Effect of facilitated harvesting and fruit cooling on extra virgin olive oil quality

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

[a]Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali (DAGRI), Università degli Studi di Firenze, Firenze, Italia

[b]Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino (NEUROFARBA), Università degli Studi di Firenze, Sesto Fiorentino, Italia

✉ CORRESPONDENCE:
E-mail address: giulia.angeloni@unifi.it;
Tel.: + 39 055 2755932

Received: January 11, 2022
Accepted: February 28, 2022

To produce high quality olive oil, best practices recommend both to avoid fruit damages during the harvesting and to avoid long storage time between harvesting and crushing. The mechanical harvesting could damage the olives, favouring pulp softening, cell breakage, increasing the fruit respiration and leading to a fast olive oil degradation. Furthermore, the working capacity of the plants is not sufficient to cover the incoming volumes of olives, and a storage period is needed. To minimise the spoilage of olives, several hand-held facilitating machines were developed and refrigerated cells for fruit storage are currently spread.

A full factorial design evaluated the combined effects of harvesting method (manual vs facilitated), storage temperature (25°C vs 6.5°C) and their interaction, aiming to understand if the storage at low temperature, applied to olives harvested using hand-held electric combs, could mitigate the potential negative effects given by the beating.

From chemical analyses of legal parameters, phenolic and aromatic fractions, the highest amounts of total phenolic compounds occurred in olive oil samples, extracted from olives harvested through the manual method. Moreover, storage at low temperature preserved secoiridoids, even if it favoured their oxidation. The mechanical stress on olives due to harvest resulted in preferably activating the oxidative reactions, including the lipoxygenase pathway, which is responsible for the production of olive oil fruity notes. The latter phenomena were enhanced by low temperatures, probably due to the higher solubility of oxygen and the selected activity of hydroperoxide lyase.

Keywords: Mechanical harvesting, Refrigeration, Phenolic compounds, Aroma profile, Mechanization, Olive growing

1. INTRODUCTION

Extra virgin olive oil (EVOO) is the highest commercial category of olive oil and is more and more appreciated both for hedonistic features and for health and nutritional properties [1]. Within the above category, chemical and sensory characteristics of oils are very different, especially in terms of phenolic and volatile compounds [2], resulting in a wide range of prices (in Italy, the wholesale price between 4 and 12 euros per kg – ISMEA data consulted in May 2021). The oil processing conditions are essential for the EVOO quality, since they can cause several positive or negative phenomena that are able to transform the qualitative characteristics of oil in the olive fruit [3]. Therefore, the capability to modulate aromatic and phenolic EVOO profiles through planned processing conditions represents a key of commercial success for the oil companies.

Among the pre-extraction factors, olive fruit storage is considered critical for the EVOO quality since incorrect conditions can lead to the fermentation of the olive fruit heaps, causing the loss of oxidative stability in the olive oil and the development of off-flavour, namely *fusty*, *musty*, *wine-vinegary* and *ran-*

cid [4, 5]. It is commonly recommended to immediately transport the olives to the mill and to crush them as quickly as possible. However, since the incoming volume of olive fruit may exceed the working capacity of the extraction plant at peak harvest time, a storage period is required [6] and time and temperature conditions should be properly managed. In this view, the decrease of olive oil quality, due to the not-avoidable storage time, could be seen as a timeliness cost for olive companies.

The manual harvesting of the olive fruit is still widespread and hand-picking followed by collecting the olives in baskets has been improved by the introduction of hand-held tools, and nets [7]. However, manual harvesting carries the highest costs for the companies, linked to both the labour and the reduction of productivity [8 - 10]. In the last decades, mechanisation of olive harvesting accelerated powerfully leading to the introduction of several harvesting tools and machines [11], which can nowadays be chosen according to the planting systems and the olive grove size [12]. Hand-held electric or pneumatic combs, hand-held vibrating rods, and arm combs are able to facilitate workers during the olive harvesting and could be easily used in traditional olive orchards. Machines, which are self-propelled or to be coupled with the tractor (i.e., trunk shakers and straddle harvesters), are also used in olive orchards that are ad-hoc designed to be mechanised. Cresti et al. [8] found an increased productivity and a reduction in unit cost per hectare or per 100 kg of olives from the use of most of the above practices compared to manual harvesting. Similarly, Sperandio et al. [9] estimated a decrease in harvesting costs which was directly proportional to the level of mechanisation; the cost of olive oil ranged from 4.7-2.7 euros per kg of oil by manual harvesting, up to 0.3-0.5 euros per kg of oil using straddle machine.

However, mechanical harvesting techniques can cause technological damages on olive fruit, for instance skin scratch or breakage, bruising and pulp softening [12 - 14], favouring the release of cell liquids and their contact with enzymes, oxygen and microorganisms [16]. The degree of damage was not merely related to the harvesting method (i.e., as much as the energy was transferred to the olive tree), but it could depend on the constructive characteristics of the mechanical harvesters as well as their setting during the use [14]. Moreover, since beating also causes a breakdown of the pulp internal cells and the activation of fruit metabolism, the fruit damage was related to a combination of physical and biochemical phenomena, potentially able to affect the olive oil quality [16, 17].

Several detrimental effects on the olive oil quality, such as the increase of acidity, the increase of oxidation indexes and the off-flavour development, are linked to senescence and microbial activity, affecting olive fruit during the storage; low storage temperature of olives was proved to be able to minimize the above phenomena [18 - 22]. The cooling of olive fruit before milling could be a useful control tool for the EVOO quality, especially when the initial conditions of olive fruit already represent a risk factor for the development of sensory defect in the olive oil. Use of refrigerated cells for storage is one of the emerging technologies adopted by the several companies and is a successful tool for the producers, in order to preserve the quality of olive fruit [19, 21, 22]. Moreover, the olive fruit cooling may also have a modulating effect on the composition of the volatile fraction of olive oil [24], even when it is limited to a pre-crushing thermal conditioning, i.e. without any storage period [25].

However, the effectiveness of refrigeration should be linked to several factors, such as olive cultivar [26, 27], ripening stage [28] and health conditions, which are affected by fly infestation rate, microbial contamination and mechanical damage of harvesting methods [15, 26 - 28].

Few works have been carried out to study the combined effect of harvesting method and storage temperature (Tab. I). Among these, the work by Yousfi et al. [13] was the only one that studied the effect of different storage time-temperature conditions (i.e., 3 and 18°C) on quality of olive fruit, which were harvested manually and mechanically through a grape harvester. Mechanical harvesting accelerated the decay of olive fruit and, consequently, caused a decrease of olive oil quality in terms of behaviour of sensory defects and decrease of tocopherols and phenolic compounds; cold storage was also able to slow down the above degradation phenomena. However, the results of Yousfi et al. [13] only referred to operating machines, and their results cannot be extended to hand-held mechanical devices, which are widespread in more than half of the Italian companies and often as complementary system to the manual harvesting [29]. The current literature still lacks a focus on the actual benefits and/or contraindications of the working practices of the small-scale companies, which produce high quality olive oil. To enhance the product's quality, short storage time are usually adopted (i.e., most of the production regulations of Protected Designation of Origin and Protected Geographical Indication Italian olive oils set a maximum number of hours from the harvest within which the olives must be processed). However, in a short storage time, temperature and harvest method could still play an important role in preserving the freshness of the olive fruit and in determining the quality of the olive oil.

To the best of the authors knowledge, a work evaluating the effects of different working chains, combining effect of harvesting methods and storage temperatures, is still lacking. Here we focus on these issues with particular regard to two of the main distinctive features of high-quality olive oil, phenolic and volatile fractions.

Table I - Main experiments on the effect of harvesting method on olive oil quality.

