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Effect of the harvesting time on the quality of olive oils produced in Calabria

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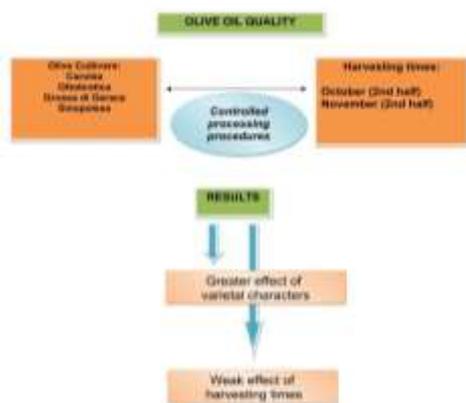
Abstract

The aim of this work was to evidence the quality of four monovarietal olive oils (Carolea, Grossa di Gerace, Ottobratica, and Sinopolese cv) produced at two crop years and at different harvesting times in Calabria region, located in the South of Italy. Qualitative parameters of oils were evaluated by analysis of major and minor components, in particular: free acidity, peroxide value, fatty acid composition, sterol composition, total polar phenolic compounds and tocopherols, and total pigments. The total antioxidant activity of olive oils was evaluated by DPPH and ABTS assays. Two variables were evaluated in the statistical data elaboration: the cultivar and the harvesting time. Their effect was studied on the chemical parameters and antioxidant components. The harvesting at October and November did not reduce the quality of two monovarietal olive oils (Carolea and Sinopolese) produced in Calabria, whereas Ottobratica and Grossa di Gerace olive oils produced at November had a lower quality. However a reduction of qualitative parameters was observed in extra virgin olive oils produced from lately harvested olives with some differences among cultivars.

Practical applications

Practical application for the study entitled 'Effect of the harvesting time on the quality of olive oils produced in Calabria' regards the study of chemical composition on olive oils produced under a controlled experimental procedure. Varietal characteristics and environmental variables affect in general the quality of the olive productions and the obtained olive oils. The results of this work showed that the considered harvesting times did not involve a decay of quality on Carolea and Sinopolese olive oils produced in the South of Italy, obtained following right processing procedures.

Graphical abstract



Under controlled processing procedures, the olive oil quality varied among cultivars mainly than the effect of the considered harvesting periods.

1 Introduction

Olive oil has been a basic element of the Mediterranean diet, particularly for its health benefits, such as its high content in mono-unsaturated fatty acids and its minor components (aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds, and several antioxidants) [1]. The intrinsic quality of olive oil is determined by its composition and could be influenced by agronomical and technological factors, by production process, and also by storage [2]. Natural antioxidants that can be found in the extra virgin olive oils are the polar phenolic compounds and tocopherols because they play an important role against cellular autoxidation and oxygen radicals [3]. In particular, the concentration and composition of phenolic compounds are strongly affected by olive cultivar, degree of maturation, crop season and processing techniques [4-7]. Excluding the original varietal characteristics, the olive oil quality is also related to the physiological conditions of the fruit from which it is extracted. Important chemical changes occur inside the drupe during ripening, related to the acidic profile, lipid oxidation [8], synthesis of organic substances, especially triglycerides, and to other enzymatic activities that may affect their characteristic after processing [9] and the virgin olive oil quality. Also the sensory characteristic of the extracted oil is influenced by the change of composition of fatty acids, levels of polar phenolic compounds, tocopherols, sterols and pigments during drupe maturation [10].

The increase of polyunsaturated fatty acids and the decrease of antioxidant components, as polar phenolic compounds, during drupe maturation reduce the olive oil shelf life. Early harvested fruits instead produce oil with high polyphenol content that contributes to the level of bitterness and pungency and to the stability for the antioxidant effect of the polar phenolic compounds [11]. The drupe maturation influences also the amounts of other biomolecules, involving a decrease of pigments [12-13]. The harvesting period more than the season conditions influences the pigment composition in the olive oil [14]. Several studies evidenced a varietal effect upon the concentration of chlorophylls and carotenoids in different olive cultivars [15-17].

Virgin olive oil is a buoyant business where blends of different varietal virgin olive oils represent a high percentage of the market, the rest being pure monovarietal virgin olive oils which is mainly sold by cooperative societies of producers [18]. Production of monovarietal olive oils has increased during the last years, due to their favourable chemical and sensorial characteristics [19]. The Italian Ministry of Agricultural, Food and Forestry Politics acknowledged in September 2015 the quality denomination of PGI (Protected Geographical Indication) to the Calabrian olive oil, obtained by drupes of different cultivars grown in Calabria. It possessed specific physico-chemical characteristics, among all free acidity $\leq 0.5\%$, peroxide value ≤ 12 mEq O₂/kg, total polar phenolic compounds ≥ 200 mg/kg. The Calabria represents the ending of the Italian peninsula, surrounded by the Tyrrhenian Sea on the West and the Ionian Sea to the East that influence directly the Mediterranean climate. Moreover, the rough nature of the region contributes to the

characteristics of the microclimate with differences on rainfall and thermal trends in the different Calabrian sites. Studies on the Calabrian olive growing has been conducted since more than ten years, focused on the qualitative aspects of the olive processing [20-22], and on the quality of olive oils from different varieties produced in Calabria [23-32].

