RESEARCH ARTICLE

Change in quality during ripening of olive fruits and related oils extracted from three minor autochthonous Sardinian cultivars

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ABSTRACT

Ripening stage is one of the key factors in determining quality of olive fruits and related oils. This research, thus, was aimed to study the influence of three different harvesting times on the quality parameters of olives and related oils of three autochthonous Sardinian cultivars, Sivigliana da olio, Semidana, and Corsicana da olio. We evaluated several parameters in olive fruits (dry matter, oil content, total soluble solids, total polyphenol and antioxidant activity) and oils (legal indices, total chlorophylls and tocopherols, single polyphenols and volatile compounds, antioxidant activity). The results obtained in olive fruits showed that all the parameters changed significantly during ripening and seem to confirm that the best harvesting time is that selected by the growers, that is when 70% of olives has just turned dark-colored and the rest is green. Results on oil evidenced that all the samples fulfilled the requirements of the European community for extra-virgin olive oils and showed a decrease in total chlorophylls and tocopherols, simple phenols and antioxidant activity during ripening, except for Sivigliana da olio oils, which evidenced an increase in simple polyphenols at the last sampling. A total of 17 volatile compounds were found on oils and those responsible for the green notes of the oils increased during ripening. PCA analysis well distinguished Sivigliana da olio olives from the other two cultivars and, in general, the genetic factor better explained variability, with respect to the ripening degree.

Keywords: Antioxidant activity; Extra virgin olive oil; Polyphenols; Ripening degree; Tocopherols; Volatile compounds

INTRODUCTION

Extra virgin olive oil (EVOO) plays a central role in the Mediterranean diet, not only for its peculiar sensory attributes, but mainly for health properties of its components, which can reduce the incidence of obesity, cardiovascular problems, and cancer (Gotsis et al., 2015; Owen et al., 2000). In fact, it is considered the best dietary fat for its unique fatty acid composition (Hill and Giacosa, 1992; Roche et al., 2000). Moreover, the mechanical extraction method used for its production results in the presence of minor compounds, like polyphenols, responsible for the nutritive and health benefits. Other important factors in regulating the EVOO quality are the cultivar, the ripening stage and the fruit health at harvest, the agronomic and the harvesting practices, and

the post-harvest care (Mansouri et al., 2018; Montedoro and Servili, 1992). None the less, EVOO found in the market is the result, in most cases, of blends, which are obtained from different olive cultivars. Monovarietal EVOO instead covers a minor part of the market, but consumers are discovering and appreciating this product for its unique characteristics. Moreover, in recent years, the increasing demand for olive oils and table olives has led to prefer cultivation on high-density or super high-density orchards with the result that only very few cultivars may be used (Allalout et al., 2011; Farinelli and Tombesi, 2015; Tous et al., 2011). On the other hand, major producing countries strongly follow the policy of using the major number of local cultivars. In this context, Italy plays a dominant role, considering that it has more than one third of European Union Protected Designation of Origin

(PDO). One of the 42 Italian EVOO DOP is "Sardegna DOP" as it can be produced only in Sardinia (Sardegna is the Italian name of Sardinia) by using the four main cultivars grown in this island (Bosana, Tonda di Cagliari, Tonda di Villacidro, Semidana and related synonyms) for at least the 80% of the whole olive blend, while the other 20% could be supplemented by other cultivars grown in the territory. While a certain bibliography can be found on the above cited four cultivars (Cerretani et al., 2006; Del Caro et al., 2006; Fadda et al., 2012; Tuberoso et al., 2016; Vacca et al., 2006) only a recent paper deals with other minor autochthonous Sardinian cultivars (Deiana et al., 2019). However, no information is available on the influence of the harvesting time on quality of olives and related oils. About the minor cultivars, are surely worth of investigation the "Sivigliana da olio" and the "Corsicana da olio", the first one as it has been reported to give oil with good polyphenolic content (Deiana et al., 2019) and olives that can be processed as table olives, the second one for the high productivity and resistance to some common diseases (Bandino et al., 2001). Moreover, we decided to extend the investigation to the Semidana cultivar too, due to the increasing interest of growers that are introducing it in all Sardinian orchards.

We aimed to investigate changes in selected quality parameters of olives and related oils of three autochthonous Sardinian cultivars, by sampling at three different ripening times.

MATERIALS AND METHODS

Plant material
Olives of cultivars Sivigliana da olio (SI), Semidana (SE), and Corsicana da olio (CO) were hand harvested from specialized non-irrigated orchards. The SE has a medium-sized fruit with pointed apex and umbo, it is normally harvested in the months of November-December. The CO has a medium-sized fruit, ovoid, with a rounded apex and absence of umbo, has normally a late harvest in November-December. The SI has a fruit of medium size, ovoid, with rounded apex and absence of umbo, it is generally harvested earlier in the month of October.

