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Characterization of phenolic and volatile composition of extra virgin olive oil extracted from six Italian cultivars using a cooling treatment of olive paste

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ABSTRACT

The effects of a cooling treatment of olive paste were studied to evaluate the impact of six Italian cultivars on the phenolic and volatile compounds of extra virgin olive oil (EVOO) strictly related to its health and sensory quality. The EVOOs, extracted using a continuous industrial system (2.5 ton/h), exhibited a significant increase in phenolic composition of Frantoio, Gentile, Leccino and San Felice cultivars. Significant modifications of the volatile profiles were obtained; the sum of aldehydes, mainly represented by the concentration of (*E*)-2-hexenal, showed increases for all the cultivar processed, while the sum of alcohols and esters appeared to be more affected by the genetic origin of the olive cultivars and their varying enzyme specific activities related to the lipoxygenase pathway.

1. Introduction

In recent years, the scientific world and the industrial olive oil sector have become increasingly linked by the main goal of improving extra virgin olive oil (EVOO) production and quality. Various technological innovations in the mechanical extraction process have been studied to improve plant working efficiency and to increase the health and sensory properties of EVOO (Bejaoui, Beltran, Aguilera, & Jimenez, 2016; Esposto et al., 2013; Puértolas & Martínez de Marañón, 2015; Veneziani et al., 2015). The health characteristics of vegetable oils are largely connected with several molecules marked by high biological activities as well as by the distinctive fatty acid composition (Gustan, 2000; Purcaro, Barp, Beccaria, & Conte, 2016) . The health-protective effects of EVOO are mainly ascribed to secoiridoid derivatives of oleuropein (hydroxytyrosol (3,4-DHPEA), the dialdehydic forms of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), and the isomer of oleuropein aglycon (3,4-DHPEA-EA)) and ligstroside (tyrosol (*p*-HPEA), the dialdehydic forms of elenolic acid linked to tyrosol (*p*-HPEA-EDA) and ligstroside aglycon) in addition to lignans ((+)-1-acetoxypinoresinol and (+)-1-pinoresinol) (Aparicio-Ruiz et al., 2016; Di Maio et al., 2013, 2011; Fregapane & Salvador, 2013; Servili et al., 2004; Vitaglione et al., 2015), which guarantee a considerable quantitative and qualitative contribution to the daily intake of phenolic compounds in the typical Mediterranean diet (Briante, Febbraio, & Nucci, 2003; Vasto et al., 2014). The bioactive properties of olive phenolic compounds such as antioxidant, anti-inflammatory, antiatherogenic, cardioprotective, and chemo-preventive properties (Casamenti et al., 2015; Khanal et al., 2011; Leri et al., 2016; Pantano et al., 2016; Reddy & Naidu, 2016; Segade et al., 2016) and also the health claim (EFSA, 2012) related to the inhibition of low density lipoproteins and protection of blood lipids from oxidative stress, have justified the enormous efforts expended by researchers and producers to obtain EVOOs richer in phenolic compounds (Reboredo-Rodríguez et al., 2017). A recent technological advance of oil processing is the cooling treatment (CT) of olive paste proposed for the first time by Veneziani et al. (2017) based on the use of a thermal exchanger, which generated a significant increase in phenols for all the cultivars processed, yielding final products with higher quality, bioactivity and health benefits.

Together with the phenolic compounds, volatile compounds

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play an important role in the production of an EVOO of high quality, helping create the olive oil flavor (Garrido-Delgado, Dobao-Prieto, Arce, & Valcárcel, 2015; Kalua, Bedgood, Jr. Bishop, & Prenzler, 2013). These volatile compounds, generated by the lipoxygenase pathway during the first steps of olive oil extraction, are highly influenced by genetic, agronomic and technological variables (Muzzalupo et al., 2012; Padilla, Martinez-Rivas, Perez, & Sanz, 2012; Perez, Luaces, Rios, Garcia, & Sanz, 2003; Sacchi, Caporaso, Paduano, & Genovese, 2015; Sanchez-Ortiz, Romero-Segura, Sanz, & Perez, 2012). The novel olive paste CT showed a variation of the main volatile molecules (saturated and unsaturated C_5 and C_6 aldehydes and alcohols as well as esters) involved in the positive aroma of EVOO (Veneziani et al., 2017). The modifications seem to be principally affected by the cultivar; however, further investigation should be conducted to understand the real impact of the CT on the qualitative and quantitative concentrations of volatile compounds in EVOO.

