Influence of the nocturnal harvesting of olives from Salento (Italy) on the quality of the extra virgin olive oil

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SUMMARY: In southern Italy, some olive cultivars such as 'Ogliarola Salentina' ripen when the day temperature is still high, at up to 28 to 30 °C. This can affect both the oil quality and the expression of its valuable characteristics. In addition, it increases the risk of undesirable fermentation that can generate off-flavors. In the present study, nocturnal olive harvesting was carried out to determine the influence that temperature can have on the characteristics of the extra virgin olive oil, and particularly on the volatile and oxidative compounds. The data obtained are encouraging: the lower temperatures that occur during the night appear to have a particular and positive impact on the organoleptic characteristics of the extra virgin olive oil produced, with enhanced formation of the volatile compounds that contribute to the 'green' notes of the flavor. In areas where daytime temperatures are very high, the nocturnal harvesting of olives at the correct stage of maturity therefore represents a good agronomic practice for the production of oils with high organoleptic and nutritional merit.

KEYWORDS: *Extra virgin olive oil; Nocturnal harvesting; Quality; Volatile substances*

RESUMEN: ***Influencia de un cosechado nocturno de aceitunas en "Salento" sobre la calidad del aceite de oliva virgen extra***. En el sur de Italia, algunas variedades de olivo maduran cuando la temperatura del día sigue siendo alta, hasta 28 °C a 30 °C. Esto puede perjudicar la calidad del aceite y a la expresión de sus valiosas características, y aumenta el riesgo de fermentación indeseable que puede generar malos sabores. En el presente estudio, la cosecha nocturna de aceitunas se llevó a cabo para determinar la influencia que la temperatura puede tener sobre las características del aceite de oliva virgen extra, y en particular sobre los compuestos volátiles y los compuestos relacionados con la oxidación. Los datos obtenidos son alentadores: las temperaturas más bajas que se producen durante la noche parecen tener un impacto positivo en las características organolépticas del aceite de oliva virgen extra producido, con una mejora de la formación de los compuestos volátiles que contribuyen a las notas "verdes" del sabor. En las zonas donde las temperaturas diurnas son muy altas, la cosecha nocturna de aceitunas, representa, por tanto, una buena práctica agronómica para la producción de aceites con grandes ventajas organoléptica y nutritivas.

PALABRAS CLAVE: *Aceite de oliva virgen extra; Calidad; Compuestos volátiles; Cosecha nocturna*

1. INTRODUCTION

The agricultural and technological choices made throughout the olive-oil sector have important impacts on the quality of the final product. Favorable climatic conditions, plant treatments, olive harvesting at the correct stage of ripeness, fruit picking and storage methods, and the extraction technology applied, definitely affect both the quality and the organoleptic and nutritional characteristics of the resulting olive oil (Angerosa et al., 2001; Angerosa, 2002; Kalua et al., 2007). To achieve the best results, it is necessary to choose the best time for harvesting, which corresponds to the phenological phase of veraison, when the olives have reached their maximum development, and they have the highest concentrations of phenolic substances, reduced water, sugar and acid contents, and maximum oil accumulation.

Furthermore, it is necessary to store olives in perforated boxes, to ensure their correct ventilation and prevent the fermentation processes that can lead to the development of organoleptic defects (i.e., off-flavors). Their processing time also has to be as rapid as possible. Indeed, even if most of the enzymes in the olive pulp are active during the phases of crushing and kneading, these can come into contact with polyunsaturated fatty acids, which are produced by lipolytic enzymes during the storage of olives. This is also promoted by high temperatures, prolonged storage periods, and lack of ventilation, which can all result in undesirable fermentation and affect the enzyme activities. This, in turn, encourages an increase in the volatile compounds that are responsible for off-flavors (Kiritsakis et al., 1998; Koprivnjak et al., 2000, 2002).

The features that make extra virgin olive oil unique among the edible fats arise partly from the saponifiable fraction (98%–99%), but also to a greater extent from the unsaponifiable fraction (1%–2%). These include compounds that contribute to the olive oil flavor, and other compounds with high nutritional and therapeutic value that are effective natural antioxidants. Among these latter compounds, there are the phenolic substances that result from the degradation of oleuropein and ligstroside, and that contribute to the antioxidant potential of the oil. These also confer the specific organoleptic properties to the oil, and in particular, its bitter and pungent characteristics (Montedoro et al., 1992; Caponio et al., 1999). The volatile compounds that arise from the lipoxygenase pathway are responsible for the aromatic notes of extra virgin olive oil (Angerosa et al., 2000).

Several agronomic parameters can modify the phenolic concentrations. The most extensively studied of these include olive cultivar, fruit ripening, pedoclimatic conditions, agronomic techniques, and the mechanical oil extraction process. In particular,

the olive crushing and malaxation are the most important and critical points in the production of oil (Montedoro et al., 1989; Servili et al., 1994, Servili and Montedoro, 2002).

