International Journal of Refrigeration 127 (2021) 59–68

Contents lists available at ScienceDirect

International Journal of Refrigeration

journal homepage: www.elsevier.com/locate/ijrefrig

Use of refrigerated cells for olive cooling and short-term storage: Qualitative effects on extra virgin olive oil

Lorenzo Guerrini[a], Ferdinando Corti[a,*], Lorenzo Cecchi[b], Nadia Mulinacci[b], Luca Calamai[a], Piernicola Masella[a], Giulia Angeloni[a], Agnese Spadi[a], Alessandro Parenti[a]

[a] Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI), Università degli Studi di Firenze, Piazzale delle Cascine 15, 50144 Florence, Italy

[b] Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino (NEUROFARBA), Università degli Studi di Firenze, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Italy

ARTICLE INFO

Article history:
Received 3 September 2020
Revised 24 February 2021
Accepted 3 March 2021
Available online 5 March 2021

Keywords:
Aroma volatiles
Off-flavor
Post-harvest temperature
Refrigeration

ABSTRACT

In extra virgin olive oil production, the time between olive harvesting and milling is a critical period that must be carefully controlled to preserve oil quality. Particularly, several detrimental phenomena can emerge during storage. Hence, a key issue for producers is to optimize conditions to preserve the quality of fruit before milling. With this requirement in mind, we tested the effect of olive cooling and short-term cold storage on olive oil quality in two experiments. The first, baseline trial was run in the laboratory, and involved storing small batches of olives at 6°C and at 25°C for 16 h. Here, the aim was to simulate a situation with a high temperature difference. The second experiment was conducted at industrial scale, using a refrigerated storage cell. One batch of fruit was stored at 6.5°C for 16–18 h, while a control batch was stored at ambient temperature (13.5 ± 1°C). Finally, the effect of the surface/volume ratio (SVR) of the storage container was evaluated in a full factorial experiment. Although an effect of SVR on olive temperature was found, no significant differences were registered in oil quality. Short-term storage after cooling slowed metabolic processes, reducing hydrolysis of phenols and slowing the development of undesirable compounds. Furthermore, it supported oxidation, evidenced by higher concentrations of the oxidized form of polyphenols and higher production of lipoxygenase pathway compounds. The latter result suggests that this system could be successfully used to modulate the aroma profile of the produced olive oil.

Utilisation de cellules frigorifiques pour le refroidissement et le stockage à court terme des olives: effets sur la qualité de l'huile d'olive vierge extra

Mots-clés: Arômes volatiles; Perte de saveur; Température après récolte; Froid [artificiel]

1. Introduction

In extra virgin olive oil production (EVOO), the period between harvesting and milling – storage – is a critical element in product quality. After harvesting, ripening continues, and microbiota remain active, degrading oil quality (Fakas et al., 2010; Garcia et al., 1995; Vichi et al., 2009), while degradation is accelerated by unhealthy or mechanically-damaged fruit (Yousfi et al., 2012). In this context, storage temperature is an important parameter, as it affects fruit metabolism (Angerosa et al., 2004;

* Corresponding author.
E-mail addresses: lorenzo.guerrini@unifi.it (L. Guerrini), ferdinando.corti@unifi.it (F. Corti), lo.cecchi@unifi.it (L. Cecchi), nadia.mulinacci@unifi.it (N. Mulinacci), luca.calamai@unifi.it (L. Calamai), piernicola.masella@unifi.it (P. Masella), giulia.angeloni@unifi.it (G. Angeloni), agnese.spadi@unifi.it (A. Spadi), alessandro.parenti@unifi.it (A. Parenti).

https://doi.org/10.1016/j.ijrefrig.2021.03.002
0140-7007/

García et al., 1996b; García and Yousfi, 2006). Many factors support fruit softening (García, Gutiérrez, Barrera et al. 1996) and the release of cellular liquids that facilitate contact between the oil, water, oxygen, olive enzymes and microbiota (Proietti, 2014). The availability of such substrates increases acidity, lipid oxidation (García et al., 1996b), and supports microbial proliferation, leading to EVOO sensory defects such as *fusty, musty, wine-vinegary*, and *rancid* (Angerosa et al., 1996, 1990; Di Giovacchino, 2010; Langstaff, 2014).

Thus, current best practice recommends milling olives as quickly as possible, to minimize the time after harvesting. However, several technical and operative barriers make this difficult. Fruit should be harvested in as short a timeframe as possible, to obtain the best technological ripeness; consequently, harvesting can exceed the working capacity of extraction plants, which are often undersized. However, to achieve an effective quality control, the time factor must be linked to the temperature factor, since the time-temperature interaction directly affect the kinetics of reactions (Peri, 2014).

The literature reports several studies of the effects of cold storage on olive fruit (Ben et al., 2012; García et al., 1996b, 1996a; Hachicha Hbaieb et al., 2016, 2015; Inarejos-García et al., 2010; Jabeur et al., 2015; Kalua et al., 2008; Luaces et al., 2005; Morales-Sillero et al., 2017; Pereira et al., 2002; Piscopo et al., 2018; Saffar Taluri et al., 2019; Vichi et al., 2009; Yousfi et al., 2012, 2008), which is often combined with controlled atmospheric conditions (Castellano et al., 1993; Clodoveo et al., 2007; Dourtoglou et al., 2006; García et al., 1994; Gutierrez et al., 1992; Kader et al., 1989; Kiritsakis et al., 1998; Maestro et al., 1993; Rinaldi et al., 2010; Yousfi et al., 2009). The available literature is resumed in the Table S1 (supplementary materials). Many studies indicate that storage temperatures between 5 and 10°C are optimal for delaying fruit degradation. On the other hand, current findings suggest there are no clear advantages due to the use of a controlled atmosphere (i.e. lowering the percentage of O_2 and/ or increasing CO_2). These earlier studies test very long storage times, with extensive periods between measurements (days or even weeks) and could be considered very interesting and impacting for some industrial realities.

The olive oil sector has a high variability in terms of prices and product quality. For example, in Italy, if we consider only the best quality categories (i.e. extra virgin olive oil and Protected Denominations of Origin), the market price ranges from 3.80 euro/kg to 22.00 euro/kg (ISMEA, 2020). For the production of higher quality olive oils, the time between harvest and extraction is usually about a few hours, and rarely exceeds one day since very small changes in oil composition could strongly decrease the product quality and price (Fiorini et al., 2018). The processing technologies and their settings can change the composition of the EVOO, especially in terms of phenolic compounds and volatile molecules, directly affecting the organoleptic quality of the product (Di Giovacchino et al., 2002). The process control and the modulation of quality characteristics can also give the producers a further premium in term of price (Carbone et al., 2018). Hence, a short cold storage could be used to further increase the quality of the extra virgin olive oil by improving the phase between olive harvesting and milling. Only two studies have examined the effect of fruit mass during the period between harvest and milling (García et al., 1994; Inarejos-García et al., 2010). For both fruit mass and storage times, previous experimental conditions were inconsistent with the production process that we examined. These observations motivated our study of the impact of cold short-term storage of fruit on olive oil quality.

The aim of the present study is to find operative conditions that can compensate for the lack of mill capacity at peak harvest. In particular, we evaluate the effect of low-temperature storage of olive fruits on EVOO quality at mill scale. Furthermore, we examine the interaction between storage volume and storage temperature. Consequently, our trials mimic the operative context, where the time between harvest and processing is short, and fruit batches are relatively small.

2. Materials and Methods

2.1. Olive fruit

Olive fruit (*Olea europaea*, a blend of cv. *Frantoio* (≃70%) and cv. *Moraiolo* (≃30%)) was harvested and processed in central Italy (Fattoria di Maiano, Fiesole, Florence, Italy – approx. 43°79' N, 11°30' E) in November 2019. Specifically, olives for Trial 1 were harvested on 7 November 2019, while fruit for Trial 2 was harvested on 7, 13, and 14 November 2019. Harvested olives were in good health (assessed by visual inspection by company technicians) with a maturity index (MI) between 3 and 4. MI was determined according to the Uceda and Frias (1975) method. Water content (45.45 ± 1.28 % w/w) was measured by weighing 20 g of olives before and after storage for 24 h at 105°C, for each processed batch.

Trials were carried out as follows.

2.2. Trial 1: Storage at laboratory scale

Olive fruits were sampled immediately after their arrival at the mill. Then, they were transported to our laboratory (Department of Agriculture, Food, Environment and Forestry, University of Florence, Florence, Italy). The distance between the olive mill and the laboratory is approximately 11 km, and the travel time is roughly 20 min. At the lab, a trial was run to reproduce the following conditions: i) a large temperature difference between ambient conditions and cold storage; and ii) fast heat exchange between fruit and the environment due to a small mass of olives. Two, homogeneous 1.5 kg batches of fruit were stored in a monolayer, in a perforated plastic box at 25°C (ambient temperature), and in a chiller at 6.5°C (Irinox MultiFresh, MF 25.1, Irinox Spa, Treviso, Italy) for 16–18 h. Three replicates were run for each condition, making a total of six olive oil micro-extractions (i.e. three at ambient temperature and three at cold temperature).

