Quality evaluation of extra-virgin olive oils from Sicilian genotypes grown in a high-density system

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Chemical and nutritional evaluation of different Sicilian olive oils from modern olive plantings

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What is it about?

We analysed chemical and sensory traits of olive oils from 14 minor Sicilian olive genotypes in comparison with oils from six major Sicilian and three international cultivars. Fatty acid composition, phenol composition, carotenoid content and antioxidant power were determined and analysed using univariate and multivariate procedures.

Why is it important?

Studying the sensory profile and chemical composition of monovarietal extra-virgin olive oils is important to define and manage their quality and uniqueness.

Perspectives

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Some Sicilian accessions used in this study may represent valid alternatives to produce high-quality extra-virgin olive oils in modern, hedgerow planting systems.

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Keywords:	Carotenoids, chlorophyll, Fatty acids, Phenols, sensory attributes, UHPLC-HESI-MS

Quality evaluation of extra-virgin olive oils from Sicilian genotypes grown in a high-density system.

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Abstract

Studying the sensory profile and chemical composition of monovarietal extra virgin olive oils (EVOO) is important to define and manage their quality and uniqueness. Chemical and sensory traits of olive oils from 14 minor Sicilian olive genotypes in comparison with oils from six major Sicilian and three international cultivars, were analysed. Oils were extracted in 2015 from fruit of the 23 genotypes grown in an experimental orchard at a planting density of 1140 trees ha^{-1}. Fatty acid composition, phenol composition, carotenoid content and antioxidant power were determined and analysed using univariate and multivariate

procedures. In particular, Nocellara Etnea along with carotenoid, phenol content and good sensory attributes, producing the best quality EVOO among the genotypes in trial. These results show that some Sicilian accessions used in this study may represent valid alternatives to produce high quality EVOOs in modern, hedgerow planting systems.

Keywords: carotenoids, chlorophyll, fatty acids, phenols, sensory attributes, UHPLC-HESI-MS

Introduction

Olive (*Olea europaea* L.) is a crop with economic and environmental relevance in the Mediterranean areas where it has been cultivated since ancient time (Loumou and Giourga 2003). Many olive genotypes in the Mediterranean area have local origin (Sarri et al. 2006), due to cross-pollination, climatic differences among sites where the species has been domesticated and the long life span of the olive tree (Lavee and Zohary 2011). In particular, a high pool of genetic variability has been recognized in Sicily (La Mantia et al. 2005). Major olive genotypes used for olive oil production result from farmers selection based on phenotypic and horticultural traits of the trees, particularly on their suitability for extensive cultivation under dry conditions (Belaj et al. 2010).

In modern intensive and irrigated orchards, the quality standards have generally declined and some of the minor, neglected genotypes may represent an opportunity to increase the diversity and improve the quality of olive oil produced in Sicily. Currently, in the international scenario only three cultivars (Arbequina, Arbosana and Koroneiki) fit the modern super intensive orchard design and management, showing good performance also in Sicily (Tous et al. 2008; Godini et al. 2011; Caruso et al. 2014a). Nevertheless, the worldwide diffusion of these genotypes could lead to a dangerous reduction of biodiversity

and to a flattening of the differences in olive oil quality, both from a chemical and an organoleptic point of view. Nowadays, 'Biancolilla', 'Cerasuola', 'Moresca', 'Nocellara del Belice', 'Nocellara Etnea', 'Ogliarola Messinese', 'Santagatese' and 'Tonda Iblea' are the predominant olive oil genotypes in the island (Caruso et al. 2014b). The recovery of Sicilian genotypes and their use in modern intensive orchards may contribute to new opportunities for olive oil production. The genetic richness of Sicilian olive germplasm has been well documented in the last decades (Motisi et al. 2006; Besnard et al. 2013; Lo Bianco et al. 2013; Caruso et al. 2014b).

Extra virgin olive oil (EVOO) is obtained exclusively by mechanical and physical processes. It is composed by a major fraction (more than 98% of the total weight) of saturated and mono- and poly-unsaturated fatty acids (mainly triacylglycerides), whereas a minor fraction (approximately 2% of the weight) is composed by minor compounds, which includes over 230 chemical compounds (terpenoids, sterols, pigments, volatile compounds and antioxidants) (Servili et al. 2014). Traditionally, the beneficial effects of extra-virgin olive oil have been attributed to the fatty acids composition and phenolic compounds. Nowadays, there is a trend to reduce saturated fat and increase the level of unsaturated/polyunsaturated fatty acids and omega 3 fatty acids for health benefits (White 2009), and this represents a new challenge for olive oil producers and the selection of new genotypes.

More recently, olive oil health benefits have been attributed to phenolic compounds, which have antioxidant, anti-inflammatory, anti-cancer, antimicrobial, antiviral, hypoglycemic, hepatic-, cardiac- and neuro-protective properties (Cicerale et al. 2012; Martín-Peláez et al. 2013; Servili et al. 2014). In general, five major classes of phenolic compounds can be found in olive oils: phenolic acids, phenolic alcohols, flavonoids, lignans and secoiridoids (Fuentes de Mendoza et al. 2013). Secoiridoids are found only within the

family of Oleaceae and they are considered the main components (50-70%) of the phenolic fraction in the extracted oil. The most abundant compounds belonging to this family are the dialdehydic forms of elenoic acid linked either to hydroxytyrosol in oleacin (3,4-DHPE-EDA) or to tyrosol in oleocanthal (p-HPEA-EDA); oleuropein aglycon (3,4-DHPEA-EA) and ligstroside aglycon (p-HPEA-EA). Oleocanthal, a secoiridoid derivative with very promising pharmacological properties, has been proposed as an agent to induce apoptosis in colon cancer cells, inhibition of proliferation in breast cancer and prostate cancer cell lines, stimulating further interest in cancer research (Elnagar et al. 2011; Abuznait et al. 2013). Several animal and in vitro studies have shown that oleocanthal possess important neuroprotective activities against Alzheimer's disease (Abuznait et al. 2013). 3,4-DHPE-EDA as a novel drug aimed to prevent or reduce inflammation of endothelium, plays an important protective role against reactive oxygen species-induced oxidative injury in red blood cells (Paiva-Martins et al. 2009).

The phenolic compounds present in olive oil are also responsible for its unique sensory properties. In this respect, 3,4-DHPEA-EDA and 3,4-DHPEA-EA are considered responsible for the "bitter" and "astringent'' attributes in EVOO (Tovar et al. 2001; Andrewes et al. 2003). Furthermore, García et al. (2001) established a correlation between the bitterness of EVOO and the related chemical compounds behind it, finding that the sum of the two secoiridoids derivatives of hydroxytyrosol, the dialdehydic form of decarboxymethyl oleuropein aglycon and the aldehydic form of oleuropein aglycon, represents a reliable estimation of the oil organoleptic characteristics.

The phenolic composition of olive oils may depend on agricultural practices, degree of fruit ripeness, soil type, climate, olive oil extraction method and storage, but firstly it depends on the olive cultivar (Inglese et al. 2011; Sinesio et al. 2015; Di Stefano et al. 2019). Moreover, some Sicilian accessions have been already recognized for a high

percentage of oleic acid or high level of phenolic compounds, regardless of the season or method of extraction (Motisi et al. 2006; Marino et al. 2017).

According to the European Food Safety Authority, dietary substitution of saturated fatty acids (SFAs) with *cis*-monounsaturated fatty acids (MUFAs) and/or *cis*-polyunsaturated fatty acids (PUFAs) contributes to the maintenance of normal LDL blood cholesterol levels (EFSA Journal 2011 a). Moreover, a cause and effect relationship has been established between the consumption of olive oil polyphenols (standardized by the content of hydroxytyrosol and its derivatives) and protection of LDL particles from oxidative damage (EFSA Journal 2011 b).

Giving the recent new perspectives of health benefits (i.e. reduction of blood cholesterol and cardiovascular diseases) related to daily consumption of unsaturated fatty acids and polyphenols content in olive and olive oil (Martini et al. 2017; Reboredo-Rodríguez et al. 2018), there is a real opportunity for the Sicilian genotypes and accessions. Thus, the objective of this research is the classification of Sicilian oils obtained both from major and minor genotypes, based on their main chemical and sensory attributes for the individuation of the best performing genotypes in intensive hedgerow orchards. Twenty-three monovarietal EVOO were analysed with the aim to evaluate their ability to obtain a health claim from EFSA and increase the chances for the Sicilian olive oils to be introduced in the international market.

Material and methods

Sampling material

Samples of monovarietal EVOO were obtained from trees grown in an experimental field located in South West Sicily, (37°53'N, 13°00'E, about 56 m a.s.l.). From previous observations, the area where this study was conducted has demonstrated to be suitable for

intensive hedgerow planting systems. The area is characterized by a long growing season that allows, with the proper cultural management, a constant (non-alternating) bearing (Marra et al., 2012). The genotypes studied, in order to be considered suitable for high density production must originate heavy production and high quality oils. High levels of monounsaturated fatty acids and phenolic compounds allow EVOO to be consider as a functional food (Stark and Madar 2002).

The orchard was planted in 2006 using one-year-old own-rooted olive cuttings of 20 Sicilian genotypes, and 3 international cultivars used in super intensive orchards (Table 1). Sicilian genotypes were classified as major or minor according with their distribution and production in Sicily. Major genotypes are largely diffused in the region and characterize the majority of Sicilian olive oils in the market, while minor genotypes are only grown in small farms and contribute to the production of very exclusive olive oils.

A total of 25 plants per genotype, spaced at 2.5 x 3.5 m (about 1140 trees ha^{-1}), were planted in single North-South oriented rows. Trees were trained to hedgerow system (free Palmette shape) to allow partial mechanization of canopy pruning and full mechanization of harvesting. From the 5^{th} year after planting trees were mechanically pruned (topping) to 2.5 m high. Trees were irrigated with an amount of water corresponding to approximately 800 m^3 ha^{-1} $year^{-1}$. Trees were harvested from October to November 2015 and fruit was weighed to calculate yield in tons per hectare and yield efficiency as kg of fruit per cm^2 of trunk cross-sectional area. After harvest, maturation index was determined based on fruit skin and pulp colour according to Hermoso et al. (1991). Olive oil was extracted with a two-phase extraction system (Pieralisi Leopard Model 6 DMF Tec Jesi, Italy) and a 30-min malaxation time at 25 °C. Oil yield was calculated as g of oil per 100 g of fruit and expressed in percent. Three samples for each monovarietal EVOO were stored in dark glass bottles and at 10 °C until analyses.

Chemicals and standards

Water, methanol, acetonitrile (LC-MS grade) were purchased from Biosolve B.V. (Valkenswaard, The Netherlands). Formic acid (LC-MS grade) used as ionization agent in the chromatographic mobile phase, was purchased from VWR International B.V. (Roden, The Netherlands). Reference phenolic compounds including caffeic acid, cinnamic acid, ferulic acid, gallic acid, p-coumaric acid, p-hydroxybenzoic acid, syringic acid, vanillic acid, apigenin, apigenin 7-glucoside, diosmetin, hydroxytyrosol, tyrosol, luteolin, oleuropein, vanillin and pinoresinol were purchased from Sigma-Aldrich (Steinheim, Germany). N-t-butyl-α-phenylnitrone (PBN) was purchased from Sigma-Aldrich (Steinheim, Germany). All solvents and other chemicals used were of analytical grade purity and were supplied by Merck (Darmstadt, Germany).

Quality attributes

Free acidity (g of oleic acid per 100 g of oil) and peroxide value (mEq O_2 kg^{-1}) were measured according to the European Union standard methods (UE, 1989/2003 modifying the ECC 2568/91). Chlorophyll and carotenoid contents were measured using a Beckman DU 640 UV spectrophotometer at 476 nm and 670 nm and dissolving 7.5 g of olive oil in 25 ml of cyclohexane, as described by Mineo et al. (2007). Pigment amounts were calculated using the specific extinction values, E0 = 613 for pheophytin 'a' and E0 = 2000 for lutein. Thus, pigment contents were calculated as follows:

$$[\text{chlorophyll}](\text{mg kg}^{-1}) = (A_{670}) / (613 \times 100 \times d)$$

$$[\text{carotenoids}](\text{mg kg}^{-1}) = (A_{476} / (2000 \times 100 \times d),$$

where A is the absorbance and d is the spectrophotometer cell thickness (1 cm). The chlorophyll or carotenoid contents are expressed as mg of chlorophyll "a" or β-carotene per

kg of oil, respectively. Total phenols content was determined using Folin-Ciocalteu method as reported by Montedoro et al. (1992) and was expressed as mg gallic acid equivalent (GAE) per kg of oil sample.