The aim of this work is to evaluate the effect of the harvesting time of olive drupes to the quality of the obtained monovarietal oils, considering in this study the cultivars more prevailing on the composition of the Calabrian olive oil PGI.

2 Materials and methods

2.1 Sampling

The studied olive cultivars were Carolea, Grossa di Gerace, Ottobratica, Sinopolese, recognized as very important for their diffusion in the Calabrian orchards. Carolea is cultivated in all the areas of the region; Ottobratica and Sinopolese are mainly present in the Tyrrhenian southern coast, while Grossa di Gerace is cultivated in the Ionian southern coast. The sampling of olives was conducted at two crop years (2012-2013 and 2013-2014): seven farms were considered for Carolea olives, while three for the Grossa di Gerace, Ottobratica and Sinopolese olives. The difference among farm number for the sampling is explained by the large diffusion of the Carolea cultivar in the territory. Six dry-farmed mature (30-35 years-old) olive (*O. europea* L.) trees of each cultivar were selected in each farm on the basis of a homogeneous development and crop load. Five kilograms of drupes (fifteen per cultivar and farm) were carefully harvested to the oil extraction at the second half of October (h-O) and the second half of November (h-N). Oil extraction was performed using a small olive oil press mill of the Company Agrimec Valpesana, Calzaiolo, San Casciano (Florence-Italy) at the laboratory of Food Technologies of the Mediterranean University of Reggio Calabria (Italy). The oil was centrifuged to separate water and then it was filtered and stored in dark bottles without headspace at room temperature prior to analyses. The results of analyses are the mean data of two years of observation on respectively twenty-four Carolea oils, nine Ottobratica oils, thirteen Grossa di Gerace oils, and eight Sinopolese oils.

2.2 Qualitative analyses

Free acidity, peroxide value, UV light absorption (K232 and K270), total sterols, total waxes, fatty acid methyl esters (FAME) and fatty acid ethyl esters (FAEE) were determined following European Community Regulation [33]. Oxidative stability was expressed as the induction time (h) in the Rancimat equipment (Metrohm, Basel, Switzerland) at 98 °C and an air flow of 10–12 L/h, as defined in AOCS Official Method Cd 12b-92 [34]. Tocopherol composition analysis was performed by HPLC, applying the IUPAC method 2432 (1987) [35]. The total polar phenolic compounds were analyzed spectrophotometrically at 725 nm using Folin-Ciocalteu reagent as reported by Baiano et al. (2009) [36], and expressed as mg/kg of gallic acid by the calibration plot of pure gallic acid as standard at different concentrations. Sterol composition was determined following the AOCS Ch 6-91 method [37]. Total chlorophyll and carotenoid contents were quantified according to Minguez-Mosquera et al. (1991) [38]. Antioxidant assays (DPPH and ABTS) were assessed according to Baiano et al. (2009) and Miller et al. (1993) [36, 39]. All the analyses were determined in duplicate for each sample. The colour parameters were measured by a tristimulus colorimeter (Konica Minolta CM-700d, Osaka, Japan) with reference to the CIELAB colour space. The L*, a*, and b* colour coordinates were measured using D65 illuminant, conducting the analysis in five replicates.

2.3 Statistical analysis

One-way analysis of variance (ANOVA) was applied to the data to determine the presence of significant differences in the chemical parameters of monovarietal olive oils between the two harvesting times (significant level for $p < 0.05$). Multivariate analysis was applied to evidence the differences attributed to several variables (harvesting time and variety). SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA) was used for data processing.

3 Results and Discussion

Free acidity, peroxide index, UV spectrophotometric indices, total waxes, total fatty acid ethyl esters.

The means and the standard deviations of results on physical and chemical analyses performed on the olive oils obtained at two harvesting times are reported in Tables 1-4. All the oils extracted at the second half of October possessed a mean free acidity lower than the limit for extra virgin olive oils [40]. The lowest values were found in Ottobratica (0.29% in October 2012) and in Sinopolese (0.33% in October 2013) (data not shown). This qualitative parameter tended to increase in the successive sampling with significance only in Ottobratica and Grossa di Gerace oils ($p=0.025$ and $p=0.005$ respectively). The peroxide mean value at both harvesting times was inside the limits of 20 mEq O₂/kg for the extra virgin olive oil in all the studied cultivars, except for Sinopolese oils that showed higher value. The high amount of peroxides as a typical characteristic of Sinopolese oils was confirmed in another study on olive oils produced in Calabria [41]. The results for K232 and K270, in oils obtained at two harvest periods were inside the limits set by the European Regulation [40]. The total wax content in Calabrian olive oils differed among cultivars, as confirmed by literature [42, 43] but no significant differences were noted between harvesting periods ($p>0.05$). Total waxes in Calabrian olive oils were larger than those quantified in some Spanish cultivars [44] and in all studied cultivars inside the limits set by the European Regulation [40]. The results on total ethyl ester content in the different oils denoted an influence of drupe ripening, because most of Ottobratica and Grossa di Gerace h-N oils exceeded the accepted maximum amount of 30 mg/kg for the extra virgin olive oil with significant differences between the two harvesting dates in particular for the last cultivar ($p<0.05$). The over-ripening and the advanced season mainly expose the olive flesh to mechanical and parasitic attacks that encourage fermentation and ethyl alcohol production with final esterification of fatty acids. The result of total ethyl ester mean content in h-O Sinopolese olive oils is explained by an observed high amount in an only sample (54.84 mg/kg).

