou and Tsimidou[28]. Oil sample (0.16 g ± 0.01)

was dissolved in a mixture of *n*-hexane/2-propanol (99/1, v/v) (2 mL). Separation was achieved on a LiChrospher-Si column (250 x 4 mm i.d., 5 µm) (MZ Analyzentechnik, Mainz, Germany). The flow rate was 1.2 mL/min and the injection volume was 20 µL. A gradient elution was used with *n*-hexane/2-propanol (99:1 v/v) (A) and 2-propanol (B) as eluents. The gradient for A was as follows: 100% (10 min); 100 – 95% (10 – 14 min); 95% (16 – 20 min); 95-100% (20 – 24 min); 100% (24 – 30 min). Detection of α-T was performed at 294 nm by DAD and by fluorescence at 294 nm (ex) and 330 nm (em). α-T was identified and quantified using the calibration curve ($y = 1E + 10^6 x - 15486$, $R^2 = 0.997$) of a standard solution at five different concentrations (7.5, 15, 30, 60, 80 mg/kg). Other tocopherols were identified using existing library data on the HPLC system. Repeatability of method was found satisfactory (CV% = 1, $n = 5$ for a mean value of 219.1 mg of α-T/kg oil). Samples were run in duplicate and periodically in triplicate in order to verify the repeatability of measurement.

2.6 RP-HPLC analysis of squalene

RP-HPLC analysis of squalene was performed on a solvent delivery system consisted of an LC 20 AD liquid chromatography pump (Shimadzu Corporation, Kyoto, Japan) and a SPD-10AV UV-VIS detector (Shimadzu, Corporation). The data were processed with the aid of the software Clarity Data Apex (Prague Czech Republic). Analysis of squalene was carried out following the saponification method described by Grigoriadou et al.[29]. Specifically, 0.1 g ± 0.02 of oil was added in a 25 mL glass stopped tube followed by the addition of 3 mL KOH (600g/L), 2 mL ethanol and 5 mL ethanolic pyrogallol solution (60 g/L). The tube was flushed with nitrogen, closed and vortexed (5 sec). Alkaline saponification was carried out in a water bath at 75 °C for 30 min. After that, 15 mL of NaCl solution (10 g/L) was added and the mixture was extracted twice (5 min) with 15 mL of *n*-hexane/ethyl acetate (9:1, v/v). Saponification was performed in triplicate and the unsaponified extracts were collected together. After evaporation of the solvent, the dry matter was diluted in acetone. Repeatability of the method was verified (CV% = 6.89, $n = 7$ for a mean value of 4216 mg squalene/kg oil) and found satisfactory. Squalene content in the samples was determined on a reversed phase LiChroCART column (125 × 4.0 mm i.d.; 4 m). The elution solvent was acetonitrile (100%); the volume of the injection was 10 µL; the flow rate was fixed at 1.2 mL/min. Detection and quantification of squalene was performed at 208 nm. Squalene was identified and quantified using the calibration curve ($y = 4.1181 x - 339.63$, $R^2 = 0.997$) of a standard solution at 5 different concentrations (25, 50, 100, 150, 250 mg/kg). Results were expressed as mg of squalene per kg of oil. Analyses were performed in duplicate, periodically the repeatability of method was tested in triplicate.

2.7 "Apparent" chlorophyll content estimation

"Apparent" chlorophyll content[28] was estimated using the following equation:

$C_{(pheophytin\ a,\ mg/kg)} = 345.3[A_{670}-(A_{630}+A_{710})/2]/L$

A_λ is the absorbance of the oil at the respective wavelength and L is the cell thickness (mm). Samples were measured in triplicate.

3. Results and discussion

3.1 Meteoclimatic conditions

The growing season was characterized by a 5 months period, from May to September, of temperatures above the average year and scarcity of precipitations (see supporting information S1). Peaks of +1.33 (Tmax) and +1.42 (Tmin), corresponding respectively to temperatures of 35 °C and 20 °C, were observed during July. At the same time this period was characterized by precipitations below the average period 2006-2017. It is well documented the influence of environmental factors on VOO composition[18,30-32]. Environmental stress conditions (high temperatures, water availability and evapotranspiration) during fruit development and pit hardening process have been proved to play an important role on phenolic compounds biosynthesis, increasing their concentration, as a defense response[33-34]. Thermal regime of summer period strongly affect fatty acid composition, increasing linoleic and palmitic acid as temperature rises, while opposite effect is observed on oleic acid[30-31]. Culeddu et al.[18] observed that monovarietal Bosana VOOs obtained in "Campidano", a southern arid areas of Sardinia (calculated with the FAO-UNEP aridity index), showed lower concentrations in monounsatured fatty acids and higher concentrations in squalene if compared to the oils from the same variety grown in more humid and colder areas of the island. Similar bioclimatic conditions are present in the area of study, and differ from the majority of olive growing areas in Sardinia, characterized by *Mesomediterranean* bioclimates[20]. On the other hand, similar climatic conditions could be found in some other areas of southern Italy (i.e. south Apulia, some coastal areas of Campania and Calabria and some areas of Sicily)[35]. At European level, it could be found in the eastern and southern coastal areas of Spain, a large part of Andalusia, southern Portugal and coastal areas of southern Greece[36,37].

3.2 Quality indices

Most of the samples were classifiable as EVOO[38]. Bosana and Semidana (3rd and 4th samples) exceeded the EU limit for K270 value, Tonda di Cagliari and Nera di Oliena exceeded the EU limit for K232 resulting classifiable as VOO. Confetto sample exceeded the limit for EVOO and VOO classification indicated by EU for peroxide index (20.37 meq O_2/kg oil).

3.3 FAMEs composition

The fatty acid composition of the 21 varieties analyzed in this study is reported in Table 2. All the values fell within the ranges indicated by EU for extra VOOs[38].This is the only requirement set

currently for the PDO "Sardegna"[5] Despite a quite wide variability observed, varieties used in the PDO Sardegna (Group A) showed some common features. Among this group, Tonda di Villacidro and similar varieties reported the highest percentage values of oleic acid (71.25% – 74.48%) and the lowest values of linoleic acid (9.53% - 11.85%), showing a monounsaturated/polyunsaturated ratio (MUFA/PUFA) close to the ones we observed for the Italian cultivars Frantoio and Itrana. MUFA/PUFA is an important parameter indicating the stability of the VOO to oxidation processes that involve first PUFAs[39]. On the contrary, Tonda di Cagliari and synonyms showed low contents of oleic acid (62.86% - 70.94%) and the highest values of linoleic acid (12.72% - 17.98%). Only the cultivar Maiorca stood out for a slightly higher MUFA/PUFA. Bosana and Semidana reported intermediate values, with a decreasing trend of this ratio during maturation. Also the minor varieties Corsicana da Olio and Sivigliana da Olio reported low values. According to our findings, with regard to fatty acid composition, Sardinian varieties might have lower oxidative stability and a shorter shelf life if compared to VOOs from some common Italian varieties (e.g. Leccino and Coratina). For this reason, in the context of a PDO Sardegna VOO production, it might be preferable to valorize cultivars belonging to Tonda di Villacidro group or Bosana and Semidana at earlier stages of ripening. If compared with data reported in literature for the same varieties, our findings showed some slight differences[39,40]. For instance, on regard to Frantoio, Leccino and Itrana, the database of Italian monovarietal EVOOs[40] reported higher values for oleic acid and lower values for linoleic acid than our findings. Differences that can be attributed to the meteoclimatic conditions of this site of study, warmer and dryer if compared to their traditional growing areas (central Italy)[35]. Same observations could be made for the Sardinian varieties grown in other areas of the island[11,12]. These remarks should be taken into consideration when the shelf life of a PDO "Sardegna" product is labeled.