Phenolic compound determination by UHPLC-HESI-MS

Identification and quantification of phenolic compounds was carried out for each EVOO sample. They were extracted from the monovarietal oils according to the COI procedure and Montedoro et al. (1992), with some modifications. Briefly, in a centrifuge tube, 2 g of EVOO were mixed with 5 ml of a solution of methanol:water (80:20 v/v). The tube was vortexed for 1 min and held in an ultrasonic bath for 15 min, centrifuged at 5000 rpm for 25 min. The surnatant was filtered over a 0.45-µm PTFE siringe filter and stored at 4°C until further analysis. Triplicate samples of olive oil were used for each genotype. Phenolic compounds were identified by ultra high performance liquid chromatography, heated electrospray, and mass spectrometry (UHPLC-HESI-MS) (Di Stefano et al. 2017). UHPLC analysis was performed using a Dionex Ultimate 3000 System (Dionex Softron GmbH, Germering, Germany) equipped with an autosampler controlled by Chromeleon 7.2 Software and coupled to a photodiode array detector (Thermo Fisher Scientific, Bremen, Germany). A UHPLC column (Phenomenex Luna C18(2) 50 x 1 mm, 2.5µ) was set for separation of the selected compounds at 35°C. The mobile phases used were 0.1% formic acid in water (A) and methanol (B). The gradient elution program was: 0-5 min 10% B; 5-50 min linear increase to 99% B, 50-56 min 10% B coming back to the initial conditions until full stabilization. The column temperature was set at 30 °C and the injection volume at 1 µl. The flow rate was 50 µl min^{-1}. MS detection was performed using a Q-Exactive accurate-mass spectrometer (Thermo Scientific, Bremen, Germany). The HESI parameters were set using negative ion mode with spectra acquired over a mass range from 180 to 2000 *m/z*. The optimum values of

HESI-MS parameters were: gas flow rate at 30 arbitrary units; auxiliary gas unit flow rate at 10 arbitrary units; capillary temperature at 250 °C; auxiliary gas heater temperature at 150 °C; spray voltage at 2.8 kV; and S lens RF level at 50%. The automatic gain control was set to a maximum injection time of 200 ms. Negative HESI-MS spectra yield the singly deprotonated ion, $[M-H]^-$, at the same time as the mode FULL-SCAN and t-SIM (targeted Selected Ion Monitoring), to increase sensitivity. The total UHPLC-HESI-MS method runtime was 60 min. Detection was based on calculated exact mass and on retention time of target compounds. The detection was evaluated by Quan/Qual browser Xcalibur 3.0 (Thermo Fisher Scientific, San Jose, CA, USA). Linearity of the MS response was verified with solutions containing all standards at six different concentration levels from 0.250 to 5 ppm. Each point of the calibration graph corresponded to the average of five independent injections.

Standard solutions of phenolic compounds

Reference phenolic stock solutions (caffeic acid, cinnamic acid, ferulic acid, gallic acid, p-coumaric acid, p-hydroxybenzoic acid, syringic acid, vanillic acid, apigenin, apigenin 7-glucoside, diosmetin, hydroxytyrosol, tyrosol, luteolin, oleuropein, vanillin and pinoresinol) were prepared individually at a concentration of approximately 0.1 mg mL^{-1} by dissolving approximately 10 mg of each standard in 20 mL of 80:20 MeOH/H_2O (v/v). A standard mix solution at 5 ppm was prepared mixing 1 mL of each individual standard solution with 100 mL volumetric flask and diluting with methanol up to the mark. The other diluted solutions (at 2.5, 1.0, 0.5, 0.25 ppm) were prepared by dilution of the standard mix. All solutions were corrected for purity and no internal standard was used in this study. Calibration curves were constructed by injecting standard mix solutions at six different concentration levels in quadruplicate. The peak areas were calculated and plotted against the corresponding

concentrations of the standard compounds using linear regression (least squares) to generate standard curves.

Electron paramagnetic resonance spectroscopy assay.

To test the antioxidant capacity and the potential of free radical formation after cooking in oil samples, the electron paramagnetic resonance (EPR) spectroscopy with spin trapping technique has been applied (Skoutasa D. et al. 2001, Ottaviani M.F. et al. 2001, Papadimitriou V. et al. 2006, Ricca M. et al. 2012), using PBN as spin traps. EPR measurements were carried out at room temperature using a Bruker e-Scan Food Analyzer spectrometer (Bruker Biospin GMBH, Rheinstetten, Germany) operated at 9.8 GHz (X-band), microwave power 3.2 mW and modulation amplitude 0.1 mT. Before treatment at 70 °C, 1 g of EVOO samples with 3 mg of spin trapper (PBN) was added to react with free radicals as they formed during the incubation period. After cooling down to 25 °C, the spectra were detected every 30 min up to an oxidation period of 180 min (Figure S1, supplementary data).

Fatty acids analysis

Fatty acids of oil samples were determined as methyl esters by gas-chromatography/mass spectrometry. Fatty acid methyl esters were prepared by alkaline trans-methylation. Aliquots of 0.1 g of sample were diluted in 1 ml of n-heptane and manually agitated for 10 s. Afterwords, 0.1 ml of a 2N KOH solution in MeOH was added and mixed in vortex for 2 min. After the solution turned clear and transparent, 500 µl of the upper phase, containing fatty acid methyl esters was decanted, diluted with n-heptane to a final volume of 1 ml and analysed in GC-MS within 12 hours from preparation. GC-MS analyses were carried out using a Thermo Scientific DSQ II single quadrupole system in EI (Electron Ionization)

mode, working in full scan. The temperature of ion source and injector were 260 °C and 270 °C, respectively. A ZB-WAX capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness, Phenomenex, Italy) was used. The column temperature was programmed to start at 165 °C for 10 min, to increase by 1.5 °C/min up to 200 °C and by 10 °C/min up to 250 °C, and finally to remain for 20 min under isothermal conditions. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. A sample of 1μl was injected with a split ratio of 1:100. The ion source temperature was 260 °C, the MS transfer line temperature was 265 °C and the injector temperature was 270 °C. Ionization voltage was 70eV and the mass range scanned was 35-550 m/z. Peak areas of 16 fatty acids and their quantification were performed using Thermo Scientific Xcalibur Data system software for Windows. Chemical identification of fatty acid methyl esters was carried out using mass spectrum libraries (NIST/EPA/NIH mass spectral Library 2.0) and a 37-component fatty acid methyl esters mix purchased from Supelco #47885-U, (Sigma Aldrich Milan, Italy). Triplicate samples of olive oil were used for each genotype. Each fatty acid was expressed as the percentage of total fatty acids.

Sensory evaluation

Sensory evaluation of the oils was performed according to the panel test method (EUC, 1991) by panelists from the Regional Office for Agriculture, Rural Development, and Mediterranean Fishing (Assessorato Regionale dell'Agricoltura, dello Sviluppo Rurale e della Pesca Mediterranea), located in Sciacca, Italy. Olive oils have been classified according to the intensity and perception of the positive attributes (fruity, bitter and pungent) as *Robust* (median of at least one attribute is more than 6), *Medium* (median of at least one attribute is between 3 and 6) and *Delicate* (median of attributes is less than 3).

Data analysis

Analysis of means (ANOM) was used to establish differences among genotypes on their yield traits, olive oil content of total and individual phenolic compounds, as well as fatty acid composition; upper and lower decision limits were plotted and used to show differences of cultivar/accession means from the grand mean. Principal component analysis (PCA) was carried out using the biplot technique to study the relationship among chemical composition and sensory traits of olive oils from different genotypes; cluster analysis on standardized components was used to separate groups of genotypes based on similar properties.

Results and Discussion

According to the limits of free acidity, peroxide value, fatty acid composition and sensory evaluation imposed by the International Olive Oil Council (IOOC 2016), all oils studied were classified as EVOOs (Tabs. 2 and 3).

Maximum oil yield was 14.95 % for CE, while the minimum was 1.64 % for EBN. BTTG and NR presented values of 3.57 and 3.99 %, respectively (Tab. 3). Free acidity ranged from a minimum of 0.24 % in AQ to a maximum of 0.59 % in OLM, while peroxide values ranged from a minimum of 0.49 meq O_2 kg^{-1} in NR to a maximum of 7.59 meq O_2 kg^{-1} in KO (Tab. 3).

Fruit yield ranged from 3.07 t ha^{-1} in CAR to 12.51 t ha^{-1} in KO (Tab. 4). ABS, BL, BTTG, CVL, KO, NE and PRC exhibited above-average yields per tree, whereas BLC, CAR, CE, CRS, GF, MO, NB, and NM exhibited below-average yields per tree (Tab. 4). Yield efficiency ranged from 0.03 kg cm^{-2} for CAR to 0.19 kg cm^{-2} for CL. ABS, BL, BTTG, CL, CVL, KO, and PRC exhibited above-average yield efficiency, whereas BLC, CAR, EBN, GF, MO, NB, and NM exhibited below-average yield efficiency (Tab. 4). Fruit yield, yield efficiency and oil yield were in the same range as previous studies from the same production

region, where KO showed the highest yield efficiency, oil and fruit yield among the international cultivars (Marino et al. 2017). Fruit was harvested in the months of October and November and fruit maturity index of the 23 genotypes ranged from 1.27 (NE) to 3.68 (CRS), with BL, CL, CRS, GF, MNT, MO, NR, OLM, and VDA showing above-average maturity index and AQ, BTTG, CE, EBN, NA, NB, NE, NM, and PRC showing below-average maturity index. No significant correlation was found between maturity index and any of the production or quality parameters. Chlorophyll content ranged from 2.23 mg kg^{-1} in AQ to 8.11 mg kg^{-1} in CAR, while carotenoids ranged from 2.43 mg kg^{-1} to 7.79 mg kg^{-1} in AQ and CRS, respectively (Table 4). Both chlorophyll and carotenoid contents of all genotypes were within decision limits indicating no significant difference among genotypes. In general, however, this study confirms previous findings on Sicilian genotypes by Mineo et al. (2007) were CL and MNT stood out for their relatively high content in pigments.

Total phenols measured by the Folin-Ciocalteu colorimetric method ranged from 148 mg kg^{-1} in GF to 630 mg kg^{-1} in EBN, with BTTG, CAR, CRS, MNT, NA, NM, PRC and VDA showing above-average total phenols and AQ, ABS, BL, BLC, GF, KO, MO, NR, and OLM showing below-average total phenols (Table 4). Values for this parameter were higher than those previously found in olive oils from Southern Italy (Barbarisi et al. 2014; Marino et al. 2017) showing significant variability of phenol content among cultivars and harvest seasons. A significant correlation was found ($R^2 = 0.643$, $p < 0.001$) between the total phenols with the Folin-Ciocalteu colorimetric method (Table 4) and the sum of phenols identified by UHPLC-HESI-MS (Table 5). Results of both methods were not exactly the same due to a low specificity of Folin-Ciocalteu reagent that, particularly in water–methanol extracts, reacts in presence of other non-phenolic compounds, such as proteins (Lowry et al. 1951). In particular, total phenol content identified by UHPLC-HESI-MS varied greatly among genotypes, ranging from 162.89 mg kg^{-1} in BL to 791.83 mg kg^{-1} in EBN (Table 5).

Two major genotypes NB and NE, together with minor genotypes, CL, CAR, CRS, EBN, MNT, NM, OLM, PRC and VDA showed above-average contents of total phenols. On the other hand, the international cultivars, KO, ABS and AQ together with the major Sicilian cultivar BL, BLC, CE, MO and the Sicilian minor genotypes CVL, GF, NA and NR had below average contents of total phenols.