Reference	Harvesting methods	Olive fruit storage temperature	Olive fruit storage time (h=hours, dd=days)	Parameters tested on olive oil	Results
(Dag et al. 2008)	Manual – hand-picking Mechanical- Hand-held machine with combs	---	0 dd	Free fatty acids Peroxide value Total phenolic compounds	Mechanical harvesting increases free fatty acids and peroxide value and reduce phenolic content of olive oils (cv. *Souri*).
(D'Imperio et al. 2010)	Mechanical - Hand-held machine with combs Mechanical - Hand-held shaking machine	---	0 dd	Free fatty acids Peroxide value UV spectrophotometric indexes (K_{232}, K_{270} and ΔK) NMR spectroscopy	Hexanal amount was higher in olive oils from olive harvested by shaking machine, whereas unsaturated fatty acids were lower in the same oil samples. This may indicate a greater level activity of lipoxygenase enzymes after shaking treatment.
(Yousfi et al. 2012)	Manual – Hand picking Mechanical – Grape straddle harvester	3 ± 1°C 18 ± 3°C	0, 4, 7, 10, 14, 21 dd	Free fatty acids Peroxide value UV spectrophotometric indexes (K_{232}, K_{270} and ΔK) Sensory evaluation Oxidative stability Pigments Tocopherol Phenolic compounds Fatty acid composition	Free fatty acids, peroxide value and K_{232} were significantly higher in oil from mechanically harvested fruit (cv. *Arbequina*). No sensory defects were detected in oils from hand harvesting, while oils from mechanical harvesting obtained significant lower grading scores after 4 days of storage at 18°C or after 7 days at 3°C. Greater concentrations of tocopherols, total phenolic compounds and secoiridoids were also found in olive oils from manual harvesting.
(Morales-Sillero and García 2014)	Manual – Hand picking Mechanical – Grape straddle harvester	5°C	< 1 dd	Free fatty acids Peroxide value UV spectrophotometric indexes (K_{232}, K_{270} and ΔK) Sensory evaluation Oxidative stability Pigments Tocopherol Phenolic compounds Fatty acid composition	Oils from mechanically harvested olives have lower acidity. Moreover, they shows a lower intensity of fruity, bitter and pungent, and, consequently, a lower overall grading. Mechanical harvesting also decreases the content of total phenols, *o*-diphenols and secoiridoids and reduces oxidative stability in oils from cv. *Manzanilla de Sevilla* and cv. *Manzanilla Cacereña*.
(Saglam et al. 2014)	Manual – Hand picking Mechanical - Hand-held shaking machine	---	0 dd	Free fatty acids Peroxide value	Acidity and peroxide value are lower in olive oil from hand-harvested olives (cv .*Gemlik* and cv. *Ayvalik*).
(Abenavoli and Proto 2015)	Manual - Wood sticks (beating) Mechanical – Hand-held shaking machine Mechanical - Trunk shaker	---	8, 24, 48 h	Free fatty acids Peroxide value UV spectrophotometric indexes (K_{232}, K_{270} and ΔK)	Olive oils (cv. *Carolea*) from mechanical harvesting through hand-held shaking machine shows lower acidity and peroxide value compared to oils from beating and trunk shaker harvesting. Both parameters also rise increasing the time of storage of olives.

Continua Tabella I

Reference	Harvesting methods	Olive fruit storage temperature	Olive fruit storage time (h=hours, dd=days)	Parameters tested on olive oil	Results
(Morales-Sillero et al. 2017)	Manual – Hand picking Mechanical – Grape straddle harvester	2 ± 0.5°C	1, 6, 11 dd	Free fatty acids Peroxide value UV spectrophotometric indexes (K_{232}, K_{270} and ΔK) Sensory evaluation Oxidative stability Pigments Tocopherol Phenolic compounds Fatty acid composition Volatile organic compounds	Mechanical harvesting increases free acidity in *Manzanilla de Sevilla* oils . In both *Manzanilla de Sevilla* and *Manzanilla Cacereña* oils, peroxide value is significantly higher after the mechanical harvesting but there are no differences among samples over the cold storage of fruit. *Manzanilla de Sevilla* oils from manual harvesting have a higher sensory score for positive attributes compared to samples from mechanical harvesting. In general, oxidative stability and total phenolic compounds are lower in oils from mechanical harvesting and decrease over the storage. After 1 day of storage oils from mechanical harvesting have lower content of C5 and C6 volatile compounds. These differences are mitigated by cold storage of olives.
(Famiani et al. 2020)	Manual - Hand picking Manual - Rakes Mechanical - Hand-held machine with combs Mechanical – Grape straddle harvester	18 ± 2°C	0 h, 48 h, 7 dd	Free fatty acids Peroxide value UV spectrophotometric indexes (K_{232}, K_{270} and ΔK) Phenolic compounds Volatile organic compounds	Fruit damage of olives (cv. *Arbequina* and cv. *Frantoio*) is higher increasing the mechanization level of the harvesting (from hand picking to straddle harvester). Total phenolic content and some secoiridoids decrease directly proportional to the mechanization level and time of storage. The contents of volatile compounds is linearly related to the level of mechanization. In general, concentration of C5, C6 and esters decreases in oils increasing the level of mechanization and the damaged index of fruit.

2. MATERIALS AND METHODS

2.1 OLIVE FRUIT HARVESTING AND ANALYSIS

2.1.1 Harvesting of olive batches

Olive oil fruits (*Olea europaea* L.) of *Frantoio* cultivar were harvested in central Italy (Fattoria di Maiano, Fiesole, Florence, Italy - approx. 43°79' N, 11°30' E) during the 2020 olive crop season. Three replicated collections were carried out in three different working days within the same harvesting week (i.e., 9-13 November 2020). For each replicate, two different methods were used for harvesting of two 5 kg-batches of olive fruit, respectively: i) the manual harvesting (MH), detaching the fruit from the plants directly by hands, or through the help of hand-held rakes ii) the facilitated harvesting (FH), using vibrating hand-held electric combs. Within the same replicate, the representative samples were obtained by collecting the olives from various canopy areas of the same olive tree, carrying out first the MH, then the FH.

2.1.2 Harvesting machines

There are various types of comb machines for harvesting on the market; they differ mainly for the driving power (electric or pneumatic) and the operating mode of teeth that can be oscillating, vibrating or rotating [30]. The devices used for the trials were hand-held electric combs with vibrating system (Olivion, T220/300, Pellenc S.A.S., Pertuis, France), since they were very suitable for the olive trees with a not too dense crown of our host company. In detail, the equipment was composed of portable battery (43.2 V), power cable, telescopic (2.20 m to 3.00 m length) rod with ergonomic handling and ON/OFF button, electric motor (380 W) and vibrating rake (38 cm width) with 8 carbon prongs. The device operated in "Continuous" mode at 840 strokes per min.

2.1.3 Olive fruit characterisation

After the harvesting, the whole batches were visually inspected for health conditions including the pres-

ence of fruit damage, examining a 100-unit sample. A damaged olive index (*DOI*) was assigned according to the method adopted by Famiani et al. [31]. A sample of N=100 olives was divided into 4 groups according to the degree of damage: no damage (i=0), damage on < 50% of the pulp (i=0.25), damage on > 50% of the pulp (i=0.75), 100% damaged (i=1). After counting the number of olives per each group (n_i), DOI was calculated as follows:

$$DOI = \sum_{i=0}^{i=1} \frac{(i \times n_i)}{N}$$

The maturity index (*MI*) was calculated following the method described by [32] that divides the olives in a 8-point-scale (range from 0 to 7), according to the colour of the skin and flesh. The water content was measured after weighing 20 g of olives before and after drying for 24 h at 105°C.

2.1.4 Storage conditions

All the olive fruit samples were immediately transported to the DAGRI (Department of Agriculture, Food, Environment and Forestry, University of Florence, Florence, Italy) laboratory that was 12 km away from the harvesting place, with a travel time of approximately 20 min. For each harvesting thesis (i.e., hand-picked and facilitated harvesting) the initial batch was divided in two homogeneous 2 kg sub-batches of fruit using mash bags (Raschel), which were stored for 24 h as follows: i) at 25°C in a controlled-temperature room; ii) at 6.5°C inside a chiller (Irinox MultiFresh, MF 25.1, Irinox Spa, Treviso, Italy). The fruit sample mass was arranged in monolayer to improve the heat exchange between fruit and storage environment and to make the samples reach promptly the setting temperatures. The latter were chosen to obtain the largest temperature range between the two different storage conditions. The 25°C-thesis was chosen to simulate the worst-case storage scenario for olive fruit, assuming masses of fruit waiting to be processed on a warm harvesting day. Moreover, olive growers anticipated the fruit harvest for either olive oil characteristics or climate change related issues. In the Mediterranean area, global warming led to the alteration of the phenological stages of olive tree and fruit ripening [31, 32] and growers often decided on an early harvest [35]. For instance, during the last 2021 olive oil season, the average maximum temperatures in Italy easily exceeded 20°C in October (Ministero delle Politiche Agricole, Alimentari e Forestali, consulted in January 2022).