Olives of SI and SE cultivars were harvested in a 4 ha orchard in Ittiri, Italy (40°37'15.7"N 8°32'20.7"E, 270 m of altitude), while samples of CO were picked in a 6 ha orchard in Usini, Italy (40°39'39.0"N 8°31'32.7"E, 185 m of altitude). Both orchards are on a soil made up of limestone and sandstone, have plants with an age of approximately 70 years, trained to vase at a distance of 8 × 8 m and are mineral fertilized once per year with a ternary formulation (NPK 20-10-10 fertilizer at a dose

of 300 kg ha^{-1} year^{-1}). The area has had in the harvesting year an annual precipitation of 460 mm and a maximum, minimum and mean annual temperatures of 28.8, 4.8 and 14.8°C for Ittiri and 29.5, 5.8 and 15.8°C, respectively.

Three samplings were carried out, one at the ripening stage (second sampling) at which growers harvest olives for oil extraction (70% of olives just turned dark-colored and the rest green), 15 days before (first sampling) and 15 days after (third sampling). Each sampling was carried out by 10 selected trees. For each cultivar and sampling 10 kg of olives were harvested and soon transported to the laboratory where they were inspected and a sample of at least 7 kg of olives free of defects was chosen for analyses (2 kg) and oil extraction (5 kg).

Oil extraction
Oils were extracted using a small laboratory plant made up of a hammer-mill (RETSCH GmbH 5657, Haan, Germania) and a bench centrifuge (Marelli Motori S.p.A., Arzignano, VI, Italia). Once extracted, oils were stored in dark glass bottles at 4 ± 1°C and analyzed in one week.

Olive analysis
The olives were pitted, homogenized and subjected to analysis of dry matter (DM) and moisture (FM), total soluble solids content (TSSC), oil content (O), total polyphenols (TP) and antioxidant activity (AA). DM (%) were determined according to official methods of analysis (AOAC, 2000), while O (%) was determined after the extraction of the oil with a Soxhlet apparatus (AOAC, 2000).

TSSC (as g of glucose kg^{-1} dm) were determined after extraction from pulp according to Kiai and Hafidi (2014), with some modifications (1 g of pulp instead of 10 g). The obtained extract was subjected to spectrophotometric determination at 480 nm.

TP (as g of gallic acid kg^{-1} dm) were determined after extraction from pulp. In particular 1 g of olive pulp was mixed with 3 mL of hexane and 15 mL of methanol: water 70:30 (v/v), centrifuged at 6000 rpm at 4°C for 10 min, then the hydro alcoholic phase was collected and further centrifuged at 9000 rpm at room temperature for 5 min and the resulting extract was filtered with nylon filters (0.45 mm). The obtained extracts, after proper dilution, were spectrophotometrically analyzed at 750 nm using Folin-Ciocalteu reagent (Singleton and Rossi, 1965).

Polyphenolic extract was used for AA using a discoloration curve of the stable radical 2,2 diphenyl-1-picrylhydrazyl (DPPH·) as previously reported by Brand-Williams et al. (1995). A 50 mL aliquot of the sample was added with

Emir. J. Food Agric ● Vol 31 ● Issue 3 ● 2019

197

950 mL of methanol, then 200 mL of the resulting solution was made to react with 2.8 mL of a 6×10^{-5} M solution of DPPH for one h at 515 nm and 22°C to obtain a decrease in absorbance by the radical DPPH. The scavenging effect was calculated as a percentage of spectrophotometric decrease in absorbance by using the following formula:

$$\% \text{ Inhibition} = [1 - (A_{sample}/A_{control})] *100$$

where $A_{control}$ is the absorbance value of the only DPPH solution. The 50% inhibition (IC_{50}) was calculated and expressed as mg g^{-1}. Data of AA with lower values correspond to a lower amount of olives necessary to obtain a 50% inhibition, thus a lower value corresponds to a higher AA.

Oil analyses

Oils were analyzed for free acidity (FA), peroxide value (PV), total chlorophylls (TCH), total tocopherols (TTO), single polyphenols (SP), antioxidant activity (AA) and volatile compounds (VC). FA (as % of oleic acid) and PV (as mEq of O_2 kg^{-1}) were determined by the methods reported in the EEC/2568/91 Regulation of the Official Journal of the European Communities.

TCH (as mg kg^{-1} pheophytin a) was determined according to Pokorny et al. (1995).

