The evolution of free acidity and oxidation related parameters in olive oil during olive ripening from cultivars grown in the region of Calabria, South Italy

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ABSTRACT

The variation in free acidity and oxidation related parameters was studied in olive oil produced from olives at different stages of ripening from cultivars growing in the Calabria region (South Italy). A three-year study was conducted on twelve cultivars growing in rainfed conditions. The number of bi-weekly samplings per each cultivar varied from 5 to 7 depending on the cultivar characteristics in this geographical area. Free acidity remained within the present legal parameters for an extra virgin olive oil (< 0.8%) in all cultivars until 1st November and was highest in Nocellara Messinese cv (1.23%) and in Sinopolese cv (1.25%) on 3rd January. Phenolic and tocopherol contents were highest in oils from Nociara cv (350 mg kg^{-1} and 223 mg kg^{-1} respectively) and in Coratina cv (342 mg kg^{-1} and 216 mg kg^{-1} respectively) on 1st October and decreased constantly during olive ripening. Oil stability index was maximum in Itrana cv, from 22.48 h to 8.82 h in two months ripening. Chlorophyll and carotene content decreased with harvest date.

Keywords: Biodiversity; Harvest date; Oil oxidation; Olive cultivar

INTRODUCTION

The olive tree (*Olea europea* L.) is one of the symbols of culture in the Mediterranean basin and, in the human diet, olive oil is by far the most consumed vegetable oil in this geographical area. More generally olive oil is one of the main products of the Mediterranean diet (Boskou, 2015). Several studies have emphasized its nutraceutical properties, and it can be considered as a functional food for its anti-hypertensive, anti-atherogenic, anti-inflammatory and anti-thrombotic properties (Carluccio et al., 2007). Olive oil's high content of oleic acid and minor components (such as phenols) could reduce oxidative damage (Fitó et al., 2007), may exert a protective effect against the development of many type of cancer, mainly breast cancer (Escrich et al., 2007) and can reduce cardiovascular disease risk factors (Huang and Sumpio, 2008). In addition, if olive oil is applied to the skin after sun exposure, it shows an inhibitory effect on sun-induced cancer development (Viola and Viola, 2009).

Studies conducted on the relationship between bioactive compounds and human health have demonstrated the beneficial effects of phenols, which were found to stimulate osteoblast cell (MG-63) proliferation (García-Martínez et al., 2016). Findings in animal experimental models (Bulotta et al., 2014) proved that oleuropein and hydroxytyrosol have both a high bioavailability (Cicerale et al., 2010; Cicerale et al., 2012), and an absolute absence of acute or sub-chronic toxicity (D'Angelo et al., 2001; Soni et al., 2006). From the organoleptic point of view, phenols in olive oil are responsible for bitterness (Dabbou et al., 2011).

Many factors were found to influence olive fruit and olive oil composition. Harvest year was found to influence the olive fruit (Giuffrè, 2017) and olive oil composition with regard to triglycerides (Giuffrè, 2013a) and some minor components such as sterols (Giuffrè 2012; Giuffrè and Louadj, 2013), waxes (Giuffrè, 2013b) and fatty alcohols (Giuffrè 2013c; Giuffrè 2014a).

Another factor influencing olive oil composition is the harvest date, which was found to affect sterols (Giuffrè et al., 2012), waxes (Giuffrè, 2014b), fatty alcohols (Giuffrè, 2014c) and triglycerides (Giuffrè, 2014d).

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During olive fruit ripening oil quantity increases (Lavee and Wodner, 2004; Desouky et al., 2010; Laila Haggag et al., 2013; Giuffrè, 2017) but olive oil quality may deteriorate, for this reason it is important to know the rate at which the chemical parameters vary, so as to decide the optimum moment to collect drupes, obtaining the best balance between olive oil quantity and chemical quality.

This work discusses findings on free acidity and the parameters related to oil oxidation during olive ripening of twelve cultivars in the region of Calabria (South Italy).

MATERIALS AND METHODS

The geographical area of production, the choice of the olive cultivars and the applied agronomic practices have been described in a previous work (Giuffrè, 2017). A 25 kg aliquot of fruits for each cultivar and for each harvest date was randomly and manually collected from ten olive trees and was quickly transferred to the laboratory. The oil extraction was conducted within four hours of olive harvesting with a laboratory mill Agrimec Valpesana, San Casciano (Florence, Italy). Olives were washed and crushed with a metallic hammer crusher, after which the olive paste was placed in a stack with metallic grids and the oil-water mixture was extracted by pressure. After extraction, the oil-water mixture was centrifuged with a laboratory centrifuge and the obtained oil was filtered with filter paper. The olive oil was stored in 100 mL amber coloured glass bottles, and closed with a screw cap polymeric material. The bottles were then stored at about 15 - 17 °C in the dark until analysis. All oils were analysed within 5 days of extraction.

Analytical methods
Free acidity
Free acidity was determined with the method proposed by the EU (2015), Annex II. The oil was dissolved in a diethyl ether/ethylic alcohol solution 1:1 and titration was carried out with a 0.1 N NaOH solution.