2.3. Trial 2: Storage at mill scale

The hosting company (Fattoria di Maiano, Fiesole, Florence, Italy) was equipped with a refrigerated storage cell (ArticStore 20', l1 6.06 x l2 2.45 x h 2.62 m; volume = 28.8 m^3, Titan Containers, Taastrup, Denmark), designed to store fruit near to the mill. The container temperature was set to 6.5°C, and continuously monitored during trials. Harvested olives (790 kg for each trial) were put into perforated plastic boxes and brought to the mill. On average, the time between harvest and arrival at the mill was 3 h. Upon arrival, fruits were merged into a batch and mixed to ensure homogeneity. Next, the batch was divided into two, homogeneous sub-batches that were stored at either ambient temperature (i.e. outdoors) or in the refrigerated storage cell (i.e. 6.5°C). We also included a variable to capture the type of container and its degree of filling – the surface/volume ratio (SVR). For each temperature treatment, olives were either stored in: filled, 250 kg perforated plastic bins (113 × 113 × 58 cm) with SVR = 7 (SVR7-bin); half-filled (i.e. 125 kg) perforated plastic bins (113 × 113 × 29 cm) with SVR = 10 (SVR10-halfbin); or 20 kg perforated plastic boxes (51 × 35 × 31 cm) with SVR = 16 (SVR16-box). Storage time was 16–18 h. This simulates working conditions, where harvesting runs from early morning to sunset, and fruit processing takes place on the following day in the laboratory.

A randomized block design was adopted in the trials. The harvest day was considered as blocking factor and treated as a replicate. Within the harvest days, 2 experimental factors (i.e. temperature and SVR) and their interaction were tested. Each day the 6 possible combinations between temperature and SVR were tested. Trials were carried out in 3 different days, for a total of 18 olive oil extractions.

For each storage condition, the temperature was constantly monitored by data-loggers located inside and outside the refrigerated container (Ebro EBI 300 PDF Data Logger, Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany), and in the middle of fruit heaps (HOBO TidbiT MX Temperature 5000′ Data Logger, Onset Computer Corporation, Bourne, MA, US).

At the beginning and end of storage, metabolic activity was evaluated by measuring O_2, CO_2 and ethanol concentration in the central part of each heap with dedicated sensors (PBI Dansensor CheckPoint O_2/CO_2, AMETEK s.r.l., Milan, Italy; Vernier Ethanol Sensor coupled with Vernier LabQuest2, Vernier, Beaverton, OR, US). This was achieved by manually introducing each probe into each heap; measures were recorded after equilibrium between the sensor and the environment was reached.

2.4. Olive oil extraction

At the end of storage, an aliquot of 1.5 kg of olive fruit was recovered from the central part of each heap and immediately transported to the laboratory. Fruit was transported in a net (Raschel) bag that was filled with olives and placed in the heap.

The oil microextraction plant consisted of a crusher, a lab-scale malaxator and a laboratory centrifuge (NEYA8, Neya centrifuges, Modena, Italy). After crushing, 1.1 kg of olive paste was mixed in a hermetically-sealed cylindrical malaxator for 20 min at a controlled temperature of 27°C. Then, oil was separated from vegetation water and solid fractions by centrifugation for 10 min at 6500 rpm, and recovered with a separatory glass funnel. This experimental device has been used in earlier studies, and is described in more detail in Masella et al. (2019).

2.5. Olive oil analysis

Oil samples obtained from laboratory and mill trials were analyzed for free fatty acids (% oleic acid), peroxide value (meq O_2 per kg of oil) and UV spectroscopic indices (K_{232}, K_{270} and ΔK) according to official methods (EC, 2008).

Biophenolic fractions were extracted, identified and determined following the International Olive Council (IOC) official method (International Olive Council, 2017). Phenolic compounds were extracted from olive oil samples through methanol:water 80:20 (v/v) solution. The HPLC analysis were performed using a HP 1100 coupled with both DAD and MS detector, the latter one equipped with HP1100 MSD API-electrospray interface (Agilent Technologies, Palo Alto, CA, USA). A Poroshell 120, EC-C18 column (150 mm x 3.0 mm id, 2.7 μm particle size; Agilent Technologies, Palo Alto, CA, USA) was used for separation. According to the official method, acetonitrile, H_2O and methanol were adopted as elution solvents following the elution gradient described by IOC. Chromatogram was recorded at 280 nm, using syringic acid as internal standard, while the phenolic concentration were expressed as mg kg^{-1} of tyrosol.

Identification and quantification of volatile organic compounds (VOC) was performed by headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) using the multiple internal standard method, as described by Fortini et al. (2017). 0.1 g of internal standard mixture (ISTD MIX) was added to 4.3 g of sample into a 20 ml vials fitted with open hole screw cap and PTFE/silicone septa. The ISTD MIX was prepared dissolving 11 molecules in refined olive oil, for a final concentration of 75 mg kg^{-1} for each ISTD. ISTDs were chosen in order to represent several molecular masses and several classes of VOCs (alcohols, aldehydes, ketones, esters, carboxylic acids and aromatic hydrocarbons). ISTDs were either deuterium-labelled or found to be absent in virgin olive oil i.e., 3,4-dimethylphenol, 4-methyl-2-pentanol, hexanoic acid-d11, 1-butanol-d10, ethyl acetate-d8, toluene-d8, ethyl hexanoate-d11, acetic acid- 2,2,2-d3, 6-chloro-2-hexanone, 3-octanone, trimethyl acetaldehyde. The same amount of ISTD MIX was added to calibration scales in order to normalize each analyte concentrations of calibration curve on those of respective ISTD. HS-SPME-GC-MS analysis was carried out after the equilibration of SPME fiber (50/30 µm DVB/ CAR/PDMS by Supelco) at 60°C for 5 min. Then, it was exposed for 20 min in the vial headspace under orbital shaking (500 rpm) and immediately desorbed for 2 min in a gas chromatograph injection port operating in splitless mode at 260°C. After that, a 15 min fiber backout at 260°C was carried out in a backout unit such to avoid carryover phenomena among subsequent specimens. The GM-MS identification of VOC was performed using a Trace CG-MS Thermo Fisher Scientific, equipped with a ZB-FFAP capillary column (Zebron) 30 m x 0.25 mm ID, 0.25 µm DF. The temperature of the column was controlled as follows: 36°C for 10 min, increase to 156°C at 4°C per min, increase to 260°C at 10°C per min, decrease to 250°C at 10°C per min, with hold time of 2 min. Helium was used as the carrier gas at constant flow of 0.8 ml per min. The temperature of both ion source and transfer line was 250°C. The mass detector was operated in scan mode within a 30–330 Th mass range at 1500 Th s^{-1}, with an IE energy of 70 eV.

VOC quantification was carried out comparing each mass spectra and retention times with those of injected authentic standards. The stock external standard mix contained 71 analytes in refined oil, which was previously verified to be free of any interferent. The analytes and their concentration ranges were chosen based on previous works on Italian virgin olive oils.

2.6. Statistical analysis

A Student's *t*-test was applied to data from Trial 1. Trial 2 data were analyzed with an ANOVA model taking into account the 2 experimental factors (temperature and SVR) and their interaction with the 3 harvest days considered as a blocking factor. Significance was set at $p < 0.05$. Tukey HSD post-hoc test was used to assess significant differences among the 3 SVRs.

3. Results

3.1. Trial 1

Trial 1 focused on a short storage period with a large temperature difference. It sought to eliminate the effect of heating of the fruit due to the mass. Oil samples obtained from olives stored at cold (C) and ambient (A) temperature were both classified as extra virgin. No significant differences were found for free fatty acids and peroxide value (Table S2), while the spectrophotometric analysis found significantly higher values for K_{232} (1.81 ± 0.05 vs 1.61 ± 0.02) and K_{270} (0.11 ± 0.00 vs 0.10 ± 0.00) for A samples compared to C samples (Table S2).

