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List of publications

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Corti, F., Parenti, A., Cecchi, L., Mulinacci, N., Masella, P., Angeloni, G., Spadi, A., Zanoni, B., Calamai, L., Guerrini, L. (2022). Effect of facilitated harvesting and fruit cooling on extra virgin olive oil quality. *La Rivista Italiana delle Sostanze Grass*e, *99*, 211-224.

Corti, F., Zanoni, B., Parenti, A., Masella, P., Breschi, C., Angeloni, G., Spadi, A., Guerrini, L. (2023). A methodological approach to estimate the overall heat transfer coefficient in olive paste malaxers. *Journal of Food Engineering*, *343*, 111377.

Guerrini, L., Corti, F., Masella, P., Calamai, L., Angeloni, A., Spadi, A., Zanoni, B., Parenti, A. (2022). Cross-batch contamination in a continuous horizontal decanter centrifuge during virgin olive oil production. *European Journal of Lipid Science and Technology*, *2200174*, 1-7.

Corti, F., Guerrini, L., Zanoni, B. (2022). Critical processing operations for extra virgin olive oil quality. Manuscript.

Participation at conferences

Evaluation of The Overall Heat Transfer Coefficient on Industrial Malaxers Using a Simple Approach (2022). *12th International AIIA Conference: "Biosystems Engineering towards the Green Deal - Improving the resilience of agriculture, forestry and food systems in the post-Covid era"*. 19-22 September 2022, Palermo, Italy.

Summary

Extra virgin olive oil (EVOO) is a product of great interest for its health, nutritional and organoleptic properties (Monteleone and Langstaff, 2014; Stark and Madar, 2013). The quality characteristics of EVOO can be affected by many factors at different stage: field, processing and post-processing (Mele et al., 2018; Peri, 2014a). However, in the olive oil chain, the processing factors are more easily controlled compared to the field and post-processing variables (Kalogianni et al., 2019).

Since the post-war period, the EVOO production underwent a deep evolution which led to increase the productivity and reduce the labor cost, both in the field and in the oil mill (Amirante et al., 2009; Vieri, 2006). The introduction of centrifugal extraction systems led to the replacement of the traditional batch processing, based on pressure and percolation extraction methods (Di Giovacchino, 2010). Significant innovations in plants and machines contributed to improve the yield and the quality of EVOO, such as the introduction of mechanical crushers and malaxers and the use of continuous centrifugal extraction systems for the oil separation and clarification (Amirante et al., 2010; Tamborrino et al., 2010). Moreover, a further step in the evolution of olive oil plant and machine aimed to improve the control of the key operative parameters, such as processing temperature, oxygen exposure, rotational speed of the devices, in order to modulate the compositional and organoleptic profile (Angeloni et al., 2022b; Catania et al., 2013; Clodoveo et al., 2014; Guerrini et al., 2017c; Leone et al., 2014a; Nucciarelli et al., 2022).

Despite in the last twenty years considerable knowledge has been achieved on the relationships between the processing variables and the oil quality, there are still critical points for the quality, which need a better control. This may arise from different reasons: combination and interaction between more variables, also related to different fundamental operations, poor effectiveness of the control systems of operative parameters and unsuitable working practices.

The first step of the present Doctoral thesis was a phase-by-phase review of the olive oil processing, in order to identify the hazards and the more critical control points for the quality of EVOO. In particular, an overview of the currently used machines and key operative

parameters involved at each fundamental operation was given, and a simplified risk analysis was carried out following a HACCP approach.

The experimental part aimed to investigated more deeply some critical issues in the EVOO production process. The experimental studies, reported in the research articles, focused on three main fundamental operations considered critical for the EVOO quality at different levels of the process: the management of the storage phase of the olives (Article #1 and Article #2), the temperature control during malaxation (Article #3) and the cross-batch contamination in the decanter centrifuge during the batch processing (Article #4).

The results of the experimental trials were discussed in the research articles.

Article #1 and Article #2 pointed out a positive effect of the cold storage of fruit in preventing the fruit heaps fermentation and warming. This led to the consequent reduction of the concentration of volatile molecules related to olive oil off-flavor in the obtained oils. Moreover, a modulating effect of the low temperature storage on phenolic and volatile fractions was observed. In particular, higher concentration of oxidized forms of secoiridoids was detected in oils obtained from cold storage of the olives. On the other hand, the cold storage seemed to favor the activation of lipoxygenase pathway, increasing in the oils the concentration of volatile compounds related to positive attributes. The latter may be promoted when facilitated harvesting methods are used due to the higher mechanical stress applied on the fruit.

Article #3 proposed a simple methodological approach for the estimation of the overall heat transfer coefficient (U) in olive paste malaxer in order to give the producers and plant manufacturers a useful tool, suitable in the plant sizing and in the choice of the operative parameters.

Preliminary malaxing trials were performed in the laboratory in order to determine the coefficient U using two different theoretical models for heat exchangers, i.e., under transient conditions and under steady state conditions. For the latter it was easier to obtain the required measurements to be applied in the model. Since no significant difference were found between the two models on lab-scale, only the model of heat exchanger under steady state conditions was applied on industrial scale for the estimation of coefficient U in

conventional and innovative malaxers. Results revealed a significant influence of the design of plants and equipment in improving the heat transfer efficiency in the oil mill.

Finally, Article #4 investigated a critical issue linked to the batch processing system in the oil mill, i.e., the cross-batch contamination. To date, no current technology in the oil mill has been implemented to avoid this drawback, which can dramatically compromise the quality and authenticity of the produced EVOO. A colorimetric and chemical characterization of oils obtained from consecutive olive batches were carried out, in order to estimate the more likely point of change between different processed batches. The final aim was to propose some practical applications to avoid the cross-batch contamination at the decanter centrifuge.

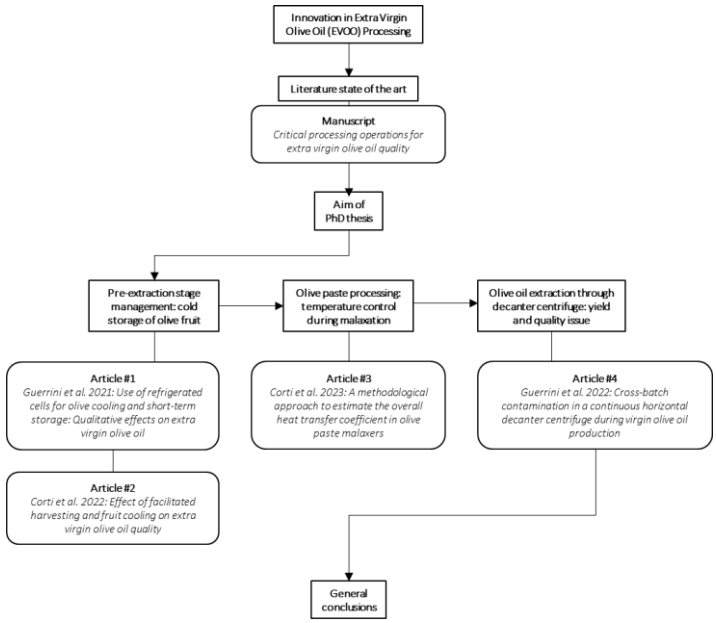

Figure I. Schematic chart of the structure of the thesis.