The secoiridoids identified as the dialdehydic forms of decarboxymethyloleuropein and ligstroside aglycones (3,4-DHPEA-EDA, or oleacin, and p-HPEA-EDA, or oleocanthal, respectively) and the aldehydic forms of oleuropein and ligstroside aglycones (3,4-DHPEA-EA, and p-HPEA-EA, respectively) were the most abundant phenolic compounds found in the samples (Tab. 5). Among all phenols present in the oil, oleuropein derivatives have the strongest antioxidant activity (Artajo et al., 2006). In this study, the sum of ligstroside derivatives (p-HPEA-EA and p-HPEA-EDA) ranged from 6.90 mg kg^{-1} in GF to 136.01 mg kg^{-1} in VDA, with CAR, CRS, EBN, NB, NE, NM, OLM, PRC, and VDA showing above-average contents and AQ, ABS, BL, BLC, BTTG, CL, CVL, CE, GF, KO, MNT, and MO showing below-average contents (Tab. 5). The sum of oleuropein derivatives (3,4-DHPEA-EA and 3,4-DHPEA-EDA) ranged between 69.03 mg kg^{-1} in GF and 347.01 mg kg^{-1} in EBN, with CL, CAR, CRS, EBN, NE, NM, OLM, and VDA showing above-average contents and AQ, ABS, BL, BLC, BTTG, CE, GF, KO, MO, NA, NR and NB, showing below-average contents (Tab. 5). Thus, the sum of secoiridoids (sum of ligstroside and oleuropein derivatives) ranged between 75.93 mg kg^{-1} in GF and 423.70 mg kg^{-1} in EBN, with CAR, CRS, MNT, NB, NE, NM, OLM, PRC, and VDA showing above-average contents and AQ, ABS, BL, BLC, BTTG, CVL, CE, GF, KO, MO, NA, and NR showing below-average contents (Tab. 5). Phenolic acids (sum of ferrulic, caffeic, p-coumeric, vanillic and p-hydroxybenzoic acids) ranged from 0.58 mg kg^{-1} in PRC to 8.70 mg kg^{-1} in NE, with GF, MO, NR, and NB showing above-average contents and ABS, BLC, CAR, CE, CRS, KO,

MNT, NM, OLM, and PRC showing below-average contents (Tab. 5). Flavonoids (apigenin, apigenin 7-glucoside, diosmetin and luteolin) ranged from 1.52 mg kg^{-1} in CE to 10.51 mg kg^{-1} in NM and PRC, with ABS, GF, MO, NA, NR, NE, NM, OLM, PRC, and VDA showing above-average flavonoid contents and AQ, BL, BLC, BTTG, CL, CAR, CVL, CE, CRS, EBN and KO showing below-average flavonoid contents (Tab. 5). Simple phenols, representing the sum of tyrosol and hydroxytyrosol, ranged from 3.40 mg kg^{-1} in BL to 73.5 mg kg^{-1} in EBN, with BTTG, CAR, CE, GF, MO, NB, PRC, and VDA showing above-average contents and AQ, ABS, BL, BLC, CL, CVL, KO, MNT, NA, NR, NE, NM, and OLM showing below-average contents (Tab. 5). Pinoresinol, belonging to the lignans family, ranged from 48.19 mg kg^{-1} in BL to 328.04 mg kg^{-1} in MNT, with BTTG, CAR, CL, CRS, EBN, MNT, NB, NE, and VDA showing above-average contents and AQ, ABS, BL, BLC, CVL, GF, KO, MO, NR, NM, and OLM showing below-average contents (Tab. 5). The EC Regulation 432/2012 established a health claim on the phenolic compounds concentration for EVOO, which states that, to provide a protective effect on human health, EVOO, should contain at least 5 mg of phenols per 20 g oil, corresponding to 250 mg kg^{-1}. The phenolic compounds contributing to these values are oleuropein and ligstroside derivatives, hydroxytyrosol and tyrosol (Tsimidou et al. 2018). The analysis of means in this study indicated a grand mean (267 mg kg^{-1}) very close to this threshold and differences among genotypes according to decision limits entirely agreed with the indications of EC Regulation 432/2012. According to our measurements, the genotypes that fulfill the EU health claim for the minimum concentration of phenolic compounds were the major genotypes NB and NE and the minor genotypes CAR, CRS, EBN, MNT, NM, OLM, PRC, and VDA, whereas all the remaining 15 genotypes did not meet the minimum requirements for the EU health claim (Tab. 5).

Figure S1 shows the EPR spectra obtained for an oil sample of ABQ with PBN untreated and after 120 min of thermal treatment at 70°C are shown. No EPR signal was detected before thermal treatment, indicating that free radicals, if any, were present at very low concentration in the sample. On the contrary, the typical EPR spectrum related to PBN spin trap was easily detected after thermal treatment (Fig. S1). To analyse the trends over time of the process and the amount of formed radicals, the intensity of peak-to-peak (H) of EPR spectrum for the 23 analysed oils was plotted against thermal treatment time (from 0 to 180 min). Figure S2 shows the EPR signal intensity (H) as a function of thermal treatment time of some analysed oils. The signal intensity (H) of central line increased as a function of the incubation time and therefore of the free radicals produced during thermal treatment and then trapped by the PBN molecule (Fig. S2). Similar results were found in all tested oils, indicating that the same processes occur.

Since free radical formation may be due to both thermal oxidation of phenols and amount of peroxides, maximum H values (Tab. 6) must be associated with a combination of ∑ phenolic compounds (Tab. 5) and peroxide values (Tab. 3); these values should provide an indication of maximum potential oxidation of oils after cooking. As a result, oils with high EPR signal H (Tab. 6) due to high levels of both ∑ phenolic compounds and peroxides, such as OLM and MNT, but also those having high levels of ∑ phenolic compounds and low levels of peroxides (CAR, CVL, EBN, CRS and VDA) should generate high amounts of free radicals after cooking. On the contrary, oils with low EPR signal H, such as NR, BTTG, NA and NM, should be more stable and produce a relatively low amount of free radicals after cooking.

The major fatty acids were oleic (C18:1), linoleic (C18:2) and palmitic (C16:0) acids (Tab. 2). Palmitoleic (C16:1), linolenic (C18:3), stearic (C18:0), arachidic (C20:0), behenic (C22:0), eicosenoic (C20:1), myristic (C14:0), lignoceric (C24:0) and heptadecenoic (C17:0)

acids were detected in little amounts. Moreover, the sum of saturated fatty acids (SFA; myristic acid, C14:0; palmitic acid, C16:0; heptadecanoic acid, C17:0; stearic acid, C18:0; arachidonic acid, C20:0; behenic acid, C22:0; and lignoceric acid, C:24:0) ranged from 12.83 % in CE to 19.41% in NA, with AQ, BTTG, MNT, MO, NA, and VDA showing above-average values and ABS, BLC, CE, KO, NM, and PRC showing below-average values (Tab. 7). The sum of unsaturated fatty acids (UFA; palmitoleic acid, C16:1; heptadecenoic acid, C17:1; oleic acid, C18:0; linoleic acid, C18:2; linolenic acid, C18:3; and eicosenoic acid, C20:1) ranged from 80.19 % in MNT to 87.22 % in CE, with ABS, BLC, KO, NM, and PRC showing above-average values and AQ, MNT, MO, NA, NR, and VDA showing below-average values (Tab. 7). The UFA/SFA ratio ranged from 4.17 in AQ to 6.79 in CE, with ABS, BLC, KO, NM, and PRC showing above-average values and AQ, BTTG, CL, MNT, MO, NA, OLM, and VDA showing below-average values (Tab. 7). The high levels of the sum of monounsaturated fatty acids (MUFA; C16:1, C17:1, C18:1, C20:1) that distinguishes olive oil from other vegetables oils (Inglese et al., 2011) ranged from 61.65 % in MO to 76.52 % in KO, with ABS, BLC, CAR, CE, NR, NM, OLM, and PRC showing above-average values and AQ, BTTG, CL, GF, MNT, MO, NE, OLM, and VDA showing below-average values (Tab. 7). Additionally, the sum of polyunsaturated fatty acids (PUFA; C18:2 and C18:3) ranged from 8.52 % in NR to 19.88 % in MO, with AQ, BL, BTTG, CL, GF, MNT, NB, NE, and VDA showing above-average values and ABS, BLC, CAR, CVL, CE, CRS, KO, NA, NR, NM, OLM, and PRC showing below-average values (Tab. 7). MUFA/PUFA ratio varied among genotypes and is also related to the environmental and growing conditions. Indeed, the decrease of MUFA/PUFA ratio has been related to advanced fruit development and/or high temperatures during fruit growth (Gutiérrez et al. 1999; Ripa et al. 2008; Inglese et al. 2011; Dag et al. 2014). Salvador et al. (1999) showed that lower MUFA/PUFA takes to a faster deterioration of the oil during storage. In this study,

MUFA/PUFA ranged from 3.10 in MO to 8.61 in NR, with ABS, BLC, CAR, CE, KO, NA, NM, OLM, and PRC showing above-average values and AQ, BL, BTTG, CL, GF, MNT, MO, NE, OLM, and VDA showing below-average values (Tab. 7).

The sensory attributes found in olive oil depend on the EVOO phenolic and volatile composition (Aparicio and Morales 1996; Angerosa et al. 2004; Servili et al. 2004). As for sensory attributes, all the examined oils belonged to the EVOO category as defined by the International Olive Oil Council (IOOC 2016). Among the genotypes, EBN and CAR produced "robust" oils, whereas MO and NR produced "delicate" oils (Fig. 2). All the international cultivars and major Sicilian genotypes produced "medium" oils. These results demonstrate the genetic influence on the sensory characteristics of the oils and agree with previous research showing that cultivar is the main factor determining the sensory attributes in EVOO; this may be in part explained by the fact that the activity of the enzymes involved in the lipoxygenase pathway is genetically regulated (Angerosa et al. 2004; Essid et al. 2016). On the other hand, differences of volatile profile during fruit development were attributed to the reduced lipoxygenase activity at advanced stages of fruit maturity (Padilla et al. 2009). In our study, there was no significant correlation across genotypes between the maturity index and sensory scores for the three main attributes, i.e. fruity, bitter and pungent. Absence of differences of maturity can be due to the limited range and lower values of maturity index of our samples compared to previous studies.

When oil yield, chlorophyll content, carotenoids content, $\sum$ phenolic compounds (detected with UHPLC-HESI-MS), fatty acid composition and sensory attributes were considered together, PCA showed that about 79.5% of the variability observed was explained by the first three components. PC1, PC2, PC3 accounted for 31.7, 27.9, 19.9% of total variability, respectively. Cluster analysis on standardized components allowed for the individuation of three main groups associating the 23 genotypes with specific chemical and

sensory properties (Tab. 8). In particular, cluster analysis indicated that the genotypes ABS, BLC, CVL, CE, KO and NM were distinguished from the other genotypes for relatively high oil yield and monounsaturated fatty acids along with the fruity sensory descriptor. A second group included CL, CAR, CRS, EBN, MNT, NA, NE, PRC and VDA distinguished for chlorophyll and carotenoid contents, total phenols and density, persistence, pungent and bitter sensory properties (Tab. 8); these genotypes produced the best quality EVOO among the genotypes in trial. A third group included AQ, BL, BTTG, GF, MO, NR, NB and OLM distinguished for their saturated and polyunsaturated fatty acid composition without any particular sensory descriptor (Tab. 8).

Conclusion

Results show that both major and minor Sicilian olive genotypes may be suitable for production of high quality EVOOs under high density planting. Olive oils extracted from Sicilian genotypes presented equivalent or higher quality than oils produced from international cultivars Arbequina, Arbosana and Koroneiki. In particular, some of the minor genotypes in trial such as CL, CAR, CRS, EBN, MNT, NA, PRC and VDA revealed significantly higher phenolic contents compared to the major Sicilian genotypes and the three international cultivars, suggesting a great potential for improving health attributes of Sicilian olive oils. In particular, oils of NA revealed also a relatively low potential for free radical formation after cooking, suggesting an additional indirect health value for the consumer. Overall, this study indicates that Sicilian genotypes can be used in modern high-density plantings and contribute to spread Sicilian high quality standards and biodiversity into the international olive oil market.

Accumulated data do indicate that olive biophenols, chiefly secoiridoids derivatives, have properties that largely explain the cardioprotective effects of diets where EVOO is the most

prominent added fat. Today, the available evidence on olive biophenols is abundant and scientifically allows suggesting the use of high-quality olive oil as the principal form of dietary fat.

Well-characterized extra virgin olive oils with significantly higher phenolic contents, could be employed as "pharma-nutritional agents" to, e.g., lessen inflammation and improve prognosis of inflammatory diseases.

Of course, results obtained in this study need to be validated by further evaluations of Sicilian EVOOS in different years as olive oil quality is influenced by weather and olive crop load, which typically follows an alternating pattern over consecutive years.

Disclosure statement

The authors report no conflicts of interest.

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EFSA Journal 2011 b ;9(4):2033 ; Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), "anti-inflammatory properties" (ID 1882), "contributes to the upper respiratory tract health" (ID 3468), "can help to maintain a normal function of gastrointestinal tract" (3779), and "contributes to body defences against external agents" (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/2006.

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Table 1. Genotypes considered in this trial. Genotypes are from Sicily (with major or minor distribution and production in the island) or already recognized internationally for high density planting.

Genotypes	Abbreviation	Geographic origin	Category
Arbequina	AQ	Spain	International
Arbosana	ABS	Spain	International
Koroneiki	KO	Greece	International
Biancolilla	BL	West Sicily	Major
Biancolilla Caltabellotta	BLC	West Sicily	Major
Cerasuola	CE	West Sicily	Major
Moresca	MO	South-East Sicily	Major
Nocellara del Belice	NB	West Sicily	Major
Nocellara etnea	NE	East Sicily	Major
Bottone di gallo	BTTG	West Sicily	Minor
Calatina	CL	Center-South Sicily	Minor
Castriciana rapparina	CAR	North-East Sicily	Minor
Cavalieri	CVL	Center-North Sicily	Minor
Crastu	CRS	North Sicily	Minor
Erbano	EBN	West Sicily	Minor
Giarraffa	GF	North-West Sicily	Minor
Minuta	MNT	North-East Sicily	Minor
Nasitana	NA	North-East Sicily	Minor
Nerba	NR	Center-North Sicily	Minor
Nocellara messinese	NM	North-East Sicily	Minor
Olivo di Mandanici	OLM	North-East Sicily	Minor
Piricuddara	PRC	North-West Sicily	Minor
Vaddarica	VDA	North-East Sicily	Minor

Table 2. Quality traits of all olive oils studied and limits imposed by the International Olive Oil Council (IOOC/T.15/NC N. 3/Rev. 11) for classification of extra virgin olive oil. Means and standard deviations (SD) of the 23 genotypes (n=69).