The volatile fraction of olive oil that is obtained from healthy, ripe and correctly stored and processed olives is mainly composed of a number of volatile compounds that are common to the aroma of many fruits and vegetables. It has been suggested that the accumulation of volatile compounds is mainly due to the olive variety, with the climate and other environmental factors apparently having indirect effects through the modification of the degree of olive ripeness (Angerosa et al., 1999). The C-6 aldehydes and alcohols and their acetyl esters that are responsible for the 'green' notes of olive oil can represent up to 80% of the volatile compounds. Their formation is stimulated in plant cells after the rupture of the cell wall, and regarding the olive fruit, this starts during the milling and continues through the malaxing. The levels of these volatile compounds that remain in the olive oil are a function of the oxidoreductase activity in the fruit, the amount of water, and the time and temperature of the malaxation (Di Giovacchino et al., 2002; Morales and Aparicio, 1999; Preziuso et al., 2009, 2010). The time and temperature of the kneading are two of the main factors that influence the activity of the enzymes that lead to the synthesis of aromas: lipoxygenase and hydroperoxide lyase. In particular, high kneading temperatures (above 30 °C) inhibit the actions of these enzymes, and consequently, the formation of volatile compounds that are produced via the lipoxygenase pathway.

In the Italian territories, the harvest period is carried out from mid-October until December, and it is influenced by several factors, including temperature, rainfall, altitude, soil type, and agronomic practices. In Puglia, in southern Italy, the harvest of some olive varieties, such as the 'Ogliarola Salentina o di Lecce' cultivar, is carried out earlier than in the other Italian regions. Here, the high daytime temperatures can affect the oil quality and the expression of certain peculiarities of precious oils, which can lead to the development of fermentation processes that generate off-flavors (Morales et al., 1997, 2005). However, there have been few studies on the effects of temperature during the harvesting, storage and extraction of olive oils, although some studies have investigated the storage of olive fruit under controlled temperatures and atmosphere (Kader et al., 1990; Garcia and Streif, 1991; Kiritsakis et al., 1998; Clodoveo et al., 2007). The storage of olives after harvesting and of the olive oil before it reaches the consumer can change the volatile composition of the olive oil. Storage of the fruit can decrease the aldehyde and ester contents that are responsible for the positive aroma. At the same time, incorrect storage of both the fruit and the oil can produce the volatile compounds that are responsible

for off-flavors (Kiritsakis *et al.*, 1998; Koprivnjak *et al.*, 2000). There can thus be an absence in the C-6 aldehydes, alcohols and esters that are produced by the lipoxygenase pathway, with increased levels of many aldehydes from chemical oxidation, including hexanal from both chemical and enzymatic reactions, and the consequent characteristic off-flavors of olive oil.

Nocturnal harvesting is a technique that involves targeted viticulture and precision oenology, and it was adopted in 1998 in particular for 'Chardonnay', the first Sicilian variety to ripen in August. During the day, the temperature is high, with it sometimes reaching 35 °C. This increases the risk of undesirable fermentation of grapes during their transport from the vineyard to the cellar, as well as the loss of some aromas during the grape pressing. At night, however, the temperature drops to 16 to 18 °C, which preserves the full aromatic content of the grapes. White grapes are cooled and pressed at a temperature of about 10 °C. This nocturnal harvesting reduces the energy used for cooling the grapes by about 70%, and it is also less tiring for the grape pickers (www.donnafugata.it/pagine/night-harvest.aspx). In Australia and California, USA, nocturnal harvesting has been practiced for 20 years or more, and recently also in southern France, with the help of harvesting machines.

In the present study, nocturnal olive harvesting was carried out to determine the influence that temperature can have on the characteristics of extra virgin olive oil and particularly on the volatile and the related compounds on the oxidation. The olives were harvested at the correct stage of ripeness using good standard practices for a quality product, and they were processed in the Forestaforte oil mill in Gagliano (Lecce, Italy).

2. MATERIALS AND METHODS

2.1. Plant material

This study was conducted at the Forestaforte oil mill (Gagliano, Lecce, Italy), which is operated in the olive-growing and oil-making sector for the production of top quality extra virgin olive oils (Protected Origin Denomination [D.O.P.] 'Terre d' Otranto') and oils from organic farming. The olives, subjected to two harvest processes, nocturnal and daytime, came from the same plot of land and belong to the cultivar 'Ogliarola Salentina', which is typical of this area. The olive grove, still under investigation, has been subjected to foliar fertilization, four pesticide treatments and one chemical weed. The plants, in addition to being of the same cultivar, were planted at the same time and have been submitted to a similar kind of pruning so as to obtain a homogeneous size of fruits. Both nocturnal and daytime harvesting were consecutively

performed when the olives were at the same stage of ripening (veraison 10% to 15%) and had not been attacked by parasites.

Daytime harvesting was carried out from 07:00 hours to 13.00 hours, and nocturnal harvesting from 20:00 hours to 02:00 hours. The average time for storage of the olives in perforated bins (300 kg each) was about 5 h, which was identical for the daytime and nocturnal harvesting. After passage through a defoliator, the total quantity of harvested and processed olives under each of these conditions was approximately 2000 kg net of impurities (twigs and leaves). The olives have been divided into three aliquots of ca 700 kg each of which was processed separately (see flowchart in Fig. 1).

To better maintain the temperature of the olives harvested at night, the wash water was cooled to around 10 °C before the introduction of the olives into the hopper for the start of the machining process. Both of these samples (daytime/ nocturnal harvesting) were processed by an extraction system that used two and half phase centrifugation. This was carried out in the afternoon for daytime harvesting, and early in the morning for nocturnal harvesting.

The olive milling was carried out using a rotary hammer crusher with an 8 mm diameter hole grid, and malaxation was then performed for a total time of 15 min with half the volume filling of the kneader (Amenduni) in a series with the hatch open and without the addition of water or heating. The oil yields obtained from each treatment were similar (13.8% for the daytime processing, 13.9% for nocturnal processing). Regarding the hourly productivity and other costs, variations between nocturnal and daytime harvesting were not recorded.