1. Introduction

1.1. Manuscript - Critical processing operations for extra virgin olive oil quality

Critical processing operations for extra virgin olive oil quality

Ferdinando Corti, Lorenzo Guerrini, Bruno Zanoni

Manuscript, 2022.

1. Introduction

The consumer interest for the extra virgin olive oil (EVOO) is growing since it is considered one of main ingredients of the Mediterranean diet and a functional food for its health and nutritional properties, due to polyunsaturated fatty acids and phenolic compounds (Cicerale et al., 2012; Foscolou et al., 2018; Stark and Madar, 2013). Moreover, the trend of the consumer preference is notably attributable to the organoleptic characteristics of olive oil, given by several attributes related to different sensory perceptions, i.e., visual, nasal, taste, chemesthesis and somesthesis (Tuorila and Recchia, 2014).

The EVOO production is characterized by great variability in terms of chemical and sensory composition which are related to the overall quality, resulting in a wide range of price (Fiorini et al., 2018).

The factors which affect the EVOO authenticity, yield and quality are attributable to different stages, which can be assimilated to the field, to the processing and post-processing, i.e., the olive storage, distribution and selling (Peri, 2014a). On field, the main contribution derives from the cultivar, the environmental conditions and the harvesting stage, that determine the quality of the raw materials, i.e., the olive fruit (Mele et al., 2018). The other great contributor is the olive fruit processing which involves several operations and variables. Between these two phases, there is a further important factor for the quality of the olive oil, including the harvesting of the olives and their handling before milling since it can altered the initial olive fruit conditions (Nasini and Proietti, 2014; Proietti, 2014). A third independent phase which can affect the quality is the olive oil storage and distribution, which also includes some other critical issues related the to the transport operation (Breschi et al., 2022; Garcia-Oliveira et al., 2021).

Since the post-war period, the EVOO production underwent a deep evolution which led to increase the productivity and reduce the labor cost, both in the field and in the mill (Amirante

et al., 2009; Vieri, 2006). However, in the olive oil supply chain, the variables linked to the processing phase, albeit numerous, can be more easily controlled by the producer than the variable of agronomic production and those affecting the EVOO shelf life, such as storage and distribution conditions (Breschi et al., 2022; Gargouri et al., 2015; Kalogianni et al., 2019; Sanmartin et al., 2018).

In the last 20 years, the virgin olive oil production has deeply changed, shifting from the traditional discontinuous processing by pressure extraction system to the modern processing consisting of centrifugal extraction system. The latter is currently composed by the following stages: leaf removal and olive cleaning and washing, olive crushing, malaxation, centrifugal separation and clarification, and filtration (Di Giovacchino, 2010). If the deepest change of extraction system seems to be completely absorbed by the oil mill, the innovation on the single machine is constantly evolving. The latest innovations and studies on oil extraction technology and engineering mainly involve that operation and machines considered relevant for the EVOO quality and yield. Nowadays, different types of crusher, i.e., knife, hammer or disc crushers, are commonly used in the process, and different types of malaxer machine as well, for instance, vertical axis or horizontal axis, closed or sealed tank (Di Giovacchino, 2010). The introduction of the decanter centrifuge in modern plants changed the virgin olive oil processing, increasing the working capacity of the plant and paying greater attention to the quality of the olive oil (Amirante et al., 2009). Thus, the traditional oil extraction system by pressure, which operated in batch and without any type of oxidative control were replaced, with significant benefits in terms of labor costs, productivity and quality (Amirante et al., 2009). Further development led to divide decanter machine into two-phase (oil is separated from a water/ pomace mixture) and three-phase (oil, water and pomace are all separated) systems, which separate the olive paste into three fractions (oil, water and pomace) or two (oil and high-moisture pomace), respectively (Baccioni and Peri, 2014). Another level of modifications also concerned the machine equipment, for instance, through the modification on the rotating device (crusher device, mixing coil) (Bianchi et al., 2020; Difonzo et al., 2021; Nucciarelli et al., 2022) or through the implementation of gas dosing systems and sensors for an advanced control of the processing parameters (Angeloni et al., 2022a, 2022b; Catania et al., 2013; Vallone et al., 2022). Finally, the application of emerging technologies such as ultrasound (US), microwave (MW) and pulsed electric field (PEF) is the latest innovation trend in oil processing, with the aim of favoring the release of oil and minor compounds from cell

tissues, promoting the separation of the oil fraction from the other phases and allowing to reduce the malaxation temperature (Clodoveo, 2013; Veneziani et al., 2019).

All these development in plant and machines led to a strong growth of the oil productivity and to the quality improvement and differentiation, even though, there are still some critical points into the processing that need to be better understood and controlled.

The present work provides a broad analysis of the EVOO extraction process, pointing out the critical operations for the quality of the product at the processing and the pre-processing stages. In particular, for each fundamental operation the relative processing parameters and the effects on the quality of the product given by their modulation were considered, also with a concise overview of the currently plant and machines and the latest innovation trends. The critical control points for the quality were identified in the EVOO processing through a risk analysis following a HACCP inspired approach according to previous works (Christaki and Tzia, 2002; El-Sayed et al., 2015). A comprehensive review of the literature was carried out in order to identify into the processing stages the hazards for the EVOO quality and to sum up the key operative parameters for preventive measures and processing control. For each fundamental operation, the relative key parameters were discussed individually or in relation to other parameters. Moreover, the contributing factors deriving from the machine setting and equipment or to the common mill practices were also considered.

2. Identification of possible hazards for extra virgin olive oil quality

The hazards for EVOO quality can be identified according to different quality characteristics on the olive oil, which were grouped and called as follows (Table 1): quality requirements - QR, commercial value – CV and organoleptic value - OV. Quality requirements (QR) grouped all the oil characteristics required for the *extra virgin* category according to EU Regulation No 2568/1991 and later modifications (European Commission, 2013), i.e., chemical and organoleptic parameters. These must to be ensured at the end of the processing chain (QRpr). Moreover, QR may also include the preservation of the same quality characteristics over the storage time, defining the shelf life of olive oil (QRsl). Thus, QRpr and QRsl can be considered the main quality requirements for EVOO quality. On the other hand, voluntary quality characteristics could be used for improve the commercial value (CV) of the product, such as health claims, certifications. These can be established by specific regulation or by product specification, according to the use of health claims and quality schemes (EFSA, 2011a; European Commission, 2012a, 2012b). Oher quality characteristics can be attributed to the improvement of organoleptic value (OV) through the modulation of olive oil sensory profile. According to Fiorini et al. (2018), a wide difference of EVOO price corresponds to a significant difference in EVOO chemical and sensory profile.

An indirect quality aspect is defined by the oil yield. This represents a basic condition to be ensured, since the achievement of the product quality standards have to be complemented by an adequate extraction yield aiming to the remuneration of the production costs. One of the main goal of the producers is to improve the oil yield without compromise the quality, trying to set the operating parameters in order to find a compromise between them (Guerrini et al., 2019).