Peroxide value
Peroxide was determined with the method proposed by the EU (2015), Annex III. The oil was dissolved in a 2:3 chloroform/acetic acid solution and titration was carried out with a 0.01 N sodium thiosulphate solution.

Spectrophotometric indices
The analysis in the ultraviolet region was conducted as suggested by the European Regulation annex IX (EU, 2015). In brief, the oil was dissolved in cyclohexane (1:100) and the solution was read at 232 and 270 nm. These absorptions are expressed as specific extinctions $E^{1\%}$ 1 cm (the extinction of 1 % solution of the fat in the specified solvent, in a thickness of 1 cm) conventionally indicated by K (also

referred to as 'extinction coefficient') (EU, 2015). A Perkin Elmer Waltham, Massachusetts, U.S.A. spectrophotometer (model Lambda 2, double ray) was used.

Carotenes and chlorophylls
Carotene and chlorophyll contents were quantified spectrophometrically using the method proposed by Minguez-Mosquera (1991).

Total phenolic content
The total phenolic content was determined according to the method proposed by Kalantzakis et al (2006).

Total tocopherol content
Tocopherol composition analysis was conducted by HPLC and the IUPAC method 2432 (1987) was applied. The HPLC analysis was carried out using a Knauer instrument (Berlin, Germany) equipped with an UV detector (model 2600) and by a 5 µm LiChrosorb RP18 (Merck) (120 x 4.6 mm), methanol/water (98/2) was the mobile phase at a flow rate of 1.5 mL/min.

Oil stability index (OSI)
OSI was determined with the method Cd-12b-92 proposed by AOAC (1997).

A Rancimat apparatus model 679 (Metrohm, Herisau - Switzerland) was used. A 5 g aliquot of oil was placed in the reaction tube and the instrument was heated to 110 °C. A 150 mL/min air flow was set and the conductivity tube was filled with 50 mL of bi-deionised water. The speed chart was 1 cm/h.

Antioxidant activity (DPPH assay)
The test allows antioxidant power to be determined by reacting the sample to be analysed with a solution of DPPH• [2,2-diphenyl-1-picrylhydrazyl, PM 394.33, C18H72N5O6] and analysing the decrease in the peak of the radical by UV analysis. The radical scavenging activity towards DPPH• was expressed as the percentage reduction in DPPH concentration by the constituents of the oils (Kalantzakis et al., 2006). The antiradical activity of olive oils was calculated using a $6 * 10^{-5}$ M DPPH·in methanol solution and reading the absorbance at 515 nm with an Agilent spectrophotometer, model 89090A (CA, USA).

Statistical analysis
All olive oils were analysed in triplicate from samples separately prepared. Excel 2010 software was used to calculate means and standard deviations. SPSS 17.0 software for Windows (SPSS Inc., Chicago, IL, USA) was used to calculate statistical differences by one-way ANOVA and Tukey test for post hoc analysis at $p < 0.05$; two variables were considered: the cultivar and the fruit harvest date.

RESULTS AND DISCUSSION

Free acidity

FA is due to the hydrolysis of triglycerides in the presence of water and by the catalytic action of the lipase enzyme. In this process, triglycerides are split into glycerol, mono , diglycerides and fatty acids. This process starts in the fruits, in particular when the skin has been broken by insects (mainly *Bactrocera oleae*) or if they are damaged by hailstorms or during drupe harvesting. FA values are presented in Table 1. Considering only this parameter, before 1st November all cultivars produced extra virgin olive oil, i.e. all oils showed a FA value below the 0.8% indicated by the EU (2015) and COI (2015). After this date EVOO continued to be extracted only from Cassanese cv until 3rd January. The oil obtained from Coratina (1.03%) and Ottobratica cvs (0.83%) were the first two (mid-November) which exceeded the maximum FA value indicated by the International standards for an EVOO. In the studied oils, FA showed a very high significant increase with harvest date for all cultivars. The cultivar effect determined very highly significant (1st October) or highly significant differences (in on all the other harvest dates).