TTO (as of α-tocopherol kg^{-1}) were detected and quantified using a HPLC Agilent 1100 Series (Agilent Technologies, Palo Alto, CA, USA), detector FLD Agilent 1100 Series set at 290 nm for excitation and 330 nm for emission, a Gemini C_{18} 100 × 4.6 mm ID, 3 mm particle size column (Phenomenex, Torrence, Usa), a mobile phase of methanol-water (98:2 v/v) and a flow rate of 2 mL min^{-1} (Gimeno et al., 2000).

SP (as mg kg^{-1}) extraction for HPLC analysis was carried out according to Caponio et al. (2018a) and Makhlouf et al. (2018). Approximately 5 g (± 0.001) of EVOO was weighted in a 50 mL plastic falcon and 1 mL of hexane and 2 mL of MeOH/water mixture (70:30 v/v) were added together with 250 mL of an internal standard solution of gallic acid (100 mg L^{-1} in MeOH/water 70:30, v/v). After mixing, samples were centrifuged for 10 min at 4°C at 3940 × *g* (SL 16R Centrifuge, Thermo Fisher Scientific Inc., Waltham, MA, USA). The methanolic fraction was collected and centrifuged for 5 min at 4°C at 8870 × *g*, then filtered with 0.45 µm nylon filters (VWR International, Center Valley, PA, USA). The chromatographic system and conditions were those reported in Tamborrino et al. (2017). Phenolic compounds were identified by comparing the retention times with those of the reference standards, or with literature data where no standards were available.

AA was determined on polyphenolic extract following the same methodology proposed for olives, with the difference that 50 mL of the sample were directly used for the analysis.

Extraction of VC was carried out as previously reported in Caponio et al. (2018b) by means of HS-SPME-GC-MS. A 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) SPME fiber was used. Samples conditioning lasts 2 min at 40°C followed by fiber exposure at the same temperature for 20 min. Desorption was made in the GC injector at 250°C for 2 min. An HP-Innowax (60 m × 0.25 mm, 0.25 µm film thickness) polar capillary column (Agilent Technologies, Santa Clara, CA, USA) was used with helium as carrier gas. VC were identified by comparison of their mass spectra with the mass spectra present in the NIST and Wiley libraries. Only those matching a quality above 70% were considered. Quantification was achieved by means of the internal standard method adding to each sample and replicate 100 mL of a 1-octanol solution in purified olive oil (60 mg kg^{-1}). Results were expressed as mg kg^{-1} of 1-octanol equivalents.

Statistical analysis

Data of olive and oil analyses were submitted to one-way analysis of variance ANOVA with harvesting time as group variable. The software Statistica 6.0 for Windows was used to process data. Means, when required, were separated according to Tukey's test, significance level $P \leq 0.05$.

Olive and olive oils dataset was explored by Principal Component Analysis (PCA) carried out on the correlation matrix by means of R-based chemometric software developed by the Group of Chemometrics of the Italian Chemical Society (R version 3.1.0, Core Team, 2014).

RESULTS AND DISCUSSION

Olive parameters

Results of analyses on olives are reported in Table 1. We decided to follow a direct approach to verify if the actual harvesting time used by growers is correct. To this end, we decided to check for O and the TSSC that are correlated (Cherubini et al., 2009; Menz et al., 2010; Migliorini et al., 2011). Both parameters were calculated on a dry basis, as recently proposed (Zipori et al., 2016), to avoid the natural fluctuation of flesh humidity that can result in unreliable data. FM tended to decrease after the grower's harvesting period and this was surely due to the increase of permeability to water of epicarp trough openings of the epidermis or cracks of protective wax (Mele et al., 2018) and contemporary absence of rainfalls. SE and CO olives have FM values ranging from 52.64 to 59.51%, while SI reached a minimum value close to 48% at the last sampling.

Values of FM are in general in agreement and in some case lower with respect to others recently reported in literature for other cultivars (Gamli and Eker, 2017; Talhaoui et al., 2015). This was probably due to the low rainfall in the growing period.

Data on O clearly showed a significant increase, especially from the second sampling that is the normal harvesting period, in agreement to what reported elsewhere (Cecchi et al., 2013; Cherubini et al., 2009; Migliorini et al., 2011; Trapani et al., 2016). It is worth to highlight the low O of SE and CO cultivars. The O increase was mirrored by an opposite decreasing trend of TSSC, as expected and reported (Trapani et al., 2016), except for CO that showed an opposite behavior.