The processing of six Italian cultivars using an industrial system with a working capacity of 2.5 ton/h will be able to provide a large amount of data concerning the impact of olive genetic origins on the minor components of EVOOs obtained with the innovative mechanical extraction process, which is based on the use of a tubular heat exchanger to cool the olive pastes after the crushing phase. The analysis of phenols and volatile compounds of EVOOs could further highlight and explain the usefulness of the new technology to preserve and improve the quality of the product.

2. Materials and methods

2.1. Chemicals

Reagents used for HPLC analysis were supplied by Sigma-Aldrich (Milan, Italy). Hydroxytyrosol (3,4-DHPEA) was purchased from Fluka (Milan, Italy), while tyrosol (*p*-HPEA) was obtained from Cabru s.a.s. (Arcore, Milan, Italy). All the other phenolic compounds (the dialdehydic forms of elenolic acid linked to hydroxytyrosol and tyrosol (3,4-DHPEA-EDA and *p*-HPEA-EDA), the isomer of oleuropein aglycon (3,4-DHPEA-EA), ligstroside aglycon, (+)-1-acetoxypinoresinol and (+)-pinoresinol) evaluated in this study were obtained as described by Veneziani et al. (2015). The pure analytical standards of the volatile compounds were supplied by Sigma-Aldrich (Milan, Italy).

2.2. EVOO mechanical extraction process

Olives of the Frantoio, Leccino, Gentile, Ogliarola garganica, Moraiolo and San Felice cultivars were used to extract the EVOO samples. Frantoio, Leccino, Moraiolo and San Felice olives came from the Umbria region, whereas the Ogliarola and Gentile growing areas were the Apulia region and Abruzzo region, respectively. The harvesting period was between the last week in September and the end of October 2015. The pigmentation index values, used to determine the ripening stage as described by Pannelli, Servili, Selvaggini, Baldioli, and Montedoro (1994), ranged from 0.91 to 0.97 for all the cultivars. The olive temperature, processed within 48 h from harvesting, was approximately 26 °C before the mechanical extraction process. The EVOO was extracted in triplicate from each olive cultivar using an Alfa Laval industrial system (Alfa Laval SpA, Tavarnelle Val di Pesa (FI), Italy) with a working capacity of 2.5 t/h. The system consisted of a 30 hp disk mill, an Alfa Laval Thermal Conditioning Module to rapidly cool the crushed olive paste, four Atmosphera RM 650round malaxers, an X6 decanter regulated at three-phase operation with the addition of a lower amount of dilution water and a vertical centrifuge model UVPX 507. The trials were carried out as reported by Veneziani et al. (2017), using a heat exchanger to regulate the temperature of olive paste, at 25 °C for the control trials and to establish a rapid cooling conditioning at 15 °C for the experimental tests with a reduction of approximately 10 °C; malaxation was performed at 25 °C for 30 min in all the trials studied.

2.3. EVOO analyses

2.3.1. Legal quality parameters

The main legal quality parameters (free acidity, peroxide value, and the UV absorption characteristics) of EVOOs were determined by the European Official Methods (OJEC, 2003).

2.3.2. Phenolic compounds

The phenolic fraction was recovered by a liquid-liquid extraction method mixing the VOOs with a solution of methanol/water (80/20 v/v) as described by Antonini et al. (2015), and the quantitative and qualitative phenolic concentration of the EVOOs was determined by high-performance liquid chromatography (HPLC) using an Agilent Technologies system, model 1100 controlled by ChemStation (Agilent Technologies, Palo Alto, CA, USA). Phenolic compounds were identified and quantified according to the procedure reported by Selvaggini et al. (2006). For the mobile phase, solvent A (water acidified with 0.2% acetic acid) and solvent B (methanol) were used. The running time of single analysis was 73 min and the gradient was changed as follows: 95% A/5% B for 2 min, 75% A/25% B in 8 min, 60% A/40% B in 10 min, 50% A/50% B in 16 min, 0% A/100% B in 14 min. This composition was maintained for 10 min before returning to the starting condition (13 min). Secoiridoid derivatives and phenolic alcohols were detected by using the DAD with a wavelength of 278 nm. Data were expressed as mg of phenols kg^{-1} of oil.