Peroxide value

PV is related to the oxidation of the unsaturated fatty acids contained in an oil with the formation of hydroperoxides. From the chemical point of view oxidation is a spontaneous reaction of fat due to free radicals of unsaturated acids in contact with oxygen in the air with a classic chain reaction that occurs through a trigger and a subsequent propagation reaction. The oxidised oils or fats develop an unpleasant odour and taste that render the oil unpalatable and, finally, inedible. Both the European (EU, 2015) and the COI (COI, 2015) standards indicate only one value (20 meq O_2/kg) to distinguish whether an oil is edible or not. Sinopolese cv was borderline after 1st December and exceeded this value from mid-December. Frantoio, Itrana, Nociara, Picholine and Roggianella cvs showed the lowest values throughout harvesting (Table 2). In the studied oils, Sinopolese cv exceeded 10 meq O_2/kg on all harvest dates, moreover, this cultivar showed the highest values for all cultivars on all harvest dates except on 1st October. In nine of the twelve studied cultivars an initial PV decrease and a subsequent increase was observed. Other Authors found the same trend in oil from fruits of a Jordan Nabali cv (Humeid et al., 1992), in oil from fruits of seven Tunisian oleasters (Baccouri et al., 2007), in oil from fruits from Arbequina cv grown in Spain (Abenoza et al., 2015) and in oil from fruits of Manzanilla cv and Frantoio cv grown in Australia (Alowaiesh et al., 2016). We found a different trend in oil from Leccino cv in which we measured an initial increase and a subsequent decrease (Table 2). We found a constant increase only in oil from Sinopolese cv, from the first to the last harvest date, similar to findings by El Sohaimy et al. (2016) who found the same increase in Manzanilla and Kalamata cvs from Egypt.

K 232 and K270

Spectrophotometric analysis in the ultraviolet can provide information about the quality of a fat, its state of preservation and changes brought about by technological processes. The presence of conjugated diene and triene systems determines the absorption at 232 nm and 270 nm respectively (EU, 2015). K232 values are listed in the Table 3. The average K232 values in olive oils showed significant variations during olive ripening ($p < 0.001$; $p < 0.01$). A decreasing trend was found in almost all cultivars except in Sinopolese, Nociara and Nocellara Messinese cvs in which a non-constant trend was detected. In many cases the K232 value of the last sampling during

Table 1: Free acidity variation in olive oil (expressed as % of oleic acid) during fruit ripening. Results are presented as the mean value ± standard deviation, n=3; ***significance at $P < 0.001$; ** significance at $P < 0.01$. Means in the same column are distinguished by small letters. Means in the same line are distinguished by capital letters

Cultivar	1st October	15th October	1st November	15th November	1st December	15th December	3rd January	Sig.	Mean	DS
Cassanese	0.29 iCD	0.31 hiC	0.34 gB	0.34 hB	0.36 mA	0.30 eC	0.37 dA	**	0.33	0.03
Coratina	0.54 aE	0.61 aD	0.66 aC	1.06 aB	1.31 aA			***	0.83	0.33
Frantoio	0.30 iE	0.33 hD	0.37 gC	0.65 eB	0.72 iA			***	0.47	0.20
Itrana	0.42 cdE	0.52 cCD	0.54 eC	0.70 dB	0.77 gA			***	0.59	0.14
Leccino	0.45 cD	0.46 dD	0.60 cC	0.73 cB	0.80 fA			***	0.61	0.16
Nocellara Messinese	0.30 iF	0.52 cE	0.67aD	0.69 dD	1.09 bC	1.19 aB	1.23 abA	***	0.81	0.36
Nociara	0.35 fgF	0.42 eE	0.55 eD	0.61 fC	0.66 lB	1.20 aA		***	0.63	0.30
Ottobratica	0.37 fF	0.53 cE	0.64 bD	0.83 bC	0.89 dB	0.95 cA	0.95 cA	**	0.74	0.23
Pendolino	0.50 bF	0.58 bE	0.65 abD	0.74 cC	0.89 dB	1.00 bA		***	0.73	0.19
Picholine	0.32 hDE	0.35 gD	0.42 fC	0.61 fB	0.75 ghA			***	0.49	0.19
Roggianella	0.27 lE	0.38 fD	0.44 fC	0.50 gB	0.99 cA			***	0.52	0.28
Sinopolese	0.40 eG	0.47 dF	0.58 dE	0.72 cD	0.86 eC	0.93 cdB	1.25 aA	***	0.74	0.29
Sign.	***	**	**	**	**	**	**			

olive ripening was in countertrend to the decreasing trend. All K232 values from October to January were within the 2.50 stated by the European regulations (EU, 2015) and the International Olive Council (COI, 2015) for an extra virgin olive oil. The cultivar effect showed significant differences ($p < 0.001$; $p < 0.01$) at each harvest date (Table 3). K270 values were significantly different during olive ripening except for Itrana cv in which no differences were found in the oil extracted from 1st October to 1st December (Table 4). Cassanese, Coratina, Roggianella and Sinopolese cvs showed a constant trend and the K270 value increased with harvest date. The other cultivars showed a non-constant trend, sometimes with an initial decrease (Nocellara Messinese, Nociara, Ottobratica, Pendolino)

and a final increase. Almost all K270 values were within the 0.22 stated by the European regulations (EU, 2015) and the International Olive Council (COI, 2015) for an extra virgin olive oil, except for Sinopolese cv when olives were picked on the last two harvest dates (Table 4).

Carotenes and Chlorophylls

Carotenes are pigments exerting an antioxidant activity in olive oil (Servili and Montedoro, 2002). Chlorophylls exert an antioxidant activity if oil is stored in the dark whereas, when oil is exposed to the light they promote the formation of oxygen radicals and speed oxidation. Carotenes and chlorophylls are also responsible for colour in olive oil, yellow-orange and green respectively.