3.4 Sterols and triterpenic dialcohols composition

Analysis of the sterolic composition is a useful tool to detect contamination with other vegetable oils[38]. Among the limits indicated by EU, the samples of Coratina, Terza Piccola, Sivigliana da olio and Itrana did not achieve the minimum limit of total sterols (1000 mg/kg), whereas, Sivigliana da Olio was the only one that exceeded the limit of 4% of campesterol (Table 3). The high amount of this compound, as well as a low total sterolic content, might be a characteristic related to the variety or to the growing season[31,41]. The other limits provisioned by the EU regulation for olive oil characteristics were met. Once again, no specific values are provisioned in the respective regulation for this particular PDO.

Within PDO varieties, Tonda di Cagliari and synonyms exhibited the highest values (1202-1624 mg/kg), while Bosana and Semidana the lowest values, close to the minimum EU limit (1077-1001 mg/kg). On this context, the presence of a small percentage of Tonda di Cagliari (or synonyms) in a

PDO Sardegna blend, might guarantee compliance with the legal minimum. It is worth emphasizing that sterolic composition has proved to be a common feature of most of the PDO varieties present in this study; β-sitosterol ranged between 83.3% and 88.6%, followed by Δ5-avenasterol (4.9% - 9.7%) and campesterol (2.5% - 3.3%). Bosana stood out from group A, exhibiting lower concentration of β-sitosterol (77% - 78%) and consequently higher values of Δ5-avenasterol (14.6% - 15.8%). Moreover, this variety was characterized by the releatively high content of the triterpenic dialcohols erythrodiol and uvaol (2.8 – 3.2 %) detected together with sterols. High quantities of Δ5-avenasterol were also observed in the three minor Sardinian varieties.

As reported by other authors on regard to other varieties, during ripening process of Semidana and Bosana, β-sitosterol relative content decreased while Δ5-avenasterol increased[42,43]. The other sterols detected seemed to be not influenced by fruit ripening, however other authors described increasing trends during fruit ripening also for 24-methylene-cholesterol and stigmasterol [43], thus this hypothesis needs further examination.

The sterols present in VOO and respective ratios have been widely proposed as indicators useful to distinguish varieties[41,42]. Some differences were found between our results and literature concerning Bosana, Frantoio, Leccino and Coratina, probably due to the influence of several factors such as growing area, fruit ripening stage or extraction conditions [31,44]. To our knowledge, this is the first time that a complete sterolic profile of VOOs from 16 Sardinian varieties, grown at similar pedo-climatic and agronomical conditions, has been described.

3.5 Antioxidant/prooxidant composition and content

3.5.1 Total polar phenolic content

The data of total phenolic content are reported in Table 4. Half of the values fell within the range 300-500 mg/kg, while the 32% between 100 mg/kg and 300 mg/kg (Fig. 1a). Our findings of total polar phenolic content with regard to the Sardinian varieties, both principal and minor ones, satisfied the minimum level requested by the application for registration document for PDO Sardegna[5] for this quality parameter (>100 mg/kg) and indicated the potential of these varieties if good practices are adopted throughout the processing and storage conditions. Moreover, 12 of the 17 Sardinian varieties analyzed reported values higher than 300 mg/kg, Bosana 1 stand out for total phenolic content above 700 mg/kg, while Bosana 2 and Semidana 2 showed values between 500 and 700 mg/kg.

The effect of fruit ripening on the total polar phenolic content was observed in Semidana and Bosana oils. In both cases, the first harvests reported the highest values, as well as reported previously in literature[45,46]. Values observed in these two cultivars were generally in the upper limits previouslypublished by other authors, as well as for Pizz'e Carroga[9-12,15,16]. The particular conditions of stress occurred during summer period of 2015 growing season might have contributed to rise

phenolic concentration. Nevertheless, such high content of polar phenols, is not always received well by the consumers, who prefer in generally mild sensory characteristics than bitter or pungent tastes[47]. Frantoio, Leccino, and Coratina are some of the most common varieties in the world and consequently extensively studied. With regard to total phenolic content, a wide range of values have been reported in literature. Our findings were in accordance with these reference ranges[34,39,46,48]. The high variability assessed in literature, within the same cultivar, might be attributed to the wide number of factors that influence the concentration of these antioxidant compounds such as the geographical origin, meteoclimatic conditions during growing season, irrigation, extraction technology[15,32,34,39,45,48,]. However, the min. amount required currently for the PDO product under study underestimates the potential of the major varieties to produce VOOs with extremely high content of polar phenols. A future revision of the respective regulation should reconsider the minimum value.

3.5.2 Individual polar phenolic compounds

Twenty-four phenolic compounds were identified (Table 5), 18 of them were quantified (see also supporting information S2). All VOOs analyzed showed a qualitatively similar phenolic profile, except for elenolic acid, the second isomer of p-HPEA-EDA, o-coumaric acid, ferulic acid and two isomers of oleuropein aglycon (peaks IV and V) that were not detected in all samples. Traces of siringaresinol, overlapped with dialdehydic form of oleuropein aglycon (peak III), were detected in Sivigliana da Olio and Bosana, while traces of pinoresinol, overlapped with p-HPEA-EDA, were detected in Corsicana da olio, Leccino, Coratina and Frantoio. The Peaks VI were characterized by the presence of two molecules co-eluted: isomers of oleuropein aglycon and ligstroside aglycon, finding already reported in literature[49].

Table 6 reports the phenolic compounds concentration (mg/kg of oil) for the 21 varieties analyzed. 3,4-DHPEA-EDA, p-HPEA-EDA and oleuropein aglycon were the most abundant compounds in both groups. In group A, a wide range of values, 9.5 – 132.6 mg/kg, 16.9 – 124.7 mg/kg, and 14.1 - 102.5 mg/kg respectively, were observed, the highest being in Bosana (3,4-DHPEA-EDA), Maiorca (p-HPEA-EDA) and Semidana at earlier stages of maturation (oleuropein aglycon). Tonda di Villacidro, and similar varieties, showed the highest concentrations of a dialdehydic form of ligstroside aglycon, as well as for elenolic acid. Bosana stood out from the other varieties for the relatively high concentration of the two isomers of p-HPEA-EDA. To our knowledge, minor secoiridoids, such as dialdehydic form of ligstroside aglycon with respective isomers, isomers of ligstroside aglycon and p-HPEA-EDA, described in this study were not previously quantified in Sardinian VOOs.