Total phenolic compound content was not significantly affected by storage temperature (Table 1). Among the 24 compounds found in the used method, statistically significant differences were observed for five individual compounds. Of these, only tyrosol was found to be in higher concentration in A samples (average difference 0.36 mg kg^{-1}). According to the literature, tyrosol results from the hydrolysis of ligstroside, and is not depleted by oxidation. Thus, it could be considered as an indicator of olive oil phenolic

Table 1
Concentration of phenolic compounds (mg kg−1) in olive oil samples taken from olives stored at cold and ambient temperature at lab scale. Letters a,b indicate significant differences ($p < 0.05$) obtained using a two-tailed student t-test. Single phenol concentrations that were not found to be significant, are not shown. Significant codes: ns = not significant; . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Biophenols (mg kg^{-1})	Ambient (25°C)	Cold (6.5°C)	p
Individual compounds			
Tyrosol	1.68 ± 0.09 [b]	1.32 ± 0.15 [a]	*
Cinnamic acid	2.13 ± 1.54 [a]	4.89 ± 1.15 [b]	*
Ligstroside aglycone, oxidized aldehyde and hydroxylic form	8.35 ± 3.90 [a]	14.00 ± 3.21 [b]	*
Methyl luteolin	0.95 ± 1.14 [a]	9.87 ± 1.58 [b]	***
Ligstroside aglycone, aldehyde and hydroxylic form	2.15 ± 1.31 [a]	9.45 ± 1.40 [b]	***
Sum of Compounds and Indexes			
Total phenolic compounds	339.58 ± 75.86	368.98 ± 75.73	*ns*
Tyrosol + Hydroxytyrosol	4.59 ± 1.12	4.45 ± 1.49	*ns*
Phenolic acids	15.68 ± 3.81	17.81 ± 3.31	*ns*
Lignans	22.89 ± 4.53	24.42 ± 4.17	*ns*
Flavones	6.94 ± 2.58 [a]	16.15 ± 2.98 [b]	**
Secoiridoids	289.48 ± 65.84	306.15 ± 65.19	*ns*
Oleuropein derivatives	178.70 ± 17.75	180.32 ± 42.69	*ns*
Ligstroside derivatives	110.78 ± 7.63	125.8 ± 22.52	*ns*
Total oxidized form	75.41 ± 5.04	96.95 ± 21.99	*ns*
Total not-oxidized form	206.14 ± 21.00	199.57 ± 47.71	*ns*
Not-Ox/Ox ratio	2.73 ± 0.22	2.07 ± 0.39	.

Table 2
Volatile organic compound profile of olive oil samples taken from olive fruits stored at cold and ambient temperature at lab-scale. Only those compounds for which significant differences ($p < 0.05$) at the two-tailed student t-test were found, are shown. Significant codes: $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Volatile organic compounds (mg kg^{-1} or µg kg^{-1})	Ambient (25°C)	Cold (6.5°C)	p
2-butanone (µg kg^{-1})	18.41 ± 4.69	9.23 ± 3.98	*
2-butanol (µg kg^{-1})	16.37 ± 1.15	14.05 ± 0.56	*
Ethanol (mg kg^{-1})	4.11 ± 0.43	2.04 ± 0.06	**
Acetic acid, butyl ester (mg kg^{-1})	0.56 ± 0.03	0.78 ± 0.03	***
Isobutanol (mg kg^{-1})	0.10 ± 0.01	0.18 ± 0.01	***
1-butanol, 2-methyl + 3-methyl (mg kg^{-1})	0.35 ± 0.01	0.21 ± 0.01	**
Propanoic acid (µg kg^{-1})	31.37 ± 2.25	38.83 ± 3.88	*
Z3-hexenal (mg kg^{-1})	0.19 ± 0.02	0.24 ± 0.02	*
E2-hexenal (mg kg^{-1})	23.85 ± 1.95	29.00 ± 2.35	*
Acetic acid, hexyl ester (mg kg^{-1})	0.03 ± 0.01	0.11 ± 0.01	***
E2-penten-1-ol (µg kg^{-1})	26.99 ± 2.65	17.16 ± 1.14	**
Z2-penten-1-ol (mg kg^{-1})	0.31 ± 0.03	0.21 ± 0.03	**
Z3-hexenyl acetate (mg kg^{-1})	0.15 ± 0.01	0.26 ± 0.01	**
1-hexanol (mg kg^{-1})	0.24 ± 0.02	0.46 ± 0.02	**
E3-hexen-1-ol (µg kg^{-1})	6.10 ± 0.42	9.53 ± 0.47	***
Z3-hexen-1-ol (mg kg^{-1})	0.45 ± 0.04	0.63 ± 0.04	**
2-nonanone (µg kg^{-1})	37.06 ± 0.47	30.72 ± 0.51	***
1-heptanol (µg kg^{-1})	8.50 ± 0.46	19.39 ± 1.54	***
Phenylethyl alcohol (µg kg^{-1})	53.55 ± 1.91	46.48 ± 3.05	*

compound hydrolysis (Guerrini et al., 2020; Migliorini et al., 2013; Pagliarini et al., 2000). Studies have found that during malaxation, and the storage of both fruit and oil, the action of β-glucosidase and esterase enzymes means that the most abundant secoiridoid glucosides (oleuropein and ligstroside) shift to aglyconic forms and simple phenol products (Brenes et al., 2001; Guerrini et al., 2017; Montedoro et al., 1992; Servili et al., 2002).

Four other biophenols were found at statistically significant higher concentrations in C samples (Table 1). These include one flavone (methyl-luteolin), two ligstroside-derived secoiridoid forms (the oxidized aldehyde and hydroxylic form of ligstroside aglycone, and its aldehyde and hydroxylic form), and one phenolic acid (cinnamic acid). Summing together the phenolic compounds according to their chemical class resulted in no statistically significant differences between C and A oils. The exception was the sum of flavones, including methyl-luteolin (Table 1). Individual sums of secoiridoid, simple phenols, phenolic acid, oleuropein-derived compounds and ligstroside-derived compounds were statistically equivalent for both oil samples.

Seventeen out of the 40 tested VOCs were significantly affected by the storage temperature (Table 2). Of these, nine are derived from the LOX pathway, and typically improve olive oil flavor (*fruity, cut-grass* or *green olive* notes) (Angerosa et al., 2004, 2000; Morales et al., 1994). Other VOCs that were significantly affected by temperature can be related to fermentative processes. When present in high concentration, they can lead to sensory defects such as *wine-vinegary* or *fusty* (Angerosa et al., 1996; Morales et al., 2005). Finally, other VOCs could be related to lipid oxidation and the *rancid* defect (López-López et al., 2019; Morales et al., 2005).

LOX-related compounds were higher in C oils than A oils (Table 2). Seven individual compounds associated with the *fruity* aroma, including Z3-hexenal, E2-hexenal, Z3-hexenyl acetate, 1-hexanol, and E3 and Z3-hexen-1-ol, were found to be higher in C oils, while two others (E-2-penten-1-ol and Z-2-penten-1-ol) were higher in A oils. The C6 branch of the LOX pathway seems to be enhanced by cold storage, while the C5 branch seems to be favored by higher temperatures. These results are consistent with other studies that report a decrease in C5 formation when olives are stored at low temperature (Hachicha Hbaieb et al., 2016;

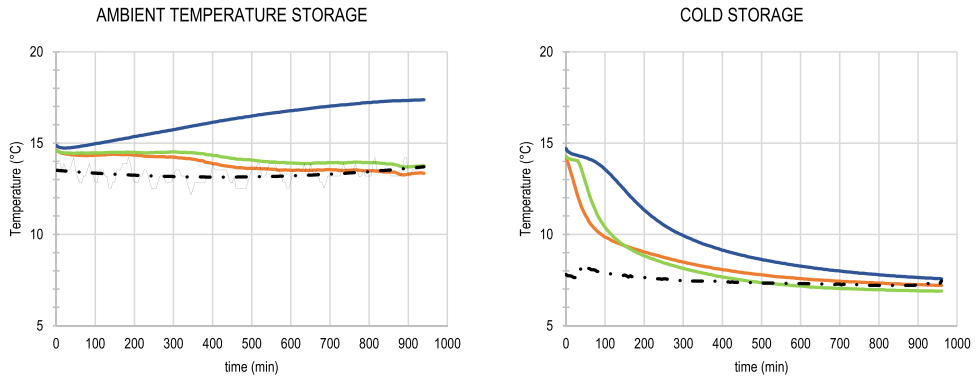

Fig. 1. Tracking plots of the inner temperature (3-replicate average values) of olive fruit heaps during cold (C) and ambient temperature (A) storage at mill scale (solid lines): filled bin – SVR = 7 (blue line), half-filled bin – SVR = 10 (orange line), and plastic box – SVR = 16 (green line). Dotted lines track the temperature of the storage medium.

Luaces et al., 2005) or cooled before oil extraction (Dourou et al., 2020). On the other hand, in our study, microbial metabolites were more abundant in A samples, with significant amounts of 2-methyl and 3-methyl butanol, ethanol, 2-butanone, 2-butanol, and phenylethyl alcohol (Table 2). These metabolites could be the result of microbial activity that begins with aminoacidic precursors (Angerosa et al., 2004, 1996; López-López et al., 2019; Qian and Wang, 2005; Vichi et al., 2011). In particular, Angerosa, Di Giacinto, and Solinas (1990) found a direct, proportional relation between 3-methyl butanol and the intensity of the *fusty* defect. Finally, we found higher concentrations of isobutanol (2-methyl-propan-1-ol) in C samples.

3.2. Trial 2

3.2.1. Storage temperature

Ambient temperature remained stable at 13.5 ± 1°C. However, the temperature of the refrigerated container never dropped below 7°C (Fig. 1). Thus, in this study, we evaluated the effect of a 5–6°C fall in temperature, for roughly 16 h.