The whole above scheme was replicated for 3 times, one for each harvesting day, making a total of 12 olive fruit samples and 12 olive oil extractions.

2.1.5 Olive oil micro-extraction

In order to exclude the effect of the whole mill operations, the olive oil samples were obtained in laboratory using a micro-extraction device as described in Masella et al. [36]. After 24 h of storage at controlled temperature, each olive sample was crushed using a lab-scale crusher that totally reproduced a knife crusher (Mori-TEM, Barberino Tavarnelle, Florence, Italy). Then, 1.1 kg of olive paste was mixed in a lab-scale cylindrical managing equipment at controlled temperature (27°C) for 20 min. The olive paste was also centrifuged at 4500 rpm (3600 xg) for 10 min to separate the oily fraction from vegetation water and solid particles through a NEYA 8 laboratory centrifuge (REMI Neya centrifuges, Modena, Italy) equipped with S 4-175 rotor (REMI Neya centrifuges, Modena, Italy). The oily fraction was recovered using a separatory glass funnel. Finally, a further centrifugation (HERMLE mod. Z 206-A, Benchmark Scientific, Sayreville, NJ, USA) at 6000 rpm for 10 min was applied to clarify the oil.

2.2 OLIVE OIL ANALYSIS

The obtained olive oil samples were analysed for quality parameters according to EU official methods [37], i.e., free fatty acids (% oleic acid), peroxide value ($meq_{O2}\ kg_{oil}^{-1}$) and UV spectroscopic indices, i.e., K_{232}, K_{270} and ΔK. Tocopherols were determined according to the ISO 9936:2016 method [38].

Phenolic compounds content were measured according to the International Olive Council (IOC) official method [39]. The extraction of the phenolic fraction through MeOh: H_2O 80:20 v/v solution was followed by the identification and quantification through an HP 1100 liquid chromatograph coupled with both the diode array detector (DAD) and mass detector (MSD). Phenol separation was carried out with the aid of a C18 SphereClone ODS column (5µm, 250 × 4.6 mm id; Phenomenex, Bologna, Italy), using acetonitrile, methanol and water (acidified to pH 2.0 with phosphoric acid) as elution solvents and following the elution gradient (1 mL min^{-1} flow rate) described by the IOC method. The chromatogram was recorded at 280 nm. Syringic acid was used as internal standard and concentrations were expressed as $mg_{tyrosol}\ kg_{oil}^{-1}$.

For the evaluation of the volatile organic compound (VOC) content, the solid-phase microextraction of the headspace (HS-SPME) coupled with gas chromatography and mass spectrometry (GC-MS) technique was used, following the multiple internal standard normalisation (MISN) method, as described by Fortini et al. [40]. To obtain the stock standard solution mix, 71 analytes were dissolved in refined oil. Then, the mix was diluted in the refined oil to obtain six levels of calibration scale. Compounds and their concentration ranges were chosen based on previous works on Italian virgin olive oils [41]. An internal standard (ISTD) mixture (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 to represent several molecular masses and several classes of VOCs, i.e., alcohols, aldehydes, ketones,

esters, carboxylic acids and hydrocarbons. ISTDs were either deuterium-labelled or found to be absent in virgin olive oils and with no interference with their volatile profile. Samples were prepared adding 0.1 g of ISTD MIX to 4.3 g of olive oil sample into a 20 ml vial fitted with open hole screw cap and PTFE/silicone septa. The same amount of ISTD MIX was added to calibration scales to normalise each compound concentrations of the calibration curve on those of the respective ISTD, assigned according to the method. The HS-SPME-GC-MS analysis was carried out using a 50/30 µm DVB/CAR/PDMS SPME fibre by Supelco for the extraction of VOCs and a Trace GC-MS Thermo Fisher Scientific, equipped with a Zebron ZB-FFAP capillary column (30 m × 0.25 mm ID, 0.25 µm DF) for the identification. Identification was achieved through a six-point linear least squares calibration of the compound peak area over the relative ISTD peak (area ratio) plotted versus the compound concentration ratio (amount ratio).

2.3 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experimental design was set up as a full factorial, with 3 replicates. Two independent variables were tested: the harvesting method (manual and facilitated) and the storage temperature (room and refrigerated). A two-way ANOVA model was applied to all the data collected from the olive oil analysis including the 2 experimental factors as fixed effect variables and considering the 2 main effects and their interaction. The harvesting day was considered as a blocking factor. Significance was set at $p < 0.05$.

3. RESULTS

The harvested olives appeared in good health conditions with no fly (*Bactrocera oleae*) infection. *DOI* values of 0.08±0.03, and 0.24±0.04 were determined in hand-picked and facilitated harvesting olives, respectively, and they can be related to bruising. Thus, the olives harvested using facilitating devices were visually more damaged than the hand-picked ones. *MI* and water content values were on average 1.6±0.2 and 52.1±0.9% w/w, respectively, without significant differences among the treatments.

All extracted olive oil samples were classified as extra virgin (Tab. II). No significant differences were found on free fatty acids (FFA), peroxide value (PV) and UV spectrophotometric indexes (K_{232}, K_{270} and ΔK) between samples obtained from different treatments. Tocopherol amounts were not significantly different between olive oil samples with an average value of 234 ± 31 mg/kg (Tab. II).

The harvesting method significantly affected the total phenolic compound content (TPC), and the phenolic profile of olive oil samples (Tab. III). Particularly, the TPC concentration resulted higher in MH oil samples (average value = 624±93 mg/kg) compared to FH oil samples (average value = 537±60 mg/kg). The MH oil samples had the significant highest amounts of phenolic acids and secoiridoids, with regards to oleuropein derivatives (Fig. 1). The MH method favoured

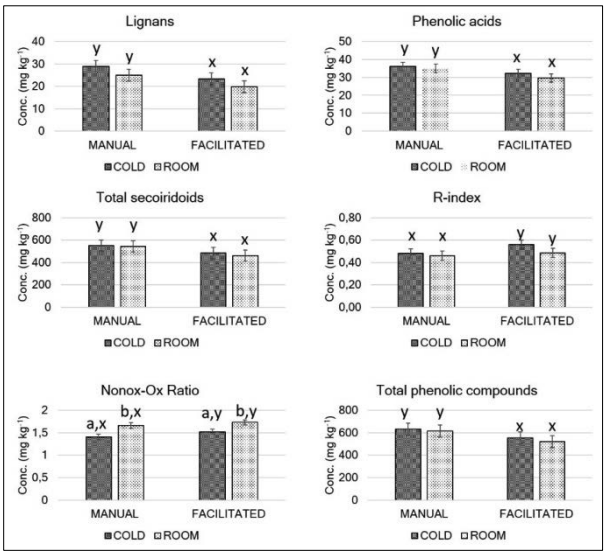

Figure 1 - Group of phenolic compounds and indexes of olive oil samples taken from manual and facilitated harvesting and stored at cold (6.5°C) and room (25°C) temperatures for 24 h. Error bars represent the Residual Standard Error of the model. Letters a,b indicate a significant difference ($p < 0.05$) for storage temperature, letters x,y indicate a significant difference ($p < 0.05$) for harvesting method.

Table II - Legal parameters and tocopherol content of olive oil samples taken from manual and facilitated harvesting and stored at cold (6.5°C) and room (25°C) temperatures for 24 h. Different letters indicate a significant difference for ($p < 0.05$) found at the ANOVA. RSE column reports the Residual Standard Error of the model. Significant codes: ns = not significant; * $p < 0.1$.