With the exception of SI olives, the TP content resulted in general the highest at the second sampling and then decreased after the grower's harvesting period, while the

AA was higher in general at the first sampling in accordance with some other reports (Damak et al., 2008; Gougoulias et al., 2017). This shift could be due to the difference in polyphenolic compounds among samplings, as in the first sampling it was probably characterized by a higher content of polyphenols with higher antioxidant activity, with respect to the second sampling. Moreover, the presence of other compounds with antioxidant activity in the methanolic extract may explain this shift.

The results obtained are, at our knowledge, the first on olives fruit of the three Sardinian cultivars and seem to confirm, at least for data on fruit parameters, that the best harvesting time is that selected by growers, even if a 15-days delayed harvest results in a higher oil yield.

Fig. 1 reports the loading plot (A) and score plot (B) of the PCA performed on olives chemical data. The first

Table 1: Physical-chemical data and antioxidant activity evolution of the three considered Sardinian olive cultivars as affected by harvesting time

Harvesting time*	Flesh humidity (%)	Flesh dry matter (%)	Oil yield (% on dm)	Total soluble solids (g kg^{-1} dm)	Total polyphenols (g kg^{-1} dm)	Antioxidant activity (EC50 mg g^{-1} dm)
Semidana
First	52.64c**	47.36a	15.62c	81b	123a	0.79b
Second	59.51a	40.49c	17.30b	89a	122a	0.83b
Third	55.96b	44.04b	25.00a	59c	115b	0.95a
Corsicana da olio
First	57.17b	42.83b	13.03b	54c	118b	0.89a
Second	57.82a	42.18c	12.45c	62b	126a	1.05a
Third	56.17c	43.83a	21.11a	87a	116b	0.95a
Sivigliana da olio
First	57.67a	42.33b	29.40b	66a	87a	1.38c
Second	47.59b	52.41a	30.31b	71a	85a	1.52b
Third	47.95b	52.05a	38.79a	50b	83a	1.78a

*The second harvesting time corresponds to the ripening stage at which grower harvest olives for oil extraction (70% of olives just turned dark-colored and the rest green), while first and third ones to 15 days before and after, respectively. **Means with different letters for each column and cultivar differ significantly according to Tukey's test, significance level P ≤ 0.05.
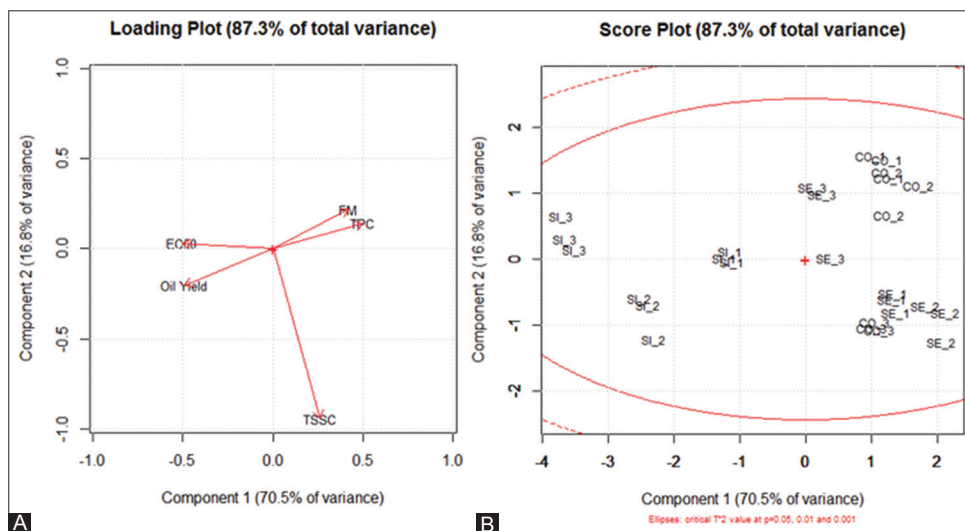

Fig 1. Loading plot (A) and score plot (B) of the PCA carried out on olives chemical data. SI, Sivigliana; SE, Semidana, CO, Corsicana. 1, 2, and 3 indicate the sampling time order.

two principal components explained about 87% of the total variability with PC1 accounting for more than 70%. On this component, olives from different cultivars were well separated. SI drupes separated well from the others two, as they were the richest in oil content and had the lowest antioxidant activity. Besides, SI fruits themselves were distinguished on the same component, highlighting that oil accumulation together with FM and TP reduction were the main changes occurring during maturation. Differently, olives from SE and CO varieties shared very similar characteristics apart from TSSC which followed a singular opposite behavior during maturation. Indeed, a dramatic reduction of TSSC was observed from the second to the third sampling time in SE fruits, while the opposite happened in CO fruits.