2.3.3. Volatile compounds

Headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME/GC-MS) was used to detect and quantify the volatile compounds in the control and CT EVOOs from different Italian cultivars. All the operative conditions of HS-SPME/GC-MS analysis, without any modifications, were set according to Veneziani et al. (2015).

2.4. Statistical analysis

All the data are the mean of three independent experiments analyzed twice. Data were statistically evaluated using SigmaPlot software 12.3 (Systat Software Inc., San Jose, CA, USA) to perform a one-way ANOVA between the control and CT samples. The mean values were considered significantly different at $p < 0.05$.

3. Results and discussion

As fully reported in previous studies (Esposto et al., 2013; Veneziani et al., 2015, 2017) and as observed in these experimental tests, the use of the heat exchanger did not cause any significant modifications to the legal parameters (free acidity, peroxide value, K_{232}, K_{270} and ΔK; data not shown).

Table 1 shows the phenolic compositions of the six Italian cultivars, demonstrating how the new technology could modify the total phenol concentrations that were strictly related to the genetic origins. The data confirm the efficiency of the heat exchanger applied for the cooling conditioning of olive paste (Veneziani et al., 2017) to increase the phenolic compounds of some cultivars. The increases are statistically significant for Frantoio, Gentile, Leccino and San Felice cultivars and represent an added value to the health and sensory properties of the EVOOs extracted (Almeida, Valli,

Table 1
Phenolic composition (mg/kg) of EVOOs extracted from six Italian cultivar with a rapid cooling conditioning of olive paste.[a]

	Control	[b]CT	Control	[b]CT
	cv. Frantoio		***cv. Ogliarola***	
3,4-DHPEA	1.2 ± 0.03a	0.8 ± 0.03b	1.8 ± 0.02a	2.4 ± 0.01b
p-HPEA	1.9 ± 0.04a	1.6 ± 0.02b	2.9 ± 0.08a	3.8 ± 0.1b
3,4-DHPEA-EDA	146.1 ± 8.2a	196.8 ± 4.4b	434.3 ± 21.7a	447.1 ± 10a
p-HPEA-EDA	39.0 ± 1.6a	49.3 ± 1.4b	106.8 ± 3.5a	115.8 ± 4b
3,4-DHPEA-EA	91.0 ± 1.2a	102.2 ± 3.2b	158.7 ± 4.2a	147.5 ± 4.1b
Ligstroside aglycon	4.9 ± 0.1a	8.1 ± 0.3b	7.2 ± 0.2a	8.4 ± 0.2b
(+)-1-Acetoxypinoresinol	40.6 ± 0.05a	40.0 ± 0.03b	46.1 ± 0.05a	46.8 ± 0.01b
(+)-Pinoresinol	11.3 ± 0.1a	11.2 ± 0.1a	18.5 ± 0.9a	18.3 ± 0.3a
Total phenols	336.0 ± 8.5a	410.0 ± 5.6b	776.3 ± 22.4a	790.1 ± 11.5a
	cv. Gentile		***cv. Leccino***	
3,4-DHPEA	0.8 ± 0.02a	1.2 ± 0.03b	4.2 ± 0.02a	1.1 ± 0.02b
p-HPEA	2.0 ± 0.02a	3.0 ± 0.03b	1.8 ± 0.01a	1.6 ± 0.04b
3,4-DHPEA-EDA	322.5 ± 16.2a	623.0 ± 13.4b	325.2 ± 17a	399.9 ± 7.9b
p-HPEA-EDA	92.7 ± 2.3a	132.9 ± 4.3b	90.3 ± 2.1a	89.4 ± 2.3a
3,4-DHPEA-EA	119.0 ± 3.1a	185.3 ± 5.2b	214.2 ± 5.6a	180.5 ± 4.6b
Ligstroside aglycon	11.8 ± 0.3a	24.1 ± 0.3b	24.9 ± 0.2a	23.4 ± 1.1a
(+)-1-Acetoxypinoresinol	24.3 ± 0.03a	26.1 ± 0.02b	16.0 ± 0.03a	16.3 ± 0.01b
(+)-Pinoresinol	10.5 ± 0.2a	10.1 ± 0.02a	16.1 ± 0.1a	15.9 ± 0.04b
Total phenols	583.5 ± 16.7a	1005.7 ± 15b	692.7 ± 18a	728.1 ± 9.5b
	cv. Moraiolo		***cv. San Felice***	
3,4-DHPEA	1.3 ± 0.02a	1.2 ± 0.02b	0.9 ± 0.04a	1.0 ± 0.03a
p-HPEA	1.3 ± 0.03a	1.6 ± 0.04b	3.5 ± 0.05a	3.2 ± 0.02b
3,4-DHPEA-EDA	614.2 ± 15.1a	625.1 ± 12.4a	405.6 ± 20.1a	522.9 ± 10.9b
p-HPEA-EDA	90.6 ± 2.3a	95.5 ± 2.3a	102.8 ± 3.1a	104.5 ± 3.6a
3,4-DHPEA-EA	171.5 ± 4.3a	178.6 ± 4.1a	144.4 ± 3.3a	158.7 ± 4b
Ligstroside aglycon	20.9 ± 0.06a	20.3 ± 1a	22.6 ± 0.2a	23.4 ± 1.2a
(+)-1-Acetoxypinoresinol	37.5 ± 0.04a	41.9 ± 0.05b	88.4 ± 0.1a	86.9 ± 0.04b
(+)-Pinoresinol	16.8 ± 0.09a	14.3 ± 0.06b	19.7 ± 0.12a	18.8 ± 0.03b
Total phenols	954.1 ± 15.9a	978.6 ± 13.4a	787.9 ± 20.6a	919.4 ± 12.2b