After the extraction, individual oil samples were collected in 250 mL bottles from the kneader, for a total of six samples (three daytime, three nocturnal), and they were stored at 17 °C. The temperature trends recorded through the collection phase to the conservation of the oil produced are shown in Figure 2.

2.2. Methods

The samples of extra virgin olive oil underwent analyses for free acidity, peroxide value, UV spectrophotometric indices, fatty acid and sterol compositions, sensory evaluation, dynamic head-space volatile compounds, alkyl esters, diglycerides, tocopherols and phenolic substances. The index of overall quality (global quality index; GQI) was also calculated, based on 13 variables that describe the nutritional and organoleptic preservation of the extra virgin olive oils (Giansante *et al.*, 2013; Finotti *et al.*, 2007). Duplicate analyses were performed on each sample.
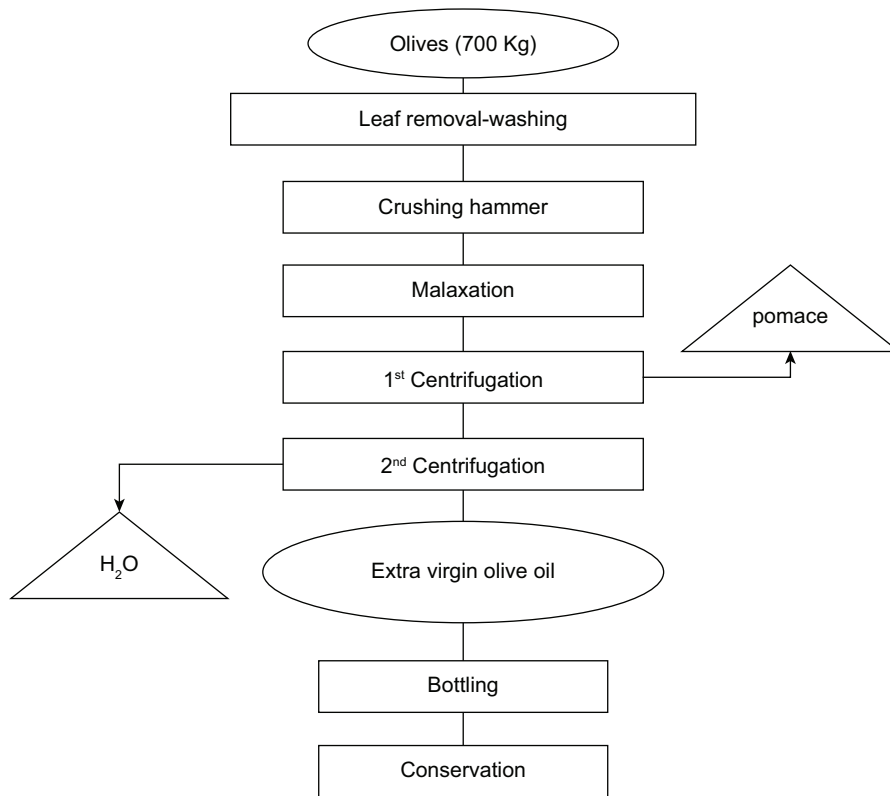
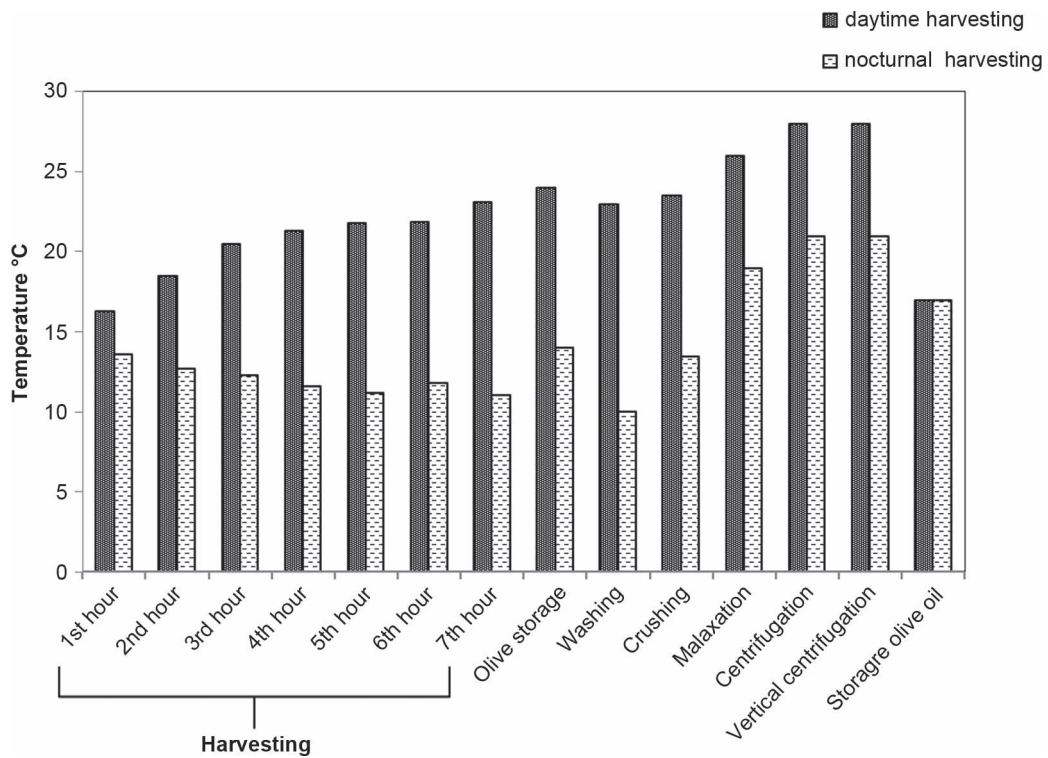FIGURE 1.   Flow diagram of the olive processing.