Temperature measurements inside olive heaps revealed very different trends as a function of SVR, both for cold and ambient temperature storage.

With respect to storage at ambient temperature, the core temperature of SVR10-halfbins and SVR16-boxes tended to reach equilibrium with the external atmosphere in a relatively short time (from about 15°C to equilibrium in 6 h). However, observations of SVR7-bins revealed an increase in temperature at the heart of the heap during storage. Specifically, we recorded a progressively increasing gap between SVR7-bin and ambient temperature, which reached, on average, over 3°C by the end of storage.

In the refrigerated treatment, all three containers reached thermal equilibrium, although the cooling rate was a function of the SVR. SVR10-halfbins and SVR16-boxes dropped to below 10°C within 2 h, while olives in the SVR7-bin took much longer to reach thermal equilibrium.

3.2.2. Olive fruit gas emissions

At the end of storage, no significant change was found for ethanol content (Table S5). CO_2 concentration was found to be significantly higher in the refrigerated storage cell, while the O_2 concentration was significantly lower (Table S5). Although the aim of the lower temperature was to reduce cellular respiration, large quantities of CO_2 accumulated in olives stored in the refrigerated container (≃1%), with a simultaneous decrease in the concentration of O_2 (≃20%). This observation could be due to the sealing of the container, which prevented gases escaping, leading to the accumulation of CO_2 and the reduction of O_2.

3.2.3. Olive oil analysis

Under European law (EC, 2008), all olive oils produced during Trial 2 could be classified as extra virgin (Table S6). No significant differences in quality parameters were found between treatments, with the exception of free fatty acids, which were higher in oil samples obtained from fruit stored at ambient temperature. Several authors have noted that an increase in free fatty acids might indicate both endogenous lipase and hydrolytic processes of microbial origin (García et al., 1996b; Kiritsakis and Markakis, 1984).

3.2.4. Phenolic compounds

No significant differences were found as a function of storage temperature or SVR for total phenols. Significant differences were found for five biophenols (Table 3), confirming the results obtained in Trial 1.

Significant interactions between storage temperature and SVR were found. Lignans and phenolic acids were found at higher concentrations in SVR7-bin cooled samples. Similarly, 3 individual compounds, namely ferulic acid, decarboxymethyl ligstroside aglycone in the oxidized dialdehyde form, and ligstroside aglycone in the oxidized aldehyde and hydroxylic form had the same higher concentration in SVR7-bin cooled samples. The significant effect of cooling on biophenols was confirmed by higher concentrations of three secoiridoids (decarboxymethyl oleuropein aglycone, oxidized dialdehyde form; decarboxymethyl ligstroside aglycone, oxidized dialdehyde form, and ligstroside aglycone, oxidized aldehyde and hydroxylic forms), which were more abundant in C oils. Two out of 3 secoiridoids showed both the significant effect of temperature and those of the interaction temperature x SVR. Overall, the sum of ligstroside derivatives was found to be higher in oils produced from olives stored at cold temperature. Finally, it is interesting to note the increase in the ratio of oxidized and non-oxidized forms of secoiridoids, related to the cold temperature.

3.2.5. Volatile organic compounds

Statistically significant differences were found for sixteen VOCs as a function of storage condition (Table 4). Of these, several individual C6 LOX-derived alcohols and esters changed significantly depending on the storage temperature. In particular, 1-hexanol, E3-hexen-1-ol, the hexyl ester of acetic acid, and E2- and Z3-hexenyl-acetate were more abundant in C oils, in Trial 1. However, but consistent with Trial 1, we found that individual C5 LOX VOCs (E2-penten-1-ol and Z2-penten-1-ol) were higher in oils produced in the ambient temperature treatment. Ambient temperature treatment oils were also more abundant in 2-heptanone, while 1-heptanol and decanal were higher in cold treatment oils (it should be noted that these differences are statistically significant, but extremely small).

Table 3
Concentration of phenolic compounds (mg kg^{-1}) in olive oil samples taken from olives stored at cold and ambient temperature with different surface/volume ratios (SVR) at mill scale. Only significant differences ($p < 0.05$) at the ANOVA are reported for individual compounds. Letters x,y indicate compound significantly different for SVR, a,b indicate a significant difference for temperature; h,i,l, indicate significant difference for temperature x SVR interaction. The latter assignments were carried out according to the Tukey HSD post-hoc test. RSE column reports the Residual Standard Error of the model. Significant codes: ns = not significant; . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Biophenols (mg kg^{-1})	Ambient SVR7-bin	SVR10-halfbin	SVR16-box	Cold SVR7-bin	SVR10-halfbin	SVR16-box	*RSE*	*pT*	*p SVR*	*pint*
Individual compounds										
Para-coumaric acid	1.32 ^{x}	0.89 ^{y}	0.84 ^{y}	1.13 ^{x}	0.83 ^{y}	0.95 ^{y}	*0.27*	*ns*	*	*ns*
Ferulic acid	2.12 ^{h}	1.73 ^{h}	1.45 ^{h}	5.10 ^{i}	2.07 ^{h}	1.71 ^{h}	*0.87*	*	**	*
Decarboxymethyl oleuropein aglycone, oxidized dialdehyde form	34.75 ^{a}	48.46 ^{a}	40.94 ^{a}	55.23 ^{b}	56.94 ^{b}	60.05 ^{b}	*11.88*	*	*ns*	*ns*
Decarboxymethyl ligstroside aglycone, oxidized dialdehyde form	31.53 ^{h}	45.54 ^{il}	36.28 ^{hi}	54.98 ^{l}	46.04 ^{il}	44.04 ^{il}	*5.58*	**	*ns*	*
Ligstroside aglycone, oxidized aldehyde and hydroxylic form	8.26 ^{h}	11.02 ^{h}	8.26 ^{h}	22.75 ^{i}	11.44 ^{h}	10.80 ^{h}	*3.94*	*	*ns*	**
Sum of Compounds and Indexes										
Total phenolic compounds	366	434	348	455	367	393	*70*	*ns*	*ns*	*ns*
Tyrosol + Hydroxytyrosol	3.95	4.16	3.73	6.36	4.07	4.01	*1.54*	*ns*	*ns*	*ns*
Phenolic acids	12.87 ^{h}	14.31 ^{hi}	9.94 ^{hi}	18.79 ^{i}	12.23 ^{hi}	11.84 ^{hi}	*3.01*	*ns*	*ns*	*
Lignans	20.38 ^{i}	26.60 ^{hi}	20.78 ^{i}	33.98 ^{h}	25.07 ^{hi}	24.89 ^{i}	*3.58*	**	*ns*	*
Flavones	11.18	15.97	12.69	15.13	13.45	12.58	*4.46*	*ns*	*ns*	*ns*
Secoiridoids	317.28	373.12	300.69	380.24	312.06	340.00	*61.33*	*ns*	*ns*	*ns*
Oleuropein derivatives	223.58	260.75	209.37	249.90	209.30	240.17	*50.65*	*ns*	*ns*	*ns*
Ligstroside derivatives	97.65 ^{a}	116.53 ^{a}	95.05 ^{a}	136.70 ^{b}	106.84 ^{b}	103.84 ^{b}	*13.67*	*	*ns*	*ns*
Not-Ox/Ox Ratio	2.77 ^{b}	2.15 ^{b}	2.02 ^{b}	1.53 ^{a}	1.40 ^{a}	1.54 ^{a}	*0.04*	*	*ns*	*ns*