Table 2: Peroxide value variation in olive oil (expressed as meq O_2/kg) during fruit ripening. Results are presented as the mean value ± standard deviation, n=3; ***significance at $P < 0.001$; ** significance at $P < 0.01$. Means in the same column are distinguished by small letters. Means in the same line are distinguished by capital letters

Cultivar	1st October	15th October	1st November	15th November	1st December	15th December	3rd January	Sig.	Mean	DS
Cassanese	8.37 fgA	5.24 mE	6.21 hD	4.17 nF	7.81 ilB	8.36 eA	7.17 dC	***	6.76	1.62
Coratina	11.43 aA	8.93 eC	10.38 dB	6.14 mD	5.20 nE			***	8.41	2.68
Frantoio	7.51 iB	6.66 hD	6.23 hE	7.22 fC	7.86 iA			***	7.10	0.65
Itrana	4.65 mC	4.45 nD	6.34 gB	6.33 eB	8.00 hA			***	5.95	1.45
Leccino	10.03 eD	11.17 bC	12.48 bA	11.47 cB	9.44 fE			***	10.92	1.20
Nocellara Messinese	10.80 cD	9.19 dE	8.89 fF	8.80 dFG	11.20 cC	11.48 bB	14.30 bA	***	10.67	1.96
Nociara	7.70 hB	6.58 iDE	5.99 ilF	6.68 iD	9.22 gA	7.52 fC		***	7.28	1.14
Ottobratica	8.42 fF	7.56 gG	11.65 cD	12.06 bC	13.40 bB	9.88 dE	13.76 cA	***	10.96	2.40
Pendolino	11.73 bA	10.43 cC	8.93 eE	6.73 hF	9.51 eD	11.05 cB		***	9.73	1.78
Picholine	7.43 lC	8.27 fB	5.75 mE	6.35 lD	9.63 dA			***	7.49	1.54
Roggianella	7.44 lB	6.12 lD	6.03 iDE	7.15 gC	7.77 mA			***	6.90	0.79
Sinopolese	10.63 dG	12.76 aF	13.50 aE	15.99 aD	17.55 aC	21.51 aB	25.33 aA	***	14.34	4.65
Sign.	***	***	***	***	***	***	***			

Table 3: K232 value variation in olive oil during fruit ripening. Results are presented as the mean value ± standard deviation, n=3; ***significance at $P < 0.001$; ** significance at $P < 0.01$. Means in the same column are distinguished by small letters. Means in the same line are distinguished by capital letters

Cultivar	1st October	15th October	1st November	15th November	1st December	15th December	3rd January	Sig.	Mean	DS
Cassanese	1.45 hB	1.35 hC	1.50 g A	1.28 iD	1.27 hD	1.34 eC	1.18 cE	**	1.34	0.11
Coratina	1.64 eA	1.65 dA	1.58 dB	1.47 eD	1.51 dC			**	1.57	0.08
Frantoio	1.43 hiC	1.64 dA	1.63 cA	1.46 efB	1.43 fC			**	1.52	0.11
Itrana	1.59 fA	1.52 eB	1.57 deA	1.41 gC	1.38 gCD			**	1.49	0.09
Leccino	1.73 cA	1.64 dB	1.59 dC	1.23 hE	1.54 cD			***	1.55	0.19
Nocellara Messinese	1.36 mF	1.45 gE	1.53 fBC	1.56 cB	1.60 bA	1.50 abD	1.56 bB	***	1.51	0.08
Nociara	1.87 aA	1.79 aB	1.69 aC	1.59 bD	1.42 fF	1.52 aE		***	1.65	0.17
Ottobratica	1.70 cdB	1.65 dC	1.62 cCD	1.60 bD	1.48 eE	1.43 dF	1.76 aA	***	1.60	0.12
Pendolino	1.80 bA	1.76 bB	1.51 ghCD	1.48 eE	1.53 cdC	1.48 bcE		***	1.59	0.15
Picholine	1.49 gB	1.50 efB	1.51 ghA	1.47 eC	1.52 dA			**	1.50	0.02
Roggianella	1.72 cA	1.69 cB	1.65 bC	1.64 aCD	1.66 aC			**	1.67	0.03
Sinopolese	1.41 ilDE	1.52 eC	1.42 iD	1.53 dBC	1.55 cB	1.43 dD	1.74 aA	***	1.51	0.12
Sign.	***	***	**	***	***	**	**			

Per our findings, both carotene and chlorophyll contents showed a gradual decrease during olive ripening. Pendolino and Sinopolese cultivars had the highest initial carotene content (40 mg kg^{-1} oil), whereas in all other cultivars a carotene content lower than 25 mg kg^{-1} was calculated. Itrana cv showed the lowest carotene content before 15th November (Table 5). Baccouri et al (2007) found the same decreasing trend in oil obtained from fruits of the Tunisian wild oleaster and a similar carotene content was found from the first to the last harvest date.