Tonda di Villacidro and similar varieties distinguished for the relatively high content in vanillic acid and vanillin. Acetoxypinoresinol was the only lignan quantified, and the highest concentrations were observed in Semidana 1. The abundance of both luteolin and apigenin characterized Tonda di Cagliari and similar varieties.

The quantitative analysis of polar phenolic compounds highlighted some common features related to Sardinian genetic groups. Moreover, all of them were able to provide high concentrations of secoiridoids, the polar phenolic compounds of VOO with the highest antioxidant properties[50]. Indeed, sum of hydroxytyrosol, tyrosol and derivatives (peaks 7, 15 and 8, 9, 10, 13, 14, 17, 18 respectively) was found to be higher than 250 mg/kg in 5 varieties of group A, i.e. Bosana, Semidana, Maiorca, Tonda di Villacidro, Terza Piccola, limit required by EFSA[51] for the only health claim approved for olive oil.

In Semidana samples, during maturation process, were observed several changes in phenolic composition, secoiridoids were the phenolic category that suffered the stronger decrease, but without a constant trend. Only luteolin and ligstroside aglycon isomer 2 reported a constant increase. Despite it might be necessary a further detailed study on this topic, a first indication might be acquired with regard to the importance of an early harvest of olives from this variety in order to obtain VOOs with high antioxidant properties.

Our findings concerning Sardinian and Italian varieties, compared to literature, showed some quantitative differences principally with regard to the main secoiridoids[10,14,34,46,48]. As well as for total polar phenolic content, quantitative differences in literature might be attributed to the numerous factors that affect polar phenolic composition.

Finally, interesting values were observed in Sivigliana da Olio (group B) sample that showed concentrations of the main secoiridoids 3,4-DHPEA-EDA (196.4 mg/kg) and p-HPEA-EDA (187.7) among the highest registered in this work.

3.5.3 α- Tocopherol content

Table 4 shows the α-T concentrations of the studied VOOs. None of the olive oil samples of group A had >400 mg α-T/kg in contrast to group B in which Leccino overcame 400 mg/kg (Fig. 1b), among the highest values reported for European oils[52].

All the PDO Sardegna varieties achieved broadly the minimum limit requested by the label regulation (100 mg/kg)[5]. High quality virgin olive oils contain >250 mg α-tocopherol/kg just after production whereas even higher levels (>350 mg/kg) has been mentioned in certain monovarietal products derived from healthy olives from different cultivars or regions or under controlled laboratory extraction conditions[52]. Moreover, α-T content in VOOs from Bosana, Semidana, Tonda di Cagliari and Tonda di Villacidro were in the upper levels reported from previous literature[10,14]. This result

strengthens the fact that the content of VOO in bioactive compounds depends, among other factors, also on environmental conditions of the growing region[31]. As well as for phenolic content, stress conditions caused by high temperatures occurred during fruit development might have contributed to increase α-T concentration[31].

In the other Sardinian and Italian cultivars, the ranges of α-T levels were generally higher than those reported in the literature[14]. High values for Leccino had also been mentioned in extra virgin olive oil extracted from unripe fruits[53].

Semidana VOO, at different maturation degree, showed a reduction of a-T content, in line with literature[45], but this was not the case for Bosana. However, in our case due to an insufficient number of samples, we could not end up in a safe conclusion.

3.5.3 Squalene content

Squalene content (Table 4) showed a wide range of values from 3619.5 mg/kg (Frantoio) to 9384.5 mg/kg (Confetto). More than 55% of samples hovered within the range 4000 – 6000 mg/kg, both for group A and B (Fig. 1c). Group A showed higher squalene concentrations (always above 5000 mg/kg) than group B. According to squalene content categories described by Beltran et al.[13], eight cultivars from Group A can be defined as varieties with "medium squalene content" (4000-6000 mg/kg), four as "high squalene content" (6000-7500 mg/kg), two as "very high squalene content" (>7500 mg/kg), bringing to light that the PDO Sardinian cultivars might be a good potential source of squalene; mainly Tonda di Cagliari and similar varieties. Semidana and Bosana VOOs showed a highest squalene content at earlier stages of maturation. Decrease in squalene content in olive oil during fruit ripening has been already described in literature, attributed to the effect of two biosynthetic pathways, which develop concurrently: the sterols and triterpenoid acids biosynthesis, in which squalene is involved as precursor, and the oil accumulation[43].

As far as we know, this is the first time that squalene content in VOOs in less known Sardinian varieties has been studied, and no previous published data have been found for VOOs from Itrana. Our findings on regard to Bosana, Semidana, Tonda di Cagliari and Tonda di Villacidro fell within the ranges described in literature[13,14], as well as Coratina, Frantoio and Leccino[54]. Squalene content in VOOs seems to be mainly related to the genetic factor[13].

3.6 Apparent total chlorophylls content

Apparent total chlorophylls content (Table 4) ranged between 1.5 mg/kg (Coratina) and 16.8 mg/kg (Itrana). The distribution frequency histogram (Fig. 1d) shows that, for group A, values are uniformly distributed between the first three categories identified. Within the same group, Tonda di Villacidro and synonyms, at similar M.I., showed higher mean values (11.1 mg/kg) than Tonda di Cagliari and synonyms (5.0 mg/kg), suggesting that total chlorophyll content might be strongly influenced by the

genetic factor related to fruits characteristics[55]. In fact, according to our field observations, the two genetic groups show different behavior in fruit changing color: when the skin of Tonda di Villacidro drupes turn color to violet (MI >3), the pulp still remains green, instead, when drupe skin of Tonda di Cagliari starts turning violet, the same process starts in pulp. Moreover, different total chlorophyll amount at similar MI was observed within group B. During ripening, in Semidana and Bosana VOO were observed a total chlorophyll decreasing trend, in line with literature[28]. Literature reported wide ranges of total chlorophylls content in VOOs from Bosana, Semidana, Tonda di Cagliari, Tonda di Villacidro and Pizz'e Carroga, our results fell within or above such ranges[10,14-16], differences also were found for Italian varieties[56] probably due to different stages of ripening or different oil extraction conditions.