Table 4
Volatile organic compound profile of olive oil samples taken from olive fruits stored at cold and ambient temperature, with different surface/volume ratios (SVR) at mill scale. Only those compounds for which significant differences ($p < 0.05$) were found at the ANOVA, are shown. Letters x,y indicate compound significantly different for SVR, a,b indicate a significant difference for temperature; h,i,l, indicate significant difference for temperature x SVR interaction. The letter assignment was carried out according to the Tukey HSD post-hoc test. RSE column reports the Residual Standard Error of the model. Significant codes: ns = not significant; . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Volatile Organic Compounds(mg kg^{-1} or µg kg^{-1})	Ambient SVR7-bin	SVR10-halfbin	SVR16-box	Cold SVR7-bin	SVR10-halfbin	SVR16-box	*RSE*	*pT*	*p SVR*	*pint*
Methyl acetate (µg kg^{-1})	78.45 ^{i}	33.60 ^{h}	29.87 ^{h}	32.76 ^{h}	31.37 ^{h}	43.81 ^{hi}	*14.49*	.	*ns*	*
Pentanal (mg kg^{-1})	0.86 ^{h}	0.90 ^{hi}	0.94 ^{i}	0.90 ^{hi}	0.89 ^{hi}	0.86 ^{h}	*0.03*	*ns*	*ns*	*
2-heptanone (mg kg^{-1})	1.58 ^{b}	1.58 ^{b}	1.58 ^{b}	1.57 ^{a}	1.57 ^{a}	1.58 ^{a}	*0.01*	*	*ns*	*ns*
1-butanol, 2-methyl + 3-methyl (mg kg^{-1})	0.40 ^{b}	0.33 ^{b}	0.35 ^{b}	0.21 ^{a}	0.27 ^{a}	0.19 ^{a}	*0.09*	**	*ns*	*ns*
Acetic acid, hexyl ester (mg kg^{-1})	0.06 ^{a}	0.10 ^{a}	0.09 ^{a}	0.15 ^{b}	0.15 ^{b}	0.15 ^{b}	*0.04*	**	*ns*	*ns*
E2-penten-1-ol (µg kg^{-1})	36.09 ^{b}	32.42 ^{b}	28.10 ^{b}	30.70 ^{a}	21.21 ^{a}	26.75 ^{a}	*7.67*	*	.	*ns*
Z2-penten-1-ol (mg kg^{-1})	0.40 ^{b}	0.36 ^{b}	0.32 ^{b}	0.35 ^{a}	0.25 ^{a}	0.30 ^{a}	*0.07*	*	*ns*	*ns*
Z3-hexenyl-acetate (mg kg^{-1})	0.26 $^{a, x}$	0.36 $^{a, y}$	0.32 $^{a, y}$	0.34 $^{b, x}$	0.49 $^{b, y}$	0.52 $^{b, y}$	*0.08*	**	*	*ns*
E2-hexenyl-acetate (µg kg^{-1})	0.00 ^{a}	0.52 ^{a}	0.16 ^{a}	3.01 ^{b}	4.31 ^{b}	4.40 ^{b}	*3.16*	*	*ns*	*ns*
1-hexanol (mg kg^{-1})	0.15 ^{a}	0.18 ^{a}	0.15 ^{a}	0.22 ^{b}	0.26 ^{b}	0.24 ^{b}	*0.05*	**	*ns*	*ns*
E3-hexen-1-ol (µg kg^{-1})	4.49 ^{a}	6.22 ^{a}	5.18 ^{a}	6.96 ^{b}	7.15 ^{b}	6.65 ^{b}	*3.16*	*	*ns*	*ns*
2,4-hexadienal (µg kg^{-1})	81.76 ^{y}	49.46 ^{x}	47.50 ^{x}	98.60 ^{y}	43.80 ^{x}	79.12 ^{x}	*18.89*	*ns*	**	*ns*
1-heptanol (µg kg^{-1})	2.11 ^{a}	4.08 ^{a}	2.39 ^{a}	6.58 ^{b}	6.79 ^{b}	5.43 ^{b}	*5.06*	*	*ns*	*ns*
Decanal (mg kg^{-1})	0.13 ^{a}	0.39 ^{a}	0.38 ^{a}	1.13 ^{b}	1.32 ^{b}	0.52 ^{b}	*0.57*	*	*ns*	*ns*
2,4-nonadienal (µg kg^{-1})	51.70 ^{hi}	60.30 ^{hi}	55.09 ^{hi}	64.34 ^{i}	59.08 ^{hi}	49.94 ^{h}	*6.99*	*ns*	*ns*	*
Phenol (µg kg^{-1})	30.13 ^{i}	22.26 ^{h}	19.61 ^{h}	21.50 ^{h}	23.31 ^{h}	25.28 ^{i}	*2.36*	*	*ns*	***

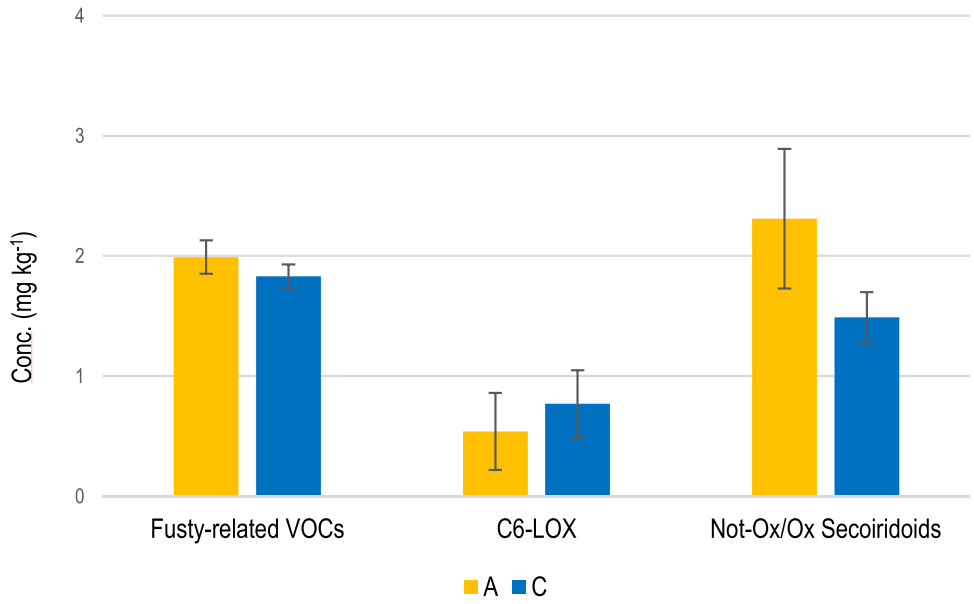

Fig. 2. Balance of positive and negative volatile organic compounds and oxidation products between the two storage treatments: cold (C) and ambient temperature storage (A). The bar reports the average values obtained from each temperature treatment, taking only those compounds that were significantly different ($p < 0.05$) at the ANOVA. Error bars represent the standard deviation.

Regarding fruit mass, only two compounds were affected by the SVR and, more specifically, in SVR7-bins. SVR7-bin concentrations of Z3-hexenyl-acetate and 2,4-hexadienal were lower and higher respectively, than other SVR conditions. A statistically significant interaction between temperature and SVR was observed for methyl-acetate, pentanal, 2,4-nonadienal and phenol.

When VOCs were grouped into similar classes, it became clear that some short chain compounds were related to microbial action (methyl-acetate, 2-methyl and 3-methyl butanol) (Angerosa et al., 1990; López-López et al., 2019; Morales et al., 2005). These molecules might also be responsible for the *fusty* defect that is often found with a low SVR (Angerosa, Di Giacinto, and Solinas 1990; Angerosa, Lanza, and Marsilio 1996).

On the other hand, all of the molecules linked to the LOX pathway had a positive correlation with cold storage. The exception was C5, where the opposite trend was found. Finally, several molecules associated with microbial or oxidative activities (Morales et al., 2005; Tuorila and Recchia, 2014), such as 2-heptanone, 1-heptanol or 2,4-nonadienal, were roughly equally distributed between all olive oil samples.

4. Discussion

The current recommendation is that harvested olives should be milled as soon as possible. In real, operative conditions this is not possible. Usually extraction cannot keep up with harvesting, resulting in unavoidable olive storage. In order to reduce the risk related to the delay in olive processing during the harvesting season, producers of high-quality EVOO have started to use refrigerating cells to store fruit between harvest and milling. This study evaluates real storage conditions, characterized by a short storage period, relatively small olive batches, and industrial mill equipment. A reference test (Trial 1), carried out in the laboratory, provided support for a second experiment at mill scale (Trial 2).

The results of Trials 1 and 2 are consistent: the industrial-scale trial confirmed the findings of the lab-scale experiment. However, at industrial scale, other logistic factors became important, such as the mass of fruit stored.

The effect of the SVR is apparent from temperature records (Fig. 1), which highlighted different trends, especially for SVR7-bins. Storage temperature was found to interact with SVR with respect to several variables. Most of the significant interactions were related to the SVR7-bin. This mass had the highest thermal capacity, and the worst heat exchange with the environment, resulting in: i) a slow temperature fall for cold storage; and ii) an increased rate of metabolic processes in olive heaps at ambient temperature. The fruit mass makes a non-negligible contribution to fruit metabolism, and the effect is more apparent at high masses, as confirmed by the literature (García and Yousfi 2006; Angerosa, Di Giacinto, and Solinas 1990). This process led to a 3–5°C increase in the internal temperature of SVR7-bin fruits stored at ambient temperature, and a slowdown in the cooling rate in the cold storage treatment.

Despite the short storage time, temperature has a marked effect on, in particular, the biophenol profile and VOC composition of oils.