The hazards for EVOO quality can be defined as particular events, processes or conditions which may produce a detrimental effect on some of the aforementioned quality characteristics. The hazard for EVOO quality can be associated to each quality characteristic according to the literature and to the community regulations. For instance, the whole events in the processing which may lead to the off-flavor development and the alteration of quality chemical parameters (free acidity, peroxide value and UV spectroscopic indexes) during the processing can be considered hazards for QR (QRpr), according the EU Regulation No 2568/1991 and later modifications (European Commission, 2013). Moreover, some

conditions deriving from unsuitable processing can become hazards for the EVOO quality in relation to the storage phase (QRsl), favoring the spoilage of chemical and sensory characteristics and lowering the shelf life of the oil, such as high water activity and dissolved oxygen concentration (Guerrini et al., 2017a; Labuza et al., 1972; Masella et al., 2009).

Hazards could also be referred to multiple quality characteristics. For instance, the loss of phenolic and volatile compounds during the processing operations may assume different quality relevance. For instance, the loss of phenolic compounds could both result in a quality depletion in terms of organoleptic properties (OV quality characteristics), due to the loss of bitterness or spicy attributes, or in terms of commercial value, due to the failure to reach the minimum concentration for labeling health claims (CV quality characteristics). Likewise, the alteration of quality chemical parameters may represent a quality hazard linked to QR for the classification as *extra virgin* category, if their value is very close to the EU Regulation's limit. On the other hand, a less serious issue involves EVOO with chemical parameters very far from the commercial limit but that have to face with only the specifications for the quality schemes, which may establish more restricted limit values. Both the latter situations may also occur for volatile aromatic compounds.

Table 1. Quality characteristics of extra virgin olive oil.

Quality characteristics	Goal	References
QRpr Quality requirements	Keep the *extra virgin* category at the production stage (end of processing)	European Commission (2013)
QRsl Quality requirements	Keep the *extra virgin* category over time (shelf life)	European Commission (2013)
CV Commercial value	Increase the commercial value by health claims and quality schemes	EFSA (2011a); European Commission (2012a, 2012b)
OV Organoleptic value	Increase the organoleptic value by the modulation of chemical and sensory profile	/

3. Risk analysis in EVOO process

The identification of the hazards for EVOO quality was a preliminary step to investigate the critical control point in the processing through a risk assessment approach. Each fundamental operation was examined in relation to the possible hazards and the processing plants and parameters involved at that stage. The risk analysis was carried out grouping the fundamental operations in two groups, as follows:

i) pre-processing: it includes the olive fruit harvesting and the other operations of olive handling before the crushing, such as transport to the mill and storage;

ii) processing: here, only the operations which are carried out in the oil mill for the extraction of the olive oil are considered. Thus, this level includes olive fruit washing and crushing, olive paste processing, centrifugal separation and clarification and filtration. Moreover, other operations and practices which can be related to all the processing phases are discussed.

3.1. Pre-processing

3.1.1. Olive fruit harvesting

Olive fruit condition at the harvesting time is one of the main variable that affect the quality of olive oil. Olive cultivar, growing area, pedoclimatic condition, irrigation, treatment and fruit ripeness at the harvest are the most important factors for the olive fruit quality at the field stage (Mele et al., 2018). The olive fruit harvesting can be considered among the field operation but it represents also the first stage for the olive oil extraction. The olive harvest can be carried out in different ways according to the geographical area, three shape and canopy features, orchard density and the company size (Ferguson, 2006; Nasini and Proietti, 2014).

Manual harvesting evolved in the history from the mere hand-picking and olive collecting in baskets to the aid of hand-held plastic rakes for fruit detachment and the use of nets for collecting them on the ground. Despite this technique is still widespread in Italian farm and Mediterranean area, due to the predominance of small and medium companies, it implies high labor costs (Bernardi et al., 2021; ISMEA, 2012).

The early research on mechanization in olive growing dates back to the post-war period leading to the implementation and development of modern harvesting machines and tools which, after their evolution and optimization led to the devices currently spread (Ferguson, 2006; Vieri, 2006).

The mechanization in olive harvest can be divided in two main categories, grouped by mechanization level: mechanical-aid harvesting and fully mechanical harvesting (Vieri et al., 2001).

The mechanical-aid harvesting, also called facilitated harvesting, is the most spread technique adopted in Italian oil production (59% of the farms) (ISMEA, 2012), since small-medium production scale and sloping soil issues represent significant hurdles for high mechanization (Vieri and Sarri, 2010). This method consists in the use of hand-held tools equipped by a telescopic rod, a motor and an operating device which can be oscillating, vibrating or turning (Nasini and Proietti, 2014). Among them, hand-held electric or pneumatic combs and vibrating rods are the most common in the olive harvest.

The fully mechanical harvesting involve the use of driving and operating machines i.e., trunk shakers, picking heads or arm combs, which can be self-propelled or being coupled with the tractor (Vieri et al., 2001). The operator has to ensure the operating and control actions of the machine. Thus, these systems are very suitable for semi-intensive and intensive olive orchards (Nasini and Proietti, 2014). The growth of super-intensive grove has also favored the development of mechanical harvesting with straddle harvester also adapted from vine harvesters (Bernardi et al., 2021; Ferguson, 2006).

In general, the harvesting costs decrease as the level of mechanization increases (Sperandio et al., 2017). However, the mechanical stress caused by the harvesting machines and devices may have significant impact on the olive fruit, especially in the form of skin scratch and laceration, bruising and pulp softening, that may cause the loss of fluid from cell tissues and the increase in fruit respiration (Gambella et al., 2013; Jiménez-Jiménez et al., 2013; Segovia-Bravo et al., 2011). The higher the mechanization level, the more severe the damage to the olive fruit, with trunk shaker and straddle machine causing greater damage compared to facilitated and manual harvesting (Famiani et al., 2020). An increased oil extraction yield may be related to the use of mechanical harvesting, due to the internal breakage of fruit tissue, regardless the storage conditions (Yousfi et al., 2012). On the other hand, the olive damage is

linked to several critical issues for EVOO quality. The breakage of olive tissues allows the oil to come into contact with oxygen and other substrates, such as fruit enzymes and natural microflora, activating chemical, biochemical and microbiological reactions which are potentially dangerous for the quality of EVOO. Despite this, the reaction rates for the off-flavor development, the lipid oxidation and the depletion of qualitative parameters depend on the time-temperature relationship, and the spoilage can be limited if the olives are crushed in a few hours (Peri, 2014b). However, even when the olive oil is extracted immediately after the harvesting or even in a few hours, significant differences can be found for some qualitative parameters such as, free acidity, peroxide value, UV spectroscopic indexes, total phenolic content and organoleptic evaluation, which can be negatively affected as a function of harvesting method and regardless the storage temperature (Corti et al., 2022; Dag et al., 2008; Morales-Sillero et al., 2017; Morales-Sillero and García, 2014; Yousfi et al., 2012). The triggering of oxidative enzymatic pathways caused by the mechanical damage on the fruit may be the main responsible for the drop of total phenolic content and oxidative stability, observed in oils from mechanically harvested olives (Corti et al., 2022; Yousfi et al., 2012). On the other hand, this process acts in an opposite way on volatile profile, increasing the concentration of volatile organic compounds derived from the lipoxygenase (LOX) pathway (Corti et al., 2022; D'Imperio et al., 2010; Morales-Sillero et al., 2017).