4. Conclusions

The VOOs of 14 varieties included in the PDO "Sardegna" (group A), obtained under the same processing and storage conditions have shown high concentrations of bioactive compounds. Minimum levels of total phenolic and tocopherols content (both 100 mg/kg) requested by the PDO "Sardegna" regulation were always achieved with a large margin, suggesting the possibility to raise the quality increasing the actual minimum requirements. Within these varieties, genetic groups expressed some specific characteristics (phenolic profile, fatty acid or sterolic composition) and may contribute in different way to the quality label: Bosana and unripe Semidana seem to be the highest sources of polar phenols, particularly secoiridoids. Tonda di Villacidro and synonyms showed a higher content in oleic acid and good MUFA/PUFA ratio, on the other hand, a medium – high phenolic and tocopherols content. Tonda di Cagliari and synonyms have proved to be a good source of squalene and sterols with relatively low levels of antioxidants.

However, due to the large number of varieties included in the PDO "Sardegna", a proper characterization of VOO needs extension of this study for more years to take into account interannual variability. Moreover, similar studies in different growing areas of the island will highlight varietal behavior at different pedoclimatic conditions. The outcome of this study is the first step of documentation of PDO "Sardegna" on the basis of compositional data for major and minor groups of compounds.

Acknowledgement

P. Deiana wish to thank Erasmus+ – Student Mobility for Traineeship program for founding his internship at Aristotle University of Thessaloniki.

Conflict of interest

The authors declare no conflict of interest.

References

[1] European Commission (EC). Reg. No 2081/92. On the protection of geographical indications and designations of origin for agricultur.al products and foodstuffs. *Off. J. Eur. Commun*. **1992**, *L208*, 1.

[2] European Commission (EC). Reg. No 1151/2012. On quality schemes for agricultural products and foodstuffs. *Off. J. Eur. Commun.* **2012**, *L343*, 1.

[3] Database Of Origin and Registration (DOOR) Agriculture and Rural development. From: http://ec.europa.eu/agriculture/quality/door/list.html.

[4] I. Muzzalupo, Olive Germplasm - *Italian Catalogue of Olive Varieties.* InTech, Rijeka (Croatia) **2012**.

[5] European Commission (EC). Publication of an application for registration pursuant to Article 6(2) of Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin. "Sardegna" *Off. J. Eur. Union* **2005**, *C224*, 7.

[6] G. Bandino, M. Mulas, P. Sedda, C. Mor, *Le varietà di olivo della Sardegna*. Consorzio interprovinciale per la Frutticoltura di Cagliari, Oristano e Nuoro, Cagliari (Italy) **2001**.

[7] P. Erre, I. Chessa, C. Muñoz-Diez, A. Belaj, L. Rallo, I. Trujillo, *Genet. Resour. Crop Ev.* **2010**, *57*, 41.

[8] L. Giansante, D. Di Vincenzo, G. Bianchi, *J. Sci. Food. Agric.* **2003**, *83*, 905.

[9] T. Gallina-Toschi, L. Cerretani, A. Bendini, M. Bonoli-Carbognin, G. Lercker, *J. Sep. Sci.* **2005**, *28*, 859.

[10] L. Cerretani, A Bendini, A. Del Caro, A. Piga, V. Vacca, M. F. Caboni, T. Gallina Toschi, *Eur. Food. Res. Technol.* **2006**, *222*, 354.

[11] L. Morrone, L. Neri, C. Cantini, B. Alfei, A. Rotondi, *Food Chem.* **2018**, *245*, 1098.

[12] M. Campus, P. Sedda, D. Delpiano, S. Secci, G. Damasco, R. Zurru, G. Bandino, *Riv. Ital. Sostanze Grasse* **2013**, *XC*, 237.

[13] G. Beltrán, M. E. Bucheli, M. P. Aguilera, A. Belaj, A. Jimenez, *Eur. J. Lipid. Sci. Technol.* **2015**, *117*, 1250.

[14] C. I. G. Tuberoso, I. Jerković, M. Maldini, G. Serreli, *J. Chem.* **2016**, 1.

[15] A. Del Caro, V. Vacca, M. Poiana, P. Fenu, A. Piga, *Food Chem.* **2006**, *98*, 311.

[16] C. Fadda, A. Del Caro, A. M. Sanguinetti, P. P. Urgeghe, V. Vacca, P. P. Arca, A. Piga, *Food Chem.* **2012**, *134*, 1542.

[17] G. Bianchi, L. Giansante, A. Shaw, D. B. Kell, *Eur. J. Lipid. Sci. Technol*. **2001**, *103*, 141.

[18] N. Culeddu, M. Chessa, G. Bandino, P. Sedda, R. Zurru, R. Anedda, A. Motroni, M. G. Molinu, S. Dettori, M. Santona, *Eur. J. Lipid Sci. Tech*. **2017**, *119.*

[19] M. Santona, M. L. Sanna, C. Multineddu, F. Fancello, S. A. de la Fuente, S. Dettori, S. Zara, *Food Microb*. **2018**, *70*, 65.

[20] S. Canu., L. Rosati, M. Fiori, A. Motroni, R. Filigheddu, E. Farris, *J. Maps*. **2015**, *11*, 711.

[21] International Olive Council (IOC). Guide for the determination of the characteristics of oil/olives. COI/OH/Doc. No 1 November **2011**.

[22] European Commission (EC) Reg. No. 2568/91. On the characteristics of olive and olive- pomace oils and on their analytical methods. *Off. J. Eur. Union* **1991**, *L24*, 1.

[23] European Commission (EC) Reg. No 1348/2013 Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Eur. Union* **2013**, *L338*, 31.

[24] European Commission (EC) Reg. 2015/1833 Amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Eur. Union* **2016**, *L266*, 29.

[25] International Olive Oil Council (IOC). Determination of biophenols in olive oils by HPLC. COI/T.20/Doc.29, **2009**.

[26] G. Bazzu, M. G. Molinu, A. Dore, P. A. Serra, *J. Agric. Food Chem.* **2017**, *65*, 1829.

[27] A. Bajoub, E. Hurtado-Fernandez, E. A. Ajal, N. Ouazzani, A. Fernández-Gutiérrez, A. Carrasco-Pancorbo, *J.Aagric. Food Chem.* **2015**, *63*, 4376.

[28] E. Psomiadou, M. Z. Tsimidou, *J. Agric. Food Chem.* **1998**, *46*, 5132.

[29] D. Grigoriadou, A. Androulaki, E. Psomiadou, M. Z. Tsimidou, *Food Chem.* **2007**, *105*, 675.

[30] N. Lombardo, E. Marone, M. Alessandrino, G. Godino, A. Madeo, P. Fiorino, *Adv. Hortic. Sci.* **2008**, 49.

[31] R. J. Mailer, J. Ayton. K., Graham, *J. Am. Oil Chem. Soc.* **2010**, *87*, 877.

[32] P. Inglese, F. Famiani, F. Galvano, M. Servili, S. Esposto, S. Urbani, *Hortic. Rev.* **2011**, 38.

[33] D. Ryan, M. Antolovich, P. Prenzler, K. Robards, S. Lavee, *Sci. Hort.* **2002**, *92*, 147.