	Room temperature storage (25°C)		Cold storage (6.5°C)		RSE	p temp	p harv.	p int
	Manual harv.	Facilitated harv.	Manual harv.	Facilitated harv.				
FFA (% oleic acid)	0.24	0.26	0.26	0.26	0.02	ns	ns	ns
PV (meq O_2 kg^{-1})	3.63	3.70	3.03	3.83	0.40	ns	*	ns
K_{232}	2.05	2.04	2.07	2.04	0.05	ns	ns	ns
K_{270}	0.24	0.25	0.24	0.25	0.03	ns	ns	ns
ΔK	0.01	0.01	0.01	0.01	0.00	ns	ns	ns
Tocopherols (mg kg^{-1})	235.67	235.33	234.00	246.67	35.83	ns	ns	ns

the best preservation of 5 derivatives of secoiridoids, namely hydroxytyrosyl acetate, decarboxymethyl oleuropein aglycone oxidised dialdehyde form, oleuropein aglycone dialdehyde form, oxidised dialdehyde form of decarboxymethyl ligstroside aglycone and aldehyde and hydroxylic form of oleuropein aglycone. The MH oil samples also showed higher lignans and cinnamic acid contents than the FH oil samples. The following indexes was considered to evaluate the oxidative degradation and overall hydrolytic status of the phenolic compounds of the olive oil samples: the ratio between non oxidised and oxidised forms of secoiridoids (Nonox-Ox ratio) and the ratio between the sum of tyrosol and hydroxytyrosol and the total content of secoiridoids derivatives (R-Index), respectively. The Nonox-Ox ratio was similarly proposed by Armaforte et al. [42]; it gives an indication on the freshness or aging of the oil from an oxidative point of view. According to the literature data, the R-index [2] was useful to monitor the hydrolytic transformation of olive oil phenolic compounds during storage, due to the release of simple phenols from secoiridoids compounds [43]. Both the highest R-Index and the highest Nonox-Ox ratio values occurred in the FH oil samples (Fig. 1).

The olive fruit refrigeration improved the amounts of six phenolic compounds, namely vanillic and caffeic acids, decarboxymethyl oleuropein aglycone oxidised dialdehyde form, oleuropein aglycone dialdehyde form, lignans, cinnamic acid and oxidised aldehyde and hydroxylic form of ligstroside aglycone. Instead, the highest amounts of vanillin, *p*-coumaric acid and ferulic acid were measured in olive oil samples which were extracted from olive fruit stored at room temperature. The Nonox-Ox ratio values showed the greatest oxidation of secoiridoids in oil samples extracted from refrigerated olive fruit (Fig. 1). It is important to point out that 4 compounds, mainly secoiridoids, were significantly affected by both the harvesting method and the storage temperature at

Table III - Concentration of phenolic compounds in olive oil samples taken from olive fruits taken from manual and facilitated harvesting and stored at cold and room temperatures for 24 h. Only significant differences ($p < 0.05$) at the ANOVA are reported for individual compounds.

	Room temperature storage (25°C)		Cold storage (6.5°C)					
Phenolic compounds (mg kg^{-1})	**Manual harv.**	**Facilitated harv.**	**Manual harv.**	**Facilitated harv.**	***RSE***	***p T***	***p H***	***p INT***
Vanillic + caffeic acid	1.29 ^{a}	1.11 ^{a}	1.82 ^{b}	1.93 ^{b}	*0.25*	**	*ns*	*ns*
Vanillin	6.25 ^{b}	6.13 ^{b}	5.65 ^{a}	4.83 ^{a}	*0.64*	*	*ns*	*ns*
p-coumaric acid	1.11 ^{b}	0.96 ^{b}	0.82 ^{a}	0.77 ^{a}	*0.10*	**	*ns*	*ns*
Hydroxytyrosyl acetate	2.23 ^{y}	1.48 ^{x}	2.35 ^{y}	1.36 ^{x}	*0.54*	*ns*	*	*ns*
Ferulic acid	3.67 ^{b}	3.13 ^{b}	2.56 ^{a}	2.47 ^{a}	*0.44*	*	*ns*	*ns*
Decarboxymethyl oleuropein aglycone, oxidized dialdehyde form	77.00 $^{a, y}$	59.68 $^{a, x}$	92.34 $^{b, y}$	70.95 $^{b, x}$	*8.62*	*	**	*ns*
Oleuropein aglycone, dialdehyde form	51.92 $^{b, y}$	40.84 $^{b, x}$	62.03 $^{a, y}$	50.47 $^{a, x}$	*5.60*	*	*	*ns*
Decarboxymethyl ligstroside aglycone, oxidized dialdehyde form	76.81 ^{y}	67.83 ^{x}	85.81 ^{y}	74.89 ^{x}	*7.66*	°	*	*ns*
Pinoresinol, 1 acetoxy-pinoresinol	25.04 $^{a, y}$	19.81 $^{a, x}$	28.82 $^{b, y}$	23.24 $^{b, x}$	*2.67*	*	**	*ns*
Cinnamic acid	19.73 $^{b, y}$	16.12 $^{b, x}$	22.09 $^{a, y}$	19.91 $^{a, x}$	*2.15*	*	*	*ns*
Oleuropein aglycone, aldehyde and hydroxylic form	24.83 ^{y}	19.90 ^{x}	21.67 ^{y}	18.41 ^{x}	*2.23*	*ns*	*	*ns*
Ligstroside aglycone, oxidized aldehyde and hydroxylic form	13.40 ^{a}	12.03 ^{a}	15.84 ^{b}	15.41 ^{b}	*2.10*	*	*ns*	*ns*

Legend: Letters a,b indicate a significant difference for storage temperature, x,y indicate compound significantly different for harvesting method. RSE column reports the Residual Standard Error of the model. Significant codes: ns = not significant; ° $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

the same time (Tab. III). However, no significant interactions between the harvesting method and storage temperature were found for all measured phenolic compounds.

After the HS-SPME-GC-MS analysis, 40 VOCs were detected and 15 of them were identified as significantly different between treatments, showing effects due to storage temperature, harvesting method and their interaction (Tab. IV and Fig. 2). Methyl propionate was the only VOC significantly increased by the MH method. The following six compounds (C6 from LOX pathway) were found to be increased by the FH method: hexyl acetate, E-2-hexenyl acetate, Z-3-hexenyl acetate, 1-hexanol, E-3-hexen-1-ol, and Z-3-hexen-1-ol.

A significant increase at room temperature storage was found for methyl acetate, 1-penten-3-one, E-2-pentenal, Z-2-penten-1-ol, (E, E)-2,4-heptadienal and propanoic acid (Tab. IV). Conversely, the amounts of hexyl acetate, E-2-hexenyl acetate, 2-heptanol, Z-3-hexenyl acetate, 1-hexanol, E-3-hexen-1-ol, Z-3-hexen-1-ol and 1-octanol were the highest in oil samples after the cooling treatment of olive fruit (Tab. IV and Fig. 2).

Significant interactions between the harvesting method and storage temperature, occurred for several C6 VOCs derived from lipoxygenase (LOX) pathway, such as hexyl acetate, E-2-hexenyl acetate, Z-3-hexenyl acetate, 1-hexanol, E-3-hexen-1-ol, Z-3-hexen-1-ol (Fig. 2). When the FH method was used instead of the MH method, the above compounds showed a considerable quantitative increase in the olive oil samples, extracted from olive fruit stored at low temperature. When the FH method was used instead of the MH method, the C6 esters (i.e., hexyl acetate, E-2-hexenyl acetate, Z-3-hexenyl acetate) also increased in the olive oil samples, extracted from olive fruit stored at room temperature; instead, the C6 alcohols (i.e.,1-hexanol, E-3-hexen-1-ol, Z-3-hexen-1-ol) decreased (Fig. 2).