Although no difference was found for total biophenol content, cold storage appears to be a good way to preserve phenolic compounds in olive oil. As reported by several authors, lower temperatures might reduce enzymatic activity (Ben et al., 2012; Clodoveo et al., 2007; García et al., 1996b), which can also derive from the microbiota of olive fruit (Zullo et al., 2014). In both of our trials, cold storage improved the preservation of the phenolic fraction of derived oils (A samples), indicated by higher concentrations of some secoiridoid aglycones, flavones, and lignans. A samples indicated greater phenolic fraction degradation than C samples, consistent with higher concentrations of tyrosol derived from the hydrolysis of ligstroside. However, oil samples from cold-stored olives were more oxidized, which may be due to an increase in dissolved oxygen at low temperature. Hachicha Hbaieb et al. (2015) described this phenomenon in the context of enzyme activity. The latter authors found that the key enzyme in phenolic compound oxidation (polyphenol oxidase), decreased during the first days of fruit storage, and that the process accelerated when olives were stored at higher temperature. In Trial 2, at ambient temperature, the temperature increase of almost 3°C could suggest greater hydrolysis of the phenolic fraction, as argued by García and Yousfi (2006). However, no significant differences were found for SVR treatments, with the exception of ferulic acid. Thus, it seems that, for short storage periods, hydrolytic, oxidative and microbial

processes that may lead to the degradation of phenolic compounds are mainly related to temperature, regardless of SVR conditions.

With respect to the VOC profile of olive oils, we found an abundance of molecules related to the *fruity* sensory attribute after cold storage, indicating a positive correlation between flavor and low temperature. However, results reported in the literature do not agree. Some studies partially confirm our finding (Dourou et al. 2020), while others note reduced development of olive oil flavor after cold storage of fruit (Luaces, Pérez, and Sanz 2005; Hachicha Hbaieb et al. 2016). The increase in LOX compounds in our data is consistent with increased oxidation, highlighted by a fall in the ratio between non-oxidized and oxidized forms of secoiridoids. VOCs linked to fermentation are more abundant after storage at ambient temperature, which clearly supports microbiological, enzymatic, and oxidative activities (Angerosa et al. 2004).

To summarize, olive cooling and short-term cold storage seems to have a positive effect on the aroma profile of olive oil, consistent with the higher sum of positive C6 LOX-related compounds (Fig. 2). In addition, it decreased the sum of the concentrations of several VOCs related to amino acid or microbial metabolism, and often linked to the *fusty* defect in olive oil (Fig. 2). In fact, the Italian word for *fusty* is *riscaldo*, which refers to the heat produced by olives during inappropriate storage, and the International Olive Council describes *fusty* as "characteristic flavour of oil obtained from olives piled or stored in such conditions as to have undergone an advanced stage of anaerobic fermentation" (International Olive Council, 2018).

It appears that low temperatures slow hydrolytic deterioration in the phenolic fraction. However, the mechanism is not completely clear, from an oxidative perspective. We could hypothesize that, on the one hand, cold storage protects against chemical oxidation, notably a rise in K_{232} and K_{270} indexes. On the other hand, cold storage seems to increase enzyme-related oxidation products. Moreover, we observed higher amounts of the oxidized form of secoiridoids, and higher concentrations of LOX-related VOCs (Fig. 2). Consistent with this hypothesis, and from a strictly chemical point of view, the LOX compounds cited above could be considered as oxidation products. It is possible that the conjugation of unsaturated lipid dienes was supported at higher temperatures (i.e. chemical oxidation) and, on the other hand, that lower temperatures facilitated the dissolution of O_2 in olive tissues (Kalua et al. 2007) leading to the oxidation of biophenols and the appearance of oxidative-related VOCs. These phenomena involve different causal mechanisms.

According to Karel (1984), in some cases, oxidative reactions may actually proceed faster at low temperatures, due to a slowing down of interfering reactions that compete for O_2. The energy require to a activate lipid oxidation is often lower than other reactions that need O_2 as a catalyzer, or act as antioxidant functions (Karel, 1984). As described for other plants, lowering the storage temperature can modify the lipid composition of the cell membrane, shifting the balance towards polyunsaturated fatty acids such as linolenic acid (C18:3), in order to maintain fluidity (Lee et al., 2005; Xu and Siegenthaler, 1997). This rearrangement leads to the production of hydroperoxides by LOX enzymes, and the initiation of the LOX pathway. Several studies have demonstrated that the enzymes involved in the LOX pathway have different optimal temperatures, which can regulate changes in the EVOO aroma profile. While hydroperoxide lyase (HPL), which breaks down 13-hydroperoxydes into aldehydes, has maximum activity at 15°C and minimum at 35°C (Salas and Sánchez, 1999a), lipoxygenase (LOX), alcohol dehydrogenase (ADH) and alcohol acyltransferase (AAT), which form hydroperoxides, alcohols and esters, respectively, have an optimum around 30–35°C (Pérez et al., 1993; Ridolfi et al., 2002).

Thus, if we consider HPL as the key LOX enzyme, high temperatures may reduce the formation of green notes in olive oil (Salas and Sánchez, 1999a), or favor the production of C5 compounds and pentene dimers, rather than C6 aldehydes (Luaces et al., 2005). However, in the present study, high temperatures were ≃15°C, which although it is the HPL optimum, could be considered relatively low compared to the other LOX enzymes that were studied (optima ≃30°C). As the reduction in green notes cannot be ascribed to the high storage temperature, it seems that flavor formation is more complex. In practice, during olive fruit storage many different factors interact, such as time, temperature, and pH (Ridolfi et al., 2002; Salas et al., 1999; Salas and Sánchez, 1999b), the fruit cultivar (Hachicha Hbaieb et al., 2016; Sánchez-Ortiz et al., 2013), and the ripening stage (Dourou et al., 2020; Hachicha Hbaieb et al., 2016; Salas et al., 1999; Salas and Sánchez, 1998). These observations make it difficult to predict the specific enzymatic and chemical mechanisms that are responsible for the evolution of phenolic and volatile profiles during short-term storage.

Finally, no interaction was found between the SVR and storage temperature, except for concentrations of a few compounds in oil. This indicates that, regardless of temperature, any type of container and any degree of filling can be considered as non-detrimental to olive oil quality for short periods of storage.

5. Conclusion

The use of a refrigerated cell to cool and store the olives between the harvest and the milling, can be considered as a new tool in the olive oil toolbox, and a new opportunity, especially for high-quality olive oil producers. Our trial showed that it was able to limit the deterioration of post-harvest fruit, and change the LOX-related VOC profile of olive oil. However, we still do not fully understand how short-term cold storage affects various biochemical and physical-chemical phenomena.

In general, fruit cooling and short-term cold storage:

- avoids fruit metabolism leading to mass warming, especially with a low SVR;
- improves the preservation of oil triglycerides and secoiridoids, from a hydrolytic point of view;
- increases production of aromatic compounds that are associated with positive EVOO attributes; and
- can reduce the production of VOCs related to *fusty, wine-vinegary* and *rancid* defects;
- allows the accumulation of small quantities of CO_2 in the storage environment.

On the other hand, low temperatures are consistent with greater oxidation in oils.

With regard to the SVR, fruit volumes should be correctly handled to ensure rapid cooling and to prevent defects in olive oil. Thus, we recommend the use of refrigerated containers for the production of high-quality EVOO, especially during the early stages of harvesting. The recent practice of early harvesting can lead to working on extremely hot days; consequently, fruit storage must necessarily be very short, and thermal conditions must be strictly controlled.

Declaration of Competing Interest

None.

Acknowledgments

The authors would like to thank Fattoria di Maiano for hosting the trials and particularly Dr. Tommaso Miari Fulcis, Dr. Roberto Rappuoli and Dr. Fiore Giovannini for their technical support.

Funding

This work was funded by the FEASR (Piano di Sviluppo Rurale 2014–2020 della Regione Toscana sottomisura 16.2) under the project title “La filiera olivo-oleicola nel distretto biologico di Fiesole: ottimizzazione dei protocolli di produzione di oli di qualità e utilizzo del frutto per nuovi prodotti nutraceutici – PIF FIESOIL”.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijrefrig.2021.03.002.

References

Angerosa, F., Di Giacinto, L., Solinas, M., 1990. Influenza dello stoccaggio in massa delle olive sull'aroma degli oli di risulta: Valutazione del difetto di “riscaldo” mediante analisis HPLC e GLC dei componenti volatili. Riv. Merceol. 29, 281–305.

Angerosa, F., Lanza, B., Marsilio, V., 1996. Biogenesis of «fusty» defect in virgin olive oils. Grasas y Aceites 47, 142–150. doi:10.3989/gya.1996.v47.i3.854.

Angerosa, F., Mostallino, R., Basti, C., Vito, R., 2000. Virgin olive oil odour notes : their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. Food Chem 68, 283–287. doi:10.1016/S0308-8146(99)00189-2.

Angerosa, F., Servili, M., Selvaggini, R., Taticchi, A., Esposto, S., Montedoro, G., 2004. Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. J. Chromatogr. A 1054, 17–31. doi:10.1016/j.chroma.2004.07.093.

Ben, Y.N., Ouni, Y., Dabbech, N., Baccouri, B., Abdelly, C., Zarrouk, M., 2012. Effect of olive storage period at two different temperatures on oil quality of two tunisian cultivars of Olea europea, Chemlali and Chtoui. African J. Biotechnol. 11, 888–895. doi:10.5897/ajb10.614.