Quality traits	**limits described by IOOC**	**mean ± SD**
Free acidity (%m/m expressed in oleic acid)	≤ 0.8	0.35 ± 0.05
Peroxide value (mEq O2 kg^{-1})	≤ 20	3.96 ± 0.51
K_{232}	≤ 2.50	0.99 ± 0.16
K_{270}	≤ 0.22	0.09 ± 0.03
ΔK	≤ 0.01	0.004 ± 0.001
Fatty acid composition (%)		
Myristic acid	< 0.03	0.01 ± 0.01
Palmitic acid	7.50 - 20.00	13.30 ± 2.19
Palmitoleic acid	0.30 - 3.50	1.69 ± 0.89
Heptadecanoic acid	< 0.40	0.09 ± 0.09
Heptadecenoic acid	< 0.60	0.17 ± 0.14
Stearic acid	0.50 - 5.00	2.23 ± 0.46
Oleic acid	55.00 - 83.00	66.50 ± 5.15
Linoleic acid	2.50 - 21.00	13.60 ± 2.88
Linolenic acid	< 1	0.90 ± 0.18
Arachidic acid	< 0.6	0.46 ± 0.09
Gadoleic acid (eicosenoic)	< 0.5	0.34 ± 0.09
Behenic acid	< 0.20	0.11 ± 0.07
Lignoceric acid	< 0.2	0.07 ± 0.02
Organoleptic characteristics:		Median
Median of the fruity	Me > 0	4.8
Median of defect	Me = 0	0.0

Table 3. Oil yield (%), free acidity (% of oleic acid), peroxide value (meq O_2 kg^{-1}), coefficient of specific extinction at 232 nm (K_{232}), and coefficient of specific extinction at 270 nm (K_{270}) in the olive oils from the 23 genotypes studied. Means ± standard deviations (n=3).

Genotypes	Oil yield	Free acidity	Peroxide value	K_{232}	K_{270}
AQ	8.42	0.24 ± 0.03	5.05 ± 0.51	0.98 ± 0.24	0.09 ± 0.03
ABS	10.01	0.27 ± 0.07	4.63 ± 1.41	1.46 ± 0.45	0.12 ± 0.08
BL	10.82	0.36 ± 0.02	5.85 ± 2.53	1.73 ± 0.40	0.16 ± 0.07
BLC	10.05	0.48 ± 0.19	3.97 ± 0.93	1.24 ± 0.12	0.15 ± 0.07
BTTG	3.57	0.25 ± 0.01	5.50 ± 0.01	1.65 ± 0.01	0.09 ± 0.01
CL	10.92	0.27 ± 0.01	2.97 ± 0.53	1.21 ± 0.12	0.08 ± 0.01
CAR	10.40	0.42 ± 0.01	1.44 ± 0.06	0.74 ± 0.12	0.08 ± 0.01
CVL	14.91	0.36 ± 0.06	1.47 ± 0.07	0.45 ± 0.09	0.05 ± 0.01
CE	14.95	0.32 ± 0.06	1.57 ± 0.12	0.33 ± 0.11	0.05 ± 0.01
CRS	8.45	0.29 ± 0.02	2.42 ± 0.51	0.88 ± 0.11	0.13 ± 0.04
EBN	1.64	0.45 ± 0.03	3.86 ± 0.51	0.64 ± 0.31	0.05 ± 0.06
GF	6.45	0.30 ± 0.03	5.40 ± 0.14	1.62 ± 0.54	0.17 ± 0.01
KO	14.43	0.39 ± 0.13	7.59 ± 0.62	1.57 ± 0.02	0.07 ± 0.01
MNT	5.64	0.31 ± 0.13	4.80 ± 0.61	1.39 ± 0.19	0.12 ± 0.01
MO	4.81	0.43 ± 0.01	3.26 ± 0.59	0.74 ± 0.02	0.05 ± 0.01
NA	6.03	0.28 ± 0.01	5.44 ± 0.01	0.64 ± 0.01	0.05 ± 0.01
NR	3.99	0.31 ± 0.01	0.49 ± 0.01	0.16 ± 0.03	0.02 ± 0.01
NB	8.10	0.39 ± 0.03	2.90 ± 0.35	0.58 ± 0.11	0.09 ± 0.04
NE	10.13	0.34 ± 0.13	2.36 ± 0.45	0.92 ± 0.58	0.08 ± 0.04
NM	14.04	0.32 ± 0.03	2.99 ± 0.01	0.52 ± 0.02	0.06 ± 0.01
OLM	9.23	0.59 ± 0.01	7.47 ± 0.67	1.99 ± 0.01	0.10 ± 0.01
PRC	10.52	0.42 ± 0.01	5.47 ± 1.01	0.64 ± 0.01	0.08 ± 0.01
VDA	8.28	0.30 ± 0.03	4.23 ± 0.14	0.62 ± 0.13	0.11 ± 0.06
average	*8.94*	*0.35 ± 0.04*	*3.96 ± 0.51*	*0.99 ± 0.16*	*0.09 ± 0.02*

Table 4. Means ± standard deviations (n=25) of fruit yield (t ha^{-1}) and yield efficiency (kg cm^{-2}). Means ± standard deviations (n=3) of maturity index (100 fruits per replicate), chlorophyll (mg kg^{-1}), carotenoids (mg kg^{-1}) and total phenols (Folin-Ciocalteau method, mg kg^{-1}) in the olive oils from the 23 genotypes studied. Grand mean (GM), upper (UDL) and lower (LDL) decision limits from analysis of means ($P < 0.05$). Numbers in boldface and italics indicate means above UDL and below LDL, respectively.

Genotypes	Yield	Yield efficiency	Maturity index	Chlorophyll	Carotenoids	Total phenols
AQ	6.39 ± 2.19	0.09 ± 0.04	*2.49 ± 0.10*	2.23 ± 0.62	2.43 ± 1.96	*238 ± 18*
ABS	**9.71 ± 1.69**	**0.12 ± 0.03**	2.58 ± 0.07	8.12 ± 3.36	7.38 ± 2.12	*173 ± 10*
BL	**11.10 ± 3.25**	**0.17 ± 0.02**	**3.17 ± 0.06**	3.01 ± 1.22	3.44 ± 1.43	*175 ± 7*
BLC	*4.15 ± 5.25*	*0.05 ± 0.04*	2.57 ± 0.06	2.40 ± 0.10	3.32 ± 0.21	*296 ± 6*
BTTG	**9.20 ± 5.19**	0.11 ± 0.05	*2.35 ± 0.05*	5.34 ± 2.46	5.20 ± 2.44	**437 ± 17**
CL	8.65 ± 2.26	**0.19 ± 0.08**	**3.47 ± 0.06**	7.80 ± 1.25	6.25 ± 1.98	424 ± 25
CAR	*3.07 ± 1.57*	*0.03 ± 0.02*	2.57 ± 0.06	8.83 ± 2.27	8.11 ± 1.31	**504 ± 21**
CVL	**9.71 ± 3.76**	**0.14 ± 0.06**	2.67 ± 0.58	3.03 ± 2.92	3.07 ± 2.63	416 ± 54
CE	*4.03 ± 2.19*	0.07 ± 0.03	*2.33 ± 0.58*	3.88 ± 2.29	3.82 ± 1.95	343 ± 3
CRS	*4.28 ± 3.44*	0.08 ± 0.04	**3.68 ± 0.07**	9.09 ± 4.65	7.79 ± 2.80	**543 ± 27**
EBN	6.58 ± 4.45	*0.05 ± 0.03*	*2.40 ± 0.01*	3.65 ± 1.40	3.48 ± 1.44	**630 ± 39**
GF	*3.62 ± 2.39*	*0.06 ± 0.04*	**3.01 ± 0.01**	2.97 ± 1.42	3.05 ± 1.52	*148 ± 18*
KO	**12.51 ± 1.52**	**0.17 ± 0.04**	2.67 ± 0.06	6.33 ± 3.31	5.26 ± 3.32	*207 ± 15*
MNT	7.73 ± 2.92	0.07 ± 0.04	**3.03 ± 0.03**	5.90 ± 4.12	5.44 ± 3.16	**604 ± 59**
MO	*3.22 ± 1.49*	*0.04 ± 0.02*	**3.44 ± 0.03**	3.66 ± 1.91	4.30 ± 1.08	*171 ± 13*
NA	6.72 ± 1.73	0.10 ± 0.02	*2.52 ± 0.03*	3.09 ± 2.30	4.77 ± 2.58	**562 ± 23**
NR	7.77 ± 3.10	0.08 ± 0.04	**3.33 ± 0.03**	6.19 ± 0.52	5.74 ± 0.82	*254 ± 22*
NB	*3.81 ± 1.62*	*0.05 ± 0.02*	*2.40 ± 0.09*	4.94 ± 0.56	4.30 ± 0.39	377 ± 28
NE	**8.97 ± 4.34**	0.09 ± 0.04	*1.27 ± 0.02*	4.93 ± 3.29	4.78 ± 2.90	330 ± 18
NM	*4.52 ± 1.63*	*0.06 ± 0.01*	*2.50 ± 0.53*	7.07 ± 2.74	5.37 ± 2.22	**604 ± 3**
OLM	7.64 ± 1.37	*0.05 ± 0.02*	**3.13 ± 0.06**	4.29 ± 2.67	3.86 ± 2.05	*298 ± 35*
PRC	**9.94 ± 2.42**	**0.12 ± 0.04**	*2.53 ± 0.06*	7.21 ± 3.82	6.14 ± 2.98	**466 ± 51**
VDA	8.46 ± 3.53	*0.06 ± 0.02*	**3.40 ± 0.10**	8.94 ± 1.62	6.96 ± 0.87	**522 ± 56**
GM	7.03 ± 2.75	0.09 ± 0.03	2.76 ± 0.12	5.34 ± 2.21	4.97 ± 1.92	379 ± 28
UDL	8.68	0.11	2.97	9.29	8.41	429
LDL	5.38	0.07	2.55	1.39	1.53	329

Table 5. Content (mg kg^{-1}) of ligstroside and oleuropein derivatives, secoiridoids (∑ ligstroside and oleuropein derivatives), phenolic acids, flavonoids (∑ apigenin, apigenin 7-glucoside, luteolin, diosmetin), simple phenols (sum of tyrosol and hydroxyltyrosol), pinoresinol and ∑ phenolic compounds in the olive oils from the 23 genotypes studied and detected by UHPLC-HESI-MS. Health claim value is given by the sum of secoiridoids and simple phenols (threshold at 250 mg kg^{-1}). Means ± standard deviation (n=3). Grand mean (GM), upper (UDL) and lower (LDL) decision limits from analysis of means ($P < 0.05$). Numbers in boldface and italics indicate means above UDL and below LDL, respectively.