[34] M. Servili, S. Esposto, E. Lodolin, R. Selvaggini, A. Taticchi, S. Urbani, G. Montedoro, M. Serravalle, R. Gucci, *J. Agric. Food Chem.* **2007**, *55*, 6609.

[35] S. Pesaresi, D. Galdenzi, E. Biondi, S. Casavecchia, *J. Maps*. **2014**, *10*, 538.

[36] S. Rivas-Martınez, A. Penas, T. E. Diaz, *Cartographic Service, University of Leon.* 2004, from http://www.globalbioclimatics.org/form/bi_med.htm.

[37] S. Rivas-Martınez, A. Penas, T. E. Diaz, *Cartographic Service, University of Leon*. 2004, from http://www.globalbioclimatics.org/form/tb_med.htm.

[38] European Commission (EC) Reg. 2016/2095 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Eur. Union* **2016**, *L326*, 1.

[39] M. Paz Aguilera, G. Beltrán, D. Ortega, A. Fernández, A. Jiménez, M. Uceda, *Food Chem.* **2005**, *89*, 387.

[40] Italian monovarietal oils database from http://www.olimonovarietali.it.

[41] A. M. Giuffrè, L. Louadj, *Czech J. Food Sci.* **2013**, *31*, 256.

[42] M. Lukić, I. Lukić, M. Krapac, B. Sladonja, V. Piližota, *Food Chem.* **2013**, *136*, 251.

[43] A. Fernández-Cuesta, L. León, L. Velasco, R. De la Rosa, *Food Res. Int.* **2013**, *54*, 1885.

[44] G. Delrio, A. Lentini, V. Vacca, G. Serra, *Riv. Ital. Sostanze Grasse.* **1995**, *72*, 5.

[45] D. Boskou, In: Olive oil: minor constituents and health. (Eds: D. Boskou), CRC Press, Boca Raton, **2008**.

[46] G. Gambacorta, M. Faccia, M. A. Previtali, S. Pati, E. L. Notte, A. Baiano, *J. Food Sci.* **2010**, *75*, C229.

[47] M. Z. Tsimidou, in: Handbook of olive oil (Eds. R. Aparicio, J. Harwood) Springer, New York, **2013**, Ch 9.

[48] G. Caruso, R. Gucci, S. Urbani, S. Esposto, A. Taticchi, I. Di Maio, R. Selvaggini, M. Servili, *Agric.Water Manag.* **2014**, *34*, 94.

[49] T. Jerman Klen, A. Golc Wondra, U. Vrhovšek, B. Mozetič Vodopivec, *J. Agric. Food Chem.* **2015**, *63*, 3859.

[50] M. Servili, S. Esposto, R. Fabiani, S. Urbani, A. Taticchi, F. Mariucci, R. Selvaggini, G. F. Montedoro, *Inflammopharmacology*, **2009**, *17*, 76.

[51] European Commission (EC) Reg. 432/2012. Establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. *Off. J. Eur. Union* **2012**, *L136*, 1.

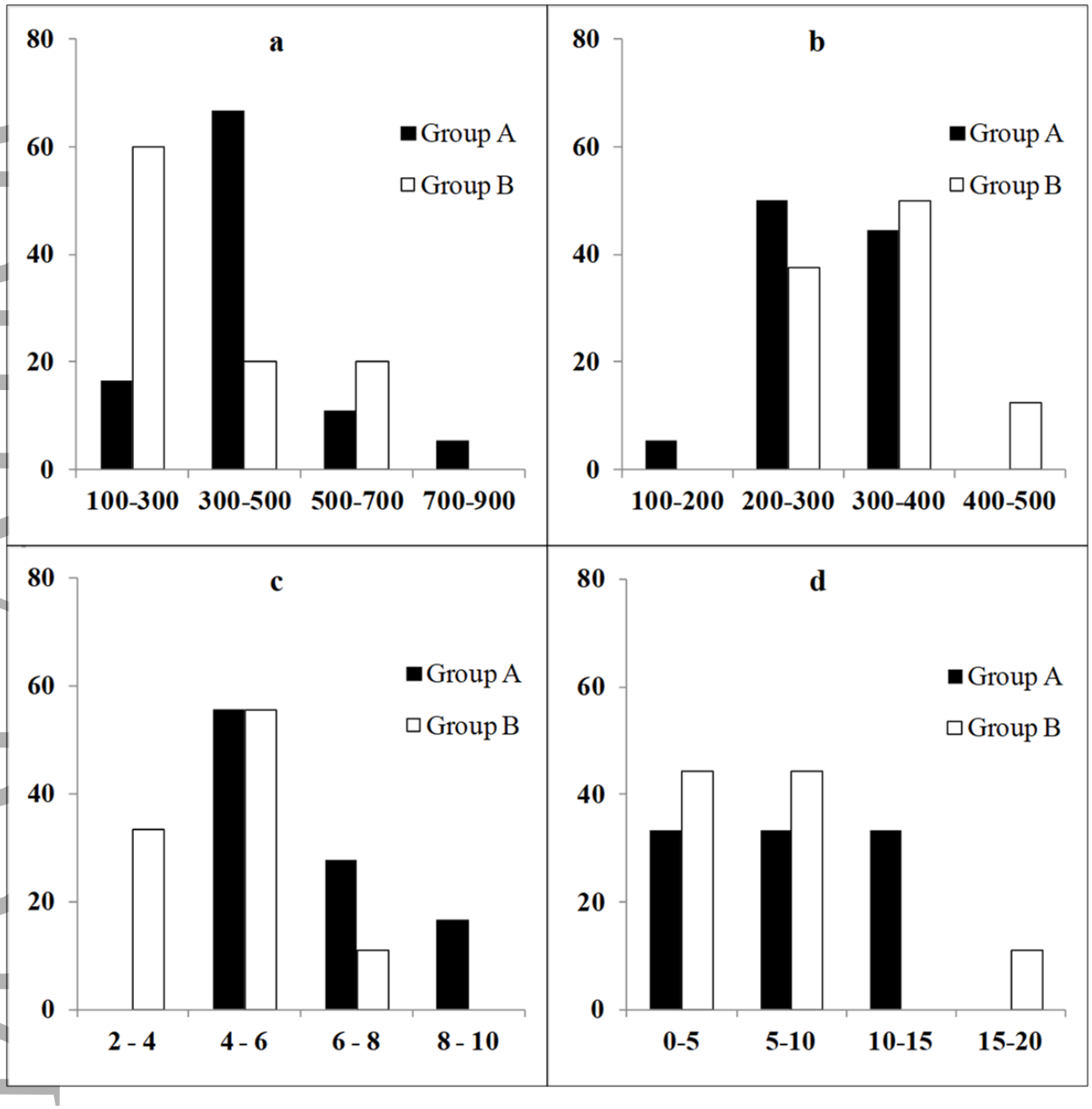
[52] M. Z. Tsimidou, in: Tocopherol: Sources, Uses and Health Benefits (Eds. A. Catala) Nova Science Publishers, Inc., New York **2012**, Ch 1

[53] V. Lavelli, L. Bondesan, *J. Agric. Food Chem.* **2005**, *53*, 1102.

