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REGULAR ARTICLE

Effect of olive storage temperature on the quality of Carolea and Ottobratica oils

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ABSTRACT

The aim of this work was to evidence the effect of the olive storage temperature on the quality of the extracted oils from the cultivars Carolea and Ottobratica, grown 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, squalene content and total content of polyphenols, tocopherols and pigments. The total antioxidant activity of olive oils was evaluated by DPPH and ABTS assays. The response to the storage conditions applied to olives in terms of oil qualitative parameters was different between the cultivars. In Carolea oils the effect of the temperature was significant, whereas also the harvesting time and the storage times affected the general quality of Ottobratica oils.

Keywords: Fatty acids; Olive oil; Quality; Sterols; Storage

INTRODUCTION

Extra virgin olive oil is the 'king' fat of the Mediterranean Diet, recognized as a cultural heritage of humanity by United Nations Educational, Scientific and Cultural Organization-UNESCO in 2010. Two principles of the Mediterranean Diet dictate a low intake of saturated fat and high intake of unsaturated fat, in particular olive oil. Its quality depends on several variables, from variety characters to processing technology among which the olive maturity stage is one of the most important ones. The olive maturation develops for few months by many metabolic processes and transformations that affect phenolic and chemical composition of extracted olive oils (Yorulmaz et al., 2013; Giuffrè, 2014a). In particular the occurrence of hydrophilic phenols is strongly affected by the agronomic aspects (Servili et al., 2004), free acidity generally increases due to the activity of lipolytic enzymes (Salvador et al., 2001), peroxide value, ultraviolet spectrophotometric indices, fatty alcohol and fatty acid composition varied depending on variety response (Giuffrè, 2014b; Giuffrè et al., 2010; Matos et al., 2007; Baccouri et al., 2008; Poiana and Mincione, 2004). A decreasing trend due to olive maturation process was instead observed in oil for total sterols and β-sitosterol and for pigments (Gutiérrez et al., 1999).

In 2016 Italy was the second world olive oil producer after Spain, despite the physiological annual drop of the production and the *Bractocera oleae* (Gmelin) attack and the *Xylella fastidiosa* disease which hit in particular the trees of the orchards in the Southern Italy. After Puglia, Calabria region was the second Italian olive producer with 31000 tonnes in the last year and it represents the principal income source for the local firms (ISMEA, 2017). The olives collected in large amounts by mechanical system are directly delivered to the oil mill and stored at room temperature in a separate warehouse or in an area in front of the processing line. So olives undergo to a qualitative decay due to various factors, such as the respiration rate, the morphological changes, the physiological modifications and maturity. The quality decay is strictly linked to the respiration rate, and the fruit conditions are affected by also external factors, such as mechanical stress, humidity, gas composition, and, principally, the temperature. An increment of alkyl esters can be observed in extra virgin olive oils obtained from olives of not good quality, damaged or stored under bad conditions. In such cases, production of ethanol from fermentation of olive sugars, and of free fatty acids from lipolysis, is a common occurrence which triggers alkyl esters synthesis (Costa et al., 2017, Grompone et al., 2016).The

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wax esters are located in the surface layer of the olive and are poorly extracted by the oil pressed from the fruit. The extracted amount is higher the more soft, possibly degraded the olives are (Biedermann et al., 2008).

Some authors evaluated the effect of olive storage before the extraction process on the olive oil quality (Yousfi et al., 2013, Clodoveo et al., 2007, Kiritsakis et al., 1998) and storage of mill olives at 5 °C has been successfully applied by the olive oil industry in Spain (Garcia et al., 1996). Storage of green Manzanillo olives at temperatures below 5 °C caused chilling injury, and thus the minimum safe storage temperature was determined at 5 °C (Maxie, 1964; Kader et al., 1990). These authors also reported that the severity of chilling injury depended on time-temperature, cultivar, maturity, and atmospheric humidity. Different studies were conducted in the last years on the qualitative characteristics of olive cultivars diffused in Calabria region (Southern Italy) (Giuffrè, 2017; Sicari, 2017; Piscopo et al., 2016a) but no previous studies exist regarding the evolution of quality in Calabrian olive oils produced from olives stored at different thermal conditions. The aim of this work was to evaluate the effect of two storage temperatures on the principal qualitative parameters of olive oils produced from Carolea and Ottobratica that are typical varieties diffused in Calabria region.

MATERIALS AND METHODS

Sampling

Olives (Carolea and Ottobratica cvs) were harvested at November and December 2015 in an olive orchard sited in Gioia Tauro province (Reggio Calabria, Italy) and transferred to the laboratory of Food Technologies of the Mediterranea University of Reggio Calabria where they were selected to eliminate defected drupes. An amount of these was directly processed for the oil extraction (0 days) and another one was stored in glass jar at 4 °C and at 25 °C and crushed after 1, 3, 6 and 12 days. Two replicates were used for each treatment. The oil extraction was performed using a small olive oil press mill of the Company Agrimec Valpesana, Calzaiolo, San Casciano (Florence-Italy). The oil was centrifuged to eliminate water by a laboratory apparatus (3000 rpm for 3 minutes) and the oil was filtered through paper, and stored in dark bottles without headspace at room temperature prior to analyses. The olive oils of each cultivar were named as follows, reporting the letter of the harvesting month followed by the number of degrees centigrade used during the storage: N-4; N-25, D-4; D-25.