The detrimental effect of the harvesting method becomes even more significant over the storage of fruit, especially without any temperature control (Famiani et al., 2020) whilst the use of cold storage can be an effective system for the control of the spoilage rate of damaged fruit over time (Morales-Sillero et al., 2017; Yousfi et al., 2012). For these reasons, the harvesting method can be considered as a co-factor for EVOO quality hazards, since the initial olive fruit conditions are one of the main factors which can affect the oil spoilage rate at the fruit storage.

Table 2. Possible causes of quality hazards at the harvesting phase. "Main effects on EVOO" are described as: alteration of quality chemical parameters - QCP alt., off-flavor development - OF dev., loss of phenolic compounds PC loss, loss of volatile organic compounds – VOC loss. "Effect on yield" codes: + increase, - decrease.

Cause of EVOO quality loss	Main effects on EVOO				Affected quality characteristics				Preventive actions	Effect on yield	References
	QCP alt.	OF dev.	PC loss	VOC loss	QRpr	QRsl	CV	OV			
Olive damage	x		x		X	x	X	X	- Choice of delicate harvesting method - Avoid storage - Temperature control during the storage	+	Corti et al. (2022); D'Imperio et al. (2010); Dag et al. (2008); Morales-Sillero et al. (2017); Morales-Sillero and García (2014); Yousfi et al. (2012)

3.1.2. Olive fruit storage

The storage of the olives can be considered an optional operation of the process since the olive fruit may also be harvested and immediately transported to the mill to be processed as quickly as possible, as commonly recommended by the good handling practices in olive oil mill (Goula et al., 2017).

However, storage operation is often very hard to avoid due to either a low working capacity of the plants, or an insufficient harvesting productivity for reach the critical mass to be processed.

Even after the olive fruit is detached from the tree, it still have a physiological and biochemical activity and in the absence of sap flow from the plant, it need to maintain the metabolic functions only with its own reserves of water and nutrients. Changes in fruit tissue permeability, respiration rate and gas exchange occur (DeEll et al., 2003; Garcia et al., 1995). In addition, the natural microflora of the olive fruit, made mainly by yeasts, bacteria and moulds (Fakas et al., 2010), finds in the breaking and bruised olives an optimal substrate for its growth. Thus fruit spoilage begins, combining ripening, senescence and microbial proliferation.

Different substrates and reactions are involved in this scenario. Oil comes in contact with water and enzymes, released from the fruit tissues, but also with microrganism and oxygen. Thus, different chemical and enzymatic pathways can start, such as the hydrolytic and oxidative processes, both from endogenous and exogenous origin, which act on triglycerides and phenolic fractions (Ciafardini et al., 2006; McClements and Decker, 2000; Migliorini et al., 2013a; Montedoro et al., 1992b; Proietti, 2014). These phenomena become more severe over the storage time until they determine irreversible damage on quality parameters, such as free acidity, peroxide value, UV spectroscopic indices, and sensory attributes, lowering the quality of the olive oil, even to the loss of *extra virgin* category (García et al., 1996a, 1996b, 1994). A lack in temperature control and unsuitable storage conditions may accelerate the rate of spoilage and favor the fermentation of the heaps due to anoxia and intense microbial spoilage (Guerrini et al., 2021). The latter may be responsible for the mill plant contamination (Mari et al., 2016) and the development of sensory defects in the oil, i.e., *fusty*, *musty*, *wine-vinegary* (Angerosa et al., 1996, 1990; S. Guerrini et al., 2015; Langstaff, 2014).

Together with the time-temperature factor, the initial health conditions of the olive fruit can be added to the factors that affect the spoilage of the olive oil during storage. Olive fruit with fly infection, microbial contamination and mechanical damage may stimulate the detrimental processes (Kyriakidis and Dourou, 2001; Yousfi et al., 2012).

One of the most used preventive and control strategies to slow down the spoilage rate is to carry out the temperature control through the use of refrigerated cells. This system allows to slow down the physicochemical and biochemical processes of the fruit, controlling the softening process, the decay index and the quality parameters of the oil (Brkić Bubola et al., 2020; Castellano et al., 1993; Clodoveo et al., 2007; García et al., 1996a, 1996b; Kader et al., 1989). Furthermore, since the low temperature can be selective for some enzymatic pathways, phenolic and volatile profiles can be modulated (Hachicha Hbaieb et al., 2016, 2015; Luaces et al., 2005). The detrimental action of uncontrolled storage temperature on phenolic content due to oxidation may also result in a reduction of oxidative stability and shelf life of the olive oil (Clodoveo et al., 2007).

Conflicting results were found for oil yield, which may increase due to a greater rupture and water loss, induced by softening during storage, (Brkić Bubola et al., 2020; Yousfi et al., 2012) or decrease due a higher incidence of fruit decay (Gutierrez et al., 1992).

However, the heat transfer could encounter some resistance due to the warming and fermentation processes inside the heaps and a low ratio between the heat transfer surface and the volume of the fruit, especially when the olives are stored in large piles or in containers with large capacity (García et al., 1994; Guerrini et al., 2021). This element may compromise the effectiveness of the use of refrigerated cells in the olive mill, since the cooling rate has to compensate the exothermal activity of the heaps, and often face with short storage time, as well.

The use of controlled atmosphere combined with cold storage was largely investigated, as well. This mainly consisted in lowering the concentration of O_2 and/ or increasing CO_2 and N_2. No satisfactory results was found using controlled atmosphere, due to the anoxic conditions and the increase in chilling injury, suggesting that only the temperature control could be the most suitable for delay the oil spoilage, also to avoid the costs for technical gases (Castellano et al., 1993; Clodoveo et al., 2007; Kiritsakis et al., 1998).

Table 3. Possible causes of quality hazards at the olive fruit storage phase. ""Main effects on EVOO" are described as: alteration of quality chemical parameters - QCP alt., off-flavor development - OF dev., loss of phenolic compounds PC loss, loss of volatile organic compounds – VOC loss. "Effect on yield" codes: + increase, - decrease.

Cause of EVOO quality loss	Main effects on EVOO				Affected quality characteristics				Preventive actions	Effect on yield	References
	QCP alt.	OF dev.	PC loss	VOC loss	QRpr	QRsl	CV	OV			
Olive decay (softening/ ripening/senescence)	x	x	x	x	X	x	X	X	- Short storage time - Arrange fruit in thick layers - Cold storage	Debated	Brkić Bubola et al. (2020); Castellano et al. (1993); Famiani et al. (2020); García et al. (1996b, 1996a, 1994); Kader et al. (1989); Morales-Sillero et al. (2017); Yousfi et al. (2012)
Microbial fermentation and warming of the heaps	X	X	X	X	X	x	X	X	- Short storage time - Arrange fruit in thick layers or small containers - Avoiding anaerobic conditions - Cold storage	Debated	Angerosa et al. (1996, 1990); García et al. (1996b, 1994); García and Yousfi (2006); Guerrini et al. (2021); Hachicha Hbaieb et al. (2016); Vichi et al. (2009)