Total phenolic content
Phenols can be found in every part of all plants, and are a fundamental part of the human diet. Three classes of

phenols are present in olives: i) lignans, ii) the simple phenol derivatives from phenylethyl alcohol, cinnamic and benzoic acids, iii) the oleuropein and ligstroside aglycons and their derivatives (Conde et al., 2008). The cultivar effect caused high and very high significant differences in the cultivars studied in this work. The total phenolic content decreased in the oil of all cultivars with drupe ripening and the calculated means showed very highly significant differences ($p < 0.001$) from the first to the last harvest date (Table 7). Six of the twelve studied cultivars had an initial total phenolic content higher than 300 mg kg^{-1}. After one month's ripening, only in the oil of two cultivars this value was exceeded, i.e. Coratina (364 mg kg^{-1}) and Nociara (312 mg kg^{-1}). The initial highest total phenolic content was

Table 4: K270 value variation in olive oil during fruit ripening. Results are presented as the mean value±standard deviation, n=3; ***significance at P<0.001; ** significance at P< 0.01; * significance at P< 0.05; n.s., not significant. Means in the same column are distinguished by small letters. Means in the same line are distinguished by capital letters.

Cultivar	1st October	15th October	1st November	15th November	1st December	15th December	3rd January	Sig.	Mean	DS
Cassanese	0.12 bC	0.12 bC	0.15 abB	0.16 aB	0.17 bcAB	0.19 bA	0.18 cA	**	0.16	0.03
Coratina	0.14 aC	0.14 aC	0.16 aB	0.15 abBC	0.18 bA			**	0.15	0.02
Frantoio	0.09 cdB	0.13 abA	0.12 deA	0.08 eBC	0.08 gBC			**	0.10	0.02
Itrana	0.09 cdA	0.08 dA	0.09 fA	0.08 eA	0.09 gA			n.s.	0.09	0.00
Leccino	0.10 cCD	0.13 abBC	0.14 bcB	0.11 cdC	0.16 dA			**	0.13	0.02
Nocellara Messinese	0.12 bAB	0.11 bBC	0.13 cdA	0.10 dBC	0.10 fBC	0.14 dA	0.10 dBC	**	0.12	0.01
Nociara	0.12 bB	0.12 bB	0.11 eBC	0.11 cdBC	0.15 dA	0.15 dA		*	0.13	0.02
Ottobratica	0.13 abD	0.11 bDE	0.12 dD	0.15 abC	0.18 bB	0.18 bcB	0.21 bA	**	0.15	0.04
Pendolino	0.11 bC	0.10 cC	0.11 eC	0.11 cdC	0.13 eB	0.17 bcA		**	0.12	0.03
Picholine	0.11 bBC	0.12 bB	0.12 deB	0.12 cB	0.14 eA			*	0.12	0.01
Roggianella	0.14 aB	0.13 abC	0.14 bcB	0.14 bB	0.18 bA			*	0.18	0.03
Sinopolese	0.12 bD	0.11 bDE	0.13 cdD	0.17 aC	0.20 aBC	0.22 aB	0.25 aA	***	0.17	0.05
Sign.	*	*	**	*	**	**	**			

Table 5: Carotene variation in olive oil during fruit ripening. Results are presented as the mean value ± standard deviation, n=3; ***significance at P < 0.001; ** significance at P < 0.01; * significance at P <0.05. Means in the same column are distinguished by small letters. Means in the same line are distinguished by capital letters

Cultivar	1st October	15th October	1st November	15th November	1st December	15th December	3rd January	Sig.	Mean	DS
Cassanese	15 deA	13 eB	10 eC	10 bcC	7 cdD	8 cD	4 bcE	***	9.45	3.63
Coratina	23 bcA	23 cA	14 bcB	8 dC	8 cC			**	15.03	7.57
Frantoio	22 cA	14 eB	15 bcB	10 bcC	8 cD			***	13.80	5.40
Itrana	9 gA	7 fgAB	4 gC	3 eC	7 cdAB			**	6.00	2.36
Leccino	25 bA	16 deB	11 dC	11 bC	8 cD			***	14.17	6.71
Nocellara Messinese	17 dA	13 eB	12 dB	7 dC	7cdC	16 bA	6 bC	**	11.14	4.53
Nociara	23 bcA	9 fB	8 fB	7dB	5 eC	5 dC		**	9.33	6.91
Ottobratica	23 bcA	18 dB	12 dC	11 bC	11 bC	9 cD	5 bE	***	12.83	6.08
Pendolino	40 aA	28 bB	16 bD	12 bE	12 bE	20 aC		**	21.39	10.87
Picholine	21 cA	17 dB	9 fD	9 cD	12 bC			**	13.37	5.15
Roggianella	11 fA	9 fAB	10 eA	7 dC	6 dC			**	8.60	2.07
Sinopolese	40 aA	31 aB	23 aC	17 aD	17 aD	17 bD	20 aC	**	23.64	8.76
Sign.	**	**	**	*	**	**	**			