Qualitative analyses

Free acidity, peroxide value (PV), spectrophotometric coefficients (K_{232} and K_{270}), total sterols, fatty acids, total waxes, fatty acid methyl esters (FAME), fatty acid ethyl esters (FAEE), and squalene content were determined following European Community Regulation (EUC, 1991). Tocopherol composition analysis was performed by HPLC, applying the IUPAC method 2432 (1987). The total phenols were analysed spectrophotometrically at 725 nm using Folin-Ciocalteau reagent as reported by Baiano et al. (2009). Total chlorophyll and carotenoid contents were quantified according to Minguez-Mosquera et al. (1991). Antioxidant assays (DPPH and ABTS) were assessed according to Baiano et al. (2009), and Miller et al.(1993). 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*b* colour coordinates were measured using D65 illuminant, conducting the analysis in five replicates. All the analyses were determined in duplicate for each sample.

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 among different variables: cultivar, harvesting times, storage days, and temperature (significant level for $P<0.05$). The Duncan's post-hoc test was used to evidence the differences during the storage times ($P<0.05$). SPSS Software (Version 15.0, SPSS Inc., Chicago, IL, USA) was used for data processing.

RESULTS AND DISCUSSIONS

Free acidity, peroxide value (PV), spectrophotometric coefficients

Tables 1-2 show the analytical results of free acidity, peroxide value (PV), K_{232} and K_{270} of Carolea and Ottobratica olive oils, obtained from olives that were processed immediately after harvesting (0 days) and after a storage of 1, 3, 6 and 12 days at 4 °C and 25 °C. After 12 days of storage at 25 °C, Ottobratica olives harvested at December showed so physical damage as they were not suitable to the oil processing. Then the qualitative parameters of these samples were reported to 6 days of olive storage. Significant differences ($P=0.000$) in the free acidity of olive oils were observed between cultivars, with oleic acid percentage upper in Ottobratica than in the Carolea t0 olive oils. Sicari et al. (2009) evidenced that Ottobratica oil possess the best qualitative parameters when olives are collected in October, month that also gives the name to the variety, and it is a peculiar characteristic of that olive cultivar. In our study Carolea oils possess a lower free acidity than Ottobratica oils, because a later olive ripening occurs. Moreover this qualitative parameter tended to increase during the olive harvesting and storage time and it was particularly influenced by the storage temperature, as confirmed by several studies (Clodoveo et al., 2007; Nabil et al., 2012; Kiritsakis et al., 1998). The values of free acidity

Table 1: Qualitative parameters of Carolea olive oils

Samples	Storage days	Free acidity (g oleic acid/100 g)	PV (mEq O2/kg)	K232	K270	Total polyphenols (mg/kg gallic acid)	ABTS (% of extinction)	DPPH (% of extinction)
N-25	0	0.28±0.00d	4.68±0.14b	1.83±0.00b	0.14±0.00	716.18±70.92b	93.66±0.57a	35.11±0.18c
	1	0.32±0.05d	5.43±0.06a	1.82±0.04b	0.15±0.04	880.43±12.94a	53.46±0.62b	48.54±0.22a
	3	0.42±0.01c	3.00±0.00d	1.75±0.00b	0.14±0.00	670.37±35.25b	20.47±0.22d	35.91±0.30c
	6	0.52±0.05b	4.15±0.02c	2.18±0.08a	0.18±0.01	721.74±6.55b	53.21±0.34b	46.11±0.21b
	12	1.31±0.01a	4.07±0.10c	1.52±0.00c	0.12±0.00	736.25±47.78b	31.27±0.45c	21.60±0.58d
	Sign.	**	**	**	n.s.	*	**	**
N-4	0	0.28±0.00c	4.68±0.14c	1.83±0.00a	0.14±0.00b	716.18±70.92ab	93.66±0.57a	35.11±0.18d
	1	0.39±0.05bc	5.65±0.08b	1.84±0.03a	0.17±0.02a	722.96±44.61ab	50.92±0.09c	40.81±0.64c
	3	0.59±0.05a	6.36±0.03a	1.85±0.06a	0.17±0.01a	842.34±72.42a	48.49±0.66d	44.59±0.53b
	6	0.37±0.02bc	4.21±0.01d	1.60±0.00b	0.14±0.01b	823.93±35.32ab	50.56±0.43c	39.82±0.30c
	12	0.51±0.13ab	4.35±0.02d	1.81±0.01a	0.13±0.00b	685.24±28.14b	72.17±0.14b	53.42±0.56a
	Sign.	*	**	**	*	n.s.	**	**
D-25	0	0.35±0.03c	5.95±0.09a	1.62±0.03b	0.12±0.01b	450.43±59.13b	28.57±0.47a	48.35±0.43a
	1	0.38±0.05c	5.41±0.18b	1.47±0.00d	0.07±0.00cd	724.28±8.05a	18.25±0.57b	14.00±0.46c
	3	0.46±0.02c	5.42±0.22b	1.54±0.00c	0.06±0.00d	710.46±59.30a	15.34±0.44c	15.60±0.61b
	6	0.76±0.01b	5.36±0.05bc	1.53±0.01c	0.11±0.01bc	387.71±49.28bc	12.81±0.07d	13.52±0.19c
	12	1.25±0.24a	5.04±0.02c	1.89±0.03a	0.23±0.03a	304.17±56.73c	14.86±0.23c	6.14±0.17d
	Sign.	**	*	**	**	**.	**	**
D-4	0	0.35±0.03c	5.95±0.09ab	1.62±0.03a	0.12±0.01a	450.43±59.13bc	28.57±0.47a	48.35±0.43a
	1	0.27±0.05d	5.01±0.20c	1.48±0.01b	0.07±0.01b	355.13±40.21cd	24.63±0.57b	19.78±0.82b
	3	0.41±0.01bc	5.69±0.08b	1.50±0.01b	0.06±0.00b	269.98±39.19d	19.21±0.47d	15.31±0.48c
	6	0.45±0.02b	4.04±0.20d	1.59±0.02a	0.11±0.00a	580.66±38.34a	28.58±0.45a	20.93±0.11b
	12	0.54±0.02a	6.42±0.35a	1.52±0.01b	0.07±0.00b	497.53±35.65ab	23.02±0.07c	12.49±0.18d
	Sign.	**	**	**	**	**.	**	**