Genotypes	Ligstroside derivatives	Oleuropein derivatives	Secoiridoids	Phenolic acids	Flavonoids	Simple phenols	Pinoresinol	∑ phenolic compounds	Health claim value
AQ	*34.43 ± 0.15*	*161.22 ± 1.28*	*195.65 ± 1.36*	2.16 ± 0.82	*2.72 ± 0.16*	*15.30 ± 0.61*	*53.11 ± 0.38*	*268.94*	*210.95*
ABS	*40.81 ± 0.02*	*166.51 ± 1.73*	*207.32 ± 1.71*	*1.11 ± 0.04*	**6.72 ± 0.50**	*18.70 ± 0.63*	*64.32 ± 1.23*	*298.17*	*226.02*
BL	*18.14 ± 0.23*	*88.00 ± 5.72*	*106.14 ± 5.84*	2.39 ± 0.07	*2.77 ± 0.09*	*3.40 ± 0.81*	*48.19 ± 0.29*	*162.89*	*109.54*
BLC	*15.52 ± 0.58*	*102.14 ± 5.70*	*117.66 ± 5.59*	*1.20 ± 0.05*	*3.03 ± 0.04*	*8.70 ± 0.55*	*111.09 ± 5.72*	*241.68*	*126.36*
BTTG	*25.26 ± 0.94*	*166.11 ± 0.73*	*191.37 ± 0.36*	2.75 ± 1.03	*4.63 ± 0.06*	**37.71 ± 0.64**	**220.11 ± 0.41**	456.57	*229.08*
CL	*31.71 ± 0.07*	**201.21 ± 2.37**	232.92 ± 2.30	2.81 ± 0.059	*4.24 ± 0.03*	*10.81 ± 0.35*	**258.03 ± 5.49**	**508.81**	*243.73*
CAR	**62.57 ± 1.53**	**315.43 ± 4.94**	**378.00 ± 4.98**	*1.03 ± 0.07*	*2.96 ± 0.29*	**43.41 ± 1.05**	**312.41 ± 0.65**	**737.81**	**421.41**
CVL	*34.91 ± 0.03*	189.21 ± 5.46	*224.12 ± 5.46*	1.84 ± 0.58	*4.64 ± 0.21*	*12.79 ± 0.11*	*166.10 ± 1.13*	*409.49*	*236.91*
CE	*18.63 ± 0.25*	*85.01 ± 1.03*	*103.64 ± 0.82*	*1.13 ± 0.04*	*1.52 ± 0.06*	**30.33 ± 0.99**	179.03 ± 0.99	*315.65*	*133.97*
CRS	**58.04 ± 0.07**	**226.13 ± 4.52**	**284.17 ± 4.46**	*1.27 ± 0.06*	*2.45 ± 0.01*	25.62 ± 0.66	**278.00 ± 0.54**	**591.51**	**309.79**
EBN	**76.69 ± 0.07**	**347.01 ± 5.95**	**423.70 ± 5.89**	2.25 ± 0.05	*2.27 ± 0.01*	**73.50 ± 0.56**	**290.11 ± 0.38**	**791.83**	**497.20**
GF	*6.90 ± 0.58*	*69.03 ± 5.77*	*75.93 ± 5.47*	**5.15 ± 0.68**	**7.38 ± 0.03**	**27.20 ± 0.51**	*81.12 ± 1.02*	*196.78*	*103.13*
KO	*30.91 ± 0.31*	*92.12 ± 1.50*	*123.03 ± 1.30*	*0.64 ± 0.09*	*1.61 ± 0.04*	*15.72 ± 0.44*	*78.59 ± 0.61*	*219.59*	*138.75*
MNT	*42.89 ± 0.70*	**265.01 ± 4.14**	**307.90 ± 4.71**	*1.75 ± 0.80*	5.89 ± 0.33	*15.91 ± 0.55*	**328.04 ± 0.30**	**659.49**	**323.81**
MO	*10.19 ± 0.54*	*136.22 ± 0.50*	*146.41 ± 0.94*	**3.71 ± 0.13**	**8.15 ± 0.61**	**28.01 ± 0.32**	*94.65 ± 0.11*	*280.93*	*174.42*
NA	53.48 ± 1.17	*108.43 ± 1.02*	*161.91 ± 0.19*	1.98 ± 0.63	**9.70 ± 1.06**	*21.89 ± 0.54*	181.06 ± 8.79	*376.54*	*183.80*
NR	55.33 ± 0.31	*112.01 ± 1.44*	*167.34 ± 1.13*	**8.20 ± 0.05**	**7.76 ± 0.70**	*9.90 ± 0.63*	*142.32 ± 5.79*	*335.52*	*177.24*
NB	**77.12 ± 0.46**	*175.07 ± 2.42*	**252.19 ± 2.33**	**5.34 ± 0.02**	5.37 ± 0.34	**33.29 ± 0.38**	**287.11 ± 3.24**	**583.30**	**285.48**
NE	**96.19 ± 4.81**	**232.24 ± 5.45**	**328.43 ± 5.06**	**8.70 ± 3.44**	**8.34 ± 0.11**	*14.35 ± 0.07*	**280.32 ± 49.7**	**640.14**	**342.78**
NM	**123.09 ± 0.06**	**280.17 ± 6.17**	**403.26 ± 6.11**	*0.61 ± 0.04*	**10.51 ± 0.17**	*11.12 ± 0.16*	*167.24 ± 10.6*	**592.74**	**414.38**

OLM	**76.64 ± 0.31**	**280.07 ± 7.06**	**356.71 ± 7.37**	*1.29 ± 0.02*	**8.26 ± 1.79**	*14.57 ± 0.11*	*161.12 ± 0.63*	**541.95**	**371.28**
PRC	**87.55 ± 2.38**	181.01 ± 1.55	**268.56 ± 3.00**	*0.58 ± 0.06*	**10.48 ± 0.04**	**63.04 ± 0.44**	192.43 ± 0.39	**535.09**	**331.60**
VDA	**136.09 ± 0.03**	**278.11 ± 5.16**	**414.20 ± 5.15**	1.93 ± 1.36	**8.40 ± 1.20**	**62.73 ± 2.32**	**262.02 ± 2.25**	**749.28**	**476.93**
GM	52.70 ± 0.68	185.01 ± 3.55	237.70 ± 3.54	2.60 ± 0.44	5.64 ± 0.34	26.02 ± 0.56	184.00 ± 4.38	455.94	263.72
UDL	53.90	191	244	3.40	6.26	27.0	192	473.56	274
LDL	51.50	179	232	1.80	5.02	24.9	176	438.22	259

Table 6. H value (decreasing order) measured at 180 minutes (Hmax) for 23 monovarietal EVOOs.

Accession	Hmax
EBN	798600
OLM	757900
CAR	673500
CVL	642900
CRS	600200
MNT	580400
VDA	575700
PRC	487903
BL	482900
ABQ	461044
ABS	461000
MO	428000
NE	414388
GF	406107
KLT	406000
NB	400300
CE	398300
BLC	398100
KO	393700
NR	294196
BTTG	290100
NA	286500
NM	266820

Table 7. Percentage of saturated fatty acids (SFA), unsaturated fatty acids (UFA), ratio of saturated and unsaturated fatty acids (UFA/SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), ratio of mono and polyunsaturated fatty acids (MUFA/PUFA) in the olive oils from the 23 genotypes studied. Means ± standard deviations (n=3). Grand mean (GM), upper (UDL) and lower (LDL) decision limits from analysis of means ($P < 0.05$). Numbers in boldface and italics indicate means above UDL and below LDL, respectively.

Genotypes	SFA	UFA	UFA/SFA	MUFA	PUFA	MUFA/PUFA
AQ	**19.32 ± 0.41**	*80.69 ± 0.41*	*4.17 ± 0.11*	*62.93 ± 0.26*	**17.82 ± 0.64**	*3.53 ± 0.143*
ABS	*14.52 ± 0.11*	**85.53 ± 1.10**	**5.89 ± 0.11**	**73.51 ± 1.07**	*11.95 ± 0.06*	**6.15 ± 0.082**
BL	15.91 ± 0.04	84.12 ± 0.04	5.29 ± 0.01	67.84 ± 0.11	**16.30 ± 0.14**	*4.16 ± 0.042*
BLC	*14.39 ± 0.49*	**85.19 ± 0.09**	**5.92 ± 0.19**	**71.92 ± 0.04**	*13.34 ± 0.12*	**5.39 ± 0.052**
BTTG	**18.19 ± 0.98**	82.78 ± 0.14	*4.55 ± 0.24*	*63.29 ± 0.16*	**19.47 ± 0.10**	*3.25 ± 0.022*
CL	17.73 ± 0.31	82.65 ± 0.76	*4.66 ± 0.12*	*67.02 ± 0.63*	**15.58 ± 0.65**	*4.30 ± 0.195*
CAR	16.01 ± 1.24	83.41 ± 1.07	5.21 ± 0.43	**70.51 ± 1.19**	*12.84 ± 0.17*	**5.49 ± 0.155**
CVL	16.05 ± 1.37	83.32 ± 1.19	5.19 ± 0.47	69.47 ± 1.11	*13.78 ± 0.40*	5.04 ± 0.164
CE	*12.83 ± 1.42*	**87.22 ± 1.35**	**6.79 ± 0.82**	**73.46 ± 0.92**	*13.65 ± 0.57*	**5.38 ± 0.184**
CRS	17.18 ± 0.90	82.49 ± 0.88	4.81 ± 0.29	68.70 ± 0.80	*13.87 ± 0.25*	4.95 ± 0.097
EBN	17.11 ± 0.48	82.27 ± 0.09	4.81 ± 0.13	67.46 ± 0.02	14.82 ± 0.07	*4.55 ± 0.021*
GF	17.24 ± 0.60	82.48 ± 0.93	4.78 ± 0.12	*63.89 ± 0.85*	**18.57 ± 0.08**	*3.44 ± 0.032*
KO	*13.48 ± 0.07*	**86.39 ± 0.84**	**6.41 ± 0.06**	**76.52 ± 0.82**	*10.00 ± 0.03*	**7.65 ± 0.065**
MNT	**19.04 ± 0.47**	*80.19 ± 0.60*	*4.21 ± 0.08*	*63.12 ± 0.57*	**17.05 ± 0.04**	*3.70 ± 0.029*
MO	**19.22 ± 0.51**	*81.45 ± 0.76*	*4.24 ± 0.08*	*61.65 ± 0.92*	**19.88 ± 0.31**	*3.10 ± 0.086*
NA	**19.41 ± 0.15**	*80.93 ± 0.35*	*4.17 ± 0.02*	67.90 ± 0.40	*13.01 ± 0.08*	**5.22 ± 0.058**
NR	17.09 ± 0.65	*81.90 ± 1.10*	4.79 ± 0.12	**73.42 ± 1.13**	*8.52 ± 0.04*	**8.61 ± 0.162**
NB	16.42 ± 0.51	83.33 ± 0.13	5.07 ± 0.16	69.00 ± 0.14	**14.28 ± 0.02**	4.83 ± 0.016
NE	17.43 ± 0.64	82.45 ± 0.09	4.73 ± 0.17	*66.23 ± 0.41*	**16.11 ± 0.33**	*4.11 ± 0.109*
NM	*14.05 ± 0.55*	**86.48 ± 0.94**	**6.15 ± 0.31**	**75.20 ± 0.90**	*11.25 ± 0.07*	**6.68 ± 0.071**
OLM	17.57 ± 0.10	82.39 ± 0.91	*4.69 ± 0.04*	**70.68 ± 0.94**	*11.76 ± 0.07*	**6.01 ± 0.102**
PRC	*15.27 ± 0.19*	**86.51 ± 1.60**	**5.66 ± 0.09**	**73.71 ± 1.53**	*12.86 ± 0.16*	**5.73 ± 0.112**
VDA	**19.23 ± 0.18**	*80.53 ± 0.17*	*4.18 ± 0.03*	*62.83 ± 0.14*	**17.69 ± 0.03**	*3.55 ± 0.002*
GM	16.67 ± 0.54	83.31 ± 0.68	4.99 ± 0.18	68.71 ± 0.65	13.76 ± 0.19	4.99 ± 0.087
UDL	17.7	84.5	5.40	69.9	14.8	5.15
LDL	15.7	82.1	4.74	67.5	14.2	4.83

Table 8. Standardized component coordinates from biplot and cluster analysis of chemical composition, oil yield and sensory traits in olive oils of the 23 genotypes in trial.

	PC1	PC2	PC3	Cluster
Oil yield	-0.258	3.769	1.312	1
MUFA	-0.881	4.347	-0.733	1
Fruity	1.689	2.312	3.204	1
ABS	-1.196	1.122	-1.525	1
BLC	-2.794	0.589	0.340	1
CVL	0.644	1.097	1.940	1
CE	-2.045	1.538	0.293	1
KO	-0.664	2.512	0.354	1
NM	0.663	2.004	-0.191	1
Chlorophyll	2.301	1.444	-3.492	2
Carotenoid	2.123	1.163	-3.617	2
Total phenols	3.584	-0.416	-1.697	2
Density	3.267	0.418	1.992	2
Persistence	3.420	0.852	1.991	2
Bitter	4.101	-0.572	-0.793	2
Pungent	3.966	0.516	1.388	2
CL	1.686	0.141	-0.071	2
CAR	1.720	0.970	-1.154	2
CRS	1.158	0.091	-1.414	2
EBN	1.583	-1.172	0.068	2
MNT	1.500	-1.265	-0.039	2
NA	1.015	-0.486	0.844	2
NE	1.343	-0.160	0.784	2
PRC	1.711	1.524	0.304	2
VDA	1.474	-1.237	-1.167	2
SFA	1.394	-4.014	-0.044	3
PUFA	0.476	-3.748	1.201	3
AQ	-0.508	-1.407	1.503	3
BL	-0.983	0.028	1.142	3
BTTG	0.563	-1.788	-0.085	3
GF	-1.317	-1.505	0.637	3
MO	-2.319	-2.588	-0.545	3
NR	-1.878	0.132	-1.477	3
NB	-0.091	-0.136	-0.114	3
OLM	-1.265	-0.005	-0.429	3

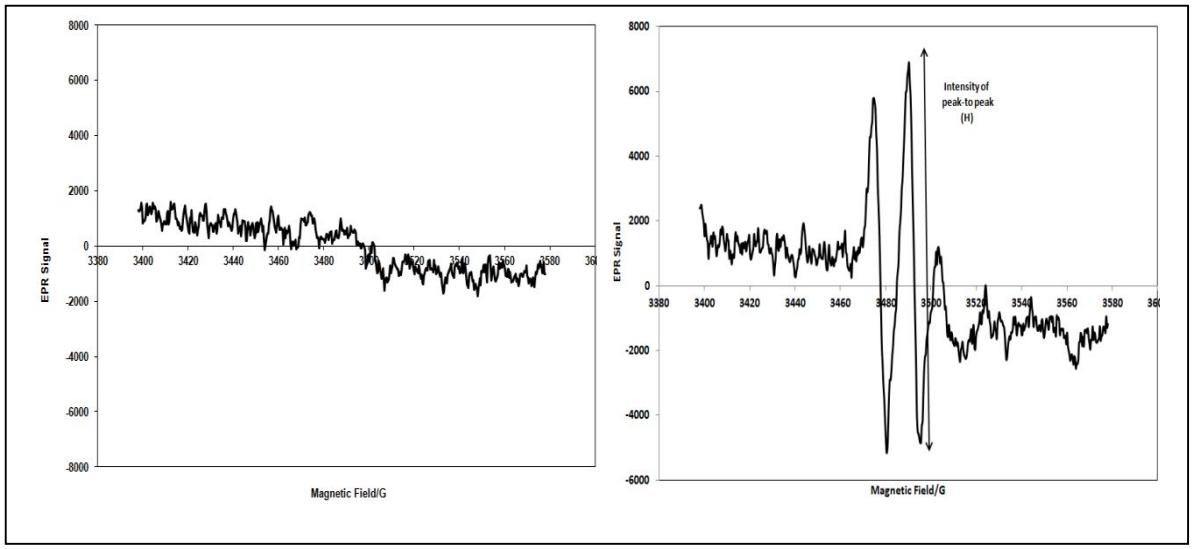

Figure S1. EPR spectra of monovarietal ABQ oil with PBN before and after thermal treatment at 70 °C.