3.2. Processing

3.2.1. Olive cleaning

The olive cleaning can be divided into two steps, i.e., the removal of leaves and other foreign material and the olive washing. The first action is carried out by means of pneumatic or mechanical separation, which are combined into dedicated machines equipped by sifters, vibrating screens and air blowing systems in order to remove leaves, small branches, sprigs, stones, and any other foreign material accidentally collected with the olive fruit (Peri, 2014c). The washing operation allows to remove the residual soil, dirt and chemicals from the fruit surface. The olive fruit are conveyed and washed into a flowing water well with bubbling air, then they are rinsed and dried in a conveyor belt equipped with clean water sprayer and air blowers. The washing operation is often considered critical by producers in terms of productivity, washing effectiveness and water consumption (Cappelli et al., 2019), rather than oil quality. The effect of washing phase on olive oil quality was poorly investigated. According to some Authors, no significant differences in oils from washed and unwashed olives can be found. As pointed out by Segura-Borrego et al. (2022), no significant alterations on quality chemical parameters, oxidative stability, volatile and sensory profile occur in oils after the washing treatment of the olive fruit. Even though, if this operation is carried out carelessly, a few possible hazards for the EVOO quality can be identified at this stage. A poor water cleanliness could increase the microbial contamination of the water and, consequently, of the olive fruit and paste. According to Vichi et al. (2011), microbiologically contaminated water can affect the chemical and sensory characteristics of the obtained olive oil, determining lower concentration of phenolic compounds and a greater production of volatile compounds associated to oxidative and fermentation pathways. On the other hand, no significant impact were found for quality chemical parameters and no sensory defects were detected on oils.

Moreover, a low effectiveness of rinsing could favor the accumulation of pro-oxidant factors on the olive fruit and paste, since transition metal ions are often contained in the dirty water (Decker et al., 2002; Peri, 2014c). However, all of these issues can be easily controlled through the preventive measures and the good manufacturing and hygiene practices commonly applied in the olive oil mill, such as water depuration and renewing, and acting the correct programs of plant disinfection and cleaning (El-Sayed et al., 2015).

Another critical issue could be represented by the washout of polar minor compounds of the olive fruit due to prolonged washing time but, any evidence was reported by the literature.

Table 4. Possible causes of quality hazards at the olive fruit washing phase. "Main effects on EVOO" are described as: alteration of quality chemical parameters - QCP alt., off-flavor development - OF dev., loss of phenolic compounds PC loss, loss of volatile organic compounds – VOC loss. "Effect on yield" codes: + increase, - decrease.

Cause of EVOO quality loss	Main effects on EVOO				Affected quality characteristics				Preventive actions	Effect on yield	References
	QCP alt.	OF dev.	PC loss	VOC loss	QRpr	QRsl	CV	OV			
Microbial and chemical contamination of water			X				X	X	- Water depuration and renewing - Rinsing and drying effectiveness - Microbiological and physicochemical monitoring - Plant cleaning	none	Vichi et al. (2011)

3.2.2. Olive crushing

The crushing operation has the function to disrupt the olive fruit into a fine homogeneous paste, in order to break the cell vacuoles and release the oil fraction, which is mixed with pit fragment, water and solid residues from skin and flesh.

The crushing process combines pressure and shearing actions, resulting from forces in an orthogonal and tangential direction, respectively. According to the type of crushing device and its setting, the contribute of pressure and shearing can be different (Leone, 2014). Several types of olive crushers can be used in the modern continuous plant, such as hammer crusher, disc crusher or blade cutter crusher. All of them replaced the granite millstones used in the traditional discontinuous extraction plant.

The hammer crusher consists in a three to six spokes ending with steel plates, or hammers, that crushes the olives against a stationary cylindrical grid. The diameter of the grid holes determines the thickness of the paste. An alternative model of hammer crusher can contain two concentric grids of different hole diameters, and a single rotor equipped with two series of spokes and hammers separated by the inner grid. The progressive size reduction of the grid holes from the inner to the second grid with smaller hole diameters, allows a more homogeneous size of the paste and prevent emulsion formations and heating effect (Leone, 2014). The disk crusher crushes the olives through the action of two toothed-disc, one stationary and one rotating in axis with the motor. The teeth arrangement on the discs is in concentrical circles at different distance from the center. Olives are fed from the central inlet placed on the fixed disc and they are pushed on the free space between discs by centrifugal force. This system ensures a more balance action between shear and pressure forces compared to hammer mills, thus the friction energy and overheating risks are lowered (Caponio et al., 2003). Thus, the phenolic extraction is less, even if the distance between the two disc can be regulated to determine the fineness of the olive paste (Leone, 2014). However, a critical issue of disk crusher is due to the possible break of teeth which can cause severe damage to the machine.

A well compromise between hammer and disk crusher features can be found in blade cutter crusher, which differs from hammer crusher only in the design of the crushing elements. The blade spokes installed on the rotor act a well balance between pressure and shearing forces, reducing the friction energy at the grid. In this way, a good disrupting action is obtained

through high rotational speed, without incurring into overheating problems (Guerrini et al., 2017c).

The main factors involved in the crushing stage are the rotational speed and the design of the crushing elements that, in combination, determine the intensity of the disruptive action. In general, the more disrupting and violent the crushing, the higher extraction of phenolic and minor compounds, due to the more complete disruption of the fruit skin and flesh fractions (Di Giovacchino et al., 2002b; Inarejos-garcía et al., 2011). According to the literature, the crushing method does not affect the quality chemical parameters of oil, such as acidity, peroxide value and UV spectroscopy index (Di Giovacchino et al., 2002b), while it is very effective on the extraction of phenolic compounds which are responsible for the nutritional properties, oxidative stability and the organoleptic attributes, i.e., bitterness and pungency, of the olive oil (Andrewes et al., 2003; Baldioli et al., 1996; Bendini et al., 2007). Thus, since the earlier researches on mill plant and machines, it was known that the choice of crushing method was useful to modulate the chemical and sensory profile of the olive oil, and, according to the olive characteristics, different procedures and machines were recommended. For instance, the mechanical hammer crusher was more suitable for olive cultivar with low content of phenolic compounds, whilst the stone mills were recommended for olive cultivar reach in phenolic compounds or unripe olives, in order to reduce the phenolic extraction and obtain a more gentle oil, preventing acute bitter and pungent notes. Therefore, on the same device, the phenolic extraction can be improved by increasing the rotating speed or reducing the grid hole diameter in hammer and blade cutter crusher (Angeloni et al., 2022a; Guerrini et al., 2017c; Inarejos-garcía et al., 2011; Polari et al., 2018). Increasing the crusher speed can also lead to rise the oil yield (Polari et al., 2018) but also no significant effects are described in the literature (Guerrini et al., 2017c). Despite this, a higher crushing speed may decrease the diameter of oil droplets increasing the oil/ water emulsion and reducing the oil separation (Amirante et al., 2010; Polari et al., 2018). No particular effect were detected on quality chemical parameters of the olive oil, as a function of type of crusher and speed setting, (Angeloni et al., 2022a; Polari et al., 2018; Preziuso et al., 2010) not even over the storage time (Guerrini et al., 2017c). However, since the crushing operations involve the contact between oxygen and fruit substrates, i.e., lipid, water and enzymes, chemical and enzymatic oxidation processes need to be controlled. Moreover, one of the main consequences of improving the disruptive performance of crushing is overheating

phenomena, deriving from the high energy dissipation at the crushing elements. Even though the temperature rise may increase the phenolic extraction during crushing improving their solubility in the oil, it also can favor the hydrolytic and oxidative processes increasing the reaction rate. According to Caponio et al. (2003) a greater temperature rise obtained using hammer crushing resulted in higher peroxide value, UV indexes and oxidized triglycerides, and lower oxidative stability. Oxidation of phenolic compounds can take place by polyphenol oxidase (PPO) and peroxidase (POD) which keep high stability at high temperature ranges (Taticchi et al., 2013).