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

Table 2: Qualitative parameters of Ottobratica olive oils

Samples	Storage days	Free acidity (g oleic acid/100 g)	PV (mEq O2/kg)	K232	K270	Total polyphenols (mg/kg)	ABTS (% of extinction)	DPPH (% of extinction)
N-25	0	0.42±0.00c	3.41±0.11b	2.07±0.00a	0.21±0.00	1521.49±25.28a	43.49±0.19d	98.03±1.09a
	1	0.31±0.04c	2.15±0.07d	1.76±0.07b	0.21±0.07	1429.74±80.89a	48.120.13bc	72.07±1.14b
	3	0.39±0.04c	2.10±0.00d	1.74±0.00b	0.20±0.00	680.88±68.68b	46.38±1.68c	47.12±0.21c
	6	0.73±0.05b	4.89±0.02a	1.59±0.03c	0.17±0.03	552.39±49.43b	55.99±2.77a	33.00±1.37d
	12	2.64±0.08a	2.55±0.03c	1.73±0.00b	0.21±0.00	296.55±9.76c	21.77±1.04	12.33±0.40e
	Sign.	**	**	**	n.s.	**	**	**
N-4	0	0.42±0.00	3.41±0.11b	2.07±0.00b	0.21±0.00a	1521.49±25.28a	43.49±0.19b	98.03±1.09a
	1	0.42±0.00	2.19±0.00c	1.87±0.02c	0.15±0.02b	1324.35±6.71b	40.43±0.15b	73.00±1.38b
	3	0.42±0.00	1.59±0.00d	1.60±0.00e	0.16±0.00b	1221.33±54.68c	41.32±1.56b	66.00±0.95c
	6	0.37±0.05	4.09±0.11a	2.10±0.01a	0.14±0.01b	931.63±50.56e	47.91±2.26a	66.77±2.20c
	12	0.46±0.01	3.97±0.02a	1.65±0.00d	0.15±0.01b	1037.40±0.00d	42.94±1.29b	37.04±1.17d
	Sign	n.s.	**	**	**	**	*	**
D-25	0	0.41±0.01d	3.11±0.04	1.61±0.02b	0.20±0.03a	376.27±91.23a	25.20±0.34a	19.99±0.47a
	1	0.54±0.06c	3.20±0.06	1.26±0.02c	0.13±0.00b	253.02±89.13a	20.44±0.29b	13.31±0.49b
	3	0.73±0.00b	3.39±0.01	1.25±0.04c	0.12±0.03b	9.25±3.48b	6.94±0.38c	4.44±0.27c
	6	1.32±0.00a	3.27±0.14	1.79±0.01a	0.20±0.01a	7.00±3.83b	7.26±0.19c	2.94±0.12d
	Sign	**	n.s.	**	*	**	**	**
D-4	0	0.41±0.01d	3.11±0.04c	1.61±0.02a	0.20±0.03	376.27±91.23a	25.20±0.34a	19.99±0.47a
	1	0.45±0.03d	3.15±0.16c	1.24±0.04c	0.17±0.03	306.21±31.78a	23.36±0.58b	8.02±0.09b
	3	0.61±0.01c	5.72±0.03a	1.21±0.01c	0.15±0.00	26.08±1.62b	3.17±0.12c	5.90±0.19c
	6	0.80±0.11b	3.21±0.04c	1.39±0.02b	0.14±0.02	20.82±0.28b	3.95±0.14c	6.37±0.48c
	12	1.25±0.04a	4.44±0.21b	1.57±0.01a	0.21±0.01	23.76±1.64b	0.93±0.13d	3.97±0.12d
	Sign	**	**	**	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

in Ottobratica oils produced at November from olives stored at 4 °C (N-4) did not evidence significant differences among times (P>0.05), with qualitative productions also after 12 days of olive storage (0.46 g % of oleic acid), inside the limit for extravirgin olive oil (EUC, 2011). Carolea olive oils showed also a good free acidity after 6 days of olive storage at 25 °C and 12 days of olive storage at 4 °C. So, the physiological deterioration of drupes was slowed down under a cold storage which preserved the quality in particular of Carolea oils regardless of the harvesting period.