Table 6: Chlorophyll variation in olive oil during fruit ripening. Results are presented as the mean value ± standard deviation, n=3; ***significance at *P* < 0.001; ** significance at *P* < 0.01; * significance at *P* < 0.05. Means in the same column are distinguished by small letters. Means in the same line are distinguished by capital letters

Cultivar	1st October	15th October	1st November	15th November	1st December	15th December	3rd January	Sig.	Mean	DS
Cassanese	17 deA	17 bA	15 dB	12 eC	10 eD	10 deD	7 cdE	***	12.57	3.87
Coratina	21 cA	20 bAB	20 aAB	18 a C	14 bD			***	18.60	2.79
Frantoio	24 bcA	22 aB	21 aBC	18 aD	15 aE			***	20.00	3.54
Itrana	12 gA	12 dA	10 fB	8 gC	8 fC			***	10.00	2.00
Leccino	22 cA	22 aA	19 bB	17 bBC	15 aD			***	19.00	3.08
Nocellara Messinese	20 cdA	20 bA	18 bcB	16 bcC	14 bD	15 aCD	9 abE	***	16.00	3.87
Nociara	25 bA	22 aB	19 bC	15 dD	15 aD	13 bE		***	18.17	4.67
Ottobratica	18 dA	15 cB	15 dB	14 dBC	13 bcC	12 bcD	8 cE	***	13.57	3.10
Pendolino	27 aA	22 aB	18 bcC	18 aC	16 aD	14 aE		***	19.17	4.67
Picholine	23 cA	23 aA	21 aB	19 aC	16 aD			***	20.40	2.97
Roggianella	15 fA	12 dB	12 eB	11 efBC	7 fD			***	11.40	2.88
Sinopolese	19 dA	18 bAB	18 bcAB	14 dC	12 dD	11 dD	10 aE	***	14.57	3.74
Sign.	***	**	**	**	***	***	***			

Table 7: Total phenol variation in olive oil during fruit ripening. Results are presented as the mean value ± standard deviation. n=3; ***significance at *P* < 0.001; ** significance at *P* < 0.01; * significance at *P* < 0.05. Means in the same column are distinguished by small letters. Means in the same line are distinguished by capital letters

Cultivar	1st October	15th October	1st November	15th November	1st December	15th December	3rd January	Sig.	Mean	DS
Cassanese	344 bA	298 eB	276 dC	267 dD	256 cE	241 bcF	193 bcG	***	267.86	47.03
Coratina	382 aA	379 aAB	364 aC	348 aD	325 aE			***	359.60	23.61
Frantoio	267 eA	258 iB	230 ghC	226 iCD	212 hE			***	238.60	23.04
Itrana	309 cA	283 fgB	277 dC	261 deD	250 cdE			***	276.00	22.58
Leccino	273 eA	257 lB	247 fC	232 ghD	208 hE			***	243.60	24.89
Nocellara Messinese	295 dA	288 fB	279 dC	270 dCD	252 cE	223 dF	209 aG	***	259.43	32.92
Nociara	350 bA	344 bAB	312 bC	292 bD	281 bE	262 aF		***	306.89	35.21
Ottobratica	345 bA	332 cB	293 cC	286 bcCD	259 cE	249 bF	199 bG	***	280.43	50.10
Pendolino	254 fA	236 nB	233 ghBC	220 iD	201 hiE	195 fEF		***	223.11	22.35
Picholine	313 cA	312 dAB	254 eC	245 fgD	233 fE			***	271.40	38.26
Roggianella	289 dA	276 hB	255 eC	253 fCD	241 eE			***	262.80	19.32
Sinopolese	255 fA	246 mAB	239 gC	240 gC	222 gD	209 eE	192 bcF	***	229.00	22.42
Sign.	**	***	**	**	***	***	***			

found in the oil from Coratina cv (382 mg kg⁻¹) in which this antioxidant fraction decreased 14.92% after two months, even if it remained the highest for all the harvest dates on which Coratina cv was picked. Pendolino and Sinopolese cvs contained the lowest phenolic values which were lower than 200 mg kg⁻¹ on their respective last sampling dates. The decreasing trend we observed was in agreement with findings of other Authors in oil from Hojoblanca and Picual cvs grown in Spain (Gutiérrez et al., 1999), and as found in oils of Ottobratica cv (Sicari et al., 2009) and Roggianella cv (Giuffrè et al., 2010) grown in a geographical area close to that of the present experiment.

Tocopherol content

Tocopherols are the second main class of antioxidants in an olive oil in which α-tocopherol is the component present in the highest quantity, from 88.5% to more than 95%, the remaining part is β+γ tocopherol and δ-tocoppherol (Fedeli, 1977; Huang et al., 2008; Asik and Özkan, 2011). In nature the total content in α-tocopherol decreases with fruit ripening (Tripoli et al. 2005).