The VOCs were grouped in 4 classes according to the number of carbon atoms (i.e., C5, C6) and their microbial or oxidative origin; the microbial metabolite VOCs included all the C5 compounds and some C > 6 compounds, whereas the oxidation VOCs grouped exclusively compounds with more than 6 carbon atoms. A detailed list of compounds grouped by the aforementioned groups is reported in Guerrini et al. [44]. No interaction between storage temperature and harvesting method emerged from the statistical analysis of the above VOCs classes; 2 of them were significantly affected by the storage temperature and 1 of them by the harvesting method (Fig. 2). Particularly, the highest amount of the C6 LOX-derived VOCs occurred in the olive oil samples, extracted from olive fruit harvested through the FH method; whereas the highest amount of the microbial metabolite VOCs and C5 were measured in the olive oil samples, extracted from olive fruit stored at room temperature. No significant difference of VOCs linked to oxidation contents occurred.

The VOCs were also grouped in their chemical species (Fig. 2). A significant effect of the olive fruit cooling was observed on ketones, which increased in the olive oil samples, extracted from olive fruit stored at room temperature. Significant interaction between storage temperature and harvesting method was detected for ester and alcohol amounts, which had a similar trend to the C6 compounds, as previously reported.

Table IV - Concentration of volatile organic compounds in olive oil samples taken from manual and facilitated harvesting and stored at cold and room temperatures for 24 h. Only significant differences ($p < 0.05$) at the ANOVA are reported for individual compounds.

	Room temperature storage (25°C)		Cold storage (6.5°C)					
Volatile Organic Compounds (µg kg^{-1})	**Manual harv.**	**Facilitated harv.**	**Manual harv.**	**Facilitated harv.**	***RSE***	***p T***	***p H***	***p INT***
Methyl acetate	59.1 ^{b}	72.22 ^{b}	43.79 ^{a}	56.69 ^{a}	*11.73*	*	°	ns
Methyl propionate	0.38 ^{y}	0.00 ^{x}	0.58 ^{y}	0.00 ^{x}	*0.30*	*ns*	*	*ns*
1-penten-3-one	676.57 ^{b}	720.02 ^{b}	349.67 ^{a}	363.50 ^{a}	*77.48*	***	*ns*	*ns*
E-2-pentenal	40.24 ^{b}	51.99 ^{b}	10.13 ^{a}	15.85 ^{a}	*9.12*	***	*ns*	*ns*
Z-2-penten-1-ol	217.43 ^{b}	226.01 ^{b}	170.94 ^{a}	169.93 ^{a}	*11.05*	***	*ns*	*ns*
2-heptanol	8.76 ^{a}	8.49 ^{a}	10.51 ^{b}	12.02 ^{b}	*0.47*	***	°	*
(E,E)-2,4-heptadienal	78.61 ^{b}	88.23 ^{b}	66.40 ^{a}	70.14 ^{a}	*11.05*	*	*ns*	*ns*
Propanoic acid	29.9 ^{b}	27.36 ^{b}	24.34 ^{a}	22.63 ^{a}	*3.40*	*	*ns*	*ns*
1-octanol	43.2 ^{a}	44.39 ^{a}	49.03 ^{b}	51.64 ^{b}	*3.69*	*	*ns*	*ns*

Legend: Letters a,b indicate a significant difference for storage temperature, x,y indicate compound significantly different for harvesting method, h,i,j, indicate significant difference for temperature x harvesting method interaction. RSE column reports the Residual Standard Error of the model. Significant codes: ns = not significant; ° $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

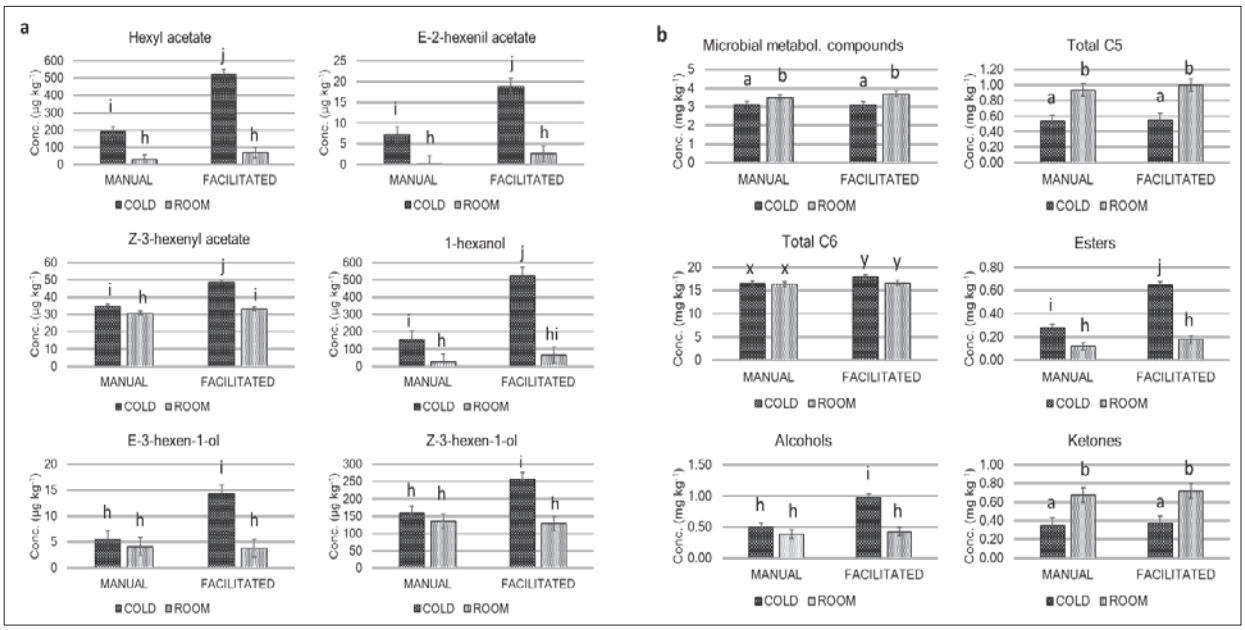

Figure 2 - Volatile organic compounds in olive oil samples obtained from manual and facilitated harvesting and stored at cold (6.5°C) and room (25°C) temperatures for 24 h. Results are reported as individual molecules for C6 VOCs (a) and as group of compounds (b). Error bars represent the Residual Standard Error of the model. Letters a,b indicate a significant difference ($p < 0.05$) for storage temperature, letters x,y indicate a significant difference ($p < 0.05$) for harvesting method, letters h,i,j indicate a significant difference ($p < 0.05$) for temperature x harvesting method interaction.

4. DISCUSSION

The cold storage of olive fruit, immediately after harvesting, was widely investigated, but it only recently starts to spread among EVOO companies. In the high quality EVOO context, the cold storage can preserve phenolic and volatile fraction and prevent degradation phenomena that are responsible for the formation of *fusty* defect in olive oil [45]. Microbial spoilage activities in the olive fruit and warming of the olive masses are the common causes of the degradation phenomena [5, 12, 20, 21, 44] and can be favoured by the fruit softening and leakage of cellular liquid [15, 19], which can be particularly accelerated in mechanically harvested fruit [13]. The mechanisation of the harvesting is spread, especially in the intensive or super-intensive olive orchard, in order to reduce the labour cost and increase the remuneration of companies [10]. It is known from the literature data that the mechanical energy transferred to olive fruit by the harvesting tools affects the physical and physiological stability of the fruit, causing some metabolic processes which are responsible for a rapid deterioration of the olive fruit components, including the oily fraction [12, 16, 17, 29]. According to Famiani et al. [31], the higher the mechanisation level of harvesting, the higher the percentage of fruit damage and, consequently, the decay of the olive oil quality.