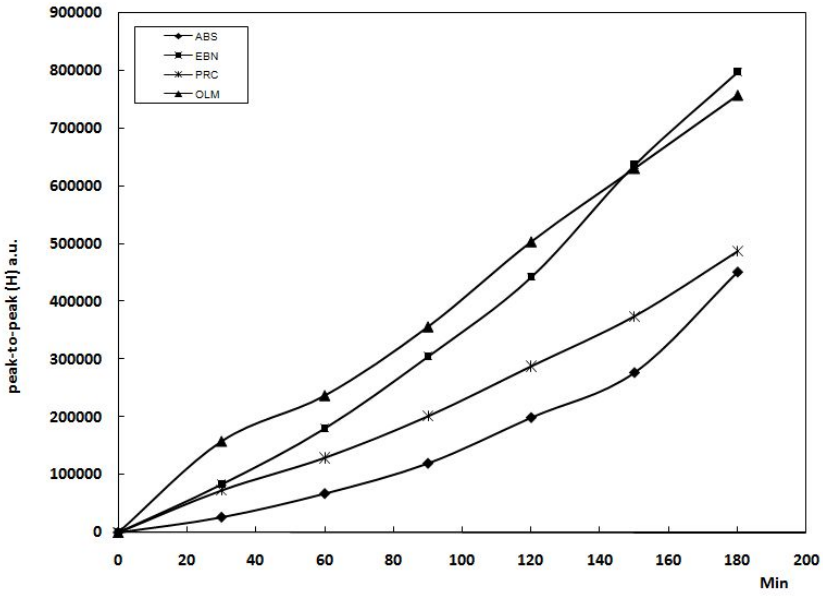

Figure S2. Intensity of peak-to-peak (H) of EPR spectrum as a function of thermal treatment times and Hmax of ABS, EBN, PRC and OLM oils.

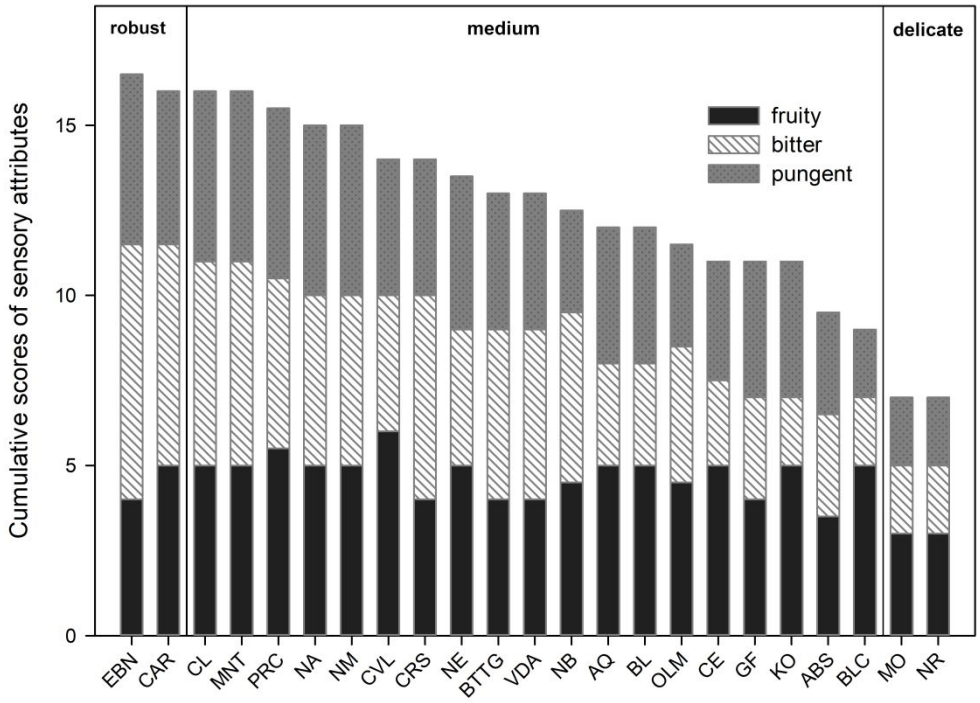

Figure 2. Cumulative scores for fruity (black bars), bitter (white stripe bars) and pungent (grey bars) sensory attributes in olive oils from the 23 genotypes. Solid vertical lines indicate group of oils classified in "robust", "medium", and "delicate" olive oils according to their cumulative sensory scores.

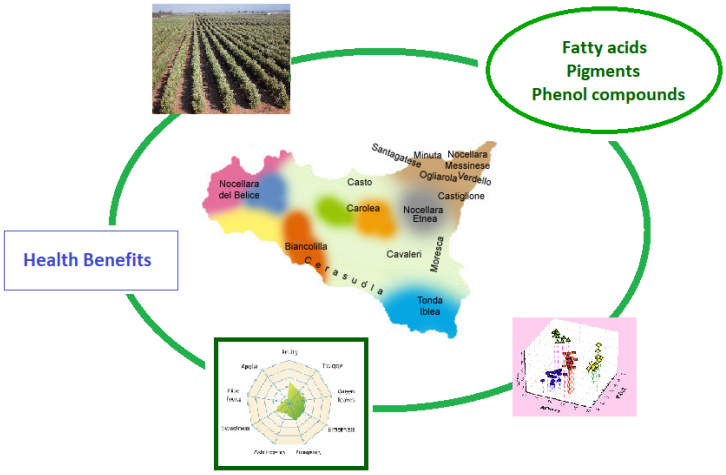
Fatty acids
Pigments
Phenol compounds
Health Benefits
Nocellara del Belice
Casto
Carolea
Biancolilla
Cerasuola
Cavaleri
Santagatese
Minuta
Nocellara Messinese
Ogliarola
Verdello
Castiglione
Nocellara Etnea
Moresca
Tonda Iblea

Table 1. Genotypes considered in this trial. Genotypes are from Sicily (with major or minor distribution and production in the island) or already recognized internationally for high density planting.

Genotypes	Abbreviation	Geographic origin	Category
Arbequina	AQ	Spain	International
Arbosana	ABS	Spain	International
Koroneiki	KO	Greece	International
Biancolilla	BL	West Sicily	Major
Biancolilla Caltabellotta	BLC	West Sicily	Major
Cerasuola	CE	West Sicily	Major
Moresca	MO	South-East Sicily	Major
Nocellara del Belice	NB	West Sicily	Major
Nocellara etnea	NE	East Sicily	Major
Bottone di gallo	BTTG	West Sicily	Minor
Calatina	CL	Center-South Sicily	Minor
Castriciana rapparina	CAR	North-East Sicily	Minor
Cavalieri	CVL	Center-North Sicily	Minor
Crastu	CRS	North Sicily	Minor
Erbano	EBN	West Sicily	Minor
Giarraffa	GF	North-West Sicily	Minor
Minuta	MNT	North-East Sicily	Minor
Nasitana	NA	North-East Sicily	Minor
Nerba	NR	Center-North Sicily	Minor
Nocellara messinese	NM	North-East Sicily	Minor
Olivo di Mandanici	OLM	North-East Sicily	Minor
Piricuddara	PRC	North-West Sicily	Minor
Vaddarica	VDA	North-East Sicily	Minor

Table 2. Quality traits of all olive oils studied and limits imposed by the International Olive Oil Council (IOOC/T.15/NC N. 3/Rev. 11) for classification of extra virgin olive oil. Means and standard deviations (SD) of the 23 genotypes (n=69).

Quality traits	**limits described by IOOC**	**mean ± SD**
Free acidity (%m/m expressed in oleic acid)	≤ 0.8	0.35 ± 0.05
Peroxide value (mEq O2 kg^{-1})	≤ 20	3.96 ± 0.51
K_{232}	≤ 2.50	0.99 ± 0.16
K_{270}	≤ 0.22	0.09 ± 0.03
ΔK	≤ 0.01	0.004 ± 0.001
Fatty acid composition (%)		
Myristic acid	< 0.03	0.01 ± 0.01
Palmitic acid	7.50 - 20.00	13.30 ± 2.19
Palmitoleic acid	0.30 - 3.50	1.69 ± 0.89
Heptadecanoic acid	< 0.40	0.09 ± 0.09
Heptadecenoic acid	< 0.60	0.17 ± 0.14
Stearic acid	0.50 - 5.00	2.23 ± 0.46
Oleic acid	55.00 - 83.00	66.50 ± 5.15
Linoleic acid	2.50 - 21.00	13.60 ± 2.88
Linolenic acid	< 1	0.90 ± 0.18
Arachidic acid	< 0.6	0.46 ± 0.09
Gadoleic acid (eicosenoic)	< 0.5	0.34 ± 0.09
Behenic acid	< 0.20	0.11 ± 0.07
Lignoceric acid	< 0.2	0.07 ± 0.02
Organoleptic characteristics:		Median
Median of the fruity	Me > 0	4.8
Median of defect	Me = 0	0.0

Table 3. Oil yield (%), free acidity (% of oleic acid), peroxide value (meq O_2 kg^{-1}), coefficient of specific extinction at 232 nm (K_{232}), and coefficient of specific extinction at 270 nm (K_{270}) in the olive oils from the 23 genotypes studied. Means ± standard deviations (n=3).

Genotypes	Oil yield	Free acidity	Peroxide value	K_{232}	K_{270}
AQ	8.42	0.24 ± 0.03	5.05 ± 0.51	0.98 ± 0.24	0.09 ± 0.03
ABS	10.01	0.27 ± 0.07	4.63 ± 1.41	1.46 ± 0.45	0.12 ± 0.08
BL	10.82	0.36 ± 0.02	5.85 ± 2.53	1.73 ± 0.40	0.16 ± 0.07
BLC	10.05	0.48 ± 0.19	3.97 ± 0.93	1.24 ± 0.12	0.15 ± 0.07
BTTG	3.57	0.25 ± 0.01	5.50 ± 0.01	1.65 ± 0.01	0.09 ± 0.01
CL	10.92	0.27 ± 0.01	2.97 ± 0.53	1.21 ± 0.12	0.08 ± 0.01
CAR	10.40	0.42 ± 0.01	1.44 ± 0.06	0.74 ± 0.12	0.08 ± 0.01
CVL	14.91	0.36 ± 0.06	1.47 ± 0.07	0.45 ± 0.09	0.05 ± 0.01
CE	14.95	0.32 ± 0.06	1.57 ± 0.12	0.33 ± 0.11	0.05 ± 0.01
CRS	8.45	0.29 ± 0.02	2.42 ± 0.51	0.88 ± 0.11	0.13 ± 0.04
EBN	1.64	0.45 ± 0.03	3.86 ± 0.51	0.64 ± 0.31	0.05 ± 0.06
GF	6.45	0.30 ± 0.03	5.40 ± 0.14	1.62 ± 0.54	0.17 ± 0.01
KO	14.43	0.39 ± 0.13	7.59 ± 0.62	1.57 ± 0.02	0.07 ± 0.01
MNT	5.64	0.31 ± 0.13	4.80 ± 0.61	1.39 ± 0.19	0.12 ± 0.01
MO	4.81	0.43 ± 0.01	3.26 ± 0.59	0.74 ± 0.02	0.05 ± 0.01
NA	6.03	0.28 ± 0.01	5.44 ± 0.01	0.64 ± 0.01	0.05 ± 0.01
NR	3.99	0.31 ± 0.01	0.49 ± 0.01	0.16 ± 0.03	0.02 ± 0.01
NB	8.10	0.39 ± 0.03	2.90 ± 0.35	0.58 ± 0.11	0.09 ± 0.04
NE	10.13	0.34 ± 0.13	2.36 ± 0.45	0.92 ± 0.58	0.08 ± 0.04
NM	14.04	0.32 ± 0.03	2.99 ± 0.01	0.52 ± 0.02	0.06 ± 0.01
OLM	9.23	0.59 ± 0.01	7.47 ± 0.67	1.99 ± 0.01	0.10 ± 0.01
PRC	10.52	0.42 ± 0.01	5.47 ± 1.01	0.64 ± 0.01	0.08 ± 0.01
VDA	8.28	0.30 ± 0.03	4.23 ± 0.14	0.62 ± 0.13	0.11 ± 0.06
average	*8.94*	*0.35 ± 0.04*	*3.96 ± 0.51*	*0.99 ± 0.16*	*0.09 ± 0.02*

Table 4. Means ± standard deviations (n=25) of fruit yield (t ha^{-1}) and yield efficiency (kg cm^{-2}). Means ± standard deviations (n=3) of maturity index (100 fruits per replicate), chlorophyll (mg kg^{-1}), carotenoids (mg kg^{-1}) and total phenols (Folin-Ciocalteau method, mg kg^{-1}) in the olive oils from the 23 genotypes studied. Grand mean (GM), upper (UDL) and lower (LDL) decision limits from analysis of means ($P < 0.05$). Numbers in boldface and italics indicate means above UDL and below LDL, respectively.