[54] P. Manzi, G. Panfili, M. Esti, L. Pizzoferrato, *J. Sci. Food Agric.* **1998**, *77*, 115.

[55] E. Psomiadou, M. Z. Tsimidou, *J. Sci. Food Agric.* **2001**, *81*, 640.

[56] D. Giuffrida, F. Salvo, A. Salvo, L. Cossignani, G. Dugo, *Food Chem.* **2011**, *124*, 1119.



Caption : **Figure 1.** Distribution frequency histogram of a) total polar phenolic content (expressed as mg of gallic acid/kg oil); b) α-tocopherol content (expressed as mg of α-T/kg oil); c) squalene content (expressed as g of squalene/kg oil); d) total apparent chlorophylls (expressed as mg of pheophytin a/kg oil), in group A and B samples

Table 1. List of the 21 varieties studied and the respective number of samples (N).

PDO Sardegna	N	Minor Sardinian	N	Italian	N
Bosana	2	Corsicana da Olio	1	Coratina	1
Semidana	4	Pizz 'e Carroga	1	Frantoio	1
Bianca di Villacidro	1	Sivigliana da Olio	1	Itrana	2
Tonda di Cagliari	1			Leccino	1
Confetto	1				
Maiorca	1				
Nera di Gonnos	1				
Sivigliana da Mensa	1				
Tonda di Villacidro	2				
Corsicana da Mensa	2				
Nera di Oliena	1				
Paschixedda	1				
Terza Grande	3				
Terza Piccola	2				

Table 2. Maturity index (MI) and fatty acid methyl ester (FAME) composition of VOOs from groups A and B

Group	Variety	MI	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	[1]MUFA/ PUFA
A	Bosana 1	2.2	13.34	0.78	n.d.	1.88	72.16	11.18	0.36	0.21	0.10	6.33
A	Bosana 2	3.8	12.90	0.76	n.d.	1.83	74.34	9.50	0.36	0.21	0.10	7.63
A	Semidana 1	1.1	14.74	0.91	0.09	2.07	70.60	10.75	0.48	0.24	0.07	6.37
A	Semidana 2	1.4	13.55	1.02	0.03	1.73	73.45	9.59	0.37	0.18	0.09	7.48
A	Semidana 3	3.0	14.62	0.84	0.02	1.74	70.51	11.71	0.35	0.18	0.02	5.92
A	Semidana 4	4.0	14.91	0.82	0.04	1.74	69.44	12.55	0.33	0.17	n.d	5.46
A	Bianca di Villacidro	3.5	13.75	0.93	n.d.	1.58	71.84	11.43	0.33	0.13	0.02	6.19
A	Tonda di Cagliari	3.3	16.51	1.58	0.08	1.59	63.19	16.44	0.37	0.17	0.09	3.86
A	Confetto	3.3	15.79	1.38	0.04	1.44	62.86	17.98	0.34	0.14	0.02	3.51
A	Maiorca	2.9	13.11	0.90	0.03	1.71	70.94	12.72	0.32	0.18	0.10	5.52
A	Nera di Gonnos	3.1	16.09	1.40	0.05	1.46	63.16	17.31	0.32	0.16	0.06	3.67
A	Sivigliana da Mensa	3.8	14.56	1.28	0.03	1.48	67.20	14.91	0.36	0.14	0.05	4.49
A	Tonda di Villacidro	3.2	13.29	0.94	n.d.	1.42	74.32	9.62	0.28	0.12	0.02	7.62
A	Corsicana da Mensa	2.6	13.03	0.98	n.d.	1.43	74.48	9.53	0.36	0.13	0.06	7.63
A	Nera di Oliena	3.1	13.89	1.15	n.d.	1.35	71.25	11.85	0.35	0.13	0.03	5.94
A	Paschixedda	3.4	13.23	1.04	n.d.	1.50	73.38	10.23	0.40	0.15	0.08	7.01
A	Terza Grande	3.0	13.09	0.88	n.d.	1.52	74.02	9.86	0.40	0.15	0.08	7.30
A	Terza Piccola	3.3	13.26	0.98	0.02	1.67	73.21	10.25	0.37	0.17	0.09	7.01
B	Corsicana da Olio	3.9	14.27	1.13	n.d.	2.32	69.78	11.87	0.33	0.20	0.10	5.82
B	Pizz 'e Carroga	2.6	14.24	1.03	0.01	1.81	72.43	9.90	0.31	0.18	0.10	7.20
B	Sivigliana da Olio	3.6	16.38	1.35	0.12	1.81	66.36	13.30	0.41	0.20	0.06	4.94
B	Coratina	3.0	9.95	0.35	n.d.	1.69	79.13	8.26	0.29	0.18	0.16	9.32
B	Frantoio	2.4	12.78	0.70	n.d.	1.33	75.34	9.41	0.27	0.10	0.07	7.86
B	Itrana	2.9	12.97	0.95	n.d.	1.62	74.59	9.32	0.34	0.14	0.07	7.86
B	Leccino	4.2	13.30	1.18	n.d.	1.65	75.93	7.41	0.30	0.14	0.08	10.02

Values are expressed as percentage of total FAMEs. [1]Monounsatured fatty acids (C16:1, C18:1 and C20:1)/Polyunsatured fatty acids (C18:2 and C18:3) ratio

Table 3. Sterolic and triterpene dialcohol composition of VOOs from groups A and B.