Since in small and medium companies, where the use of trunk shaker or straddle harvester is not feasible due to the cultivation systems or unsuitable soils, the transition to facilitated harvesting with the aids of hand-held machinery is now well-established [8], the effects of harvesting methods on olive oil quality is useful to study in order to process high-quality EVOO. In this work, the initial characterisation of the olive fruit showed a significant increase in mechanical damage, due to the use of hand-held combs. The experimental data showed no significant differences for the legal parameters of the olive oil samples, extracted in the laboratory, and the values of FFA, PV, UV, K_{232}, K_{270} and ΔK were consistent with the "extra virgin" category. Although the literature data are contradictory [12, 17, 44-48], the above results may be explained by the short storage time between harvesting and milling, capable to well-preserve the olive oil commercial quality. Moreover, the study of Famiani et al. [31] did not detect changes in the legal quality parameters of olive oil, extracted from cv. *Frantoio* olive fruit harvested through hand-held machines and after 48 h of fruit storage. Consistently with Yousfi et al. [13], even the tocopherol contents of olive oil samples did not show significant differences; the applied experimental conditions (i.e., cultivar, ripening degree, content in other antioxidants, etc.) may explained the above results, according to the study of Morales-Sillero and García [18], in which the tocopherol contents decreased by more than 50% in *Manzanilla de Sivilla* oils after mechanical harvesting, whereas non-significant changes were found in *Manzanilla Cacereña* oils during the same trials.

Instead, the phenolic and volatile compound contents were deeply affected by both the harvesting method and the storage temperature. The main changes on olive oil quality due to the harvesting method was

detected on the phenolic fraction, revealing a better preservation of secoiridoids, phenolic acids, and lignans after the MH method, with a TPC about 80-90 mg kg^{-1} more than in the olive oil samples, extracted from olive fruit harvested through the FH method. This is consistent with all the previous studies indicating a low content of total phenols and secoiridoids as the mechanisation level and storage time increase [12, 17, 29, 44, 45]. In addition, the R-index showed the highest extent of hydrolytic degradation of the secoiridoids in the olive oil samples, extracted from olive fruit harvested through the FH method. The beating of olive fruit during the facilitated harvesting may cause the rupture of the cell wall and the release of esterase and β-glucosidase enzymes [51], leading to an increase in simple phenols and a decrease in total secoiridoids. In addition, the highest amounts of LOX-derived VOCs and total C6 compounds were found in secoiridoids in the olive oil samples, extracted from olive fruit harvested through the FH method. According to Morales-Sillero et al. [46], the damages, caused by mechanical harvesting, may involve a premature activation of the LOX pathway, which lead to the production of fruity notes. Famiani et al. [31] also pointed out an increase of volatile content directly proportional to olive damage, since the rupture of fruit tissues releases the LOX enzymes [52]. This was also described in Masella et al. [53], showing a significant reduction of several C5 and C6 compounds in oils from olive fruit frozen prior storage. In the latter, the ice crystallization causes the rupture of tissues and the contact between substrates and the LOX-enzymes, which may be inactivated before the milling operations with prolonged storage. In summary, the above events mean that the vibrational stress, given to the olive fruit harvested by the FH method, was capable to trigger immediately the enzymatic reactions of olive fruit. Consistently with previous work [24], the effect of cooling resulted in the preservation of the individual phenolic and aromatic compounds. The volatile profile did not reveal particular differences regarding VOCs that are related to olive oil defect, because no marker of *fusty*, *musty* or *wine-vinegary* defects expressed significant variation. The short storage time of olive fruit could favour the above phenomena, but the significant highest amounts of some microbial metabolites, which were measured in the olive oil samples extracted from olive fruit stored at room temperature, suggests that these phenomena were at an early stage. The cooling treatment caused some significant differences linked to oxidation, probably due to both the highest solubility of oxygen at low temperature and the enzymatic selection. On the one hand, the cold storage preserved the individual phenolic compound contents, but shifted the Nonox-Ox ratio of secoiridoids towards the oxidation; it also favoured the formation of C6 VOCs by the LOX pathway, as also confirmed by the previous study [24]. On the other hand, the effect of cooling decreased the formation of C5 VOCs, confirming that this branch of LOX pathway was not favoured by low temperatures, as also described by Luaces et al. [54] and Dourou et al. [25].

An interesting result for the olive oil aroma profile was observed with the combination of mechanical harvesting and refrigerated storage of olive fruit. For several C6-LOX related VOCs, an interaction between temperature and harvesting methods was observed. The mechanical stress induced by the FH method pushed the enzymatic activity of the fruit including the LOX activity; then, the following olive fruit cooling enhanced the LOX pathway, increasing the formation of the C6 VOCs responsible for the positive fruity attribute in the EVOO. The above phenomena may be explained by the different optimal temperatures of the key enzymes of LOX pathway. For instance, the hydroperoxide lyase enzyme (HPL) slows down above 15°C [55], whereas the LOX enzyme has its optimum at 30°C [56]. Therefore, room temperature storage may activate preferably the LOX-mediated homolytic cleavage of linolenic acids (LNA), that forms the C5 alcohol and 13-alcoxyl radical [57, 58]. The above hypothesis was related to the study of Morales-Sillero et al. [46] that conversely reported the highest amounts of C6 compounds after manual harvesting and a 40% reduction of C6 compounds in mechanical harvesting with olive straddle machines, explained as a premature activation of the LOX enzymes. However, the same Authors pointed out that the differences of the C6 compound contents was mitigated by cold storage with a flattening of the differences between the two harvesting treatments during storage. In summary, cooling storage and FH method appeared additional to achieve the best results in terms of olive oil aromatic profile. It should be highlighted that the above results were related to a short-term storage; therefore, the potential negative effects of the enzymatic pathways, occurring after FH method and during storage at room temperature, were not detectable and consequently did not lead to the formation of oil sensory defects.

The results obtained at laboratory scale, could be extended to a small industrial scale if the refrigerated cells are able to control olive temperature during the fruits storage prior to milling. Guerrini et al. [24] found that cells were able to control and decrease the temperature of fruits stored in 250 kg bins. However, for the same fruit mass, storing at room temperature may cause a rise of temperature in the core part. On the other hand, the use of a refrigerated cell is not very suitable for a large industrial scale, where the storage masses exceed 250 kg and may not reach the desired temperature in the inner part causing the worsening of the olive oil quality [6, 19].

Thus, in the high-quality EVOO processing, the most correct management strategies for temperature control should be applied as a function of the plants and equipment, as well as the climatic conditions.

5. CONCLUSIONS

This work studied the effects of the following pre-extraction factors on extra virgin olive oil quality: the facilitated harvesting and the olive fruit storage at low temperature. Application of vibrating hand-held electric combs for the facilitated harvesting of olive fruit represents a useful tool for improving the production efficiency of olive oil companies, reducing labour costs. However, it is a common thought, based also on scientific evidence, that the above technique has detrimental impact on the physical-chemical integrity of the olive fruit before milling, compared to the manual harvesting, and that it can be a potential factor for the bad quality of extracted olive oil. At present, the post-harvest cooling of the olive fruit is drawing interest among the techniques used to prevent deterioration and sensory defects of olive oil, even if it involves an additional cost, albeit relatively small.

Experimental data showed that the short-term storage of olive fruit is the best practice to prevent the downgrading of the olive oil "extra virgin" category, since no significant differences on legal quality parameters were detected regardless the harvesting method and storage temperature. The combined action of FH method and storage at low temperature during the storage of olive fruit, provided an opportunity for olive oil companies to obtain a modulating effect on the oil aromatic profile, favouring both the release of LOX enzymes and the solubility of oxygen. Indeed, the above factors may contribute to a "good" and controlled oxidation, leading to the production of positive molecules for the EVOO flavour.

To sum up, the facilitated harvesting using hand-held vibrating combs seems to have reached a sufficient technological evolution to minimise the side effect on olives and olive oil, especially if it is supported by storage of olive fruit at low temperature. Nevertheless, the facilitated harvesting must be included in an integrated post-harvest organisational approach that ensures the correct handling, transport and hygiene practices, combined with a short-term storage and small olive heaps during storage.

Authors' contributions

A. Parenti and L. Guerrini, provided the concept and the design of the experiment. L. Guerrini and F. Corti, carried out the experiment, the analysis and interpretation of the data. L. Guerrini and F. Corti, drafted the article with the important help offered by B. Zanoni. A. Parenti, P. Masella, L. Cecchi, N. Mulinacci, G. Angeloni, A. Spadi, L. Calamai and B. Zanoni gave their generous contribution to the trials, data acquisition, manuscript revision and final approval.