Total polar phenolic compound and tocopherol content, oxidative stability, antioxidant assays, total pigments.

The total polar phenolic compounds varied among cultivar from 202 to 488 mg/kg of gallic acid. Even though no statistically differences were observed, a decreasing trend of the mean total phenol content of the oils produced at the two harvesting time was observed, in particular in Sinopolese olive oils when produced at half November decreased with significance ($p=0.009$) (Table 4). The other antioxidant class, represented in the olive oil by the tocopherols, varied from 197 to 365 mg/kg in the different samples, with significant differences in the Carolea and Grossa di Gerace oils extracted at two harvest periods. Ottobratica and Sinopolese oils preserved instead their amounts at both harvesting times, demonstrating an environmental effect on these chemical compounds due to the climatic and agronomical characteristics of the growing area. In general a trend of a reduction of the mean tocopherol content was also observed at the second harvest, as observed for the total polar phenolic compounds. Also concerning the pigment content in the studied oils, a varietal effect was observed among the different cultivars with a range of 6.8-15.4 mg/kg of chlorophylls and 3.3-7.9 mg/kg of carotenoids ($p<0.01$) with amounts comparable to other olive varieties according to Giuffrida et al. (2011) [45]. The harvesting time affected in particular the Ottobratica oils that manifested higher amount of chlorophylls and carotenoids when produced at November, whereas the other samples did not show great differences between samples. The olive oils did not manifest significant variations of colorimetric parameters at both sampling times with the exception of L* in Ottobratica oils and a* in Carolea oils.

Virgin olive oil results more stable than other edible oils because of its high content of phenolic compounds, tocopherols, carotenoids and monounsaturated fatty acids [46]. Pearson coefficient correlation was used to evidence the possible influence of the various compounds on oxidative stability of olive oils. Different results were obtained among varieties: in particular an exclusive correlation between total phenols and induction time was observed as follows: $p=0.66$ in Carolea oils, $p=0.77$ in Ottobratica oils, and $p=0.95$ in Grossa di Gerace. A various contribution to the oxidative stability of Sinopolese oils was instead manifested by total tocopherols ($p=0.94$), total carotenoids ($p=0.88$), and total polar phenolic compounds ($p=0.65$). The antioxidant assays denoted results higher for the ABTS method than the DPPH,

with values ranging from 24.62 to 41.00 % of ABTS extinction and from 16.68 to 37.55 % of DPPH-extinction with some results similar to Algerian and Tunisian cultivars [47].

Total sterol content, sterol composition, campesterol/stigmasterol ratio.

The content of sterols in the monovarietal olive oils obtained at two harvesting dates is presented in Table 5. All the samples possessed a sterol composition in compliance with the limits fixed for the extra virgin olive oils by European Regulation [40]. Moreover most of these compounds are not significantly affected by the maturation stage of the fruit. Only the Δ^7 -stigmastenol and campesterol/stigmasterol ratio, reported as a quality index [48], decreased with significance ($p < 0.05$) respectively in Carolea and Ottobratica oils produced at November. The multivariate statistical analysis revealed no influence of cultivar variable in the sterol composition and a light influence of harvesting time only in the Δ^7 -stigmastenol content. The total content of sterols ranged from about 1520 mg/kg in Ottobratica olive oils produced at October to 1830 mg/kg in Grossa di Gerace produced as well at October with no significant statistical differences. In other papers [49, 50] higher amounts were observed in olive oils from Picholine marocaine and Zalmati grown respectively in Morocco and in Spain, whereas similar results were reported for Sinopolese olive oil previously produced in Calabria [51]. In our study, this quality index was not affected by the cultivar and the fruit ripeness.

Fatty Acid composition.

The fatty acid composition is a quality parameter and authenticity indicator of virgin olive oils and particularly the MUFAs are of great importance because of their high nutritional value and positive effect on oxidative stability of oils [11]. The Table 6 reports the results on fatty acids composition of the different olive oil samples. The prevalent fatty acid in the olive oil is the oleic that varied among samples ($p = 0.02$) with the highest percentage in h-N Sinopolese oils (74.72%) and the highest correspondent oleic acid/linoleic acid ratio ($p < 0.05$). This last index was higher than the results observed in other Mediterranean olive oil productions [52]. The specific amounts of fatty acids were inside the qualitative limits fixed by European Regulation concerning the commodity classification of extra virgin olive oil [40]. The results of ANOVA did not evidence significant differences among the oils produced at two harvesting periods. Results of multivariate analysis of variance of several important indexes of olive oil quality with cultivar and harvesting time as variables are shown in Table 7. A significant ($p < 0.05$) effect of cultivar was observed in the principal qualitative parameters, except for Δ^7 -Stigmastenol content. Harvesting time did not influence with significance ($p > 0.05$) the qualitative parameters of the olive oils by multivariate analysis.