From olives of the Nociara cv the oil with the highest initial tocopherol content (223 mg/kg) was extracted, which gradually decreased 2.56 times to 87 mg/kg in olives collected on 15th December (Table 8). On 1st October a tocopherol content higher than 200 mg/kg was found in the oil of 5 of the 12 studied cultivars: Coratina, Itrana, Nociara, Ottobratica and Picholine. After one month, no cultivar produced an oil exceeding this quantity. The cultivar effect produced highly significant or very highly significant differences during olive ripening. The harvest date produced significant, highly significant and very highly significant differences in the same oils. Gutierrez et al (1999), in Spanish cultivars a decreasing

544

Emir. J. Food Agric ● Vol 30 ● Issue 7 ● 2018

Table 8: Total tocopherol variation in olive oil during fruit ripening. Results are presented as the mean value ± standard deviation. n=3; ***significance at $P < 0.001$; ** significance at $P < 0.01$; * significance at $P < 0.05$. Means in the same column are distinguished by small letters. Means in the same line are distinguished by capital letters

Cultivar	1st October	15th October	1st November	15th November	1st December	15th December	3rd January	Sig.	Mean	DS
Cassanese	189 eA	174 cB	141 cV	111 fD	85 eE	70 dF	71 bF	***	120.14	48.86
Coratina	216 abA	203 aB	178 abC	165 bD	144 aE			***	180.60	28.37
Frantoio	168 fA	150 dB	133 cdC	120 eD	80 fE			***	130.20	33.36
Itrana	202 cdA	188 bB	173 bC	106 gD	94 dE			***	152.60	49.28
Leccino	156 gA	131 eB	128 dB	127 dB	90 deC			*	126.40	23.59
Nocellara Messinese	104 hA	106 gA	98 fB	90 hC	86 eCD	77 cE	50 cF	**	87.29	19.36
Nociara	223 aA	207 aB	181 aC	174 aD	100 cE	87 abF		***	162.00	56.07
Ottobratica	204 cdA	200 aA	187 aB	155 cC	108 bD	91 aE	88 aF	***	147.57	51.41
Pendolino	132 hA	127 efAB	111 eC	89 hD	85 eDE	83 bE		***	104.50	21.85
Picholine	209 cA	152 dB	116 eC	80 lD	73 gE			***	126.00	56.10
Roggianella	153 gA	150 dA	133 cdB	85 iC	84 eC			**	121.00	34.18
Sinopolese	112 iA	105 gB	94 fC	83 iD	84 eD	72 dE	70 bE	**	88.57	15.91
Sign.	***	***	**	***	**	**	***			

Table 9: OSI variation in olive oil during fruit ripening. Results are presented as the mean value ± standard deviation. n=3; ***significance at $P < 0.001$; ** significance at $P < 0.01$; * significance at $P < 0.05$. Means in the same column are distinguished by small letters. Means in the same line are distinguished by capital letters

Cultivar	1st October	15th October	1st November	15th November	1st December	15th December	3rd January	Sig.	Mean	DS
Cassanese	8.77 fA	8.29 eB	6.91 eE	7.73 cC	6.60 cdE	7.12 aD	5.55 aF	***	7.28	1.08
Coratina	11.27 eA	10.34 bcB	8.21 cC	7.59 cE	8.00 bCD			***	9.08	1.62
Frantoio	8.08 hA	7.15 fB	6.66 efC	5.33 eD	4.74 hiE			***	6.39	1.36
Itrana	22.48 aA	18.40 aB	16.72 aC	11.65 aD	8.82 aE			***	15.62	5.43
Leccino	8.19 hA	7.08 fB	5.43 hC	5.11 eD	5.16 fgD			***	6.19	1.38
Nocellara Messinese	6.58 mA	6.47 gB	5.50 hC	5.02 efD	5.33 fC	4.98 cD	4.42 bE	***	5.47	0.80
Nociara	11.67 dA	10.58 bcB	9.59 bC	8.27 bD	6.73 cE	5.69 bF		***	8.76	2.29
Ottobratica	12.21 cA	10.04 dB	7.51 dC	4.71 gD	4.22 lmE	3.37 eF	2.12 dG	***	6.31	3.73
Pendolino	7.18 lA	6.41 gB	5.46 hC	4.78 gD	4.35 lE	4.00 dF		***	5.36	1.24
Picholine	13.49 bA	10.89 bB	9.17 bC	8.34 bD	5.53 eE			***	9.48	2.96
Roggianella	7.79 iA	6.23 hB	5.89 gC	4.31 hD	3.16 nE			***	5.48	1.79
Sinopolese	8.35 gA	6.95 fB	5.23 iCD	5.66 dC	4.82 hE	4.02 dF	2.70 cG	***	5.39	1.86
Sign.	***	***	**	**	***	***	***			

α-tocopherol content was found in oil from Picual cv and an initial α-tocopherol constant content with a subsequent decreasing content during ripening in oil from Hojiblanca cv (Sakouhi et al., 2008). Neves, 2011; Gutierrez 1999; Salvador, 2001