The PV observed in all the oil samples did not exceed the European limit for extra virgin category (EUC, 2011) and the highest value (below 7 mEq O_2/kg) was observed in Carolea olive oils. Confirming the previous results of a low oil oxidation, the coefficients of extinction K_{232} and K_{237} rarely tended to increase during the olive storage times with no great influence by the temperature, in contrast with some studies (Kalua et al., 2006; Ben Yahia et al., 2012). All the observed coefficients were inside the limit for extra virgin olive oil with values that were similar to Arbequina olive oil obtained in different times of olive cold storage (Yousfi et al., 2013).

Antioxidant components and radical scavenging activity of olive oils

In Tables 1-2 are also reported the results on antioxidant content of olive oils. The total polyphenols were significantly affected by the variables: cultivar and storage temperature. All the oils from olives collected at November possessed higher content and olive oils from Ottobratica cultivar were richer on these constituents than Carolea oils. At December, Carolea oils possessed instead more abundant amount after olive storage at both temperatures. The storage time affected significantly (P<0.01) the total polyphenols of Ottobratica olive oils which decreased, in particular at December, whereas Carolea oils denoted a good maintenance of these antioxidants, despite the storage times and the different temperatures, as also referred by Yousfi et al. (2013).

The α-tocopherol content and the total tocopherols in oils produced at November followed the trend previously observed for the polyphenols. This fact can be considered rather normal, since the tocopherols play an antioxidant role in the oils (Marmesat et al., 2010). Their amounts were higher in Ottobratica than in Carolea olive oils, without differences among olive storage temperatures in each harvesting time. For these components, a general decrease was observed during the storage times with the lowest values after 12 days of storage at 25 °C and both harvesting times (Fig. 1).

As illustrated in Fig. 2, also the squalene content decreased during the storage time, and this trend could depend on

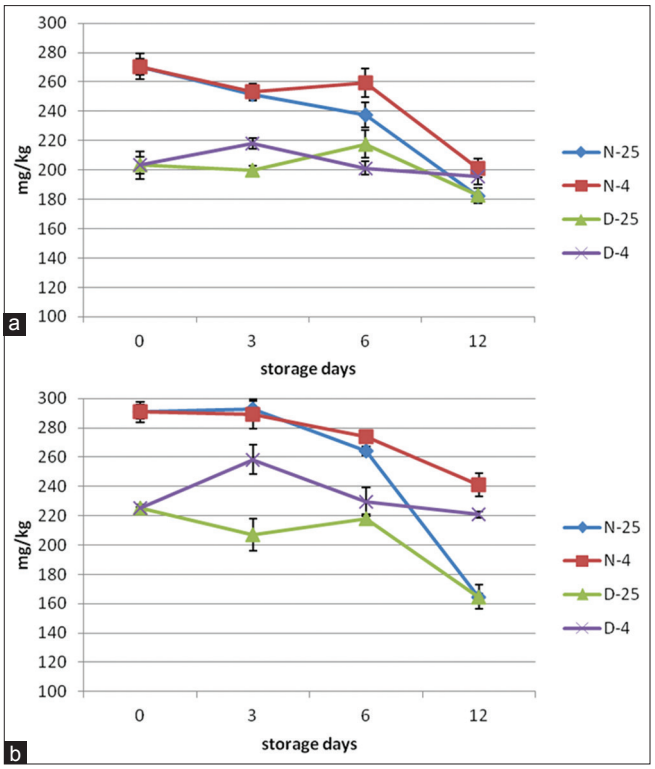

Fig 1. Variation of α-tocopherol content in Carolea (a) and Ottobratica (b) olive oil samples

oxidative reactions started in the stored olives. In Carolea oils from olives harvested at December and stored for 12 days at both temperatures the total squalene amount was lower than those observed in the N-oils, probably because of the advanced olive ripeness degree as also observed in literature for other cultivars (Manzi et al., 1998). No effect of storage temperature was observed in Carolea olive oils produced at both harvesting times, whereas the highest reduction in Ottobratica olive oils was observed after the olive storage at 25 °C.

The DPPH and ABTS assays on the oils revealed a decrease of the total antioxidant capacity with percentages of extinction that followed the previously discussed antioxidant contents. In particular different correlations were evidenced among the components for each olive cultivars: in Carolea oils the α-tocopherol (and so the total tocopherols) was better correlated with ABTS assay (P=0.64) than with the DPPH assay (P=0.41). Different correlations with higher coefficients resulted instead in Ottobratica: 0.83 for α-tocopherol/DPPH, and 0.94 for total polyphenols/DPPH. The squalene content was correlated with antioxidant activity only in Carolea olive oils (P= 0.79 with DPPH assays).

FAME, FAEE and total waxes

Results of FAME and FAEE contents in olive oils are illustrated in Fig. 3. In olive oils produced immediately

after the olive collecting (0 days of storage), no differences were observed due to harvesting time except for the FAME amounts in Carolea oils. The effect on FAME and FAEE contents of the olive storage temperature was instead manifested with significant differences ($P< 0.05$). Moreover an increasing trend of these components was correlated to storage times and the highest amounts of both alkyl esters and ethyl esters were possessed by all olive oils produced at December after 12 days of storage at 25 °C. Considering the limits of FAEE for extra virgin olive oil category (<30 mg/kg) (EUC, 2013), the storage for 12 days at 25 °C was not suitable for Carolea reflecting the results obtained in the free acidity values. Moreover, also the results of FAEE in olive oils obtained at December after 12 days at 4 °C revealed no qualitative productions, demonstrating a not optimal physical state of drupes and an aptitude to deterioration during storage. It is interesting to note that the amounts of FAEE in Carolea olive oils extracted after a cold olive storage were similar at both harvesting times.