Group	Variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	Bosana 1	0.1	0.2	2.6	0.1	0.8	1.1	78.1	0.3	14.6	1.0	0.3	0.8	95.1	1068	3.2
A	Bosana 2	0.1	0.3	3.0	0.1	0.7	1.1	77.0	0.2	15.8	0.9	0.2	0.7	95.0	1018	2.8
A	Semidana 1	0.1	0.1	3.3	0.1	0.9	1.1	87.6	0.5	4.9	0.5	0.2	0.6	94.6	1015	1.0
A	Semidana 2	0.1	0.1	2.8	0.1	0.7	1.1	87.9	0.5	5.3	0.5	0.3	0.6	95.3	1010	1.3
A	Semidana 3	0.2	0.1	3.1	0.1	0.9	1.1	85.1	0.4	7.6	0.5	0.2	0.7	94.7	1001	1.1
A	Semidana 4	0.1	0.2	3.0	0.1	0.8	1.1	83.3	0.3	9.7	0.5	0.2	0.7	94.9	1004	1.1
A	Bianca di Villacidro	0.1	0.1	2.7	0.1	0.7	1.1	86.4	0.4	6.8	0.5	0.3	0.8	95.2	1077	2.2
A	Tonda di Cagliari	0.1	0.1	2.5	0.1	0.7	1.0	87.3	0.4	5.6	0.8	0.5	1.1	95.1	1529	1.2
A	Confetto	0.1	0.1	2.6	0.1	0.8	1.0	87.1	0.4	5.4	0.9	0.5	1.0	94.8	1624	1.4
A	Maiorca	0.1	0.1	2.6	0.1	0.7	1.1	86.8	0.5	5.9	0.7	0.5	0.9	95.0	1202	1.5
A	Nera di Gonnos	0.1	0.1	2.5	0.1	0.7	1.0	87.0	0.4	5.6	1.0	0.5	1.0	95.0	1581	1.3
A	Sivigliana da Mensa	0.1	0.1	2.6	0.1	0.8	1.0	87.6	0.4	5.3	0.8	0.4	0.8	95.1	1340	1.0
A	Tonda di Villacidro	0.1	0.1	2.6	0.1	0.6	1.0	86.8	0.4	6.7	0.5	0.4	0.8	95.4	1014	1.6
A	Corsicana da Mensa	0.1	0.1	2.6	0.1	0.6	1.0	88.0	0.4	5.7	0.6	0.3	0.6	95.7	1191	1.6
A	Nera di Oliena	0.1	0.1	2.6	0.1	0.7	1.0	87.3	0.4	6.0	0.6	0.3	0.8	95.3	1292	1.3
A	Paschixedda	0.1	0.1	2.7	0.1	0.7	1.1	88.1	0.5	5.3	0.4	0.3	0.6	95.4	1072	1.5
A	Terza Grande	0.1	0.1	2.6	0.1	0.6	1.1	88.6	0.5	4.9	0.5	0.3	0.5	95.6	1109	1.5
A	Terza Piccola	0.1	0.1	2.6	0.1	0.6	1.1	87.8	0.6	5.4	0.6	0.4	0.9	95.3	934	1.5
B	Corsicana da Olio	0.1	0.1	2.6	0.1	0.6	1.0	80.7	0.5	11.6	1.3	0.5	0.9	95.1	1500	1.3
B	Pizz 'e Carroga	0.1	0.1	3.0	0.1	0.7	1.0	83.7	0.4	8.9	1.1	0.3	0.7	95.1	1302	1.4
B	Sivigliana da Olio	0.2	0.4	4.6	0.1	0.4	1.1	81.1	1.3	9.0	0.8	0.2	0.8	93.3	844	1.2
B	Coratina	0.2	0.3	3.1	0.1	0.8	1.1	84.5	0.8	7.5	1.0	0.2	0.4	94.9	696	1.9
B	Frantoio	0.1	0.1	2.8	0.1	0.6	1.0	83.4	0.4	9.6	0.9	0.3	0.7	95.3	1612	1.0
B	Itrana	0.1	0.1	2.6	0.1	0.7	1.1	88.0	0.6	5.6	0.5	0.4	0.5	95.7	993	1.8
B	Leccino	0.1	0.1	2.2	0.1	0.9	1.0	78.6	0.6	13.3	1.0	0.5	1.6	94.5	1637	0.7

Values are expressed as percentage of total sterols. Total sterols are expressed as mg/kg of oil. 1 = cholesterol; 2 = 24-methylencholesterol; 3 = campesterol; 4 = campestanol; 5 = stigmasterol; 6 = chlerosterol; 7 = β-sitosterol; 8 = sitostanol; 9 = Δ-5-avenasterol; 10 = Δ-5,24- stigmastadienol; 11 = Δ-7-stigmastenol; 12 = Δ-7-avenasterol; 13 = apparent β-sitosterol (6 + 7 +8 + 9 + 10) ; 14 = total sterols (mg/kg); 15 = erythrodiol + uvaol

Table 4. Total polar phenolic content (mg gallic acid/kg oil), α-Tocopherol (mg α-T/kg oil), squalene (mg squalene/kg oil) and total apparent chlorophylls (mg of pheophytin a/kg oil), of VOOs from groups A and B.

Group	Variety	Total polar phenols	α-Tocopherol	Squalene	Total apparent chlorophylls
A	Bosana 1	746.9	340.7	7050.5	7.4
A	Bosana 2	604.7	353.8	5887.9	6.0
A	Semidana 1	467.4	291.7	8256.5	13.6
A	Semidana 2	563.7	297.9	5837.3	9.6
A	Semidana 3	327.1	219.1	6585.9	3.6
A	Semidana 4	359.9	227.4	6654.4	3.3
A	Bianca di Villacidro	413.4	327.9	5565.6	10.9
A	Tonda di Cagliari	366.6	246.6	9220.8	4.5
A	Confetto	308.9	248.7	9384.5	4.3
A	Maiorca	468.0	307.7	5726.1	4.5
A	Nera di Gonnos	387.9	210.4	5831.4	3.9
A	Sivigliana da Mensa	270.4	257.6	6897.6	8.0
A	Tonda di Villacidro	430.6	289.5	5336.3	7.9
A	Corsicana da Mensa	374.5	314.7	5401.8	11.4
A	Nera di Oliena	155.2	198.9	6824.0	5.5
A	Paschixedda	285.2	377.5	5112.3	13.2
A	Terza Grande	342.7	306.9	5106.7	14.8
A	Terza Piccola	427.0	313.1	5397.0	13.5
B	Corsicana da Olio	290.7	291.8	3682.1	1.9
B	Sivigliana da Olio	465.2	337.2	4950.5	8.4
B	Pizz 'e Carroga	236.2	216.7	4062.2	5.8
B	Coratina	659.6	300.5	4658.9	1.5
B	Frantoio	202.1	249.0	3619.5	7.7
B	Itrana	426.2	344.6	5705.1	16.8
B	Leccino	220.6	505.9	4369.2	4.1

Table 5. Phenolic compounds identified in VOO samples by HPLC-MS.

Peak n	Phenolic Compound	Molecular Weight	M-H	Fragments
1	Hydroxytyrosol	154.17	153.054	
2	Tyrosol	138.17	137.06	
3	Vanillic acid	168.14	167.054	
4	Vanillin	152.15	151.054	
5	p-Coumaric acid	164.16	163.038	
I	Ferulic acid	194.19	193.049	
6	Elenolic acid	241.07		
II	o-Coumaric acid	164.16	163.038	
7	3.4-DHPEA-EDA	320.00	319.118	195.065; 337.124; 639.245
III	Oleuropein aglycon dialdehydic form	378.13	377.240	307.082; 755.255
8	Ligstroside aglycon dialdehydic form 1	362.14	361.129	291.087; 723.266
9	Ligstroside aglycon dialdehydic form 2	362.14	361.129	291.087; 723.266
10	p-HPEA-EDA	304.13	303.123	165.054; 285.113; 607.255
11	Acetoxypinoresinol	416.426	415.15	
12	Luteolin	286.05	285.050	
13	p-HPEA-EDA Isomer 1	304.13	303.123	165.054; 285.113; 607.255
14	p-HPEA-EDA Isomer 2	304.13	303.123	165.054; 285.113; 607.255
IV	Oleuropein aglycon Isomer 1	378.13	377.240	307.082; 755.255
V	Oleuropein aglycon Isomer 2	378.13	377.240	307.082; 755.255
15	Oleuropein aglycon	378.13	377.240	307.082; 755.255
16	Apigenin	270.06	269.040	
VI	Ol aglycon + Lig aglycon	378.13 + 362.14		
17	Ligstroside aglycon 1	362.14	361.129	291.087; 723.266
18	Ligstroside aglycon 2	362.14	361.129	291.087; 723.266

Table 6. Polar phenolic composition of VOOs from groups A and B.