4 Conclusions

This study was conducted in a controlled experimental procedure that permitted to process olives carefully selected and harvested. From a final observation, the variations on chemical parameters due to the varietal effect are well clear. The harvesting time affected some constituents of the studied olive oils: free acidity in Ottobratica and Grossa di Gerace oils, the FAEE in Grossa di Gerace oils, the amounts of antioxidants (polar phenolic compounds and tocopherols) in Sinopolese, Carolea and Grossa di Gerace oils and finally the total content of pigments in Ottobratica oils. The results of this study evidence that the quality of Carolea and Sinopolese oils produced in Calabria was not negatively affected by the harvesting period. The best productions of Ottobratica and Grossa di Gerace oil were instead obtained in olives harvested at October. Therefore, from our observations, the production of high-quality olive oils in Calabria is more dependent on right agronomical and processing practices than the considered harvesting periods.

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The authors have declared no conflict of interest.

References

- [1] Ocakoglu, D., Tokatli, F., Ozen, B., Korel, B., Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. *Food Chem.* 2009, 113, 401–410.
- [2] Vacca, V., Del Caro, A., Poiana, M., Piga, A., Effect of storage period and exposure conditions on the quality of Bosana extra-virgin olive oil. *J Food quality.* 2006, 29, 139–150.
- [3] Aguilera, M.P., Beltran, G., Ortega, D., Fernandez, A., Jimenez, A., Uceda, M., Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. *Food Chem.* 2005, 89, 387–391.
- [4] Baccouri, O., Guerfel, M., Baccouri, B., Cerretani, L., Bendini, A., Lercker, G., Zarrouk, M., Daoud Ben Miled, D. Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. *Food Chem.* 2008, 109, 743–754.
- [5] Sicari, V., Giuffrè, A.M., Piscopo, A., Poiana, M., Effect of the 'Ottobratica' variety ripening stage on the phenolic profile of the obtained olive oil. *Riv. Ital.Sostanze Gr.* 2009, 82, 215-221.
- [6] Gómez-Alonso, S., Salvador, M.D., Fregapane, G., Phenolic compounds profile of Cornicabra virgin olive oil. *J. Agr. Food Chem.* 2002, 50, 6812–6817.
- [7] Cerretani, L., Bendini, A., Del Caro, A., Piga, A., Vacca, V., Caboni, M.F., Gallina Toschi, T., Preliminary characterisation of virgin olive oils obtained from different cultivars in Sardinia. *Eur. Food Res. Technol.* 2006, 222, 354-361.
- [8] Giuffrè, A.M., Piscopo, A., Sicari, V., Poiana, M., The effects of harvesting on phenolic compounds and fatty acids content in virgin olive oil (cv Roggianella). *Riv.Ital. Sostanze Gr.*2010, 87, 14-23.
- [9] Romeo, F.V., Piscopo, A., Poiana, M., Effect of acidification and salt concentration on two black brined olives from Sicily (cv Moresca and Giarraffa). *Grasas Aceites.* 2010, 61, 251-260.
- [10] Dag, A., Kerem, Z., Yogev, N., Zipori, I., Lavee, S., Ben-David, E., Influence of time of harvest and maturity index on olive oil yield and quality. *Sci Hortic-Amsterdam*, 2011, 127, 358–366.
- [11] Diraman, H., Dibeklioglu, H. Characterization of Turkish virgin olive oils produced from early harvest olives. *J. Am. Oil Chem.* 2009, 86, 663–674.
- [12] Criado, M.N., Motilva, M.J., Goñi, M., Romero, M.P., Comparative study of the effect of the maturation process of the olive fruit on the chlorophyll and carotenoid fractions of drupes and virgin oils from Arbequina and Farga cultivars. *Food Chem.* 2007, 100, 748-755.
- [13] Youssef, N.B., Zarrouk, W., Carrasco-Pancorbo, A., Ouni, Y., Segura-Carretero, A., Fernández-Gutiérrez, A., Daoud, D., Zarrouk, M., Effect of olive ripeness on chemical properties and phenolic composition of Chétoui virgin olive oil. *J. Sci. Food Agr.*2010, 90, 199-204.
- [14] Criado, M.N., Romero, M.P., Casanovas, M., Motilva, M.J., Pigment profile and color of monovarietal virgin oils from Arbequina cultivar obtained during two consecutive crop seasons. *Food Chem.* 2008, 110, 873-880.