[a] The data are the mean values of three independent experiments analyzed in duplicate, ± standard deviation. For each cultivar the values in each row having different letters (a-b) are significantly different from one another ($p < 0.05$).
[b] CT = cooling treatment.

Bendini, & Gallina Toschi, 2016; Reboredo-Rodríguez et al., 2017), according to health claims referring to olive oil phenolic compounds that "may be used only for olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil" (EFSA, 2012). Qualitatively, the lowest variability is ascribed to those compounds that are only slightly affected by the technological process, such as lignans and to a lesser extent ligstroside derivatives. In contrast, the highest increases correspond to the sum of oleuropein derivatives that are more sensitive to processing parameters and are also the most abundant compounds in the EVOO. The only exceptions were the Gentile and Frantoio cultivars, which also showed a very high percentage increase in the phenolic fraction for the sum of ligstroside derivatives (50.4% and 28,8%, respectively) with the lignans, further confirming a very limited concentration variability (Fig. 1). Each cultivar is characterized by a different enzymatic activity level of polyphenoloxidase (PPO) that could be influenced by the CT-processed olives, capable of guaranteeing a specific impact on the EVOO phenolic concentration of different genetic origins. As described by other authors (Garcia-Rodriguez, Romero-Segura, Sanz, Sanchez-Ortiz, & Perez, 2011; Hbaieb et al., 2016; Taticchi et al., 2013), the low temperature was able to modulate the PPO activities, which are involved in the oxidative degradation of phenolic compounds, thereby creating a total or partial enzymatic inhibition in relation to the cultivar, with a positive impact on the nutritional and organoleptic properties of the EVOO due to an increase in phenolic compounds. The possibility of a major or minor level of PPO inhibition due to the different genetic origins could modify the cultivar phenolic concentration when the olive oil extracted using the CT is obtained, while maintaining the other conditions of the mechanical extraction process (Garcia-Rodriguez, Romero-Segura, Sanz, & Perez, 2015). These other conditions, such as time and temperature of malaxation, can affect not only the dynamics of phenolic partition coefficients in the water-oil system but also the enzymatic activities (Selvaggini et al., 2014). The inhibition of the enzymatic activities produces different percentage increases, also of high importance, in oil from Gentile cultivar (72.4%) and, as reported by a previous study (Veneziani et al., 2017), from Peranzana olives (61.2%) processed at the same malaxation temperature (25 °C).