Acknowledgments

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

REFERENCES

[1] M. Servili, R. Selvaggini, S. Esposto, A. Taticchi, G.F. Montedoro, G. Morozzi, Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that affect their occurrence in the oil, J. Chromatogr. A. 1054 (2004) 113-127. https://doi.org/10.1016/j.chroma.2004.08.070.

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

[3] B. Zanoni, Which processing markers are recommended for measuring and monitoring the transformation pathways of main components of olive oil?, Ital. J. Food Sci. 26 (2014) 3-12.

[4] M.T. Morales, G. Luna, R. Aparicio, Comparative study of virgin olive oil sensory defects, Food Chem. 91 (2005) 293-301. https://doi.org/10.1016/j.foodchem.2004.06.011.

[5] S. Vichi, A. Romero, J. Gallardo-Chacón, J. Tous, E. López-Tamames, S. Buxaderas, 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 (2009) 1449-1455. https://doi.org/10.1021/jf803242z.

[6] J.M. García, K. Yousfi, The postharvest of mill olives, Grasas y Aceites. 57 (2006) 16-24. https://doi.org/10.3989/gya.2006.v57.i1.18.

[7] L. Ferguson, Trends in olive fruit handling previous to its industrial transformation, Grasas y Aceites. 57 (2006) 1-7. https://doi.org/10.3989/gya.2006.v57.i1.17.

[8] G. Cresti, R. Gucci, L. Omodei Zorini, R. Polidori, M. Vieri, Progetto MATEO - Modelli tecnici ed economici per la riduzione dei costi di produzione nelle realtà olivicole della toscana, (2009).

[9] G. Sperandio, M. Biocca, M. Fedrizzi, P. Toscano, Economic and Technical Features of Different Levels of Mechanization in Olive Harvesting, Chem. Eng. Trans. 58 (2017) 853-858. https://doi.org/10.3303/CET1758143.

[10] H.A. Mansour, T.A. Elmesiry, A.A. Abdelhady, Cost Analysis of Olive Harvesting By Hand-Held Machine, World Wide J. Multidiscip. Res. Dev. 4 (2018) 361-363.

[11] M. Vieri, Progressi della meccanizzazione - Evoluzione in atto per la olivicoltura, in: Atti Dei Georg. 2006, Ser. VIII, Florence, 2006.

[12] P.P. Miglietta, R. Micale, R. Sciortino, T. Caruso, A. Giallanza, G. La Scalia, The sustainability of

olive orchard planting management for different harvesting techniques: An integrated methodology, J. Clean. Prod. 238 (2019) 117989. https://doi.org/10.1016/j.jclepro.2019.117989.
[13] K. Yousfi, C.M. Weiland, J.M. García, 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 (2012) 4743-4750. https://doi.org/10.1021/jf300331q.
[14] F. Gambella, C. Dimauro, F. Paschino, Evaluation of Fruit Damage Caused by Mechanical Harvesting of Table Olives, Am. Soc. Agric. Biol. Eng. 56 (2013) 1267-1272.
[15] F. Jiménez-Jiménez, S. Castro-García, G.L. Blanco-Roldán, E.J. González-Sánchez, J.A. Gil-Ribes, Isolation of table olive damage causes and bruise time evolution during fruit detachment with trunk shaker, Spanish J. Agric. Res. 11 (2013) 65-71. https://doi.org/10.5424/sjar/2013111-3399.
[16] P. Proietti, Olive handling, storage and transportation, in: C. Peri (Ed.), Extra-Virgin Olive Oil Handb., First, John Wiley & Sons, Hoboken, NJ, 2014: pp. 107-112. https://doi.org/10.1002/9781118460412.ch9.
[17] K.A. Segovia-Bravo, P. García-García, L. Antonio, A. Garrido-Fernández, Effect of Bruising on Respiration , Superficial Color, and Phenolic Changes in Fresh Manzanilla Olives (Olea europaea pomiformis): Development of Treatments To Mitigate Browning, J. Agric. Food Chem. 59 (2011) 5456–5464. https://doi.org/10.1021/jf200219u.
[18] A. Morales-Sillero, J.M. García, Impact assessment of mechanical harvest on fruit physiology and consequences on oil physicochemical and sensory quality from “Manzanilla de Sevilla” and “Manzanilla Cacereña” super-high-density hedgerows. A preliminary study, J. Sci. Food Agric. 95 (2014) 2445–2453. https://doi.org/10.1002/jsfa.6971.
[19] J.M. García, F. Gutiérriez, J.M. Castellano, S. Perdiguero, A. Morilla, M.A. Albi, Storage of Olives Destined For oil Extraction, Acta Hortic. 368 (1994) 673-681. https://doi.org/10.17660/ActaHortic.1994.368.80.
[20] J.M. García, F. Gutiérrez, M.J. Barrera, M.A. Albi, Storage of mill olives on an industrial scale, J. Agric. Food Chem. 44 (1996) 590-593. https://doi.org/10.1021/jf950479s.
[21] J.M. García, F. Gutiérrez, J.M. Castellano, S. Perdiguero, A. Morilla, M.A. Albi, Influence of Storage Temperature on Fruit Ripening and Olive Oil Quality, J. Agric. Food Chem. 44 (1996) 264-267. https://doi.org/10.1021/jf950399o.
[22] A. Dag, S. Boim, Y. Sobotin, I. Zipori, Effect of Mechanically Harvested Olive Storage Temperature and Duration on Oil Quality, Horttechnology. 22 (2012) 528-533. https://doi.org/10.21273/HORTTECH.22.4.528.
[23] K. Brkić Bubola, M. Lukić, A. Novoselić, M. Krapac, I. Lukić, Olive Fruit Refrigeration during Prolonged Storage Preserves the Quality of Virgin Olive Oil Extracted Therefrom, Foods. 9 (2020).
[24] L. Guerrini, F. Corti, L. Cecchi, N. Mulinacci, L. Calamai, P. Masella, G. Angeloni, A. Spadi, A. Parenti, Use of refrigerated cells for olive cooling and short-term storage: Qualitative effects on extra virgin olive oil, Int. J. Refrig. 127 (2021) 59-68. https://doi.org/10.1016/j.ijrefrig.2021.03.002.
[25] A.M. Dourou, S. Brizzolara, G. Meoni, L. Tenori, F. Famiani, C. Luchinat, P. Tonutti, The inner temperature of the olives (cv. Leccino) before processing affects the volatile profile and the composition of the oil, Food Res. Int. 129 (2020). https://doi.org/10.1016/j.foodres.2019.108861.
[26] A. Piscopo, A. De Bruno, A. Zappia, G. Gioffrè, N. Grillone, R. Mafrica, M. Poiana, Effect of olive storage temperature on the quality of Carolea and Ottobratica oils, Emirates J. Food Agric. 30 (2018) 563-572. https://doi.org/10.9755/ejfa.2018.v30.i7.1739.
[27] E. Plasquy, M.C. Florido, R.R. Sola-Guirado, J.M. García, Effects of a Harvesting and Conservation Method for Small Producers on the Quality of the Produced Olive Oil, Agriculture. 11 (2021) 1-22. https://doi.org/10.3390/ agriculture11050417.
[28] R. Hachicha Hbaieb, F. Kotti, M. Gargouri, M. Msallem, S. Vichi, Ripening and storage conditions of Chétoui and Arbequina olives: Part I. Effect on olive oils volatiles profile, Food Chem. 203 (2016) 548–558. https://doi.org/10.1016/j.foodchem.2016.01.089.
[29] ISMEA, Piano di settore Olivicolo-oleario: Indagine sui costi di produzione delle olive da olio, (2012) 1-53.
[30] L. Nasini, P. Proietti, Olive harvesting, in: C. Peri (Ed.), Extra-Virgin Olive Oil Handb., First Edit, John Wiley & Sons, Hoboken, NJ, 2014: pp. 87-105. https://doi.org/10.1002/9781118460412.ch8.
[31] F. Famiani, D. Farinelli, S. Urbani, R. Al Hariri, A. Paoletti, A. Rosati, S. Esposto, R. Selvaggini, A. Taticchi, M. Servili, Harvesting system and fruit storage affect basic quality parameters and phenolic and volatile compounds of oils from intensive and super-intensive olive orchards, Sci. Hortic. (Amsterdam). 263 (2020). https://doi.org/10.1016/j.scienta.2019.109045.
[32] M. Uceda, L. Frias, Harvest Dates. Evolution of the Fruit Oil Content, Oil Composition and Oil Quality, in: Proc. II Semin. Oleícola Int. Int. Olive Oil Counc., Cordoba, Spain, 1975: pp. 125-130.