Genotypes	Yield	Yield efficiency	Maturity index	Chlorophyll	Carotenoids	Total phenols
AQ	6.39 ± 2.19	0.09 ± 0.04	*2.49 ± 0.10*	2.23 ± 0.62	2.43 ± 1.96	*238 ± 18*
ABS	**9.71 ± 1.69**	**0.12 ± 0.03**	2.58 ± 0.07	8.12 ± 3.36	7.38 ± 2.12	*173 ± 10*
BL	**11.10 ± 3.25**	**0.17 ± 0.02**	**3.17 ± 0.06**	3.01 ± 1.22	3.44 ± 1.43	*175 ± 7*
BLC	*4.15 ± 5.25*	*0.05 ± 0.04*	2.57 ± 0.06	2.40 ± 0.10	3.32 ± 0.21	*296 ± 6*
BTTG	**9.20 ± 5.19**	0.11 ± 0.05	*2.35 ± 0.05*	5.34 ± 2.46	5.20 ± 2.44	**437 ± 17**
CL	8.65 ± 2.26	**0.19 ± 0.08**	**3.47 ± 0.06**	7.80 ± 1.25	6.25 ± 1.98	424 ± 25
CAR	*3.07 ± 1.57*	*0.03 ± 0.02*	2.57 ± 0.06	8.83 ± 2.27	8.11 ± 1.31	**504 ± 21**
CVL	**9.71 ± 3.76**	**0.14 ± 0.06**	2.67 ± 0.58	3.03 ± 2.92	3.07 ± 2.63	416 ± 54
CE	*4.03 ± 2.19*	0.07 ± 0.03	*2.33 ± 0.58*	3.88 ± 2.29	3.82 ± 1.95	343 ± 3
CRS	*4.28 ± 3.44*	0.08 ± 0.04	**3.68 ± 0.07**	9.09 ± 4.65	7.79 ± 2.80	**543 ± 27**
EBN	6.58 ± 4.45	*0.05 ± 0.03*	*2.40 ± 0.01*	3.65 ± 1.40	3.48 ± 1.44	**630 ± 39**
GF	*3.62 ± 2.39*	*0.06 ± 0.04*	**3.01 ± 0.01**	2.97 ± 1.42	3.05 ± 1.52	*148 ± 18*
KO	**12.51 ± 1.52**	**0.17 ± 0.04**	2.67 ± 0.06	6.33 ± 3.31	5.26 ± 3.32	*207 ± 15*
MNT	7.73 ± 2.92	0.07 ± 0.04	**3.03 ± 0.03**	5.90 ± 4.12	5.44 ± 3.16	**604 ± 59**
MO	*3.22 ± 1.49*	*0.04 ± 0.02*	**3.44 ± 0.03**	3.66 ± 1.91	4.30 ± 1.08	*171 ± 13*
NA	6.72 ± 1.73	0.10 ± 0.02	*2.52 ± 0.03*	3.09 ± 2.30	4.77 ± 2.58	**562 ± 23**
NR	7.77 ± 3.10	0.08 ± 0.04	**3.33 ± 0.03**	6.19 ± 0.52	5.74 ± 0.82	*254 ± 22*
NB	*3.81 ± 1.62*	*0.05 ± 0.02*	*2.40 ± 0.09*	4.94 ± 0.56	4.30 ± 0.39	377 ± 28
NE	**8.97 ± 4.34**	0.09 ± 0.04	*1.27 ± 0.02*	4.93 ± 3.29	4.78 ± 2.90	330 ± 18
NM	*4.52 ± 1.63*	*0.06 ± 0.01*	*2.50 ± 0.53*	7.07 ± 2.74	5.37 ± 2.22	**604 ± 3**
OLM	7.64 ± 1.37	*0.05 ± 0.02*	**3.13 ± 0.06**	4.29 ± 2.67	3.86 ± 2.05	*298 ± 35*
PRC	**9.94 ± 2.42**	**0.12 ± 0.04**	*2.53 ± 0.06*	7.21 ± 3.82	6.14 ± 2.98	**466 ± 51**
VDA	8.46 ± 3.53	*0.06 ± 0.02*	**3.40 ± 0.10**	8.94 ± 1.62	6.96 ± 0.87	**522 ± 56**
GM	7.03 ± 2.75	0.09 ± 0.03	2.76 ± 0.12	5.34 ± 2.21	4.97 ± 1.92	379 ± 28
UDL	8.68	0.11	2.97	9.29	8.41	429
LDL	5.38	0.07	2.55	1.39	1.53	329

Table 5. Content (mg kg^{-1}) of ligstroside and oleuropein derivatives, secoiridoids (∑ ligstroside and oleuropein derivatives), phenolic acids, flavonoids (∑ apigenin, apigenin 7-glucoside, luteolin, diosmetin), simple phenols (sum of tyrosol and hydroxyltyrosol), pinoresinol and ∑ phenolic compounds in the olive oils from the 23 genotypes studied and detected by UHPLC-HESI-MS. Health claim value is given by the sum of secoiridoids and simple phenols (threshold at 250 mg kg^{-1}). Means ± standard deviation (n=3). Grand mean (GM), upper (UDL) and lower (LDL) decision limits from analysis of means ($P < 0.05$). Numbers in boldface and italics indicate means above UDL and below LDL, respectively.

Genotypes	Ligstroside derivatives	Oleuropein derivatives	Secoiridoids	Phenolic acids	Flavonoids	Simple phenols	Pinoresinol	∑ phenolic compounds	Health claim value
AQ	*34.43 ± 0.15*	*161.22 ± 1.28*	*195.65 ± 1.36*	2.16 ± 0.82	*2.72 ± 0.16*	*15.30 ± 0.61*	*53.11 ± 0.38*	*268.94*	*210.95*
ABS	*40.81 ± 0.02*	*166.51 ± 1.73*	*207.32 ± 1.71*	*1.11 ± 0.04*	**6.72 ± 0.50**	*18.70 ± 0.63*	*64.32 ± 1.23*	*298.17*	*226.02*
BL	*18.14 ± 0.23*	*88.00 ± 5.72*	*106.14 ± 5.84*	2.39 ± 0.07	*2.77 ± 0.09*	*3.40 ± 0.81*	*48.19 ± 0.29*	*162.89*	*109.54*
BLC	*15.52 ± 0.58*	*102.14 ± 5.70*	*117.66 ± 5.59*	*1.20 ± 0.05*	*3.03 ± 0.04*	*8.70 ± 0.55*	*111.09 ± 5.72*	*241.68*	*126.36*
BTTG	*25.26 ± 0.94*	*166.11 ± 0.73*	*191.37 ± 0.36*	2.75 ± 1.03	*4.63 ± 0.06*	**37.71 ± 0.64**	**220.11 ± 0.41**	456.57	*229.08*
CL	*31.71 ± 0.07*	**201.21 ± 2.37**	232.92 ± 2.30	2.81 ± 0.059	*4.24 ± 0.03*	*10.81 ± 0.35*	**258.03 ± 5.49**	**508.81**	*243.73*
CAR	**62.57 ± 1.53**	**315.43 ± 4.94**	**378.00 ± 4.98**	*1.03 ± 0.07*	*2.96 ± 0.29*	**43.41 ± 1.05**	**312.41 ± 0.65**	**737.81**	**421.41**
CVL	*34.91 ± 0.03*	189.21 ± 5.46	*224.12 ± 5.46*	1.84 ± 0.58	*4.64 ± 0.21*	*12.79 ± 0.11*	*166.10 ± 1.13*	*409.49*	*236.91*
CE	*18.63 ± 0.25*	*85.01 ± 1.03*	*103.64 ± 0.82*	*1.13 ± 0.04*	*1.52 ± 0.06*	**30.33 ± 0.99**	179.03 ± 0.99	*315.65*	*133.97*
CRS	**58.04 ± 0.07**	**226.13 ± 4.52**	**284.17 ± 4.46**	*1.27 ± 0.06*	*2.45 ± 0.01*	25.62 ± 0.66	**278.00 ± 0.54**	**591.51**	**309.79**
EBN	**76.69 ± 0.07**	**347.01 ± 5.95**	**423.70 ± 5.89**	2.25 ± 0.05	*2.27 ± 0.01*	**73.50 ± 0.56**	**290.11 ± 0.38**	**791.83**	**497.20**
GF	*6.90 ± 0.58*	*69.03 ± 5.77*	*75.93 ± 5.47*	**5.15 ± 0.68**	**7.38 ± 0.03**	**27.20 ± 0.51**	*81.12 ± 1.02*	*196.78*	*103.13*
KO	*30.91 ± 0.31*	*92.12 ± 1.50*	*123.03 ± 1.30*	*0.64 ± 0.09*	*1.61 ± 0.04*	*15.72 ± 0.44*	*78.59 ± 0.61*	*219.59*	*138.75*
MNT	*42.89 ± 0.70*	**265.01 ± 4.14**	**307.90 ± 4.71**	*1.75 ± 0.80*	5.89 ± 0.33	*15.91 ± 0.55*	**328.04 ± 0.30**	**659.49**	**323.81**
MO	*10.19 ± 0.54*	*136.22 ± 0.50*	*146.41 ± 0.94*	**3.71 ± 0.13**	**8.15 ± 0.61**	**28.01 ± 0.32**	*94.65 ± 0.11*	*280.93*	*174.42*
NA	53.48 ± 1.17	*108.43 ± 1.02*	*161.91 ± 0.19*	1.98 ± 0.63	**9.70 ± 1.06**	*21.89 ± 0.54*	181.06 ± 8.79	*376.54*	*183.80*
NR	55.33 ± 0.31	*112.01 ± 1.44*	*167.34 ± 1.13*	**8.20 ± 0.05**	**7.76 ± 0.70**	*9.90 ± 0.63*	*142.32 ± 5.79*	*335.52*	*177.24*
NB	**77.12 ± 0.46**	*175.07 ± 2.42*	**252.19 ± 2.33**	**5.34 ± 0.02**	5.37 ± 0.34	**33.29 ± 0.38**	**287.11 ± 3.24**	**583.30**	**285.48**
NE	**96.19 ± 4.81**	**232.24 ± 5.45**	**328.43 ± 5.06**	**8.70 ± 3.44**	**8.34 ± 0.11**	*14.35 ± 0.07*	**280.32 ± 49.7**	**640.14**	**342.78**
NM	**123.09 ± 0.06**	**280.17 ± 6.17**	**403.26 ± 6.11**	*0.61 ± 0.04*	**10.51 ± 0.17**	*11.12 ± 0.16*	*167.24 ± 10.6*	**592.74**	**414.38**

OLM	**76.64 ± 0.31**	**280.07 ± 7.06**	**356.71 ± 7.37**	*1.29 ± 0.02*	**8.26 ± 1.79**	*14.57 ± 0.11*	*161.12 ± 0.63*	**541.95**	**371.28**
PRC	**87.55 ± 2.38**	181.01 ± 1.55	**268.56 ± 3.00**	*0.58 ± 0.06*	**10.48 ± 0.04**	**63.04 ± 0.44**	192.43 ± 0.39	**535.09**	**331.60**
VDA	**136.09 ± 0.03**	**278.11 ± 5.16**	**414.20 ± 5.15**	1.93 ± 1.36	**8.40 ± 1.20**	**62.73 ± 2.32**	**262.02 ± 2.25**	**749.28**	**476.93**
GM	52.70 ± 0.68	185.01 ± 3.55	237.70 ± 3.54	2.60 ± 0.44	5.64 ± 0.34	26.02 ± 0.56	184.00 ± 4.38	455.94	263.72
UDL	53.90	191	244	3.40	6.26	27.0	192	473.56	274
LDL	51.50	179	232	1.80	5.02	24.9	176	438.22	259

Table 6. H value (decreasing order) measured at 180 minutes (Hmax) for 23 monovarietal EVOOs.