Olive oil main chemical parameters
Table 2 shows the legal quality indices, TCH, TTO, and AA of the oil obtained by the three Sardinian cultivars at the three ripening stages. FA and PV were below the legal limits for EVOO category. The FA was very low in SE and CO, with values ranging from 0.11 to 0.23 and dropped significantly after the grower's normal harvest date, while the exact opposite happened for SI cultivar. FA has been reported to increase during ripening due to enhancement of lipase activity (Ben Youssef et al., 2010; Nsir et al., 2017; Piscopo et al., 2018) thus agreeing with results obtained with SI, while for the other two cultivars it may be supposed that values could increase at later harvesting. The PV ranged from 5.8 to 9.0 and increased significantly at the third sampling as reported in literature, due to a probable increase in lipoxygenase activity (Baccouri et al., 2008; Salvador et al., 2001).

TCH impart to the EVOO the characteristic green color that is appreciated by consumers. Data obtained clearly

show a significant reduction in agreement with literature data (Bengana et al., 2013; Ben-Youssef et al., 2010; Nsir et al., 2017), especially for CO and SI, as they are converted during maturation to pheophytins. Values agree with those reported by Deiana et al. (2019) for the same cultivar, except for SE. A similar trend was registered for TTO, that evidenced a significant decrease, with the exception, again, of SE cultivar. The TTO evolution agrees with data reported in previous papers (Bengana et al., 2013; Gambacorta et al., 2010; Giuffrè et al., 2018; Matos et al., 2017), who always found a decrease in TTO content during the ripening period. Values of TTO agree with those reported by Deiana et al. (2019) for the same cultivar, except for SI and are, in general, in accordance with recent literature data (Slim et al., 2018).

The AA evolution agrees with that found on olives, with lower values and thus higher antioxidant activity, at the first sampling.

Phenolic composition
Fifteen SP were identified and quantified (Table 3). We detected six secoiridoids (compounds 7-10, 12, and 14) that accounted quantitatively from 46 up to 75% of total polyphenols detected. The most abundant secoiridoid was the dialdehydic form of the elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), while the dialdehydic form of elenolic acid linked to the tyrosol (*p*-HPEA-EDA), known as oleocanthal and responsible for the pungency of olive oil as well as having anti-inflammatory activity, was the second most abundant in CO cultivar. On the contrary, the oleuropein aglycon isomer (3,4-DHPEA-EA) and the ligstroside aglycon (*p*-HPEA-EA) were the second most important secoiridoids in SE and SI, respectively. Data on secoiridoids are quite different from those recently reported by Deiana et al. (2019) on the same cultivar. It is well known

Table 2: Influence of harvesting time on acidity, peroxide value, total chlorophylls, total tocopherols, and antioxidant activity of Semidana, Corsicana da olio, and Sivigliana da olio extra virgin olive oils

Harvesting time*	Free acidity (% oleic acid)	Peroxide value (meq O$_2$ kg^{-1} oil)	Total chlorophylls (mg kg^{-1} oil of pheophytin a)	Total tocopherols (mg kg^{-1} oil of tocopherol α)	Antioxidant activity (EC50 mg g-1 oil)
Semidana					
First	0.23a**	6.5b	50.7b	247a	41.5c
Second	0.23a	6.3b	55.1a	270a	56.7a
Third	0.17b	7.5a	51.9b	260a	48.5b
Corsicana da olio					
First	0.18a	7.0b	23.7a	275a	42.4b
Second	0.18a	6.9b	15.1b	231b	45.1b
Third	0.11b	9.0a	5.9c	214c	51.7a
Sivigliana da olio					
First	0.41b	5.8c	16.9a	172a	34.6c
Second	0.43b	6.6b	3.0c	162b	36.6b
Third	0.55a	7.8a	5.3b	146c	38.3a

*The second harvesting time corresponds to the ripening stage at which grower harvest olives for oil extraction (70% of olives just turned dark-colored and the rest green), while first and third ones to 15 days before and after, respectively. **Means with different letters for each column and cultivar differ significantly according to Tukey's test, significance level P≤0.05.