The study also concerned the volatile compounds involved in the expression of positive sensory note of EVOOs released by the activation of lipoxygenase pathways immediately after the crushing phase: aldehydes ((*E*)-2-pentenal, hexanal, (*E*)-2-hexenal, (*E,E*)-2,4-hexadienal and 2,4-hexadienal (i)), alcohols (1-penten-3-ol, (*E*)-2-penten-1-ol, 1-hexanol, (Z)-3-hexen-1-ol and (E)-2-hexen-1-ol) and esters (hexyl acetate and (Z)-3-hexenyl acetate). The rapid cooling conditioning of olive paste caused a general increase in the sum of aldehydes of all the cultivars processed, with variability ranging from 1.2 to 20.3% (Fig. 2). The main share was due to the (*E*)-2-hexenal responsible of green and cut grass sensory note (Angerosa et al., 2004; Kalua, Bedgood, Bishop, & Prenzler, 2013), which increased in all the cultivars and represented over 90% of the total aldehydes, whereas the other saturated and unsaturated C_5 and C_6 aldehydes showed major or minor values in any given cultivar compared to the control trials (Table 2). Alcohol and ester compounds appear to be more influenced by the genetic origin of the olives, without following the same trend compared to the control. Indeed, the sum of alcohols concentration increased by 4.5% and 9.2% for the Leccino and Moraiolo cultivars and decreased by 7.4%, 23.3%, 4.4% and 30.7% for the Frantoio, Ogliarola, Gentile

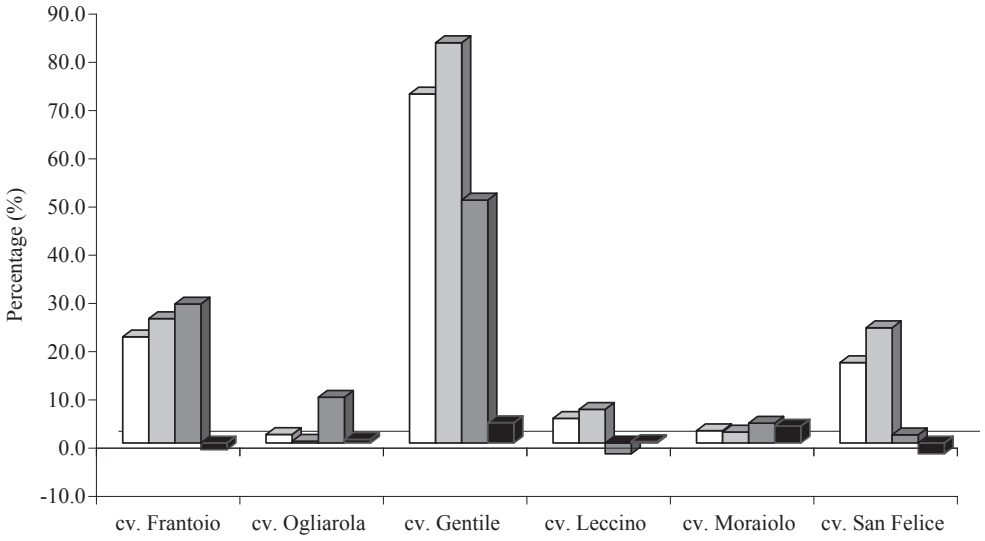

Fig. 1. Percentage variability of phenolic compounds of EVOOs extracted with rapid cooling treatment of olive paste (white, total phenols; light grey, sum of oleuropein derivatives; dark grey, sum of ligstroside derivatives; black, sum of lignans).

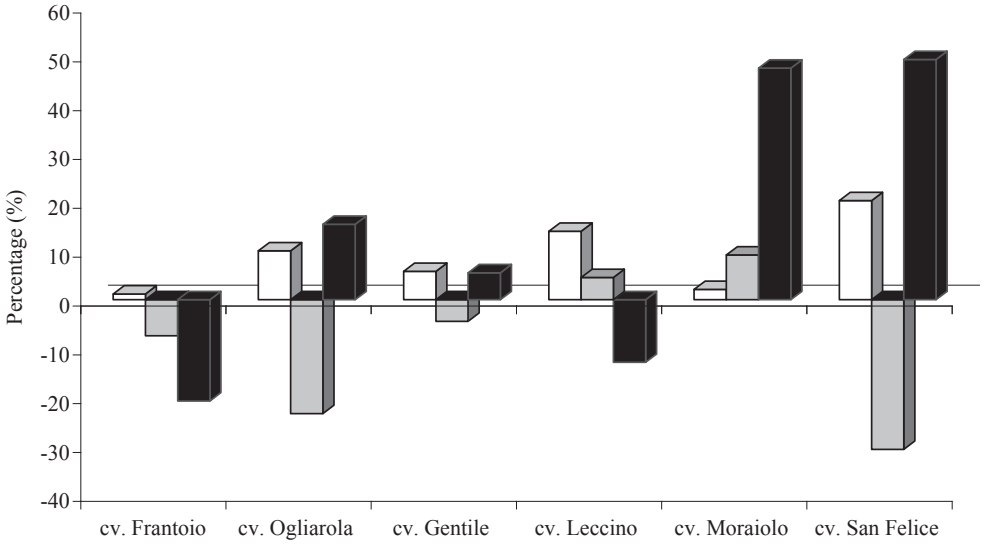

Fig. 2. Percentage variability of volatile compounds of EVOOs extracted with rapid cooling treatment of olive paste (white, sum of aldeyhdes; light grey, sum of alcohols; black, sum of esters).