- [15] Cerretani, L., Motilva, N.J., Romero, M.P., Bendini, A., Lerker, G., Pigment profile and chromatic properties of monovarietal virgin olive oils from different Italian cultivars. *Eur. Food Res. Technol.* 2008, 226, 1251-1258.
- [16] Giuffrida, D., Salvo, F., Salvo, A., La Pera, L., Dugo, G., Pigments composition in monovarietal virgin olive oils from various Sicilian olive varieties. *Food Chem.* 2007, 101, 833-837.
- [17] Oueslati, I., Anniva, C., Daoud, D., Tsimidou, M.Z., Zarrouk, M., Virgin olive oil (VOO) production in Tunisia: The commercial potential of the major olive varieties from the arid Tataouine zone. *Food Chem.* 2009, 112, 733-741.
- [18] Aparicio, R., Luna, G., Characterisation of monovarietal virgin olive oils, *Eur. J. Lipid Sci. Technol.* 2002, 104, 614-627.
- [19] Salvador, M.D., Aranda, F., Gómez-Alonso, S., Fregapane, G., Influence of extraction system, production year and area on Cornicabra virgin olive oil: A study of five crop seasons. *Food Chem.* 2003, 80, 359-366.
- [20] Piga, A., Mincione, B., Runcio, A., Pinna, I., Agabbio, M., Poiana, M., Response to hot air drying of some olive cultivars of the south of Italy. *Acta Aliment.* 2005, 34, 427-440.
- [21] Piscopo, A., De Bruno, A., Zappia, A., Poiana, M., Antioxidant activity of dried green olives (Carolea cv.). *Lebensm. Wiss. Technol.* 2014, 58, 49-54.
- [22] Piscopo, A., De Bruno, A., Zappia, A., Poiana, M., Increase in antioxidant activity of brined olives (Carolea cv.) thermally treated in different packaging types. *Eur. J. Lipid Sci. Technol.* 2016, 118, 1132-1140.
- [23] Di Serio, M.G., Di Giacinto, L., Di Loreto, G., Giansante, L., Pellegrino, M., Vito, R., Perri, E., Chemical and sensory characteristics of Italian virgin olive oils from Grossa di Gerace cv. *Eur. J. Lipid Sci. Technol.* 2016, 118, 288-298.
- [24] Sicari, V., Antioxidant potential of extra virgin olive oils extracted from three different varieties cultivated in the Italian province of Reggio Calabria. *J. Appl. Bot. Food Qual.* 2017, 90, 76-82.
- [25] Giuffrè, A.M., Influence of cultivar and harvest year on triglyceride composition of olive oils produced in Calabria (Southern Italy). *Eur. J. Lipid Sci. Tech.* 2013, 115, 928-934.
- [26] Giuffrè, A.M., Variation in triacylglycerols of olive oils produced in Calabria (Southern Italy) during olive ripening. *Riv. Ital. Sostanze Gr.* 2014, 91, 221-240.
- [27] Giuffrè, A.M., The effects of cultivar and harvest year on the fatty alcohol composition of olive oils from Southwest Calabria (Italy). *Grasas Aceites.* 2014, 65, e011.
- [28] Giuffrè, A.M., Evolution of Fatty Alcohols in Olive Oils produced in Calabria (Southern Italy) during Fruit Ripening. A.M. Giuffrè. *J. Oleo Sci.* 2014, 63, 485-496.
- [29] Giuffrè, A.M., Louadj, L., Poiana, M., Macario, A., Sterol composition of oils extracted from olives cultivars of the province of Reggio Calabria (South of Italy). *Riv. Ital. Sostanze Gr.* 2012, 89, 177-183.
- [30] Piscopo, A., De Bruno, A., Zappia, A., Ventre, C., Poiana, M., Data on some qualitative parameters of Carolea olive oils obtained in different areas of Calabria (Southern Italy). *Data Brief.* 2016, 9, 78-80.

- [31] Poiana, M., Mincione, A., Fatty acids evolution and composition of olive oils extracted from different olive cultivars grown in Calabrian area. *Grasas Aceites*.2004, 55, 282-290.
- [32] Runcio, A., Sorgonà, L., Mincione, A., Santacaterina, S., Poiana, M., Volatile compounds of virgin olive oil obtained from Italian cultivars grown in Calabria: Effect of processing methods, cultivar, stone removal, and antracnose attack. *Food Chem*. 2008, 106, 735–740.
- [33] EUC, European Union Commission regulation 2568/91, Characteristics of olive and olive pomaceoils and their analytical methods. *Official J Eur. Communities*.1991, L248, 1-82.
- [34] AOCS, Official methods and recommended practices of the American oil chemists' society (4th edn.).1993, Champaign, Illinois: American Oil Chemists' Society, ed. D. Firestone.
- [35] IUPAC, International Union of Pure and Applied Chemistry. Determination of Tocopherol and Tocotrienols in Vegetable Oils and Fats by HPLC: Method 2.432. In Paquot C, Haufenne A, (Eds.) *Standard Methods for the Analysis of Oils, Fats and Derivatives*. Blackwell Scientific Publications, Oxford, 1997, 2432/1-2432/7.
- [36] Baiano, A., Terracone, C., Gambacorta, G., La Notte, E., Changes in quality indices, phenolic content and antioxidant activity of flavored olive oils during storage. *J. Am. Oil Chem*.2009, 86, 1083-1092.
- [37] AOCS Official Method Ch 6-91 Determination of the composition of the sterol fraction of animal and vegetable oils and fats by TLC and capillary GLC. In: *Official Methods and Recommended Practices of the AOCS, 6th Edition*, D. Firestone (ed.), AOCS, Urbana, IL. 2009.
- [38] Minguez-Mosquera, M.I., Rejano, L., Gandul, B., Sanchez, A.H., Garrido, J., Color-pigment correlation in virgin olive oil. *J. Am. Oil Chem*. 1991, 68, 332–336.
- [39] Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V., Milner, A., A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci*.1993, 84, 407–412.
- [40] EUC, European Union Commission, Regulation No 1830/2015 *Official J Eur. Communities*. 2015, 266, 9-13.
- [41] Piscopo, A., De Bruno, A., Zappia, A., Ventre, C., Poiana, M., Characterization of monovarietal olive oils obtained from mills of Calabria region (Southern Italy). *Food Chem*. 2016, 213, 313-318.
- [42] Giuffrè, A.M., Influence of harvest year and cultivar on wax composition of olive oils. *Eur. J. Lipid Sci. Technol*. 2013, 115 (5), 549-555.
- [43] Giuffrè, A.M., Wax ester variation in olive oils produced in Calabria (Southern Italy) during olive ripening. *J Am Oil Chem Soc*. 2014, 91 (8), 1355-1366.
- [44] Aragón, A., Toledano, R.M., Cortés, J.M., Villén, J., Vázquez, A., Wax ester composition of monovarietal olive oils from Designation of Origin (DO) “Campos de Hellin”. *Food Chem*. 2011, 129, 71–76.
- [45] Giuffrida, D., Salvo, F., Salvo, A., Cossignani, L., Dugo, G., Pigments profile in monovarietal virgin olive oils from various Italian olive varieties. *Food Chem*. 2011, 124, 1119-1123.