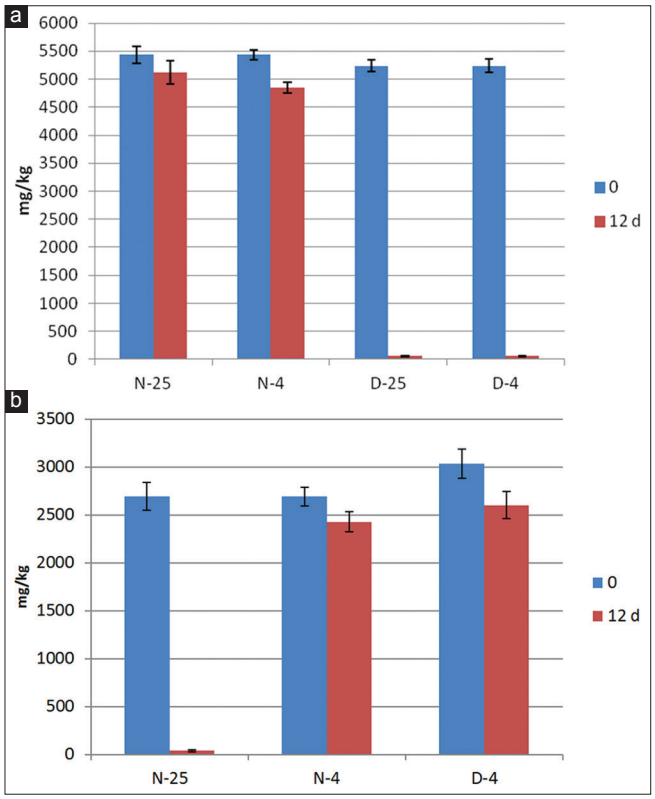

Fig 2. Variation of squalene content after 12 days of olive storage in the Carolea (a) and Ottobratica (b) olive oils samples.

The wax esters are quality indicators for olives and oils (Mariani and Fedeli, 1986; Grob et al., 1990; Bianchi et al., 1994). The total waxes quantified in olive oils immediately extracted after olive collecting differed significantly between cultivars (Fig. 4), with the most abundant amounts in Ottobratica at both harvesting times, with results confirmed by literature (Piscopo et al., 2016b).The storage at 25 °C involved after 12 days a general increase of total waxes in all samples. The cold storage for 12 days of olives preserved the content of total waxes in Carolea oils