and San Felice cultivars, whereas the sum of esters decreased by 20.8% and 12.8% for the Frantoio and Leccino cultivars and increased by 15.4%, 5.5%, 47.4% and 49.2% for the Ogliarola, Gentile, Moraiolo and San Felice cultivars, respectively (Fig. 2). As explained for the different percentage increases of phenolic compounds, the different volatile profiles of the EVOOs obtained with the CT may be related to a dissimilar modulation in each cultivar of the activity levels of the enzymes belonging to the LOX pathways (Angerosa et al., 2004) (lipoxygenases (LOXs), hydroperoxide lyases (HPLs), alcohol dehydrogenases (ADHs), isomerases and alcohol acetyltransferases (AATs)). Padilla et al. (2012) showed different thermal inactivation parameters for three LOX isoforms and HPL, involved in the synthesis of EVOO aroma, demonstrating a significant decrease in volatile compounds and changes in the quantitative and qualitative volatile composition as a function of the temperature during the crushing phase. The low temperature might influence the LOX activity level of each cultivar, modifying its own LOX activity load, which represents a limiting factor for the synthesis of volatile compounds (Sanchez-Ortiz et al., 2012) and can also be influenced by environmental parameters of the growing season such as temperature, soil fertility, and tree water status, among others. (Chiappetta, Benincasa, & Muzzalupo, 2015; Muzzalupo et al., 2012; Patui et al., 2010; Servili et al., 2007). In accordance with these research studies, the different genetic origins that characterize the enzymes of the LOX pathways of each olive cultivar and their different thermal stabilities (Luaces, Perez, & Sanz, 2007) can create a specific impact on the volatile profile of EVOOs obtained by the olive paste CT at 15 °C, highlighting an overall increase in the sum of aldehydes for all the cultivars analyzed (Fig. 2).

4. Conclusion

After a complex study of the impact of the new technology, is possible to infer that the use of the heat exchanger after the milling step, to rapidly reduce the olive paste temperature to 15 °C,

Table 2
Volatile composition (μg/kg) of EVOOs extracted from six Italian cultivar with a rapid cooling conditioning of olive paste.[a]