Table 3: Influence of harvesting time on phenolic profile of Semidana, Corsicana da olio, and Sivigliana da olio extra virgin olive oils

N	Polyphenols (mg kg^{-1} oil of gallic acid)	Cultivar								
		Corsicana da olio			Sivigliana da olio			Semidana		
		Harvesting time*								
		First	Second	Third	First	Second	Third	First	Second	Third
1	Hydroxytyrosol	0.14b**	0.27a	0.13b	0.91a	0.70b	1.14a	0.81a	0.74a	0.91a
2	Tyrosol	0.32c	0.36b	0.43a	1.55a	0.55c	0.83b	1.18b	1.32b	1.55a
3	Vanillic acid	0.25b	0.37a	0.35a	0.70a	0.25b	0.26b	0.37b	0.75a	0.70a
4	Syringic acid	0.47a	0.30b	0.25c	1.13a	0.64b	0.65b	1.65a	1.06b	1.13b
5	*p*-cumaric acid	0.07a	0.11a	0.17a	0.06a	0.24b	0.08a	0.44a	0.17a	0.06a
6	*trans*-Ferulic acid	0.09a	0.10a	0.10a	0.23a	0.15a	0.14a	0.21a	0.22a	0.23a
7	3,4-DHPEA-EDA	33.95a	30.06b	9.20c	7.78b	7.88b	12.72a	13.32a	9.69a	7.78b
8	3,4-DHPEA-EDA-ox	0.00a	0.00a	0.00a	3.21b	4.38a	5.42a	2.85b	3.41a	3.21a
9	3,4-DHPEA-EDA-carb	0.29c	1.64a	0.50b	3.82a	4.79a	5.13a	4.17a	4.05a	3.82a
10	*p*-HPEA-EDA	13.42a	9.97b	6.00c	3.26b	3.52b	6.13a	5.78a	4.00a	3.26b
11	Pinoresinol	17.50a	17.77a	16.14b	5.87a	5.89a	6.07a	7.05a	5.89a	5.87a
12	3,4-DHPEA-EA	1.71c	2.21b	2.51a	6.60a	1.09b	0.92c	6.21a	6.70a	6.60a
13	Luteolin	1.99c	4.06b	4.66a	3.14a	0.00a	0.00a	3.80a	2.87a	3.14a
14	*p*-HPEA-EA	4.36a	4.18a	4.20a	4.85c	6.80b	9.37a	5.34a	4.45a	4.85a
15	Apigenin	2.47b	2.74b	3.30a	2.78a	3.15a	3.67a	3.26a	2.73a	2.78a
	Total	77.03a	74.14a	47.94b	45.89b	40.03b	52.53a	56.44a	48.05b	45.89b

3,4-DHPEA-EDA, decarboxymethyl oleuropein aglycon, dialdehydic form; 3,4-DHPEA-EDA-ox, decarboxymethyl oleuropein aglycon, oxidased dialdehydic form; 3,4-DHPEA-EDA-carb, carboxymethyl oleuropein aglycone, dialdehydic form; p-HPEA-EDA, decarboxymethyl ligstroside aglycon, dialdehydic form; 3,4-DHPEA-EA, oleuropein aglycon; *p*-HPEA-EA, ligstroside aglycon. *The second harvesting time corresponds to the ripening stage at which grower harvest olives for oil extraction (70% of olives just turned dark-colored and the rest green), while first and third ones to 15 days before and after, respectively. **Means with different letters for each column and cultivar differ significantly according to Tukey's test, significance level P ≤ 0.05.

that multiple factors, out of the cultivar, may affect the polyphenolic composition, as stated in the introduction section. An important contribute to the polyphenolic fraction was also exerted by pinoresinol that was the second most abundant polyphenol in CO and was not detected by Deiana et al. (2019). Pinoresinol has been recently addressed to potentially have antitumor activity in human breast cancer cells (López-Biedma et al., 2016), although it has recently been demonstrated that pinoresinol is responsible for decreasing vitamin D intestinal absorption in rats (Goncalves et al., 2018). Phenolic acids and alcohols were detected in low amounts, while a good content of the flavonoids luteolin and apigenin, which was comparable to that reported by Deiana et al. (2019), was detected. The evolution of the total polyphenols, calculated by summing the single compounds, evidenced a decrease, except for SI cultivar that showed a significant increase at the last sampling period.

Volatile compounds

The evolution of volatile compounds of the olive oils is reported in Table 4. A total of 17 compounds have been detected and quantified, 6 aldehydes, 5 alcohols, 3 esters, 1 acid, 1 ketone, and 1 alkane. CO and SE oils were mainly characterized by the presence of C5 and C6 aldehydes, which ranged from 70 to 88%. The predominant aldehyde, as reported in other papers (Caponio et al., 2016; Kalua et al., 2007; Vekiari et al., 2010), was *trans*-2-hexenal, a C6 volatile deriving from autoxidation of linoleic acid