Accession	Hmax
EBN	798600
OLM	757900
CAR	673500
CVL	642900
CRS	600200
MNT	580400
VDA	575700
PRC	487903
BL	482900
ABQ	461044
ABS	461000
MO	428000
NE	414388
GF	406107
KLT	406000
NB	400300
CE	398300
BLC	398100
KO	393700
NR	294196
BTTG	290100
NA	286500
NM	266820

Table 7. Percentage of saturated fatty acids (SFA), unsaturated fatty acids (UFA), ratio of saturated and unsaturated fatty acids (UFA/SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), ratio of mono and polyunsaturated fatty acids (MUFA/PUFA) in the olive oils from the 23 genotypes studied. Means ± standard deviations (n=3). Grand mean (GM), upper (UDL) and lower (LDL) decision limits from analysis of means ($P < 0.05$). Numbers in boldface and italics indicate means above UDL and below LDL, respectively.

Genotypes	SFA	UFA	UFA/SFA	MUFA	PUFA	MUFA/PUFA
AQ	**19.32 ± 0.41**	*80.69 ± 0.41*	*4.17 ± 0.11*	*62.93 ± 0.26*	**17.82 ± 0.64**	*3.53 ± 0.143*
ABS	*14.52 ± 0.11*	**85.53 ± 1.10**	**5.89 ± 0.11**	**73.51 ± 1.07**	*11.95 ± 0.06*	**6.15 ± 0.082**
BL	15.91 ± 0.04	84.12 ± 0.04	5.29 ± 0.01	67.84 ± 0.11	**16.30 ± 0.14**	*4.16 ± 0.042*
BLC	*14.39 ± 0.49*	**85.19 ± 0.09**	**5.92 ± 0.19**	**71.92 ± 0.04**	*13.34 ± 0.12*	**5.39 ± 0.052**
BTTG	**18.19 ± 0.98**	82.78 ± 0.14	*4.55 ± 0.24*	*63.29 ± 0.16*	**19.47 ± 0.10**	*3.25 ± 0.022*
CL	17.73 ± 0.31	82.65 ± 0.76	*4.66 ± 0.12*	*67.02 ± 0.63*	**15.58 ± 0.65**	*4.30 ± 0.195*
CAR	16.01 ± 1.24	83.41 ± 1.07	5.21 ± 0.43	**70.51 ± 1.19**	*12.84 ± 0.17*	**5.49 ± 0.155**
CVL	16.05 ± 1.37	83.32 ± 1.19	5.19 ± 0.47	69.47 ± 1.11	*13.78 ± 0.40*	5.04 ± 0.164
CE	*12.83 ± 1.42*	**87.22 ± 1.35**	**6.79 ± 0.82**	**73.46 ± 0.92**	*13.65 ± 0.57*	**5.38 ± 0.184**
CRS	17.18 ± 0.90	82.49 ± 0.88	4.81 ± 0.29	68.70 ± 0.80	*13.87 ± 0.25*	4.95 ± 0.097
EBN	17.11 ± 0.48	82.27 ± 0.09	4.81 ± 0.13	67.46 ± 0.02	14.82 ± 0.07	*4.55 ± 0.021*
GF	17.24 ± 0.60	82.48 ± 0.93	4.78 ± 0.12	*63.89 ± 0.85*	**18.57 ± 0.08**	*3.44 ± 0.032*
KO	*13.48 ± 0.07*	**86.39 ± 0.84**	**6.41 ± 0.06**	**76.52 ± 0.82**	*10.00 ± 0.03*	**7.65 ± 0.065**
MNT	**19.04 ± 0.47**	*80.19 ± 0.60*	*4.21 ± 0.08*	*63.12 ± 0.57*	**17.05 ± 0.04**	*3.70 ± 0.029*
MO	**19.22 ± 0.51**	*81.45 ± 0.76*	*4.24 ± 0.08*	*61.65 ± 0.92*	**19.88 ± 0.31**	*3.10 ± 0.086*
NA	**19.41 ± 0.15**	*80.93 ± 0.35*	*4.17 ± 0.02*	67.90 ± 0.40	*13.01 ± 0.08*	**5.22 ± 0.058**
NR	17.09 ± 0.65	*81.90 ± 1.10*	4.79 ± 0.12	**73.42 ± 1.13**	*8.52 ± 0.04*	**8.61 ± 0.162**
NB	16.42 ± 0.51	83.33 ± 0.13	5.07 ± 0.16	69.00 ± 0.14	**14.28 ± 0.02**	4.83 ± 0.016
NE	17.43 ± 0.64	82.45 ± 0.09	4.73 ± 0.17	*66.23 ± 0.41*	**16.11 ± 0.33**	*4.11 ± 0.109*
NM	*14.05 ± 0.55*	**86.48 ± 0.94**	**6.15 ± 0.31**	**75.20 ± 0.90**	*11.25 ± 0.07*	**6.68 ± 0.071**
OLM	17.57 ± 0.10	82.39 ± 0.91	*4.69 ± 0.04*	**70.68 ± 0.94**	*11.76 ± 0.07*	**6.01 ± 0.102**
PRC	*15.27 ± 0.19*	**86.51 ± 1.60**	**5.66 ± 0.09**	**73.71 ± 1.53**	*12.86 ± 0.16*	**5.73 ± 0.112**
VDA	**19.23 ± 0.18**	*80.53 ± 0.17*	*4.18 ± 0.03*	*62.83 ± 0.14*	**17.69 ± 0.03**	*3.55 ± 0.002*
GM	16.67 ± 0.54	83.31 ± 0.68	4.99 ± 0.18	68.71 ± 0.65	13.76 ± 0.19	4.99 ± 0.087
UDL	17.7	84.5	5.40	69.9	14.8	5.15
LDL	15.7	82.1	4.74	67.5	14.2	4.83

Table 8. Standardized component coordinates from biplot and cluster analysis of chemical composition, oil yield and sensory traits in olive oils of the 23 genotypes in trial.

	PC1	PC2	PC3	Cluster
Oil yield	-0.258	3.769	1.312	1
MUFA	-0.881	4.347	-0.733	1
Fruity	1.689	2.312	3.204	1
ABS	-1.196	1.122	-1.525	1
BLC	-2.794	0.589	0.340	1
CVL	0.644	1.097	1.940	1
CE	-2.045	1.538	0.293	1
KO	-0.664	2.512	0.354	1
NM	0.663	2.004	-0.191	1
Chlorophyll	2.301	1.444	-3.492	2
Carotenoid	2.123	1.163	-3.617	2
Total phenols	3.584	-0.416	-1.697	2
Density	3.267	0.418	1.992	2
Persistence	3.420	0.852	1.991	2
Bitter	4.101	-0.572	-0.793	2
Pungent	3.966	0.516	1.388	2
CL	1.686	0.141	-0.071	2
CAR	1.720	0.970	-1.154	2
CRS	1.158	0.091	-1.414	2
EBN	1.583	-1.172	0.068	2
MNT	1.500	-1.265	-0.039	2
NA	1.015	-0.486	0.844	2
NE	1.343	-0.160	0.784	2
PRC	1.711	1.524	0.304	2
VDA	1.474	-1.237	-1.167	2
SFA	1.394	-4.014	-0.044	3
PUFA	0.476	-3.748	1.201	3
AQ	-0.508	-1.407	1.503	3
BL	-0.983	0.028	1.142	3
BTTG	0.563	-1.788	-0.085	3
GF	-1.317	-1.505	0.637	3
MO	-2.319	-2.588	-0.545	3
NR	-1.878	0.132	-1.477	3
NB	-0.091	-0.136	-0.114	3
OLM	-1.265	-0.005	-0.429	3

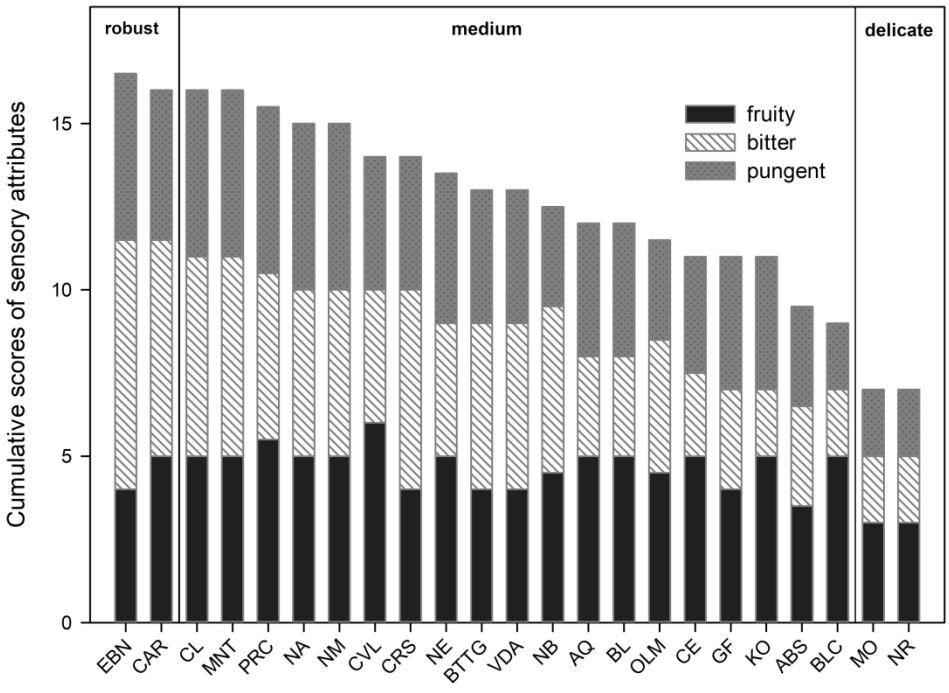

Figure 1. Cumulative scores for fruity (black bars), bitter (white stripe bars) and pungent (grey bars) sensory attributes in olive oils from the 23 genotypes. Solid vertical lines indicate group of oils classified in "robust", "medium", and "delicate" olive oils according to their cumulative sensory scores.

146x118mm (600 x 600 DPI)

Figure S1. EPR spectra of monovarietal ABQ oil with PBN before and after thermal treatment at 70 °C.

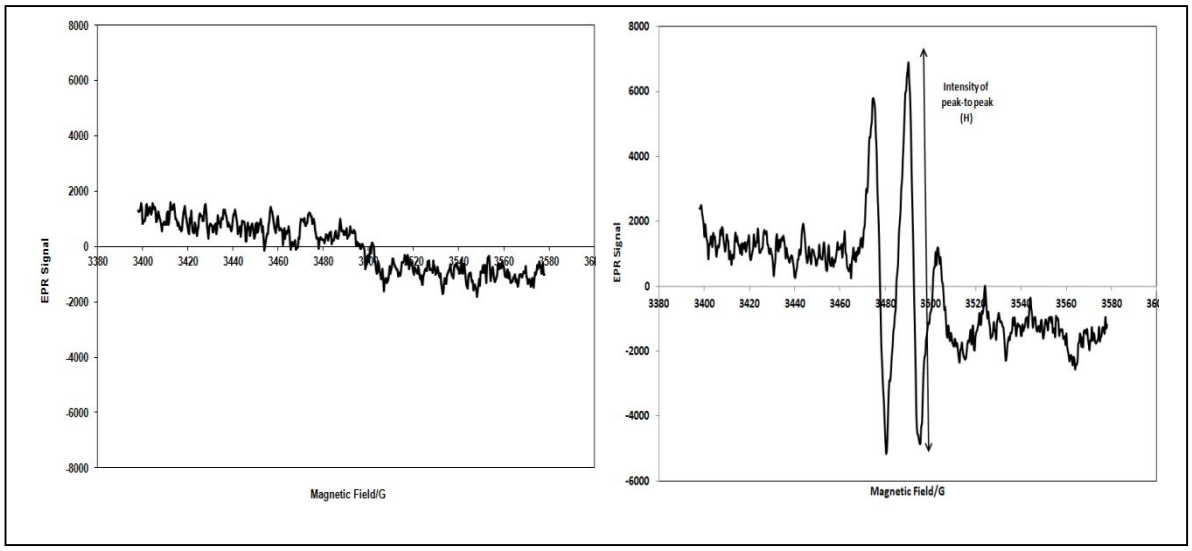

Figure S2. Intensity of peak-to-peak (H) of EPR spectrum as a function of thermal treatment times and Hmax of ABS, EBN, PRC and OLM oils.