Moreover, since at this stage also the formation of EVOO positive volatile compounds can start through the triggering of LOX pathway (Angerosa et al., 2000; Salas et al., 1999), the crushing temperature may be selective for the enzymes involved in the formation of oil flavor, such as LOX and hydroperoxide lyase (HPL). The latter has an optimal range of activity around 15 °C, which can be significantly reduced over 30 °C (Salas and Sánchez, 1999a). Therefore, the latest innovation trends on crushing stage aim to reach an advanced control of the crushing setting and parameters, such as crushing temperature and oxygen concentration, in order to control the oxidative damage and to modulate the organoleptic profile with high precision. According to Nucciarelli et al. (2022), the implementation of cooling system on crushing can control the phenolic oxidation and increase the concentration of LOX-derived volatile compounds, verifying the positive effect of low temperature in the production of EVOO flavor, as also described by other Authors (Dourou et al., 2020; Guerrini et al., 2021). In addition, Angeloni et al. (2022a) investigated the effect on EVOO quality of a dosing oxygen system installed on blade cutter crusher, showing for both levels of added oxygen (2.2 and 5.5 mg O_2/ kg of paste) an increase in positive volatile compound from LOX pathway and *fruity* sensory score, regardless the speed crusher. Only slight differences in sensory and volatile profiles were found by Guerrini et al. (2017b), as a function of crusher speed.

Table 5. Possible causes of quality hazards at the crushing phase. "Main effects on EVOO" are described as: alteration of quality chemical parameters - QCP alt., off-flavor development - OF dev., loss of phenolic compounds PC loss, loss of volatile organic compounds – VOC loss. "Effect on yield" codes: + increase, - decrease.

Cause of EVOO quality loss	Main effects on EVOO				Affected quality characteristics				Preventive actions	Effect on yield	References
	QCP alt.	OF dev.	PC loss	VOC loss	QRpr	QRsl	CV	OV			
Low crushing disruption			X				X	X	- Correct choice of crusher type - Correct crusher setting (rotational speed/ grid hole diameter) - Maintenance	+ , none	Angeloni et al. (2022a); Di Giovacchino et al. (2002b); Guerrini et al. (2017c); Inarejos-garcía et al. (2011); Polari et al. (2018)
Oxidation	X		X		X	X	X	X	- Correct choice of crusher type, avoid mill-stone crusher - Oxygen control - Correct crusher setting (rotational speed/ grid hole diameter) - Maintenance	none	Angeloni et al. (2022a); Caponio et al. (2003)
Temperature rise/ overheating	X		X		X	X	X	X	- Temperature control - Olive fruit conditioning	Debated	Caponio et al. (2003); Dourou et al. (2020); Nucciarelli et al. (2022)

3.2.3. Malaxation

After the olives are crushed to break the oil from vacuoles and extract the minor component from the fruit tissues, a slow kneading is needed to allow the coalescence of oil droplets in order to aid the separation process by centrifugation (Kalogianni et al., 2019). In modern mills this function is achieved by malaxer machine, consisting in a stainless steel chamber, or tank, equipped by a shaft with vertical or horizontal axis with blades and coil which perform the paste kneading and the removal of paste layer at the tank wall. The external surface of the tank is covered by a jacket in which the service fluid, i.e., water, flows for the thermal conditioning of the olive paste during the operation (Clodoveo, 2012).

In general, the greater the coalescence process, the better the oil separation and thus, the higher the oil yield. Both prolonging the kneading time and increasing the temperature promote the coalescence, also through the reduction of the olive paste viscosity (Boncinelli et al., 2013; Clodoveo, 2012; Di Giovacchino et al., 2002a; Inarejos-García et al., 2009). However, the coalescence is considered a prerequisite to obtain a high potential extraction yield, which has to be ensure by the more suitable time-temperature combinations (Trapani et al., 2017b).

In addition, uncontrolled time-temperature conditions and atmosphere composition, may have negative effect on the overall oil quality, since during this operation several chemical and biochemical processes occur on the olive paste, also due to the presence of oxygen and enzymes released from the fruit. Therefore, a strict control of operative parameters at this stage is needed to successfully assemble the nutritional and organoleptic features of the olive oil.

Inside the intact olive fruit, the oil fraction is enclosed into cell vacuoles, and it is composed mainly by triglycerides. Immediately after the crushing, the oil is released from the plant cells and it comes into contact with the other fraction of the disrupted olive, including vegetation water and solid fractions of flesh and skin. Thus, the following malaxing stage is also useful to allow the distribution of different molecules with biological and sensory activity from other fractions into the oil, and vice versa, i.e., phenolic compounds, tocopherols, pigments, alcohols, sterols, terpenes, hydrocarbons and volatile organic compounds (Bendini et al., 2007; Servili et al., 2004). Moreover, the triggering of the enzymes of the LOX pathway starts the formation of volatile organic compounds with 5 and 6 carbon atoms, responsible for the

positive attributes of the olive oil, i.e., *fruity* and other *green* notes. On the other hand, polyphenol oxidase and peroxidase may determine the loss of phenolic compounds, with negative consequences on the nutritional and sensory properties of the oil.

The main parameters involved in the malaxing stage are time, temperature and oxygen exposure, which should be considered in their interaction, rather than an individual perspective (Peri, 2014b; Trapani et al., 2017a, 2017b).

Several research on the effect of malaxing parameters on the yield and quality of EVOO are reported in the literature.

Even though the malaxing time is useful for promoting the extraction yield, it is considered critical in relation to the kinetic of the reactions of coalescence and oil spoilage and to the oxygen exposure (Leone et al., 2014a; Servili et al., 2003a, 2003b; Trapani et al., 2017a, 2017b). The use of hermetically sealed malaxer either the control of the head space through dosing systems for oxygen and technical gases, prevent lipid hydrolysis and oxidation and preserve phenolic compounds and organoleptic properties, also prolonging the EVOO shelf life (Leone et al., 2014a; Macaluso et al., 2021; Yorulmaz et al., 2011). Even though, the significant role of oxygen in the formation of the oil flavor through the LOX cascade is also known (Catania et al., 2016; Migliorini et al., 2006). Thus, the natural emission of carbon dioxide from the olive paste in malaxation can be exploited to improve the oil quality through a natural control of oxygen, without using inert gases (Masella et al., 2011; Parenti et al., 2006). The application of high-vacuum conditions during malaxation may lead to significant depletion of volatile compounds linked to the EVOO flavor due to stripping phenomena, which occurs even with mild and low temperature of malaxation (Taticchi et al., 2021; Veneziani et al., 2022).

Increasing the malaxation temperature affects positively the oil extraction yield but negative effects on phenolic and lipidic fractions can occur, promoting both hydrolytic and oxidative processes, which may compromise the quality parameters and lead to the production of compounds related to *rancid* (Guerrini et al., 2019; Inarejos-García et al., 2009; Ranalli et al., 2001). A reduction of phenolic content can be obtained for high malaxation temperature due to the action of oxidoreductase enzymes, i.e., polyphenol oxidase (PPO) and peroxidase (POD), which keep high stability even above 40 °C (Clodoveo et al., 2014; Ranalli et al., 2001; Taticchi et al., 2013). On the other hand, an increase in temperature till 27 °C may promote

the β-glucosidases activity, favoring the migration of polar phenolic compounds from solid and water phase to oil (Parenti et al., 2008; Veneziani et al., 2021). Then, oxidative phenomena prevail over 27 °C under the action of oxidoreductases.