throughout the lipoxygenase pathway (Olias et al., 2003), which is responsible for the positive green-cut grass odor notes of EVOO. *Trans*-2-hexenal ranged from 61 to 83% in the 2 cited cultivars. The *trans*-2-hexenal tended to increase significantly during the ripening, in accordance with what reported by other authors (Amanpour et al., 2018; Karagoz et al., 2017). Other two important aldehydes in CO and SE were hexanal (green note) and pentanal, a C5 aldehyde deriving from linoleic acid trough a secondary metabolic pathway of lipoxygenase, which contributes positively to the aroma of olive oils and that was already found in considerable amounts in Sicilian EVOO (Baccouri et al., 2008). Hexanal was higher in the third sampling, with respect to the first one, while the opposite happened for pentanal. Alcohols accounted for 4 to 27% in CO and SE oils, and apart from those deriving from the lipoxygenase pathway (1-penten-3-ol, *cis*-2-penten-1-ol, 1-hexanol, and *cis*-3-hexen-1-ol), which were at a concentration well below the odor threshold value (Kalua et al., 2007; Morales et al., 2005), we found important amounts of ethyl alcohol, which may be associated to fermentation and thus development of negative sensory attributes during storage. However, it has been demonstrated that ethanol may accumulate in sound fruits during the ripening phase while on the tree, thus it is not just deriving from fermentation (Beltran et al., 2015) and this behavior is triggered by adverse environmental conditions, such as drought or cold (Moyano et al., 2004) and by genetic variability (García-Vico et al., 2018). Considering that during the pre-harvest period there

Table 4: Influence of harvesting time on volatile compounds of Semidana, Corsicana da olio and Sivigliana da olio extra virgin olive oils

Volatile compounds (mg kg⁻¹ oil)	Cultivar								
	Corsicana da olio			Sivigliana da olio			Semidana		
	Harvesting time*								
	First	Second	Third	First	Second	Third	First	Second	Third
Octane	1.18b	0.88c	1.39a	1.09b	2.04a	1.74a	0.31b	0.69a	0.63a
Methyl acetate	1.46a	0.37b	0.63b	0.33b	0.52a	0.61a	0.17a	0.25a	0.25a
Ethyl acetate	2.10a	1.31b	0.95b	1.98b	2.99a	0.97c	1.25a	0.64b	0.58b
2-Methyl butanal	1.68a	0.56b	0.70b	0.56b	1.02a	0.89a	1.06b	1.42a	0.70c
3-Methyl butanal	1.69a	0.64b	0.76b	0.38c	0.76a	0.67b	0.83b	1.63a	0.66b
Ethyl isobutyrate	0.57a	0.19c	0.42b	0.54b	1.65a	0.21c	0.57a	0.20b	0.18b
3-Pentanone	0.30a	0.30a	0.36a	1.14b	1.62a	1.43a	0.36a	0.28a	0.30a
Pentanal	9.57a	7.99a	3.50b	8.77a	4.62b	4.38b	5.36b	7.20a	4.10c
Ethyl alcohol	8.33b	22.44a	1.01c	64.21b	153.61a	28.51c	23.60a	4.77b	2.81c
Hexanal	6.23b	4.43c	7.11a	6.31b	5.39b	13.94a	3.27c	16.11a	7.19b
trans-2-Pentenal	0.30b	0.19c	0.42a	0.32b	0.97a	1.27a	0.71a	0.58a	0.50b
1-Penten-3-ol	1.30a	0.52b	0.69b	2.27a	0.14c	0.33b	1.09b	4.13a	1.76b
trans-2-Hexenal	99.68b	94.32b	126.46a	18.86a	3.29c	12.53b	77.19b	99.60a	94.67a
cis-2-Penten-1-ol	2.45a	1.25b	2.22a	3.79a	0.62c	0.98b	6.90a	2.93c	5.23b
Acetic acid	1.61a	2.53a	1.39b	0.70a	0.26b	0.90a	1.11a	1.54a	0.68b
1-Hexanol	2.20a	0.55c	1.06b	1.49c	2.10b	3.23a	0.77a	0.77a	0.25b
cis-3-Hexen-1-ol	1.75a	1.41b	1.68a	2.97b	2.75b	5.05a	2.61a	1.90a	1.17b