- [46] Tura, D., Gigliotti, C., Pedò, S., Failla, O., Bassi, D., Serraiocco, A., Influence of cultivar and site of cultivation on levels of lipophilic and hydrophilic antioxidants in virgin olive oils (*Olea Europea L.*) and correlations with oxidative stability. *Sci. Hort.* 2007, 112, 108–119.
- [47] Laincer, F., Laribi, R., Tamendjari, A., Arrar, L., Rovellini, P., Venturini, S., Olive oils from Algeria: Phenolic compounds, antioxidant and antibacterial activities. *Grasas Aceites*. 2014, 65, e001.
- [48] Ranalli, A., Angerosa, F., Integral Centrifuges for Olive Oil Extraction. The Qualitative Characteristics of Products. *J. Am. Oil Chem.* 1996, 73, 417–422.
- [49] Mansouri, F., Ben moumen, A., Richard, G., Fauconnier, M.L., Sindic, M., Serghini-Caid, H., Elamrani, A., Phytosterols composition of virgin olive oils from cultivars introduced in eastern Morocco in comparison to Picholine Marocaine. *J. Mater. Environ. Sci.* 2015, 6 (8), 2322-2329.
- [50] Kycyk, O., Aguilera, M.P., Gaforio, J.J, Jiménez, A., Beltrán, G., Sterol composition of virgin olive oil of forty-three olive cultivars from the World Collection Olive Germplasm Bank of Cordoba. *J Sci Food Agric.* 2016, 96(12), 4143-50.
- [51] Giuffrè, A.M., Louadj, L., Influence of crop season and cultivar on sterol composition of monovarietal olive oils in Reggio Calabria (Italy). *Czech J. Food Sci.* 2013, 31 (3), 256-263.
- [52] Louadj, L., Giuffrè, A.M., Analytical characteristics of olive oil produced with three different processes in Algeria. *Riv. Ital. Sostanze Gr.* 2010, 87, 186-195.

Table 1. Physical and chemical parameters of Carolea olive oils obtained at two harvesting times

Harvesting time	h-O	h-N	Sign.
Free acidity (%)	0.36±0.18	0.39±0.20	n.s.
PV (mEqO ₂ /kg)	3.56±1.40	4.05±2.13	n.s.
K ₂₃₂	1.80±0.24	1.65±0.12	n.s.
K ₂₇₀	0.12±0.03	0.10±0.02	n.s.
Total waxes (mg/kg)	49.48±17.79	68.57±27.18	n.s.
Σ FAME (mg/kg)	10.02±11.35	12.60±9.57	n.s.
Σ FAEE (mg/kg)	2.64±2.96	8.10±7.41	n.s.
Σ FAME+FAEE (mg/kg)	12.66±13.62	20.70±14.79	n.s.
Polar phenolic compounds (mg/kg)	453±131	353±143	n.s.
Tocopherols (mg/kg)	224±25	197±28	*
Oxidative stability (h)	16.90±6.19	16.40±4.05	n.s.
ABTS (% extinction)	33.57±14.69	37.92±14.16	n.s.
DPPH (% extinction)	33.66±24.20	23.13±17.99	n.s.
Chlorophylls (mg/kg)	9.29±1.96	10.87±4.21	n.s.
Carotenoids (mg/kg)	4.83±1.20	5.27±1.21	n.s.
L*	7.67±0.64	7.65±1.25	n.s.
a*	0.63±0.11	0.52±0.09	**
b*	2.67±0.27	2.45±0.36	n.s.

Results are presented as the mean value ± standard deviation, n=2; **Significance at P < 0.01; * Significance at P < 0.05; n.s. not significant

Table 2. Physical and chemical parameters of Ottobratica olive oils obtained at two harvesting times

Harvesting time	h-O	h-N	
Free acidity (%)	0.30±0.09	0.88±0.45	*
PV (mEqO ₂ /kg)	8.12±5.49	8.58±5.36	n.s.
K ₂₃₂	1.88±0.28	1.78±0.28	n.s.
K ₂₇₀	0.13±0.03	0.13±0.03	n.s.
Total waxes (mg/kg)	109.64±69.73	140.02±45.26	n.s.
Σ FAME (mg/kg)	10.61±6.13	10.63±5.52	n.s.
Σ FAEE (mg/kg)	9.48±12.20	20.13±20.83	n.s.
Σ FAME+FAEE (mg/kg)	20.09±17.40	30.75±22.15	n.s.
Polar phenolic compounds (mg/kg)	469±238	326±146	n.s.
Tocopherols (mg/kg)	330±69	278±112	n.s.
Oxidative stability (h)	12.25±1.77	11.20±2.83	n.s.
ABTS (% extinction)	41.00±14.76	33.79±17.06	n.s.
DPPH (% extinction)	37.55±5.41	25.94±31.84	n.s.
Chlorophylls (mg/kg)	6.79±0.80	11.65±1.32	**
Carotenoids (mg/kg)	3.79±0.62	5.95±0.94	**
L*	7.68±0.52	8.30±0.45	*
a*	0.60±0.09	0.60±0.06	n.s.
b*	2.53±0.15	2.64±0.20	n.s.