Moreover, the rise in malaxation temperature may inhibit the LOX cascade and the development of flavor by the inactivation of the key enzymes LOX and HPL, the latter shows a reduced activity over 20 °C (Ranalli et al., 2001; Salas and Sánchez, 1999a). Thus, low malaxation temperature are recommended for the development EVOO LOX-derived positive note (Veneziani et al., 2021). However, the temperature control in malaxation has to cope with effectiveness issues due to the poor heat transfer performance of the industrial malaxer (Corti et al., 2023). Low thermal transmittance of malaxer and small ratio between the heat transfer surface and the olive paste volume are combined to a high viscous matrix, which struggles to reach a homogeneous temperature profile during the kneading phase (Ayr et al., 2015). Thus, modifications on the malaxer machine design and setting were proposed in order to improve the heat transfer efficiency. In particular, improvements to the malaxer shape and in the configuration of the reel were made in order to ensure a better kneading process and reduce the heat transfer resistance (Amirante et al., 2012; Ayr et al., 2015; Bianchi et al., 2020; Corti et al., 2023; Difonzo et al., 2021). Moreover, since the choose of the malaxing time is very dependent from the temperature gradient to obtain on the paste and from the effectiveness of the kneading action of the shaft, a preliminary conditioning of the olive paste seems appropriate in order to reduce the malaxing time and improve the EVOO quality and shelf life (Fiori et al., 2014). Different solutions for the thermal and rheological conditioning of olive paste before malaxation have been proposed in latest research (Fiori et al., 2014; Leone et al., 2015a; Tamborrino et al., 2021a). The common aim of these solutions is to reduce the malaxing time till the ultimate goal of avoid holding phase for the kneading, substituting batch operation with a continuous processing from the crushing to the centrifugal separation. The application of ultrasounds (US), pulsed electric fields (PEF) and microwave (MW) has largely investigated in the last few years. All of this technologies applied to the olive paste processing may favor the disruption of cell walls favoring the oil extraction and coalescence, allowing to reduce the malaxing time and temperature and ensuring a more continuous process (Clodoveo, 2013). Several prototype application on the oil plant and machines, mainly on pilot-scale, have been carried out, combined to heat exchanger and malaxing systems with significant result in reducing malaxing time (Almeida et al., 2017;

Amirante et al., 2017; Clodoveo et al., 2017; Leone et al., 2018, 2015c, 2014b; Tamborrino et al., 2021a, 2021b, 2019; Veneziani et al., 2019). Despite several experimental researches on emerging technologies show clear advantages in terms of olive oil extraction and quality, continuity of the process and temperature control, the relatively higher costs for plant installation are often the main critical issue that discourage the implementation on industrial-scale (Clodoveo, 2013).

To sum up, the malaxing stage has undergone significant evolution in the latest years, but it still needs further improvement. Even if the relationship between the individual factors and the effect on the quality are quiet understood, the overall interactions between factors are far to be governed during the processing. The use of sensors for monitoring and control the malaxing parameters, for instance the head space compositions, seems to be suitable for improve the EVOO quality acting an in-line management of the processing setting (Catania et al., 2013; Vallone et al., 2022).

Table 6. Possible causes of quality hazards at the malaxing phase. "Main effects on EVOO" are described as: alteration of quality chemical parameters - QCP alt., off-flavor development - OF dev., loss of phenolic compounds PC loss, loss of volatile organic compounds – VOC loss. "Effect on yield" codes: + increase, - decrease.

Cause of EVOO quality loss	Main effects on EVOO				Affected quality characteristics				Preventive actions	Effect on yield	References
	QCP alt.	OF dev.	PC loss	VOC loss	QRpr	QRsl	CV	OV			
High time	X	X	X	X	X	X	X	X	- Adequate time-temperature conditions - Temperature control - Head space control - Pre-conditioning of olive paste (heat-exchanger, application of innovative technologies)	+	Boncinelli et al. (2013); Clodoveo (2012); Di Giovacchino et al. (2002a); Inarejos-García et al. (2009); Leone et al. (2015a); Trapani et al. (2017a, 2017b)
High temperature	X	X	X	X	X	X	X	X	- Adequate time-temperature conditions - Temperature control - Head space control - Pre-conditioning of olive paste (heat-exchanger, application of innovative technologies)	+	Guerrini et al. (2019); Inarejos-García et al. (2009); Parenti et al. (2008); Ranalli et al. (2001); Salas and Sánchez (1999a); Taticchi et al. (2013); Trapani et al. (2017a, 2017b); Veneziani et al. (2021)

Table 6 (continued)

Cause of EVOO quality loss	Main effects on EVOO				Affected quality characteristics				Preventive actions	Effect on yield	References
	QCP alt.	OF dev.	PC loss	VOC loss	QRpr	QRsl	CV	OV			
Oxygen exposure	X	X	X	X	X	X	X	X	- Sealed conditions - Inert gases and oxygen dosing	none	Catania et al. (2016); Macaluso et al. (2021); Masella et al. (2011); Parenti et al. (2006); Yorulmaz et al. (2011)

3.2.4. Centrifugation operations

The use of the centrifugation system in the oil mill has replaced over the years other methods of oil extraction, such as by pressure or percolation, increasing the productivity, improving the olive oil quality and reducing the labor cost (Di Giovacchino, 2010).

Centrifugation techniques are based on the separation of different phase with different density using centrifugal acceleration, according to the Stokes law (Berk, 2009a). In the olive oil processing, centrifugation operation can perform relatively different separation functions, according to the phase composition of the matrix to be treated, i.e., liquid-liquid or solid-liquid, to which different machines are dedicated:

1) Separation: removal of the oil fraction from a viscous matrix, i.e., the olive paste, where solid and water fractions prevail.

2) Clarification: removal of fine solid residues and micro-drops of water from a mainly lipid liquid matrix, i.e., the oil must, in which they are suspended or emulsified.

3.2.4.1. Oil separation with decanter centrifuge

Decanter centrifuge is a horizontal axis bowl with cylindrical and conical shape in which the olive paste from malaxer is separated into its phases through the rotation at high speed, i.e., about 3500 rpm. A similar shape screw conveyor rotates inside the bowl at slight lower speed creating a speed gradient responsible for the discharge of the pomace to one end (Baccioni and Peri, 2014). The light phases, i.e., oil and vegetation water (only in three-phase decanter), are discharged via gravity through an adjustable system of overflows pushed by new paste income. The first model of decanter launched in the oil mill in the 60's was the three-phase decanters, which separate the oil from vegetation water and olive pomace. This system requires a significant water addition, about the 50% w/w of the paste, causing the dilution of hydrophilic minor component of the olive fruit, including the phenolic compounds. The more recent two-phase decanters separate the oil from a mixture of water and pomace, and no particular amount of added water are required. Thus, a better preservation of phenolic fraction is ensured by the use of two-phase decanter without any loss in oil yield. Moreover, this system allows to reduce the operative costs linked to water consumptions and the disposal of vegetation water (Baccioni and Peri, 2014).