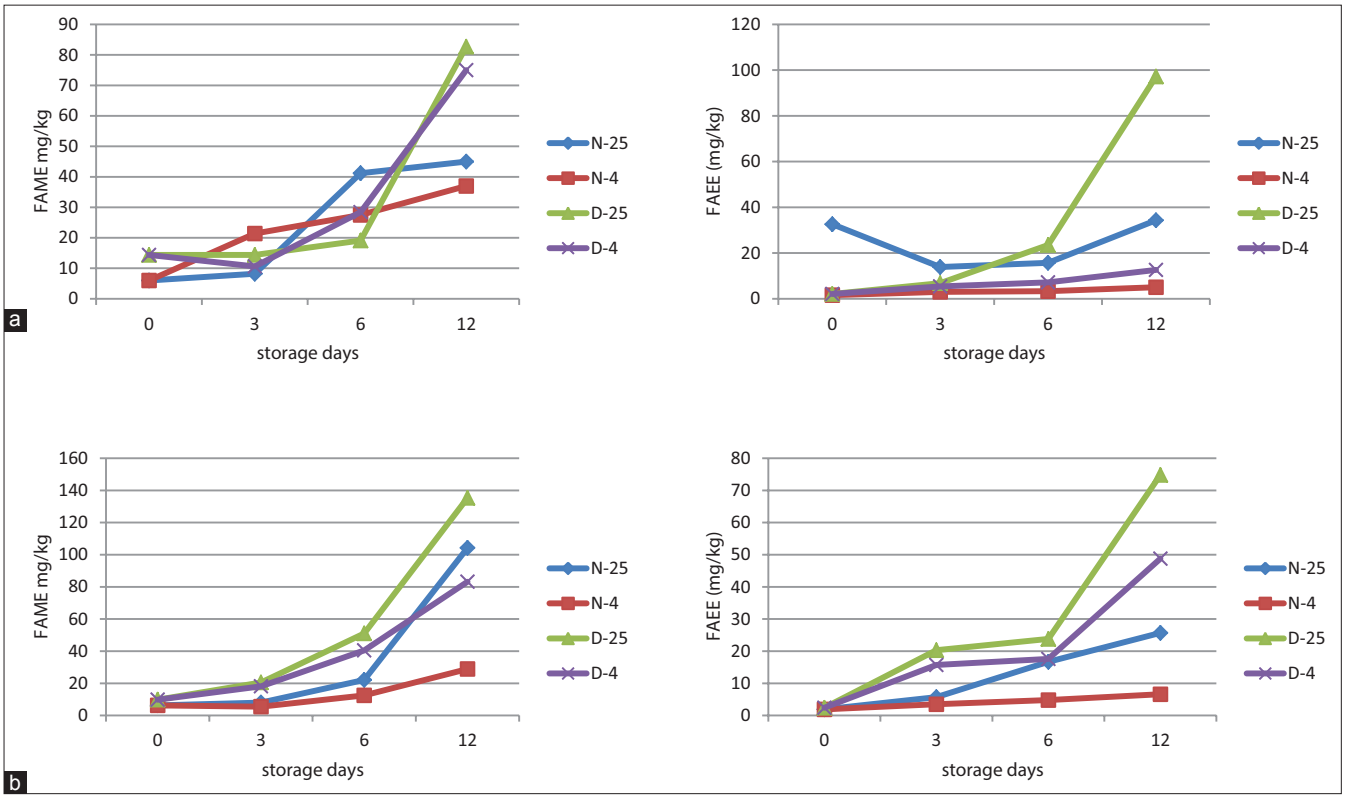

Fig 3. Changes of FAME and FAEE content in olive oils Carolea (a) and Ottobratica cv. (b)

produced at November, whereas it reflected an increase in the other samples, with the highest amount quantified in Ottobratica oils produced at December (71 mg/kg). These results are confirmed by Giuffrè (2014c) who evidenced that the wax content in olive oil produced in South West Calabria was significantly influenced both by the effect of harvesting date and olive cultivar. In that study, Sinopolese, Cassanese, Ottobratica, and especially Leccino olive oil, showed a higher variability in waxes during ripening.

Pigments and colour parameters

The total content of pigments (chlorophylls and carotenoids) varied between the cultivars and among samples with differences due to the applied temperatures and olive storage times. In particular, Ottobratica olive oils produced at November immediately after olive collecting denoted higher chlorophyll content (6.48 mg/kg) respect to the Carolea olive oils (5.18 mg/kg), whereas the carotenoids did not vary with significance ($P<0.05$) between the cultivars (3.74-3.99 mg/kg) (Tables 3-4). Concerning the olive harvesting of December, the initial contents of pigments were similar between Carolea and Ottobratica. The room temperature of storage (25 °C) involved changes during the time in the total pigments of Carolea oils. The cold storage maintained instead constant both the total chlorophylls of oils produced at the two harvesting times and at different days of storage, and the total carotenoids

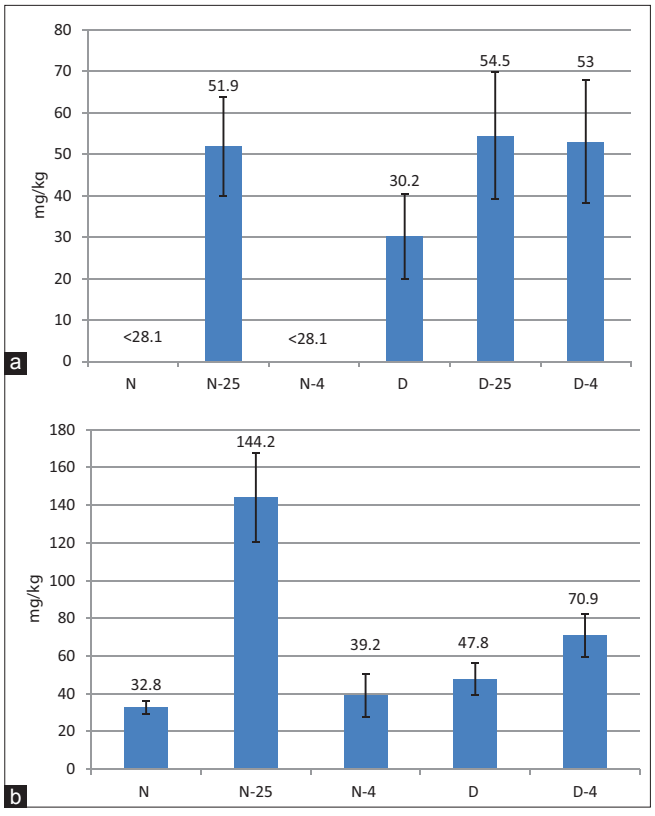

Fig 4. Variation after 12 days of olive storage of total wax esters in Carolea (a) and Ottobratica (b) olive oil samples cv.)

of olive oils produced at November. The general trend of pigments was to increase during the olive storage time, as confirmed by Yousfi et al. (2013). These authors found a rising trend in oils extracted from Arbequina olives and interpreted the release of these pigments in the olive oil due to a decrease in the chloroplast consistency. Concerning the colour parameters, a general decrease of L* value was observed with the advanced harvesting time and after the olive storage. The effect of this variable was also denoted for a* and b* parameters by statistical data elaboration. Summarizing, with the progress of storage time of olives, the oils tended to lose lightness and yellow tone, and to become darkener. This event was observed in both cultivar and at both temperatures of storage.

Sterol and fatty acids composition

In table 5 results of sterol and acid composition of olive oil obtained immediately after olive harvesting and after 12 days of storage are reported. All of these samples possessed a total content of sterols upper than 1000 mg/kg, conforming to the EUC limits (EUC, 2013). The fatty acid composition revealed a decreasing of monounsaturated acids as oleic to the rise of polyunsaturated as linoleic acid, in particular under storage at 25 °C. The results revealed that Carolea olive oil better maintained their nutritional value after an olive storage respect the Ottobratica oils which manifested lower and more variable amounts of oleic acid.