FIGURE 2.   Trends in daytime and nocturnal temperature during the olive harvesting and olive oil processing.

2.2.1. Free fatty acid content

The free fatty acid content was determined as the percentage of oleic acid, according to Annex II of the European Union Commission Regulation EEC/2568/91 and its subsequent modifications.

2.2.2. Peroxide index

The peroxide index was measured as milli-equivalents of active oxygen per kilogram of oil (i.e., meqO$_2$/kg oil), as determined according to Annex III of the European Union Commission Regulation EEC/2568/91 and its subsequent modifications (REG. EEC/2568, 1991).

2.2.3. UV absorption characteristics

The UV absorption characteristics (K_{232}, K_{270}, ΔK) were determined according to Annex IX of the European Union Commission Regulation EEC/2568/91 and its subsequent modifications (REG. EEC/2568, 1991).

2.2.4. Fatty acid composition

The fatty acid composition was determined according to the method described in the European Union Commission Regulation EEC/2568/91 and its subsequent modifications (Annex X.B) (REG. EEC/2568, 1991).

2.2.5. Sterol composition

The sterol composition was determined according to the method described in the European Union Commission Regulation EEC/2568/91 and its subsequent modifications (Annex V) (REG. EEC/2568, 1991).

2.2.6. Sensory evaluation

The sensory evaluation was carried out by ten tasters of the CRA-OLI Panel, according to Annex XII of the European Union Commission Regulation EEC/2568/91 and its subsequent modifications (REG. EEC/2568, 1991). The sensorial profile was determined according to COI/T.20/DOC. 22–2005. Nine sensory attributes which can be considered typical for the variety "Ogliarola Salentina" were considered: fruity, bitter, pungent, leafy, grassy, artichoke, bitter almond and chicory. The intensity scale ranged from zero to 10.

2.2.7. Dynamic headspace volatile compounds

The volatile compounds were stripped from 50 g of oil sample with N$_2$ (1.2 dm^{3}min^{-1}, for 2 h at 37 °C), using 10 mg nonan-1-ol added as the internal standard. The samples were adsorbed onto 50 mg of activated charcoal and eluted with 1 mL of diethyl ether. A quantitative analysis was carried out using gas chromatography, with a Carlo Erba Mega Series 5160 gas chromatograph equipped with a Restek Stabil-wax silica capillary column (length, 60 m; i.d., 0.32 mm; film thickness, 0.5 µm), and an on-column injection system, a CO$_2$ cryogenic accessory to hold the oven at 28 °C, with the flame Ionization detector set at 230 °C. The oven temperature programme was run at 28 °C for 6 min, increasing at 1.0 °C/min to 33 °C (no hold), then at 1.8 °C/min to 110 °C (no hold), and then 2.5 °C/min to 215 °C, where it was held for 10 min. The carrier gas was H$_2$ at 40 kPa. The injection volume was 0.5 µL. Quantification was by peak area integration with a Carlo Erba Mega Series integrator. Concentrations are expressed as mg/kg nonan-1-ol.

2.2.8. Alkyl esters

The alkyl esters were determined according to Annex II of the European Union Commission Regulation EU/61/2011 (REG. EU/61, 2011).

2.2.9. Diglycerides

The diglycerides were determined according to COI/T.20/Doc. No 32/2013.

2.2.10. Tocopherols

For the determination of the tocopherols (Rovellini *et al*., 1997), a solution of 1 g olive oil in 10 mL acetone was used directly for HPLC. α-Tocopherol (0.32 µg/10 µL) was used as the external standard. The HPLC analysis was conducted using a high-resolution LC 200 liquid chromatograph, equipped with a Series 200 UV/Vis detector (Perkin Elmer, Waltham, Massachusetts, USA), a 7725 Rheodyne injector, a 20 µL sample loop, and a Totralchrom workstation for data acquisition (Perkin Elmer, Waltham, Massachusetts, USA). Separation on Spherisorb ODS2 (250 x 4.6 mm I.D., 5µm; Waters, Milford, MA, USA) was performed at 25 °C under a constant flow rate of 1 mL/min with a mobile phase of 0.2% (v/v) H$_3$PO$_4$ in water/methanol/ acetonitrile (2/49/49, v/v/v). The eluted compounds were detected at 292 nm.