[33] M. Benlloch-González, R. Sánchez-Lucas, M.A. Bejaoui, M. Benlloch, R. Fernández-Escobar, Global warming effects on yield and fruit maturation of olive trees growing under field conditions, Sci. Hortic. (Amsterdam). 249 (2019) 162-167. https://doi.org/10.1016/j.scienta.2019.01.046.
[34] C. Gabaldón-Leal, M. Ruiz-Ramos, R. de la Rosa, L. León, A. Belaj, A. Rodríguez, C. Santos, I.J. Lorite, Impact of changes in mean and extreme temperatures caused by climate change on olive flowering in southern Spain, Int. J. Climatol. 37 (2017) 940-957. https://doi.org/10.1002/joc.5048.
[35] A. Dag, G. Harlev, S. Lavee, I. Zipori, Z. Kerem, Optimizing olive harvest time under hot climatic conditions of Jordan Valley, Israel, Eur. J. Lipid Sci. Technol. 116 (2014) 169-176. https://doi.org/10.1002/ejlt.201300211.
[36] P. Masella, L. Guerrini, G. Angeloni, B. Zanoni, A. Parenti, Ethanol From Olive Paste During Malaxation, Exploratory Experiments, Eur. J. Lipid Sci. Technol. 121 (2019) 1-7. https://doi.org/10.1002/ejlt.201800238.
[37] EC, 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 (2008) 11-16.
[38] International Organization for Standardization, ISO 9936:2016, Animal and vegetable fats and oils. Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography, Geneva, Switzerland, 2016.
[39] International Olive Council, Determination of Biophenols in Olive Oils By Hplc, COI/T.20/Doc No 29/Rev.1. (2017) 1-8. http://www.internationaloliveoil.org/.
[40] M. Fortini, M. Migliorini, C. Cherubini, L. Cecchi, L. Calamai, Multiple internal standard normalization for improving HS-SPME-GC-MS quantitation in virgin olive oil volatile organic compounds (VOO-VOCs) profile, Talanta. 165 (2017) 641-652. https://doi.org/10.1016/j.talanta.2016.12.082.
[41] L. Di Giacinto, G. Di Loreto, C. Di Natale, G. Gianni, S. Guasti, M. Migliorini, M. Pellegrino, E. Perri, M. Santonico, Caratterizzazione analitica degli attributi sensoriali degli oli di oliva vergini (AROMOLIO) - Risultati dell'attività sperimentale 2006-2010, (2011).
[42] E. Armaforte, V. Mancebo-Campos, A. Bendini, M. Desamparados Salvador, G. Fregapane, L. Cerretani, Retention effects of oxidized polyphenols during analytical extraction of phenolic compounds of virgin olive oil, J. Sep. Sci. 30 (2007) 2401-2406. https://doi.org/10.1002/jssc.200700130.
[43] M. Migliorini, C. Cherubini, L. Cecchi, B. Zanoni, Degradation of phenolic compounds during extra virgin olive oil shelf-life, Riv. Ital. Sostanze Grasse 90, 71-80, (2013).
[44] L. Guerrini, C. Breschi, B. Zanoni, L. Calamai, G. Angeloni, P. Masella, A. Parenti, Filtration Scheduling : Quality Changes in Freshly Produced Virgin Olive Oil, Foods. 9 (2020) 1-14. https://doi.org/doi:10.3390/foods9081067.
[45] F. Angerosa, B. Lanza, V. Marsilio, Biogenesis of «fusty» defect in virgin olive oils, Grasas y Aceites. 47 (1996) 142-150. https://doi.org/10.3989/gya.1996.v47.i3.854.
[46] A. Morales-Sillero, A.G. Pérez, L. Casanova, J.M. García, Cold storage of 'Manzanilla de Sevilla' and 'Manzanilla Cacereña' mill olives from super-high density orchards, Food Chem. 237 (2017) 1216-1225. https://doi.org/10.1016/j.foodchem.2017.06.073.
[47] A. Dag, A. Ben-gal, U. Yermiyahu, L. Basheer, Y. Nir, Z. Kerem, The effect of irrigation level and harvest mechanization on virgin olive oil quality in a traditional rain-fed 'Souri' olive orchard converted to irrigation, J. Sci. Food Agric. 88 (2008) 1524-1528. https://doi.org/10.1002/jsfa.3243.
[48] M. D'Imperio, M. Gobbino, A. Picanza, S. Costanzo, A. Della Corte, L. Mannina, Influence of harvest method and period on olive oil composition: An NMR and statistical study, J. Agric. Food Chem. 58 (2010) 11043-11051. https://doi.org/10.1021/jf1026982.
[49] C. Saglam, Y.T. Tuna, U. Gecgel, E.S. Atar, Effects of Olive Harvesting Methods on Oil Quality, APCBEE Procedia. 8 (2014) 334-342. https://doi.org/10.1016/j.apcbee.2014.03.050.
[50] L.M. Abenavoli, A.R. Proto, Effects of the divers olive harvesting systems on oil quality, Agron. Res. 13 (2015) 7-16.
[51] J. Fernández-Bolaños, R. Rodríguez, R. Guillén, A. Jiménez, A. Heredia, Activity of cell wall-associated enzymes in ripening olive fruit, Physiol. Plant. 93 (1995) 651-658. https://doi.org/10.1111/j.1399-3054.1995.tb05113.x.
[52] F. Angerosa, M. Servili, R. Selvaggini, A. Taticchi, S. Esposto, G. Montedoro, Volatile compounds in virgin olive oil: Occurrence and their relationship with the quality, J. Chromatogr. A. 1054 (2004) 17-31. https://doi.org/10.1016/j.chroma.2004.07.093.
[53] P. Masella, L. Guerrini, G. Angeloni, A. Spadi, F. Baldi, A. Parenti, Freezing/storing olives, consequences for extra virgin olive oil quality, Int. J. Refrig. 106 (2019) 24-32. https://doi.org/10.1016/j.ijrefrig.2019.06.035.
[54] P. Luaces, A.G. Pérez, C. Sanz, Effect of cold storage of olive fruits on the lipoxygenase pathway and volatile composition of virgin olive oil, Acta Hortic. 682 (2005) 993-998. https://doi.org/10.17660/ActaHortic.2005.682.129.
[55] J.J. Salas, J. Sánchez, Hydroperoxide lyase

from olive (Olea europaea) fruits, Plant Sci. 143 (1999) 19–26. https://doi.org/10.1016/S0168-9452(99)00027-8.
[56] M. Ridolfi, S. Terenziani, M. Patumi, G. Fontanazza, Characterization of the lipoxygenases in some olive cultivars and determination of their role in volatile compounds formation, J. Agric. Food Chem. 50 (2002) 835-839. https://doi.org/10.1021/jf0109118.
[57] Y.P. Salch, M.J. Grove, H. Takamura, H.W. Gardner, Characterization of a C-5 , 13-Cleaving Enzyme of 13(S)-Hydroperoxide of Linolenic Acid by Soybean Seed, Plant Physiol. 108 (1995) 1211-1218. https://doi.org/10.1104/pp.108.3.1211.
[58] H.W. Gardner, M.J. Grove, Y.P. Salch, Enzymic Pathway to Ethyl Vinyl Ketone and 2-Pentenal in Soybean Preparations, J. Agric. Food Chem. 44 (1996) 882-886. https://doi.org/10.1021/jf950509r.