CONCLUSIONS

The qualitative investigation on olive oils of Carolea and Ottobratica cv. performed by this work denoted some peculiar results. The response of the cultivars to the storage conditions was different. Considering the principal qualitative index for an olive oil, the free acidity, it was more influenced by the temperature than the storage time in Carolea olives. In fact, this cultivar resulted suitable to produce high quality olive oils (extra virgin) from 1 to 6 days of olive storage at room temperature, whereas it is possible to prolong the time to 12 days at 4 °C. After these periods the oils were classified as 'virgin'. For Ottobratica cultivar, a similar result was observed, but also an effect of harvesting time was showed. In fact, the olives collected at December are subjected to a faster deteriorations respect the Carolea ones, so extravirgin olive oil production was achieved till 3 days of storage at 25 °C and 6 days at 4 °C. The successive production was classified as virgin olive oils. Concerning the antioxidant components in the different samples, it is interesting to note that Carolea oils maintained constant their total polyphenols despite the storage time and temperatures. Ottobratica oils showed to possess the highest amount when produced at November from olives

Table 3: Total pigments content and colour parameters of Carolea olive oils

Samples	Storage days	Total chlorophylls (mg/kg)	Total carotenoids (mg/kg)	L*	a*	b*
N-25	0	5.18±0.33c	3.99±0.17bc	15.27±0.32a	0.51±0.06b	1.69±0.14a
	1	10.05±0.49a	5.89±0.10a	14.12±0.19b	0.45±0.02b	1.29±0.08b
	3	6.07±0.87c	3.86±0.46bc	8.21±0.48d	0.76±0.06a	1.77±0.09a
	6	5.90±0.58c	3.59±0.19c	7.87±0.34d	0.81±0.04a	1.73±0.10a
	12	7.48±0.05b	4.45±0.03b	9.57±0.36c	0.80±0.06a	1.25±0.11b
	Sign.	**	**	**	**	**
N-4	0	5.18±0.33	3.99±0.17	15.27±0.32a	0.51±0.06cd	1.69±0.14a
	1	6.53±0.69	3.75±0.12	14.18±0.19b	0.47±0.01d	1.61±0.04a
	3	4.87±0.24	3.81±0.14	8.17±0.55c	0.83±0.07a	1.65±0.09a
	6	6.21±1.55	4.62±0.67	7.87±0.45c	0.76±0.04b	1.61±0.07a
	12	6.29±1.27	4.57±0.36	14.54±0.13b	0.54±0.06c	1.45±0.13b
	Sign.	n.s.	n.s.	**	**	*
D-25	0	3.56±0.28c	2.68±0.06d	11.83±0.33c	0.61±0.09b	1.40±0.11bc
	1	5.21±0.40b	3.43±0.07c	12.36±0.57c	0.56±0.04bc	1.51±0.10ab
	3	6.11±0.04b	3.74±0.00b	13.45±4.99b	0.52±4.92cd	1.25±4.81c
	6	4.05±0.34c	2.68±0.07d	15.01±0.41a	0.47±0.05d	1.51±0.17ab
	12	13.98±0.54a	6.52±0.13a	10.27±0.37d	0.82±0.07a	1.63±0.13a
	Sign.	**	**	**	**	**
D-4	0	3.56±0.28	2.68±0.06b	11.83±0.33a	0.61±0.09bc	1.40±0.11a
	1	4.54±0.23	3.28±0.06a	7.48±0.43b	0.79±0.06a	1.22±0.07b
	3	3.99±0.40	3.21±0.09a	14.93±0.49a	0.53±0.09bc	1.94±0.05a
	6	3.61±0.69	3.27±0.25a	15.87±0.34a	0.45±0.04c	1.71±0.16a
	12	3.59±0.46	3.50±0.19a	11.74±2.40a	0.67±0.18b	1.37±0.34a
	Sign.	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 4: Total pigments content and colour parameters of Ottobratica olive oils

Samples	Storage days	Total chlorophylls (mg/kg)	Total carotenoids (mg/kg)	L*	a*	b*
N-25	0	6.48±0.14c	3.74±0.03c	14.58±0.47a	0.50±0.40d	1.81±0.23a
	1	19.04±1.19a	8.02±0.66a	14.37±0.19a	0.37±0.04e	1.77±0.09a
	3	13.96±3.35b	5.71±0.30b	7.49±0.69b	0.73±0.19b	1.22±0.09b
	6	14.77±0.05b	5.65±0.78b	7.99±0.57b	0.84±0.03a	1.69±0.13a
	12	11.75±0.41b	5.20±0.15b	4.17±0.35c	0.66±0.04c	1.15±0.07b
	Sign.	**	**	**	**	**
N-4	0	6.48±0.14bc	3.74±0.03b	14.58±0.47a	0.50±0.04d	1.81±0.23a
	1	5.46±2.48c	2.83±0.56c	14.32±0.20a	0.44±0.02e	1.77±0.08a
	3	10.62±0.93a	5.47±0.37a	8.32±0.55b	0.71±0.04c	1.90±0.07a
	6	7.51±0.99abc	4.07±0.23b	7.85±0.43b	0.78±0.01b	1.81±0.10a
	12	9.56±0.20ab	5.14±0.17a	6.06±0.57c	0.89±0.02a	0.74±0.11b
	Sign.	*	**	**	**	**
D-25	0	3.01±0.15c	2.38±0.05bc	10.36±0.47b	0.70±0.04cd	1.37±0.23b
	1	2.09±0.23c	2.12±0.08c	9.26±0.19c	0.84±0.04a	1.69±0.10a
	3	4.50±0.30b	2.95±0.09b	13.15±0.69a	0.65±0.02d	1.40±0.09b
	6	8.35±1.25a	3.99±0.30a	9.07±3.28c	0.78±0.21bc	0.99±0.27c
	Sign.	**	**	**	**	**
D-4	0	3.01±0.15c	2.38±0.05c	10.36±0.73c	0.70±0.07b	1.37±0.04b
	1	4.58±0.74bc	3.25±0.13b	11.68±0.57b	0.68±0.07b	1.18±0.09c
	3	4.28±0.92bc	3.16±0.27b	13.42±0.27a	0.69±0.07b	1.38±0.19ab
	6	6.50±0.59a	4.02±0.30a	13.38±0.36a	0.63±0.10b	1.16±0.13c
	12	5.73±0.35ab	3.66±0.16ab	9.90±0.62c	0.84±0.09a	1.53±0.08a
	Sign.	*	**	**	*	**