OSI

The resistance to oxidation of an oil or fat depends on natural or added antioxidants, saturation degree, pro-oxidants or prior abuse. Oxidation is slow until this resistance is overcome, at which point oxidation accelerates and becomes very fast. The time before this fast acceleration of oxidation is called the 'induction period' (AOAC, 1997). The results of our study can classify the oils into three groups. Itrana cv (first group) showed the highest resistance to oxidation, on 1st October the oil extracted from this cultivar resisted oxidation for 22.48 h, decreasing to 8.82 h in oil produced 2 months later. Picholine, Coratina

and Nociara were in the second group and resisted 10-12 h during October. All other cultivars (third group) presented a resistance 10 h from the start of the study. The harvest date caused very highly significant differences (p < 0.001) in the OSI decreasing trend during olive ripening in all cultivars. The cultivar effect caused highly significant (p < 0.01) and very highly significant differences (p < 0.001). Our results are in accordance with findings of Sicari (2017) who found significant differences between oils extracted from different cultivars.

Antiradical activity (DPPH assay)

The antiradical activity (AA) in olive oil was evaluated with the DPPH assay. Many Authors have found a positive correlation between the radical scavenging activity measured as DPPH percentage of inhibition and the total phenolic content in olive oil (Samaniego Sánchez et al., 2007; Asik and Özkan, 2011; Goldsmith et al., 2014).

Table 10: Antioxidant activity (DPPH test) variation in olive oil during fruit ripening. Results are presented as the mean value ± standard deviation. n=3; ***significance at *P* < 0.001; ** significance at *P* < 0.01; * significance at *P* < 0.05. Means in the same column are distinguished by small letters. Means in the same line are distinguished by capital letters

Cultivar	1st October	15th October	1st November	15th November	1st December	15th December	3rd January	Sig.	Mean	DS
Cassanese	83.12 dA	81.34 eB	79.25 eC	75.15 iD	74.02 iE	73.06 dF	71.11 dG	***	76.72	4.53
Coratina	86.65 aA	85.59 aB	85.33 aBC	84.26 aE	84.81 aD			***	85.33	0.90
Frantoio	81.67 gA	81.20 efB	80.22 dC	78.38 ghD	78.53 fE			***	80.00	1.51
Itrana	82.02 eA	81.16 efB	81.09 cB	80.43 eC	80.45 dC			*	81.03	0.65
Leccino	80.07 ilA	79.17 lB	78.87 fC	78.44 gD	77.65 hE			***	78.84	0.89
Nocellara Messinese	81.88 fA	81.05 gB	81.06 cB	80.87 dC	80.64 dCD	80.06 bE	78.54 bF	***	80.59	1.05
Nociara	84.99 bA	84.77 bAB	84.12 bC	83.74 bD	83.21 bE	82.12 aF		***	83.83	1.06
Ottobratica	84.56 bcA	84.17 cAB	84.10 bB	83.46 bcC	82.82 cD	82.15 aE	81.38 aF	***	83.23	1.17
Pendolino	80.22 iA	79.55 iB	79.20 eBC	78.68 gD	78.05 gE	77.77 cF		***	78.91	0.93
Picholine	82.31 eA	81.98 dAB	81.39 cC	80.47 eD	79.50 eE			***	81.13	1.15
Roggianella	81.37 hA	80.27 hB	79.23 eC	79.07 fCD	78.43 fE			***	79.67	1.16
Sinopolese	80.19 iA	79.33 ilB	78.54 gC	78.26 ghCD	78.12 gD	77.55 cE	77.09 cEF	***	78.44	1.05
Sign.	***	***	***	***	***	***	***			

This correlation was also found in our results; the AA values (Table 10) decreased with the decrease of the total phenolic content. Coratina cv showed the highest initial AA value according to the total phenolic content and the total tocopherol content of this cultivar (Table 7). The cultivar effect showed very highly significant differences between cultivars (Table 10) even if the AA was high also in the oils produced during the final part of the productive seasons. The harvest date had a significant influence (p < 0.05) on the oil obtained from Coratina cv and a very highly significant effect (p < 0.001) on all other oils.

CONCLUSION

The purpose of this study was to assess free acidity and oxidation related parameters in olive oil during fruits ripening of twelve olive cultivars grown in Calabria (South Italy). Findings can be used to predict the harvest date to obtain olive oils with the best chemical characteristics and to preserve their bioactive molecules. Free acidity increased significantly during olive ripening whereas in the oil of many cultivars the peroxide value showed an initial decrease and a subsequent increase.

K232 presented a tendency to decrease from October to January whereas K270 presented an inverse trend, in both cases their values always remained within the legal parameters for an extra virgin olive oil. Pigments such as chlorophylls and carotenes decreased constantly with the harvest date. Bioactive compounds, such as phenols and tocopherols, were studied and Coratina and Nociara showed the highest initial content; in all cultivars a constant decreasing content was found. The resistance to oxidation

measured as oil stability index decreased with harvest date and at the same time a reduction in the antiradical activity expressed as DPPH assay was found.

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