2.2.11. Biophenols

The phenolic compounds were extracted from the olive oil according to the following method: 2.5 g of oil were added to 500 µL of an internal standard solution (0.015 mg/mL syringic acid in methanol). After removal of the methanol under reduced pressure at <35 °C, the samples were dissolved in 6 mL hexane and loaded onto solid-phase extraction columns

(Discovery DSC-DIOL 500 mg, 3 mL; Supelco, Bellefonte, PA, USA), which were previously conditioned with 6 mL methanol and 6 mL hexane. The samples were then washed with 2×3 mL hexane and 4 mL hexane/ ethyl acetate (90/10, v/v), and then extracted with 10 mL methanol. After removal of the methanol in a rotary evaporator at a temperature <35 °C, the dry residue was taken up with 1 mL methanol/ water (1:1, v/v), filtered through a 0.45 µm PVDF membrane, and then injected into the HPLC system. The HPLC analysis of the phenolic extraction was carried out using a high-resolution LC 200 liquid chromatograph, which was equipped with a Series 200 UV/Vis detector (Perkin Elmer, Waltham, Massachusetts, USA), a 7725 Rheodyne injector, and a 20 µL sample loop, using a Totralchrom workstation for data acquisition (Perkin Elmer, Waltham, Massachusetts, USA). Separation on Spherisorb ODS2 (250×4.6 mm I.D., 5 µm; Waters, Milford, MA, USA) was performed at 25 °C at a constant flow rate of 1 mL/min, with mobile phase mixtures formed from A (0.2% [v/v] H_3PO_4 in water), B (methanol) and C (acetonitrile), as the following ternary gradient programme (as A/B/C, v/v/v): 0 min, 96/2/2; 40 min, 50/25/25; 45 min, 40/30/30; 60 min, 0/50/50; 70 min, 0/50/50; 72 min, 96/2/2; 82 min, 96/2/2. The eluted compounds were detected at 280 nm. Previously, a 20 µL external calibration standard (solution of tyrosol 0.030 mg/ml and syringic acid 0.015 mg/ml in methanol/water 80/20 (V/V) was injected into the HPLC system, to calculate the response factor of syringic acid to tyrosol. Then 20 µL samples were injected. Quantification of the phenolic compounds, expressed as tyrosol according to COI/T.20/Doc No 29/2009, was performed according to the concentration of the internal standard and on the basis of the response factor of syringic acid to tyrosol.

2.3. Global quality index

The variables extracted from the original data matrix were those most correlated to the organoleptic properties (fruity, bitter, pungency, 1-penten-3-one), conservative properties (phenolic substances, tocopherols, peroxide value, free acidity), and nutritional properties (palmitic acid [C16:0], stearic acid [C18:0], oleic acid [C18:1], linoleic acid [C18:2], and the campesterol/ stigmasterol ratio). The GQI was calculated through these selected variables, and ranged between 0.0 and 2.0. Three levels of GQI assessment were defined for the olive oils: 0.0 <GQI≤1.0, 'excellent'; 1.0 <GQI≤1.6 'good'; and 1.6<GQI≤2.0 'sufficient' (Giansante *et al*., 2013; Finotti *et al*., 2007).

2.4. Statistical analysis

The full chemical, chemical-physical, and sensorial data were subjected to statistical analysis using the hierarchical cluster analysis of all of the variables simultaneously. Successively, each variable was analyzed using the Student's t-tests. Data calculations were carried out using the SPSS® software, release 11.5.

3. RESULTS AND DISCUSSION

All of the analytical data from the chemical, chemical-physical and sensory analyses were organized into a matrix of $n \times m$, where $n = 6$ oils, and $m = 77$ analytical variables. Without an *a-priori* hypothesis, the most straightforward method of hierarchical cluster analysis was applied to the *Z-scores* from the original data. The proximity matrix was calculated according to the squared Euclidean distance, with the furthest neighbor method (or complete linkage) used to combine the clusters. The visual representation of the distances at which the clusters are combined is shown in the dendrogram in Figure 3. The position of the line on the scale indicates the distances at which the clusters are joined. The first vertical line in the dendrogram corresponds to the smallest rescaled distance of oils A1 and A2, which together with oil A3, form a homogeneous cluster of daytime harvesting oils that are well separated from the cluster of the nocturnal harvesting oils B1, B2, B3. This cluster analysis demonstrates the natural conformations of these groups of olive oils (A, B), and in particular the homogeneity within the daytime harvesting samples (A1, A2, A3) and the nocturnal harvesting samples (B1, B2, B3), and the distance between the two groups (A, B), as expected.

3.1. Quality parameters

Table 1 gives the mean data for the quality parameters of the two replicates for each of the three samples from each harvesting (daytime, nocturnal). As expected, given the optimal conditions used for their production, the data obtained classify both these oils as top quality extra virgin olive oils, with a high content of natural antioxidants.

For the parameters considered, the 'daytime' and 'nocturnal' oils were not significantly different, except for the diglycerides and the 1,2-diglyceride content, which are parameters that are related to the 'freshness' of olive oil, and to oxidative stress. Indeed, comparing the 'daytime' and 'nocturnal' oils, there were significant differences ($p \le 0.05$) for the diglycerides (1.4 g·100 g^{-1} *vs.* 1.2 g·100 g^{-1}, respectively), and the 1,2-diglycerides (95.6% *vs.* 98.9%, respectively). Diglycerides are naturally present in extra virgin olive oils, and they derive from the incomplete formation or the partial hydrolysis of triglycerides. The lower value for the nocturnal oil is indicative of lower lipase activity. Conversely, the higher 1,2-diglyceride content (with respect to 1.3-diglycerides) is linked to the greater freshness of the nocturnal oil, due to the olives undergoing less
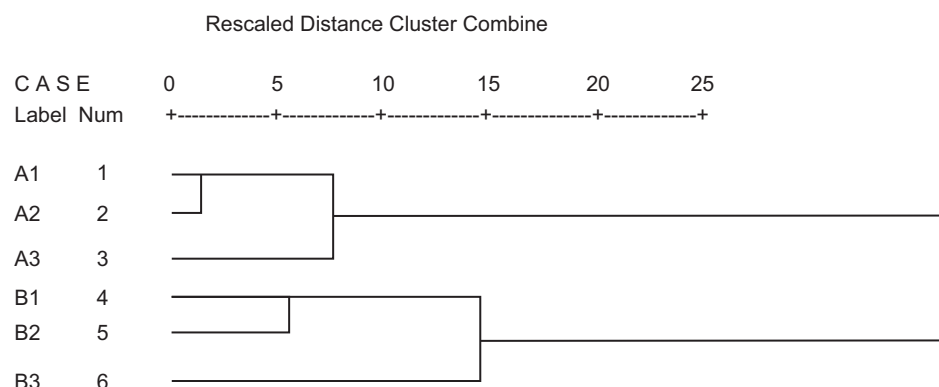
Dendrogram using Complete Linkage