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 5: Sterol and fatty acids composition of Carolea and Ottobratica olive oil samples

	cv	Carolea						Ottobratica				
	Samples	N	N-25	N-4	D	D-25	D-4	N	N-25	N-4	D	D-4
	Storage days	0	12	12	0	12	12	0	12	12	0	12
Sterols (%)	Cholesterol	0.08	0.10	0.18	0.10	0.07	0.07	0.08	0.14	0.09	0.08	0.06
	2,4-Methylene cholesterol	0.18	0.10	0.18	0.19	0.18	0.16	0.07	0.09	0.08	0.07	0.03
	Campesterol	1.86	1.76	1.74	1.92	1.76	1.69	3.17	3.28	3.29	3.23	3.24
	Campestanol	0.14	0.07	0.08	0.1	0.09	0.06	0.28	0.31	0.24	0.25	0.27
	Stigmasterol	0.62	1.16	0.71	0.81	1.42	0.98	0.54	1.61	0.89	0.09	1.24
	Clerosterol	0.99	0.59	0.90	0.99	0.83	0.91	0.77	0.66	0.69	0.68	0.60
	b-Sitosterol	78.98	82.55	78.42	79.44	79.37	79.32	83.7	85.97	85.36	84.74	85.52
	Sitostanol	0.36	1.05	1.05	0.41	1.05	1.04	1.19	0.94	1.03	1.06	1.06
	D5-Avenasterol	15.09	11.37	15.43	14.38	13.64	14.45	7.74	5.51	6.63	7.13	6.17
	D5,24 Stigmastadienol	0.89	0.71	0.65	0.69	0.80	0.67	1.37	0.79	0.96	0.99	0.93
	D7-Stigmastenol	0.23	0.19	0.18	0.45	0.35	0.19	0.48	0.29	0.28	0.37	0.40
	D7-Avenasterol	0.58	0.34	0.49	0.51	0.44	0.45	0.60	0.42	0.44	0.5	0.47
	Total b-Sitosterol	96.31	96.27	96.45	95.9	95.69	96.39	94.77	93.87	94.67	94.6	94.29
	Total sterols (mg/Kg)	1413	1602	1382	1449	1515	1479	1116	1234	1093	1041	1166
Fatty acids (%)	C16:0	14.14	14.32	14.48	13.93	13.65	13.85	14.64	14.61	14.83	12.68	12.7
	C16:1	1.61	1.67	1.7	1.73	1.78	1.71	1.32	1.31	1.31	0.97	0.99
	C17:0	0.23	0.24	0.23	0.19	0.19	0.21	0.16	0.18	0.17	0.19	0.21
	C17:1	0.53	0.50	0.51	0.42	0.42	0.43	0.26	0.26	0.27	0.3	0.28
	C18:0	2.57	2.60	2.56	2.60	2.60	2.57	2.38	2.41	2.39	2.49	2.51
	C18:1	73.34	72.51	72.65	73.35	73.17	73.43	71.25	70.73	70.8	74.1	73.76
	C18:2	6.16	6.67	6.46	6.36	6.53	6.36	8.57	9.01	8.77	7.73	8.12
	C20:0	0.44	0.46	0.44	0.45	0.45	0.45	0.42	0.45	0.44	0.45	0.45
	C18:3	0.47	0.5	0.46	0.45	0.51	0.47	0.52	0.57	0.54	0.46	0.47
	C20:1	0.3	0.3	0.3	0.29	0.28	0.29	0.26	0.26	0.26	0.27	0.27
	C22:0	0.12	0.13	0.13	0.13	0.13	0.13	0.14	0.16	0.15	0.15	0.15
	C24:0	0.07	0.08	0.07	0.08	0.07	0.07	0.06	0.06	0.07	0.08	0.06

stored at 4 °C, so the cultivar is affected by all the variables (harvesting, storage time and temperature).

The authors have declared no conflict of interest.

AUTHOR'S CONTRIBUTIONS

Rocco Mafrica and Nicola Grillone conceived of the presented idea. Marco Poiana developed the theory. Alessandra de Bruno, Angela Zappia and Giuseppina Gioffrè performed the computations. Amalia Piscopo verified the analytical methods, and with Marco Poiana she supervised the findings of this work. Amalia Piscopo discussed the results and wrote the final manuscript.

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