Brenes, M., García, A., García, P., Garrido, A., 2001. Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. J. Agric. Food Chem. 49, 5609–5614. doi:10.1021/jf0107860.

Carbone, A., Cacchiarelli, L., Sabbatini, V., 2018. Exploring quality and its value in the Italian olive oil market : a panel data analysis. Agric. Food Econ. 6, 1–15. doi:10.1186/s40100-018-0102-8, https://doi.org/https://doi.org/.

Castellano, J.M., García, J.M., Morilla, A., Perdiguero, S., Gutierrez, F., 1993. Quality of picual olive fruits stored under controlled atmospheres. J. Agric. Food Chem. 41, 537–539. doi:10.1021/jf00028a005.

Clodoveo, M.L., Delcuratolo, D., Gomes, T., Colelli, G., 2007. Food chemistry effect of different temperatures and storage atmospheres on Coratina olive oil quality. Food Chem 102, 571–576. doi:10.1016/j.foodchem.2006.05.035.

Di Giovacchino, L., 2010. Tecnologie di lavorazione delle olive in frantoio. Rese di estrazione e qualità dell'olio.

Di Giovacchino, L., Sestili, S., Di Vincenzo, D., 2002. Influence of olive processing on virgin olive oil quality. Eur. J. 104, 587–601.

Dourou, A.M., Brizzolara, S., Meoni, G., Tenori, L., Famiani, F., Luchinat, C., Tonutti, P., 2020. The inner temperature of the olives (cv. Leccino) before processing affects the volatile profile and the composition of the oil. Food Res. Int. 129. doi:10.1016/j.foodres.2019.108861.

Dourtoglou, V.G., Mamalos, A., Makris, D.P., 2006. Storage of olives (Olea europaea) under CO2 atmosphere: Effect on anthocyanins, phenolics, sensory attributes and in vitro antioxidant properties. Food Chem 99, 342–349. doi:10.1016/j.foodchem.2005.07.051.

EC, 2008. COMMISSION REGULATION (EC) No 640/2008 of 4 July 2008 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Off. J. Eur. Union L178, 11–16.

Fakas, S., Kefalogianni, I., Makri, A., Tsoumpeli, G., Rouni, G., Gardeli, C., Papanikolaou, S., Aggelis, G., 2010. Characterization of olive fruit microflora and its effect on olive oil volatile compounds biogenesis. Eur. J. Lipid Sci. Technol. 112, 1024–1032. doi:10.1002/ejlt.201000043.

Fiorini, D., Boarelli, M.C., Conti, P., Alfei, B., Caprioli, G., Ricciutelli, M., Sagratini, G., Fedeli, D., Gabbianelli, R., Pacetti, D., 2018. Chemical and sensory differences between high price and low price extra virgin olive oils. Food Res. Int. 105, 65–75. doi:10.1016/j.foodres.2017.11.005.

Fortini, M., Migliorini, M., Cherubini, C., Cecchi, L., Calamai, L., 2017. Multiple internal standard normalization for improving HS-SPME-GC-MS quantitation in virgin olive oil volatile organic compounds (VOO-VOCs) profile. Talanta 165, 641–652. doi:10.1016/j.talanta.2016.12.082.

García, J.M., Gutiérrez, F., Barrera, M.J., Albi, M.A., 1996a. Storage of mill olives on an industrial scale. J. Agric. Food Chem. 44, 590–593. doi:10.1021/jf950479s.

García, J.M., Gutiérrez, F., Castellano, J.M., Perdiguero, S., Morilla, A., Albi, M.A., 1996b. Influence of storage temperature on fruit ripening and olive oil quality. J. Agric. Food Chem. 44, 264–267. doi:10.1021/jf950399o.

García, J.M., Gutiérriez, F., Castellano, J.M., Perdiguero, S., Morilla, A., Albi, M.A., 1994. Storage of olives destined for oil extraction. Acta Hortic 368, 673–681. doi:10.17660/ActaHortic.1994.368.80.

García, J.M., Yousfi, K., 2006. The postharvest of mill olives. Grasas y Aceites 57, 16–24. doi:10.3989/gya.2006.v57.i1.18.

Garcia, P., Brenes, M., Romero, C., Garrido, A., 1995. Respiration and physicochemical changes in harvested olive fruits. J. Hortic. Sci. 70, 925–933. doi:10.1080/14620316.1995.11515368.

Guerrini, L., Migliorini, M., Giusti, M., Parenti, A., 2017. The influence of crusher speed on extra virgin olive oil characteristics. Eur. J. Lipid Sci. Technol. 119, 1–7. doi:10.1002/ejlt.201600156.

Guerrini, L., Zanoni, B., Breschi, C., Angeloni, G., Masella, P., Calamai, L., Parenti, A., 2020. Understanding olive oil stability using filtration and high hydrostatic pressure. Molecules 25, 420. doi:10.3390/molecules25020420.

Gutierrez, F., Perdiguero, S., García, J.M., Castellano, J.M., 1992. Quality of oils from olives stored under controlled atmosphere. J. Am. Oil Chem. Soc. 69, 1215–1218. doi:10.1007/BF02637683.

Hachicha Hbaieb, R., Kotti, F., García-Rodríguez, R., Gargouri, M., Sanz, C., Pérez, A.G., 2015. Monitoring endogenous enzymes during olive fruit ripening and storage: Correlation with virgin olive oil phenolic profiles. Food Chem 174, 240–247. doi:10.1016/j.foodchem.2014.11.033.

Hachicha Hbaieb, R., Kotti, F., Gargouri, M., Msallem, M., Vichi, S., 2016. Ripening and storage conditions of Chétoui and Arbequina olives: Part I. Effect on olive oils volatiles profile. Food Chem 203, 548–558. doi:10.1016/j.foodchem.2016.01.089.

Inarejos-García, A.M., Gómez-Rico, A., Desamparados Salvador, M., Fregapane, G., 2010. Effect of preprocessing olive storage conditions on virgin olive oil quality and composition. J. Agric. Food Chem. 58, 4858–4865. doi:10.1021/jf902486f.

International Olive Council, 2018. Sensory analysis of olive oil - method for the organoleptic assessment of virgin olive oil. COI/T.20/Doc No 15/Rev. 10.

International Olive Council, 2017. Determination of biophenols in olive oils by HPLC. COI/T.20/Doc No 29/Rev. 1.

ISMEA, 2020. Oli vegetali - News mercati n.33/2020 settimana n.49/2020 - dal 30 novembre al 6 dicembre 2020.

Jabeur, H., Zribi, A., Abdelhedi, R., Bouaziz, M., 2015. Effect of olive storage conditions on Chemlali olive oil quality and the effective role of fatty acids alkyl esters in checking olive oils authenticity. Food Chem 169, 289–296. doi:10.1016/j.foodchem.2014.07.118.

Kader, A.A., Nanos, G.D., Kerbel, E.L., 1989. Responses of “Manzanillo” Olives To Controlled Atmospheres Storage. In: Proceedings of the Fifth International Controlled Atmosphere Research Conference, pp. 119–125.

Kalua, C.M., Allen, M.S., Bedgood, D.R., Bishop, A.G., Prenzler, P.D., Robards, K., 2007. Olive oil volatile compounds, flavour development and quality: A critical review. Food Chem 100, 273–286. doi:10.1016/j.foodchem.2005.09.059.

Kalua, C.M., Bedgood, D.R., Bishop, A.G., Prenzler, P.D., 2008. Changes in virgin olive oil quality during low-temperature fruit storage. J. Agric. Food Chem. 56, 2415–2422. doi:10.1021/jf073027b.

Karel, M., 1984. Chemical effects in food stored at room temperature. J. Chem. Educ. 61, 335–339. doi:10.1021/ed061p335.

Kiritsakis, A., Markakis, P., 1984. Effect of olive collection regime on olive oil quality. J. Sci. Food Agric. 35, 677–678. doi:10.1002/jsfa.2740350614.

Kiritsakis, A., Nanos, G.D., Polymenopoulos, Z., Thomai, T., Sfakiotakis, E.M., 1998. Effect of Fruit Storage Conditions on Olive Oil Quality 721–724. https://doi.org/10.1007/s11746-998-0212-7

Langstaff, S., 2014. Sensory Quality Control, in: Monteleone, E., Langstaff, S. (Eds.). Olive Oil Sensory Science 81–108. doi:10.1002/9781118332511.ch4.

Lee, S.H., Ahn, S.J., Im, Y.J., Cho, K., Chung, G.C., Cho, B.H., Han, O., 2005. Differential impact of low temperature on fatty acid unsaturation and lipoxygenase activity in figleaf gourd and cucumber roots. Biochem. Biophys. Res. Commun. 330, 1194–1198. doi:10.1016/j.bbrc.2005.03.098.

López-López, A., Cortés-Delgado, A., de Castro, A., Sánchez, A.H., Montaño, A., 2019. Changes in volatile composition during the processing and storage of black ripe olives. Food Res. Int. 125, 108568. doi:10.1016/j.foodres.2019.108568.