	Control	[b]CT	Control	[b]CT
	cv. Frantoio		***cv. Ogliarola***	
Aldehydes				
(*E*)-2-Pentenal	35±2a	31 ± 1b	74 ± 2a	70 ± 2a
Hexanal	534 ± 16a	434 ± 12b	412 ± 6a	589 ± 14b
(*E*)-2-Hexenal	85400 ± 404a	86835 ± 361b	83568 ± 3230a	91758 ± 2777b
(*E,E*)-2,4-Hexadienal	2651 ± 59a	2388 ± 52b	2758 ± 205a	3082 ± 88a
2,4-hexadienal (i)	269 ± 15a	238 ± 1b	276 ± 17a	299 ± 16a
Alcohols				
1-Penten-3-ol	381 ± 21a	312 ± 14b	273 ± 14a	256 ± 12a
(*E*)-2-Penten-1-ol	24 ± 1a	20 ± 1b	27 ± 3a	26 ± 2a
1-Hexanol	739 ± 50a	614 ± 20b	821 ± 70a	725 ± 39a
(*Z*)-3-Hexen-1-ol	866 ± 55a	1039 ± 38b	1750 ± 192a	1145 ± 123b
(*E*)-2-Hexen-1-ol	763 ± 59a	583 ± 24b	215 ± 18a	214 ± 1a
Esters				
Hexyl acetate	403 ± 9a	330 ± 1b	457 ± 19a	544 ± 25b
(*Z*)-3-Hexenyl acetate	758 ± 51a	590 ± 21b	373 ± 13a	414 ± 18b
	cv. Gentile		***cv. Leccino***	
Aldehydes				
(*E*)-2-Pentenal	54 ± 1a	44 ± 1b	54 ± 5a	55 ± 3a
Hexanal	424 ± 6a	370 ± 5b	87 ± 6a	142 ± 6b
(*E*)-2-Hexenal	64495 ± 870a	68430 ± 594b	38155 ± 1695a	43610 ± 1276b
(*E,E*)-2,4-Hexadienal	1509 ± 172a	1496 ± 111a	143 ± 11a	150 ± 7a
2,4-hexadienal (i)	146 ± 16a	147 ± 10a	1428 ± 155a	1494 ± 98a
Alcohols				
1-Penten-3-ol	510 ± 21a	536 ± 16a	464 ± 4a	472 ± 13a
(*E*)-2-Penten-1-ol	362 ± 6a	358 ± 8a	274 ± 9a	275 ± 6a
1-Hexanol	1818 ± 2a	1711 ± 4b	471 ± 38a	635 ± 23b
(*Z*)-3-Hexen-1-ol	744 ± 26a	987 ± 14b	446 ± 40a	335 ± 7b
(*E*)-2-Hexen-1-ol	1069 ± 11a	711 ± 14b	525 ± 26a	562 ± 35a
Esters				
Hexyl acetate	594 ± 24a	439 ± 18b	7 ± 3a	10 ± 2a
(*Z*)-3-Hexenyl acetate	1388 ± 43a	1652 ± 45b	32 ± 4a	24 ± 5a
	cv. Moraiolo		***cv. San Felice***	
Aldehydes				
(*E*)-2-Pentenal	71 ± 3a	64 ± 1b	59 ± 1a	82 ± 1b
Hexanal	322 ± 3a	405 ± 15b	350 ± 6a	488 ± 6b
(*E*)-2-Hexenal	40930 ± 311a	41925 ± 389b	74610 ± 1170a	89760 ± 1485b
(*E,E*)-2,4-Hexadienal	2946 ± 62a	2818 ± 26b	1869 ± 66a	2159 ± 21b
2,4-hexadienal (i)	306 ± 7a	294 ± 2b	183 ± 8a	213 ± 1b
Alcohols				
1-Penten-3-ol	611 ± 13a	651 ± 18b	635 ± 1a	659 ± 1b
(*E*)-2-Penten-1-ol	400 ± 1a	377 ± 2b	333 ± 5a	404 ± 1b
1-Hexanol	1444 ± 16a	1659 ± 5b	5422 ± 33a	3078 ± 23b
(*Z*)-3-Hexen-1-ol	1683 ± 24a	1497 ± 23b	361 ± 9a	464 ± 1b
(*E*)-2-Hexen-1-ol	1465 ± 161a	1932 ± 104b	4345 ± 180a	3090 ± 67b
Esters				
Hexyl acetate	13 ± 1a	21 ± 3b	136 ± 1a	214 ± 7b
(*Z*)-3-Hexenyl acetate	84 ± 4a	122 ± 4a	110 ± 1a	153 ± 1b

[a] The data are the mean values of three independent experiments analyzed in duplicate, ± standard deviation. For each cultivar the values in each row having different letters (a-b) are significantly different from one another ($p < 0.05$).
[b] CT = cooling treatment.

guarantees a significant increase in the phenolic fraction of four cultivar and a general increase of the sum of aldehydes, with a positive effect on the health and sensory properties of the EVOOs. In particular, a higher concentration of the phenolic fraction grants the same beneficial effect of "protection of blood lipids from oxidative stress" as regulated by health claims made on olive oil phenolic compounds (EFSA, 2012), reducing the necessary daily EVOO intake (Reboredo-Rodríguez et al., 2017). The percentage variability of the increases in these important quality parameters are strictly linked with the cultivar and its specific enzyme activity load, which could be mainly related to the level of PPO and enzymes of LOX pathways. The other volatile compounds that take part in the formation of olive oil aroma (alcohols and esters) did not show a specific trend, but their concentration did increase or decrease in relation to a given cultivar. Specific enzymatic studies on PPO and LOX activities of different genetic origin could be useful to better understand how the cooling conditioning of olive paste influences the phenolic and volatile concentration of EVOOs.

It is furthermore necessary to highlight how the six Italian olive cultivars were processed with an industrial system with a working capacity of 2.5 ton/h, yielding added value to the scientific results as it allowed a more tangible and direct evaluation of the innovative technology applied to the mechanical extraction process used by the EVOO industrial sector.

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