*The second harvesting time corresponds to the ripening stage at which grower harvest olives for oil extraction (70% of olives just turned dark-colored and the rest green), while first and third ones to 15 days before and after, respectively. **Means with different letters for each column and cultivar differ significantly according to Tukey's test, significance level P ≤ 0.05.
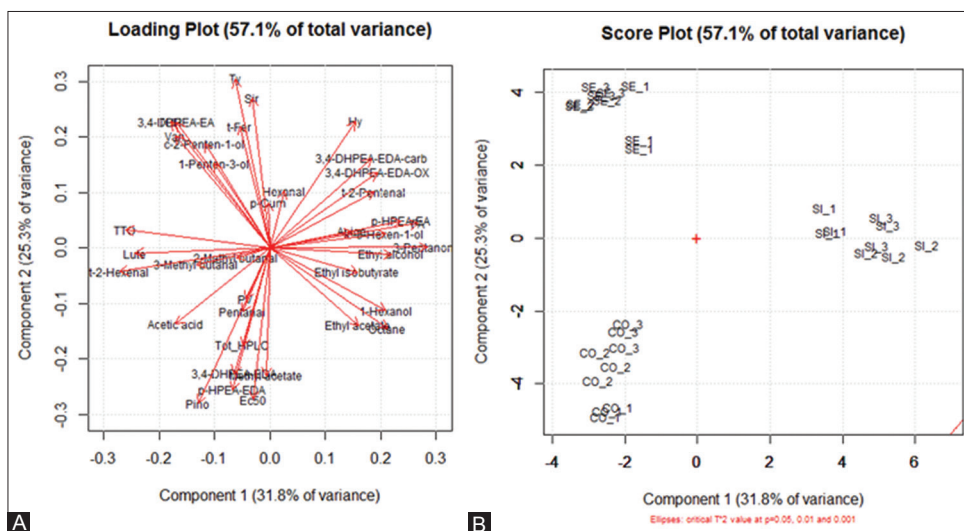

Fig 2. Loading plot (A) and score plot (B) of the PCA carried out on EVOOs chemical parameters, phenolic profile and volatile composition. SI, Sivigliana; SE, Semidana, CO, Corsicana. 1, 2, and 3 indicate the sampling time order. Hy, hydroxytyrosol; Ty, tyrosol; Van, vanillic acid; Sir, siringic acid; p-Cum, *p*-cumaric acid; t-Fer, *trans*-ferulic acid; Pino, pinoresinol; Lute, luteonin; Apige, apigenin.

was a prolonged time of drought due to absence of rainfall, we may hypothesize that a physiological build-up occurred in olives, especially in the first and second harvest, while it dropped considerably at the last sampling. The ethanol, moreover, was the most present compound in SI oils and peaked at 153 mg kg⁻¹ at the second sampling, thus if we consider that the olives were sound we can think that this cultivar has a propensity to produce non-fermentative ethanol. The profile of the other volatile compounds or the SI was like that of SE and CO.

The results obtained are, at our knowledge, the first on oils of the three Sardinian cultivars harvested at three different ripening times and seem to confirm, analogously to what already seen for olives, that the best harvesting time is that selected by growers.

EVOOs exploratory analysis

PCA carried out on oils chemical parameters, phenolic profile, and volatile composition is reported in Fig. 2. Overall, the first two principal components accounted for about 57%

of the total data variability. Oils from different cultivars were clearly separated in the space of the PCs, regardless of the olives sampling time. PC1 allowed to separate SI oils from the others that, differently, were distinguished along the second component. The main variables positively affecting PC1, and thus SI oils, were FA, hydroxytyrosol, 3,4-DHPEA-EDA-ox, 3,4-DHPEA-EDA-carb, *p*-HPEA-EA, octane, 3-pentanone, ethyl alcohol, 1-hexanol, and *cis*-3-hexen-1-ol which depicted a EVOO characterized by hydroxytyrosol derivatives as principal phenolic compounds as well as alcohols from the LOX pathway but, on the other hand, even by higher acidity and ethanol content likely due to the agronomic factors (season) or a peculiar genetic attitude as previously commented. EVOOs from SE and CO lied in the opposite half of the PCA, with respect to SI. SE oils were the richest in simple phenols, while CO oils were characterized by Ec50, 3,4-DHPEA-EDA, *p*-HPEA-EDA, pinoresinol, methyl acetate. Overall, the multivariate approach clearly showed as the variability among the studied varieties due to the genetic factors was quite higher than that due to the maturation degree, overcoming the latter. Such findings open perspective for Sardinian minor cultivar oils valorization.

CONCLUSIONS

This paper deals for the first time with the influence of the harvesting time on the quality and chemical composition of the olive fruit and related oils from three minor autochthonous Sardinian cultivars. The results obtained from this study indicate that both olive fruits and olive oil composition are statistically influenced by the ripening time and give good indications about the optimal harvesting time to obtain the best oil quality. The main variations observed in olives were the increase in O and the decrease in TP, while major changes in oil were TCH and SP. The multivariate statistical approach was a valid tool to differentiate the three olive cultivars, that differed mainly for genetic factors. All the three minor cultivars, however, produced oils of excellent quality, thus can be taken in consideration.

Author contributions
Authors contributed equally to this study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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