Luaces, P., Pérez, A.G., Sanz, C., 2005. Effect of cold storage of olive fruits on the lipoxygenase pathway and volatile composition of virgin olive oil. Acta Hortic 682, 993–998. doi:10.17660/ActaHortic.2005.682.129.

Maestro, R., García, J.M., Castellano, J.M., 1993. Changes in polyphenol content of olives stored in modified atmospheres. HortScience 28, 1993. doi:10.21273/HORTSCI.28.7.749.

Masella, P., Guerrini, L., Angeloni, G., Zanoni, B., Parenti, A., 2019. Ethanol from olive paste during malaxation, exploratory experiments. Eur. J. Lipid Sci. Technol. 121, 1–7. doi:10.1002/ejlt.201800238.

Migliorini, M., Cherubini, C., Cecchi, L., Zanoni, B., 2013. Degradazione dei composti fenolici durante la conservazione dell'olio extra vergine di oliva. Riv. Ital. delle Sostanze Grasse 90, 71–80.

Montedoro, G., Servili, F., Baldioli, M., Miniati, E., 1992. Simple and hidrolyzable phenolic compounds in virgin olive oil 2.initial characterization of the hidrolyzable fraction. J. Agric. Food Chem. 40, 1577–1580. doi:10.1021/jf00021a020.

Morales-Sillero, A., Pérez, A.G., Casanova, L., García, J.M., 2017. Cold storage of ‘Manzanilla de Sevilla’ and ‘Manzanilla Cacereña’ mill olives from super-high density orchards. Food Chem 237, 1216–1225. doi:10.1016/j.foodchem.2017.06.073.

Morales, M.T., Aparicio, R., Rios, J.J., 1994. Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil. J. Chromatogr. A 668, 455–462. doi:10.1016/0021-9673(94)80139-8.

Morales, M.T., Luna, G., Aparicio, R., 2005. Comparative study of virgin olive oil sensory defects. Food Chem 91, 293–301. doi:10.1016/j.foodchem.2004.06.011.

Pagliarini, E., Zanoni, B., Giovanelli, G., 2000. Predictive study on tuscan extra virgin olive oil stability under several commercial conditions. J. Agric. Food Chem. 48, 1345–1351. doi:10.1021/jf990372v.

Pereira, J.A., Casal, S., Bento, A., Oliveira, M.B.P.P., 2002. Influence of olive storage period on oil quality of three Portuguese cultivars of Olea europea, Co-

brançosa, Madural, and Verdeal Transmontana. J. Agric. Food Chem. 50, 6335–6340. doi:10.1021/jf011661y.
Pérez, A.G., Sanz, C., Olías, J.M., 1993. Partial purification and some properties of alcohol acyltransferase from strawberry fruits. J. Agric. Food Chem. 41, 1462–1466. doi:10.1021/jf00033a021.
Peri, C., 2014. Quality excellence in extra virgin olive oils, in: Monteleone, E., Langstaff, S. (Eds.). Olive Oil Sensory Science 4–32. doi:10.1002/9781118332511.ch1, https://doi.org/https://doi.org/.
Piscopo, A., De Bruno, A., Zappia, A., Gioffrè, G., Grillone, N., Mafrica, R., Poiana, M., 2018. Effect of olive storage temperature on the quality of Carolea and Ottobratica oils. Emirates J. Food Agric. 30, 563–572. doi:10.9755/ejfa.2018.v30.i7.1739.
in: Proietti, P., 2014. Olive handling, storage and transportation. In: Peri, C. (Ed.), The Extra-Virgin Olive Oil Handbook. John Wiley & Sons, pp. 107–112. doi:10.1002/9781118460412.ch9.
Qian, M.C., Wang, Y., 2005. Seasonal variation of volatile composition and odor activity value of "Marion" (Rubus spp. hyb) and "Thornless Evergreen" (R. laciniatus L.) blackberries. J. Food Sci. 70, 13–20. doi:10.1111/j.1365-2621.2005.tb09013.x.
Ridolfi, M., Terenziani, S., Patumi, M., Fontanazza, G., 2002. Characterization of the lipoxygenases in some olive cultivars and determination of their role in volatile compounds formation. J. Agric. Food Chem. 50, 835–839. doi:10.1021/jf0109118.
Rinaldi, R., Amodio, M.L., Colelli, G., Clodoveo, M.L., 2010. Controlled atmosphere storage of 3 Italian cultivars of olives for oil production. Acta Hortic 857, 97–106. doi:10.17660/ActaHortic.2010.857.9.
Saffar Taluri, S., Jafari, S.M., Bahrami, A., 2019. Evaluation of changes in the quality of extracted oil from olive fruits stored under different temperatures and time intervals. Sci. Rep. 9, 1–8. doi:10.1038/s41598-019-54088-z.
Salas, J.J., Sánchez, J., 1999a. The decrease of virgin olive oil flavor produced by high malaxation temperature is due to inactivation of hydroperoxide Lyase. J. Agric. Food Chem. 47, 809–812. doi:10.1021/jf981261j.
Salas, J.J., Sánchez, J., 1999b. Hydroperoxide lyase from olive (Olea europaea) fruits. Plant Sci 143, 19–26. doi:10.1016/S0168-9452(99)00027-8.
Salas, J.J., Sánchez, J., 1998. Alcohol dehydrogenases from olive (Olea Europaea) fruit. Phytochemistry 48, 35–40. doi:10.1016/S0031-9422(97)01097-2.
Salas, J.J., Williams, M., Harwood, J.L., Sánchez, J., 1999. Lipoxygenase activity in olive (Olea europaea) fruit. JAOCS. J. Am. Oil Chem. Soc. 76, 1163–1168. doi:10.1007/s11746-999-0090-7.
Sánchez-Ortiz, A., Pérez, A.G., Sanz, C., 2013. Synthesis of aroma compounds of virgin olive oil: significance of the cleavage of polyunsaturated fatty acid hydroperoxides during the oil extraction process. Food Res. Int. 54, 1972–1978. doi:10.1016/j.foodres.2013.03.045.
Servili, M., Selvaggini, R., Taticchi, A., Baldioli, M., Montedoro, G.F., 2002. The use of biotechnology means during oil mechanical extraction process: relationship with sensory and nutritional parameters of virgin olive oil quality. Acta Hortic 586, 557–560. doi:10.17660/ActaHortic.2002.586.116.
Tuorila, H., Recchia, A., 2014. Sensory perception and other factors affecting consumer choice of olive oil, in: monteleone, E., Langstaff, S. (Eds.). Olive Oil Sensory Science 55–80 https://doi.org/ePDF 9781444339901.
Uceda, M., Frias, L., 1975. Harvest dates. evolution of the fruit oil content, oil composition and oil quality. In: Proceedings of the II Seminario Oleícola Internacional, International Olive Oil Council, Cordoba, Spain, pp. 125–130.
Vichi, S., Romero, A., Gallardo-Chacón, J., Tous, J., López-Tamames, E., Buxaderas, S., 2009. Influence of olives' storage conditions on the formation of volatile phenols and their role in off-odor formation in the oil. J. Agric. Food Chem. 57, 1449–1455. doi:10.1021/jf803242z.
Vichi, S., Romero, A., Tous, J., Caixach, J., 2011. The activity of healthy olive microbiota during virgin olive oil extraction influences oil chemical composition. J. Agric. Food Chem. 59, 4705–4714. doi:10.1021/jf200642s.
Xu, Y., Siegenthaler, P.A., 1997. Low Temperature Treatments Induce an Increase in the Relative Content of Both Linolenic and Δ3-trans-Hexadecenoic Acids in Thylakoid Membrane Phosphatidylglycerol of Squash Cotyledons. Plant Cell Physiol 38, 611–618. doi:10.1093/oxfordjournals.pcp.a029211.
Yousfi, K., Cayuela, J.A., García, J.M., 2009. Effect of Temperature, modified atmosphere and ethylene during olive storage on quality and bitterness level of the oil. JAOCS, J. Am. Oil Chem. Soc. 86, 291–296. doi:10.1007/s11746-009-1348-9.
Yousfi, K., Cayuela, J.A., García, J.M., 2008. Reduction of virgin olive oil bitterness by fruit cold storage. J. Agric. Food Chem. 56, 10085–10091. doi:10.1021/jf801951g.
Yousfi, K., Weiland, C.M., García, J.M., 2012. Effect of harvesting system and fruit cold storage on virgin olive oil chemical composition and quality of superintensive cultivated arbequina olives. J. Agric. Food Chem. 60, 4743–4750. doi:10.1021/jf300331q.
Zullo, B.A., Di Stefano, M.G., Cioccia, G., Ciafardini, G., 2014. Evaluation of polyphenol decay in the oily fraction of olive fruit during storage using a mild sample handling method. Eur. J. Lipid Sci. Technol. 116, 160–168. doi:10.1002/ejlt.201300287.