Results are presented as the mean value ± standard deviation, n=2; **, *, n.s. see Table 1

Table 3. Physical and chemical parameters of Grossa di Gerace olive oils obtained at two harvesting times

Harvest time	h-O	h-N	Sign.
Free acidity (%)	0.39±0.13	1.01±0.45	**
PV (mEqO ₂ /kg)	5.77±3.46	7.96±3.85	n.s.
K ₂₃₂	1.85±0.17	1.91±0.10	n.s.
K ₂₇₀	0.10±0.01	0.11±0.01	n.s.
Total waxes (mg/kg)	105.68±27.97	136.02±39.50	n.s.
Σ FAME (mg/kg)	15.76±11.55	50.25±39.87	n.s.
Σ FAEE (mg/kg)	4.14±3.51	64.75±72.68	*
Σ FAME+FAEE (mg/kg)	19.90±14.21	115.00±111.65	*
Polar phenolic compounds (mg/kg)	337±78	269±137	n.s.
Tocopherols (mg/kg)	365±47	284±70	*
Oxidative stability (h)	9.08±1.07	5.43±1.21	**
ABTS (% extinction)	26.79±12.33	24.62±6.19	n.s.
DPPH (% extinction)	24.47±19.74	16.68±10.38	n.s.
Chlorophylls (mg/kg)	6.84±1.77	8.60±1.79	n.s.
Carotenoids (mg/kg)	3.33±0.81	3.80±0.41	n.s.
L*	7.36±1.52	7.81±0.95	n.s.
a*	0.83±0.21	0.78±0.11	n.s.
b*	3.19±0.38	2.90±0.70	n.s.

Results are presented as the mean value ± standard deviation, n=2; **, *, n.s. see Table 1

Table 4. Physical and chemical parameters of Sinopolese olive oils obtained at two harvesting times

Harvest time	h-O	h-N	Sign.
Free acidity (%)	0.36±0.25	0.39±0.27	n.s.
PV (mEqO ₂ /kg)	13.01±6.31	17.58±14.83	n.s.
K ₂₃₂	1.94±0.25	1.74±0.15	n.s.
K ₂₇₀	0.15±0.02	0.11±0.04	n.s.
Total waxes (mg/kg)	96.75±46.68	77.41±28.82	n.s.
Σ FAME (mg/kg)	17.75±9.33	27.48±27.33	n.s.
Σ FAEE (mg/kg)	14.37±22.74	9.54±9.34	n.s.
Σ FAME+FAEE (mg/kg)	32.11±28.92	34.85±35.89	n.s.
Polar phenolic compounds (mg/kg)	488±144	202±70	**
Tocopherols (mg/kg)	332±60	274±30	n.s.
Oxidative stability (h)	12.00±1.13	5.73±1.52	*
ABTS (% extinction)	33.81±17.52	33.23±25.80	n.s.
DPPH (% extinction)	23.37±12.96	13.72±9.84	n.s.
Chlorophylls (mg/kg)	15.39±1.45	13.28±4.08	n.s.
Carotenoids (mg/kg)	7.94±1.48	6.71±1.80	n.s.
L*	7.70±0.34	6.56±1.73	n.s.
a*	0.54±0.09	0.75±0.11	n.s.
b*	2.30±0.24	2.78±0.13	n.s.

Results are presented as the mean value ± standard deviation, n=2; **, *, n.s. see Table 1

Table 5. Sterol composition of monovarietal olive oils obtained at two harvesting times