FIGURE 3. Dendrogram from the hierarchical cluster analysis as applied to the Z-scores for the original data from the chemical and sensory analyses, showing the clusters of the olive oil samples.

TABLE 1. Qualitative characteristics of the extra virgin olive oils obtained following daytime and nocturnal harvesting of the olives

	Harvesting time	
Characteristic	**Daytime**	**Nocturnal**
Free acidity (% oleic acid)	0.21 ± 0.02	0.19 ± 0.03
Peroxide value (meqO$_2 \cdot$kg^{-1})	6.2 ± 0.5	5.4 ± 0.5
K$_{232}$	1.81 ± 0.06	1.80 ± 0.06
K$_{270}$	0.10 ± 0.02	0.12 ± 0.02
ΔK	-0.002 ± 0.001	0.000 ± 0.001
Alkyl esters (mg$\cdot$kg^{-1})	5 ± 3	6 ± 3
Diglycerides (g$\cdot$100 g^{-1})	1.4 ± 0.1*	1.2 ± 0.2*
1,2-diglycerides (%)	95.6 ± 0.4*	98.9 ± 0.4*
Tocopherols (mg$\cdot$kg^{-1})	631 ± 20	639 ± 14
QGI	1.1 ± 0.1	1.0 ± 0.1
Organoleptic assessment		
median of the fruity attribute	3.3 ± 0.3	3.6 ± 0.3
median of the bitter attribute	5.2 ± 0.1	5.3 ± 0.3
median of the pungent attribute	5.1 ± 0.3	5.0 ± 0.3
median of the defect	0.0 ± 0.0	0.0 ± 0.0
Total phenols (mg$\cdot$kg^{-1})	746 ± 81	723 ± 18
Secoiridoids (mg$\cdot$kg^{-1})	650 ± 73	629 ± 36
- Low molecular weight (mg$\cdot$kg^{-1})	12 ± 3	7 ± 3
- Medium molecular weight (mg$\cdot$kg^{-1})	313 ± 39	276 ± 24
Ox-forms (mg$\cdot$kg^{-1})	64 ± 11*	33 ± 10*
- High molecular weight (mg$\cdot$kg^{-1})	325 ± 27	346 ± 58
Oleuropein (mg$\cdot$kg^{-1})	167 ± 6*	215 ± 44*
Ox-forms (mg$\cdot$kg^{-1})	9 ± 2	8 ± 2
Lignans (mg$\cdot$kg^{-1})	10 ± 7	10 ± 7
Other phenols (mg$\cdot$kg^{-1})	86 ± 13	84 ± 31
Total Ox-phenols (mg$\cdot$kg^{-1})	673 ± 68	682 ± 29

*significantly different (2-tailed) for daytime *versus* nocturnal olive oils, at $p \leq 0.05$.

oxidative stress and to fewer negative exogenous factors, and in particular, to the lower temperature and the shorter storage time.

These factors also influenced the phenol content of these oils. The phenols were grouped in order to highlight the differences by means of statistical processing of content for the two processes in the following way: low molecular weight secoiridoids (hydroxytyrosol, tyrosol); medium molecular weight secoiridoids (decarboxymethyl oleuropein aglycone dialdehyde form, decarboxymethyl ligstroside aglycone dialdehyde form); oxidized forms of medium molecular weight secoiridoids (decarboxymethyl oleuropein aglycone oxidised dialdehyde form, decarboxymethyl ligstroside aglycone oxidised dialdehyde form); high molecular weight secoiridoids (oleuropein; oleuropein aglycone dialdehyde, aldehyde and hydroxylic forms; ligstroside aglycone dialdehyde, aldehyde and hydroxylic forms); oxidized forms of high molecular weight secoiridoids (oleuropein aglycone oxidized aldehyde and hydroxylic forms; ligstroside aglycone oxidized aldehyde and hydroxylic forms); lignans (pinoresinol, 1 acetoxypinoresinol); other phenols (vanillic acid, caffeic acid, vanillin, *para*-coumaric acid, hidroxytyrosyl acetate, ferulic acid, *ortho*-coumaric acid, tyrosyl acetate, cinnamic acid, luteolin, apigenin, methylluteolin). Although the mean total phenol contents of the daytime and nocturnal oils were not statistically different, the mean content of the oxidative form of secoiridoids of medium molecular weight (64 mg·kg^{-1} *vs*. 33 mg·kg^{-1}, respectively) and of high molecular weight (as oleuropein; 167 mg·kg^{-1} *vs*. 215 mg·kg^{-1}, respectively) were significantly different ($p \leq 0.05$) between these harvesting times.