Group	Variety	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A	Bosana 1	1.6	5.6	0.5	0.2	0.1	2.3	132.6	0.7	0.4	79.6	7.5	5.7	19.9	9.4	85.2	3.1	14.7	1.4
A	Bosana 2	1.2	6.2	0.4	0.3	0.2	2.8	128.3	0.9	0.5	82.0	14.8	5.1	12.2	6.4	63.3	2.7	9.6	3.3
A	Semidana 1	1.1	4.0	0.7	0.6	0.2	1.6	58.9	0.7	0.3	48.1	49.4	1.9	8.3	4.5	34.4	1.0	6.8	1.1
A	Semidana 2	2.1	4.7	1.5	1.0	0.5	3.3	100.8	2.7	2.0	54.8	10.9	3.3	9.0	6.5	102.5	1.9	7.2	1.4
A	Semidana 3	7.8	13.5	1.1	0.4	0.3	1.4	9.5	1.6	0.7	16.9	13.9	3.6	6.7	2.6	30.8	1.7	1.9	1.5
A	Semidana 4	4.1	8.0	0.9	0.4	0.3	1.5	22.2	1.7	0.6	17.9	13.6	4.4	5.1	4.5	50.8	1.9	1.8	2.3
A	Bianca di Villacidro	2.6	6.1	1.5	1.0	0.3	5.4	81.4	2.9	1.9	48.2	9.4	3.4	4.6	5.2	66.7	1.6	4.5	1.5
A	Tonda di Cagliari	1.2	5.6	0.3	0.4	0.3	2.5	88.4	3.6	0.8	66.0	1.1	9.5	3.7	2.5	27.3	6.3	2.5	2.5
A	Confetto	1.2	5.6	0.4	0.6	0.4	2.5	71.3	3.0	0.9	52.9	1.1	6.6	2.0	2.5	26.0	3.9	2.1	3.0
A	Maiorca	1.0	5.1	0.7	0.8	0.3	2.3	128.0	1.8	1.2	124.7	19.9	3.4	3.7	2.7	41.8	2.1	7.1	1.6
A	Nera di Gonnos	1.5	5.1	0.3	0.4	0.3	3.4	75.2	3.7	0.8	53.5	1.4	9.2	4.8	3.4	35.1	5.6	2.1	2.4
A	Sivigliana da Mensa	1.3	3.6	0.7	0.8	0.5	3.2	61.5	3.3	1.9	48.7	3.0	4.4	1.3	3.2	25.3	2.4	2.6	3.9
A	Tonda di Villacidro	2.0	3.7	1.9	1.2	0.8	7.7	115.5	3.9	3.4	65.8	7.3	3.9	4.	5.7	60.4	2.3	5.0	1.8
A	Corsicana da Mensa	2.5	4.0	2.5	1.7	0.4	10.1	97.0	3.7	3.0	57.9	7.4	3.1	3.6	5.3	46.7	1.8	3.5	1.0
A	Nera di Oliena	0.5	4.1	2.4	0.5	0.7	3.3	17.7	3.6	3.1	48.3	4.1	3.4	1.6	3.3	14.1	2.6	2.7	1.3
A	Paschixedda	0.8	2.6	1.8	1.5	0.6	11.7	75.6	3.5	2.7	65.3	21.5	3.0	n.d.	4.4	28.4	1.8	3.4	1.1
A	Terza Grande	0.9	2.6	1.8	1.4	0.3	9.2	70.2	3.3	2.7	62.1	23.3	2.4	n.d.	4.8	35.1	1.6	3.8	1.0
A	Terza Piccola	1.6	3.8	1.6	1.5	0.4	8.3	131.3	3.0	2.5	83.6	22.5	3.0	n.d.	3.6	52.2	2.1	6.9	4.6
B	Corsicana da Olio	0.5	5.2	0.4	0.5	0.1	2.1	17.1	1.0	0.4	70.5	56.9	3.1	3.6	0.7	35.1	1.8	8.2	1.1
B	Pizz 'e Carroga	1.0	6.8	1.4	0.7	0.1	4.9	21.9	2.1	1.0	29.1	17.5	5.6	3.5	1.8	24.7	1.5	3.6	2.1
B	Sivigliana da Olio	1.1	2.7	1.1	0.6	0.2	4.1	196.4	2.3	4.0	187.7	36.5	3.8	n.d.	3.0	17.3	3.0	3.1	0.9
B	Coratina	1.9	10.0	0.4	0.5	0.2	n.d.	178.7	0.4	0.2	246.7	33.9	2.3	6.0	2.6	62.4	1.2	20.5	2.3
B	Frantoio	1.2	7.8	1.5	0.7	0.1	3.1	11.5	1.2	1.0	31.8	23.1	2.8	4.3	1.6	20.0	1.1	3.8	2.1
B	Itrana	1.2	2.5	1.6	1.1	0.4	7.2	131.6	3.3	3.0	75.6	6.5	3.0	3.5	4.8	51.3	1.8	3.7	1.2
B	Leccino	1.5	8.1	0.9	0.7	0.1	3.3	58.1	2.2	0.5	65.3	8.4	2.17	2.3	2.0	10.9	1.6	1.3	2.0

Values are expressed as mg of the corresponding standard/kg of oil; compounds n. 6-10, 13-15 and 17-18 as mg of oleuropein/kg oil. 1 = hydroxytyrosol; 2 = tyrosol; 3 = vanillic acid; 4 = vanillin; 5 = p-coumaric acid; 6 = elenolic acid; 7 = 3,4DHPEA-EDA; 8 = ligstroside aglycon dialdehydic form 1; 9 = ligstroside aglycon dialdehydic form 2; 10 = p-HPEA-EDA; 11 = acetoxypinoresinol; 12 = luteolin; 13 = p-HPEA-EDA isomer 1; 14 = p-HPEA-EDA isomer 2; 15 = oleuropein aglycon; 16 = apigenin; 17 =ligstroside aglycon isomer 1; 18 = ligstroside aglycon isomer 2