Cultivar	Carolea		Ottobratica		Grossa di Gerace		Sinopolese	
	h-O	h-N	h-O	h-N	h-O	h-N	h-O	h-N
Cholesterol (%)	0.08±0.16	0.09±0.02	0.10±0.03	0.09±0.03	0.12±0.04	0.10±0.04	0.08±0.02	0.08±0.02
24-Methylene cholesterol (%)	0.11±0.01	0.12±0.03	0.09±0.05	0.11±0.01	0.10±0.04	0.09±0.05	0.11±0.02	0.11±0.01
Campesterol (%)	2.60±0.52	2.81±0.69	3.23±0.34	2.47±0.74	2.84±0.58	2.72±0.35	3.11±0.43	2.49±0.53
Campestanol (%)	0.14±0.18	0.14±0.02	0.16±0.03	0.15±0.02	0.14±0.02	0.14±0.02	0.14±0.01	0.16±0.03
Stigmasterol (%)	0.98±0.38	1.21±0.33	0.84±0.28	1.52±0.82	1.05±0.46	0.99±0.49	0.78±0.40	0.79±0.34
Campesterol/Stigmasterol	2.95±1.10	2.54±1.10	4.24±1.56	1.89±0.70	3.48±2.20	3.45±2.14	4.52±1.44	3.76±2.23
Clerosterol (%)	0.93±0.11	0.92±0.11	0.94±0.12	0.91±0.10	0.92±0.11	0.88±0.10	0.91±0.14	0.89±0.10
β-Sitosterol (%)	85.70±2.25	85.14±2.98	85.55±1.66	85.50±3.28	86.64±1.79	87.20±1.73	85.81±1.92	86.46±1.80
Sitosteranol (%)	1.11±0.22	1.10±0.25	1.30±0.32	1.20±0.18	1.05±0.27	1.00±0.25	1.09±0.21	1.08±0.17
Δ ⁵ -Avenasterol (%)	6.59±2.54	6.84±3.30	5.90±0.74	5.98±0.86	5.52±0.82	5.30±0.74	6.29±1.41	6.37±1.48
Δ ^{5,24} -Stigmastadienol (%)	0.93±0.11	0.91±0.13	1.14±0.46	1.32±0.83	0.96±0.17	0.94±0.20	0.92±0.13	0.87±0.13
Δ ⁷ -Stigmastenol (%)	0.28±0.05	0.21±0.07	0.26±0.99	0.21±0.08	0.20±0.11	0.20±0.06	0.26±0.72	0.19±0.04
Δ ⁷ -Avenasterol (%)	0.53±0.22	0.49±0.11	0.47±0.59	0.51±0.13	0.45±0.06	0.42±0.06	0.48±0.07	0.47±0.06
Total β-Sitosterol (%)	95.26±0.75	94.91±0.62	94.84±0.33	94.91±1.65	95.09±0.72	95.32±0.75	95.02±0.85	95.69±0.62
Total sterols (mg/kg)	1734±326	1727±277	1516±262	1655±357	1832±340	1721±228	1725±228	1527±231

Results are presented as the mean value ± standard deviation, n=2

Table 6. Fatty acid composition of monovarietal olive oils obtained at two harvesting times

Cultivar	Carolea		Ottobratica		Grossa di Gerace		Sinopolese	
	h-O	h-N	h-O	h-N	h-O	h-N	h-O	h-N
C16:0 (%)	15.68±1.59	15.06±1.42	16.81±1.60	16.69±1.15	15.42±1.06	14.98±1.03	15.93±1.84	14.62±0.70
C16:1 (%)	1.69±0.4	1.71±0.48	1.39±0.35	1.51±0.35	1.39±0.35	1.38±0.28	1.33±0.50	1.27±0.26
C17:0 (%)	0.20±0.03	0.20±0.04	0.24±0.07	0.19±0.07	0.04±0.01	0.04±0.00	0.19±0.05	0.17±0.06
C17:1 (%)	0.38±0.07	0.38±0.07	0.32±0.08	0.27±0.09	0.06±0.03	0.06±0.01	0.27±0.08	0.31±0.10
C18:0 (%)	2.67±0.32	2.76±0.44	2.69±0.65	2.50±0.68	2.35±0.73	2.33±0.62	2.54±0.52	2.64±0.68
C18:1 (%)	70.85±4.21	71.79±3.56	67.58±4.03	66.22±3.03	62.82±4.30	64.04±4.15	71.305.39±	74.02±2.13
C18:2 (%)	7.19±2.34	6.78±1.23	9.58±1.91	11.37±0.92	16.39±2.27	15.71±2.18	7.02±3.02	5.41±1.03
C20:0 (%)	0.43±0.07	0.44±0.08	0.44±0.09	0.39±0.09	0.39±0.08	0.39±0.08	0.43±0.09	0.46±0.11
C18:3 (%)	0.45±0.09	0.44±0.09	0.52±0.10	0.48±0.08	0.74±0.18	0.70±0.11	0.56±0.10	0.62±0.13
C20:1 (%)	0.26±0.04	0.25±0.04	0.22±0.05	0.21±0.03	0.22±0.03	0.21±0.02	0.24±0.05	0.26±0.05
C22:0 (%)	0.11±0.03	0.12±0.03	0.12±0.03	0.11±0.04	0.10±0.02	0.10±0.02	0.13±0.04	0.14±0.03
C24:0 (%)	0.06±0.02	0.06±0.02	0.06±0.02	0.05±0.02	0.05±0.01	0.05±0.01	0.05±0.02	0.06±0.03
C18:1/C18:2	9.85	10.59	7.06	5.82	3.83	4.08	10.15	13.69
MUFA/PUFA	0.24	0.23	0.26	0.25	0.22	0.22	0.24	0.22

Results are presented as the mean value ± standard deviation, n=2

Table 7. *P-values* of qualitative indexes in the olive oils from multivariate analysis ($p < 0.05$) related to the effect of cultivar and harvesting time

	Cultivar	Harvesting time
Free acidity	0.090	0.147
Peroxide value	0.000	0.125
ΔK	0.007	0.304
C17:1	0.000	0.799
C18:1	0.018	0.806
Total waxes	0.007	0.640
Σ FAEE	0.035	0.645
Total polar phenolic compounds	0.000	0.815
Total Tocopherols	0.005	0.181
Oxidative stability	0.000	0.000
Δ^7 -Stigmastenol	0.285	0.257