The QGI provides a definition of three levels of the overall quality of extra virgin olive oil, as 'excellent' ($0.0 < QGI \leq 1.0$), 'good' ($1.0 < QGI \leq 1.6$), and 'sufficient' ($1.6 < QGI \leq 2.0$). The extra virgin olive oil from the daytime harvesting was classified as 'good', while the extra virgin olive oil from nocturnal harvesting was classified as 'excellent'.

3.2. Volatile substances and sensory evaluation

Table 2 shows the mean data obtained from the analysis of the volatile compounds of the head-space from the oils obtained from the daytime harvesting and nocturnal harvesting. Both of these extra virgin olive oils had a high content of 'pleasant' volatile compounds, although these were significantly different (443 *vs*. 557 mg·kg^{-1}, respectively, $p \leq 0.05$). This further confirms the good quality of the olives from which these extractions were made, and the optimal storage conditions used. For the daytime harvesting, the lower content of positive volatile compounds in the olive oil (compared to the nocturnal olive oil) will be due to the negative influence of high temperatures during the

harvesting and storage of the olives (Fig. 2). Most of the volatile compounds are formed through the action of the enzymes that are released when the olives are crushed, and these continue to form during malaxation (Olias *et al.*, 1993; Tressl and Drawert, 1973), from enzymatic oxidation through the lipoxygenase pathway. The lipoxygenase pathway is a biochemical reaction scheme for the C-6 aldehydes, alcohols and esters, which are the aroma compounds (Angerosa, 2001; Kiritsakis,1998). The difference in temperature between the daytime and nocturnal harvesting times, and especially during the malaxation (26 °C *vs*. 19 °C, respectively), is reflected in the levels of the main volatile compounds that are responsible for the green notes of the flavor of extra virgin olive oil. There were significant differences among these main volatile compounds, in terms of 3-methylbutanal, 2-butanone, *trans*-2-penten-1-ol, *cis*-2-penten-1-ol, *trans*-2-hexenyl acetate, 1-hexanol and *cis*-3-hexen-1-ol. As can be seen from the data in Table 2, these principal compounds of the lipoxygenase pathway were at higher levels for the nocturnal harvesting because these are influenced by the activity of the enzymes in the olives.

The maximum activities of the lipoxygenase enzymes occur in the temperature range from 25 to 35 °C (Ridolfi *et al.*, 2002), while hydroperoxide lyase is relatively labile, with an optimum activity at around 15 °C, and maximum activity at about 35 °C (Anthon and Barret, 2003, Salas and Sánchez, 1999). These factors result in the loss of flavor for oil obtained when the processing is performed at higher temperatures. These differences in the compounds responsible for the green notes also resulted in different sensory profiles for these two types of extra virgin olive oil (Fig. 4): for the olive oil produced following night-time harvesting (the nocturnal oil), the perception of fruity, leafy, grassy, artichoke, bitter almond and chicory notes was greater. There were also significant differences ($p \leq 0.05$) between the daytime and nocturnal oils for some of the compounds responsible for off-flavors: propyl acetate, pentanal and 2-penthylfuran (Table 2). As it appears that olive storage at higher temperatures before oil processing promotes an increase in such undesirable compounds (Koprivnjak *et al.*, 2000), this is to be avoided in the production of extra virgin olive oil. Also, good practice in olive handling recommends that the olives are processed as soon as possible after harvesting, and to avoid prolonged storage periods (Di Giovacchino *et al.*, 2002; Kalua *et al.*, 2007).

4. CONCLUSIONS

The data obtained in the present study for the daytime and nocturnal harvesting of olives demonstrate that:

TABLE 2.   Levels of volatile compounds and sensory descriptors of the extra virgin olive oils
obtained following daytime and nocturnal harvesting of the olives

Volatile compound	Harvesting time		Sensory description**
	Daytime	Nocturnal	
Ethyl acetate	10±2	10±1	Slightly bitter/ pungent, sweet, aromatic
2-Butanone	4.0±0.4*	1.4±0.4*	Tomato, apple
2-Methylbutanal	0.50±0.01	0.29±0.01	Apple, malty, sweet, fruity
3-Methylbutanal	0.5±0.1*	0.2±0.1*	Apple, malty, sweet, fruity
Iso-propanol	0.3±0.2	0.3±0.2	
2-Ethylfuran	4.2±0.3	4.7±0.8	Sweet
Pentene dimers	3.7±0.6	3.9±0.5	
2-Pentanone	18±3*	19±3*	
Propyl acetate	1.7±0.50*	0.6±0.5*	
Pentanal	0.17±0.02*	0.09±0.01*	Woody, bitter, oily
alfa-Pinene	11.8±0.7	13.1±1.7	
Iso-butyl acetate	0.7±0.1	0.9±0.2	
1-Penten-3-one	20±1	22±2	Green, pungent
2-Butanol	17±3	16±5	Fusty, muddy, winey
3-Hexanone	0.20±0.09	0.37±0.06	
Hexanal	28±5	36±5	Sweet, apple, green, fruity, grassy
3-Pentanol	0.10±0.05	0.11±0.02	
1-Penten-3-ol	32±4	37±5	Fruity, wet earth
cis-3-Hexenal	1.7±0.8	2.2±0.8	Green, leaf-like
3-Methyl-1-butanol	4.2±0.3	4.6±0.9	Fusty
trans-2-Hexenal	175±17	200±32	Bitter almonds, green, apple-like, green astringent
2-Penthylfuran	12.5±0.8*	14.8±0.9*	
1-Pentanol	2.5±0.7	2.3±0.8	
3-Octanone	0.10±0.04	0.02±0.02	Rancid
Hexyl acetate	7.6±0.7	9.30.7	Green grassy, sweet, fruity
2-Octanone	0.10±0.01	0.16±0.5	Undesirable, mould, green
Octanal	2.8±0.5	2.8.5	Fatty, sharp, citrus-like
trans-2-Penten-1-ol	0.47±0.06*	0.23±0.09*	Desirable
cis-3-Hexenyl acetate	2.1±0.3	2.3±0.2	Green, banana-like
cis-2-Penten-1-ol	60±1*	96±8*	Green grass, banana-like
trans-2-Eptenal	0.4±0.2	0.30±0.1	Rancid, tallowy, pungent
trans-2-Hexenyl acetate	1.0±0.2*	2.3±0.3*	
6-Methyl-5-hepten-2-one	0.13±0.04	0.12±0.06	Rancid, Musty, Bitter taste (dried green herbs), pungent, green
1-Hexanol	12.3±0.5*	17.6±0.5*	Fruity, banana, soft
trans-3-Hexen-1-ol	0.35±0.05	0.53±0.09	Green leafy, nuts
cis-3-Hexen-1-ol	35±8*	66±8*	Green banana, leaf-like
Nonanal	2.9±0.5	2.0±0.5	Fatty, waxy, pungent
trans-2-Hexen-1-ol	11±2	9±1	Green (cut green grassy)
Acetic acid	0.24±0.09	0.1±0.1	Sour, vinegary, vinegar-like
trans-2-Nonenal	0.30±0.02	0.28±0.07	Paper-like, fatty, undesirable
Propionic acid	4.7±0.8	2.3±0.8	Undesirable
Total 'positive flavors'	443±28*	557±22*	
Total 'off-flavors'	173±11	143±12	

*significantly different (2-tailed) for daytime *versus* nocturnal olive oils, at $p \leq 0.05$.
**(Aparicio *et al*., 1994, 1996 and Aparicio and Luna 2002; Blekas *et al*., 1993; Di Giacinto *et al*., 2010; Morales *et al*., 1995; Reiners and Grosch 1998).
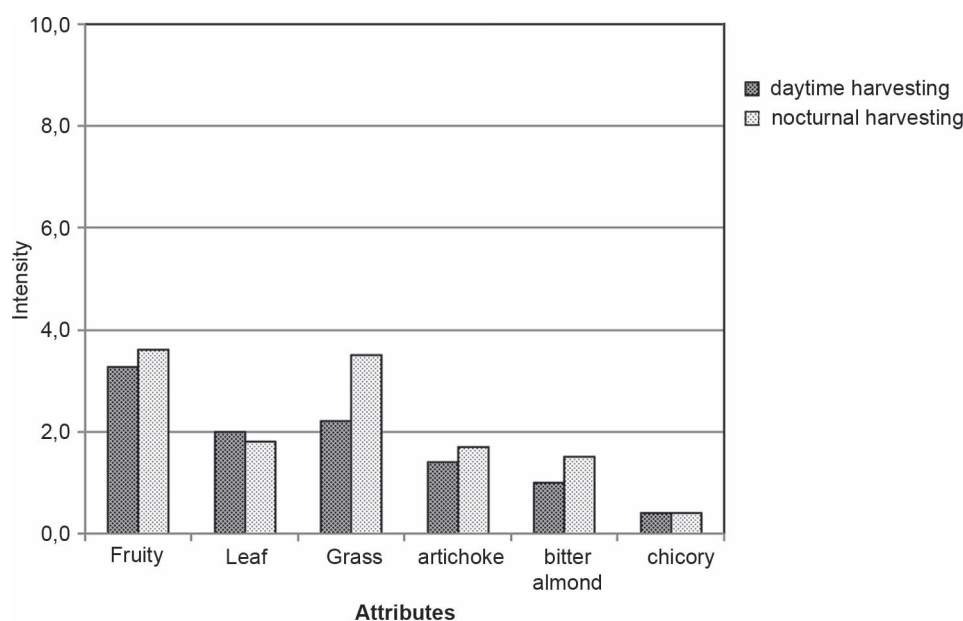
FIGURE 4.   Sensory profile of the extra virgin olive oils derived following daytime and nocturnal harvesting.

(i) The oil yields obtained by each treatment are similar (13.8% by the daytime processing, 13.9% by nocturnal processing). Regarding the hourly productivity and other costs, variations between nocturnal and daytime harvesting were not recorded.

(ii) The time and temperature of the storage of olives, and the extraction temperature for the olive oil, are the most important aspects for the quality and the particular characteristics of extra virgin olive oil.

(iii) The lower temperatures during nocturnal harvesting of the olives and during the oil extraction have positive effects on some of the quality characteristics of extra virgin olive oil, and in particular, on compounds that are negatively related to oxidative processes.

(iv) The time and temperature of the storage of olives after harvesting and during the extraction of the oil also influence the levels of volatile compounds from the lipoxygenase pathway (the green flavors) and the off-flavor composition of the extra virgin olive oil.

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