Several works in literature reported a reduction in the total phenolic compounds deriving from the use of three-phase decanter and, thus, in the oxidative stability of the obtained oils (Amirante et al., 2010; Caponio et al., 2014; Di Giovacchino et al., 2001). No significant difference or slight difference, on quality chemical parameters were found in the literature due to the type of decanter (Amirante et al., 2010; Di Giovacchino et al., 2001; Ranalli and Angerosa, 1996), while, significant improvement of volatile and sensory profile were obtained by the use of two-phase decanter (Ranalli and Angerosa, 1996).

An evolution of the three-phase decanter was reached with the so called three-phase water-saving decanter, also called ARA. This innovative device allows to reduce the adding of water through the modification in the bowl geometry and internal design, and the regulation of screw/ bowl differential speed, obtaining low dilution of phenolic compounds (Amirante et al., 2010).

The optimization of differential speed, overflow levels and olive paste throughput combinations, is the more effective way to control the extraction efficiency and the olive oil quality at the centrifugation stage (Altieri et al., 2013; Caponio et al., 2014). This, can also be aided by the implementation of sensors and the use of software to reach an on-line control of the processing parameters (Altieri et al., 2013).

Even though, there are still a few studies of the effect of decanter operating parameters on olive oil quality.

3.2.4.2. Oil clarification with vertical centrifuge

Oils obtained directly from decanter are characterized by high content of solid particles and water, both above 0.2% w/w, and microbial contamination (Breschi et al., 2019). Thus, they need to be clarified by vertical centrifugation or filtration in order to be suitable for trade and more stable during shelf life (International Olive Council, 2018a).

Centrifugal clarification is achieved through vertical centrifuge consisting in a solid bowl fitted on a vertical axis spindle rotating at 5000-7000 rpm inside a stationary housing. The bowl contains a stack of conical disks which reduce the turbulence and improve the separation between solid and liquid phases of the turbid oil. The latter enters from the central feed tube on the bottom of the bowl. After the separation, lighter liquid phases, i.e., oil and water, are discharged from the top overflows of the bowl under the pressure of the incoming turbid,

whilst the heavier phase exits form the external nozzles located at the solid deposit chamber (Baccioni and Peri, 2014; Berk, 2009a).

This operation is considered optional since sometimes the filtration can be applied directly to the oil must exiting from decanter. The critical points discouraging the use of vertical centrifuge in the olive mill are linked to an increased risk of quality deterioration in terms of phenolic content and oxidative parameters, due to water adding and high mechanical stress on the matrix (Guerrini et al., 2017a). In fact, the high rotational speed (i.e., approximately 6000-6500 rpm), combined to a lack in the control of temperature and oxygen factors, may promote the formation of water-in-oil emulsion, oxygen solubility and temperature rise (Altieri et al., 2014; Gila et al., 2022; Masella et al., 2012, 2009). In particular, the greatest damaging effect of the increase of dissolved oxygen in the oil is on phenolic compounds (increase of oxidized secoiridoids) and quality chemical parameters (peroxide value and UV spectroscopic indexes) in the obtained oils, both after the processing and during the storage (Altieri et al., 2014; Guerrini et al., 2017a; Parenti et al., 2007). Innovative mill plant solutions for the reduction of dissolved oxygen by dosing nitrogen inside the vertical centrifuge had positive results, increasing the phenolic and volatile content and fruity and bitter notes (Angeloni et al., 2022b).

Natural settling in decantation tank, under static or dynamic conditions, has recently been proposed as an alternative to clarification by centrifugation (Altieri et al., 2020, 2014; Gila et al., 2018, 2016, 2017). However, this system still needs to be optimized to achieve a clarification efficiency comparable to vertical centrifugation. Furthermore, a more efficient purge system of water and solid settled impurities is needed to avoid anaerobic fermentations and off-flavor development in the olive oil.

However, after the clarification processes by settling or centrifugation, some amount of insoluble solids, micro-drops of water and microrganism may remain in the oil into colloidal structures, representing a severe risk for EVOO quality during storage (Chaiyasit et al., 2007; Fortini et al., 2016; Fregapane et al., 2006; Guerrini et al., 2020a; Koidis et al., 2008). As for decanter, the optimal balance between oil must feed rate, water ring size and rotational speed, must be set in vertical centrifuge, in order to achieve the maximum effectiveness in the removal of solid fractions and moisture (Berk, 2009a) and keep water activity below 0.6

to avoid microbial growth (Koidis et al., 2008; Labuza et al., 1972). This issue can be completely avoided only adding the filtration step.

Table 7. Possible causes of quality hazards at the oil separation phase with decanter centrifuge. "Main effect on EVOO" are described as: alteration of quality chemical parameters - QCP alt., off-flavor development - OF dev., loss of phenolic compounds PC loss, loss of volatile organic compounds – VOC loss. "Effect on yield" codes: + increase, - decrease.

Cause of EVOO quality loss	Main effects on EVOO				Affected quality characteristics				Preventive actions	Effect on yield	References
	QCP alt.	OF dev.	PC loss	VOC loss	QRpr	QRsl	CV	OV			
Water addition			X	X		X	X	X	- Avoid three-phase decanter - Use two-phase or three-phase ARA decanter	+	Amirante et al. (2010); Di Giovacchino et al. (2001); Ranalli and Angerosa (1996)

Table 8. Possible causes of quality hazards at the clarification phase with vertical centrifuge. "Main effect on EVOO" are described as: alteration of quality chemical parameters - QCP alt., off-flavor development - OF dev., loss of phenolic compounds PC loss, loss of volatile organic compounds – VOC loss. "Effect on yield" codes: + increase, - decrease.

Cause of EVOO quality loss	Main effects on EVOO				Affected quality characteristics				Preventive actions	Effect on yield	References
	QCP alt.	OF dev.	PC loss	VOC loss	QRpr	QRsl	CV	OV			
Water addition			X			X	X	X	- Reduce the water addition - Avoid vertical centrifugation using filtration	+	Di Giovacchino et al. (1994); Fortini et al. (2016)
Dissolved oxygen	X		X	X	X	X	X	X	- Use of inert gases	none	Angeloni et al. (2022b); Masella et al. (2012, 2009); Parenti et al. (2007)
Water and solid suspended (bad clarification efficiency)	X	X	X	X	X	X	X	X	- Filtration	none	Ciafardini and Zullo (2018); Fortini et al. (2016); Fregapane et al. (2006); Guerrini et al. (2020a); Labuza et al. (1972); Zullo et al. (2013)

3.2.5. Filtration

Olive oil after centrifugation can be considered a colloidal phase due to the presence of emulsified micro-drops of water and dispersed solid impurities which form the turbid appearance of the olive oil (Gila et al., 2022). This metastable condition in the olive oil may last for several months before the natural separation of the phases by settling, which creates a brown-colored residue to the bottom of the oil containers (Lercker et al., 1994). The filtration process aims to obtain a fully limpid oil through the completely removal of moisture and suspended solid, increasing the EVOO shelf life and improving its visual appearance, as well (Bakhouche et al., 2014; Fregapane et al., 2006).

However, EVOO can also be packaged and commercialized without any filtration treatment, as veiled EVOO, including some critical issue in terms of shelf life. The presence of water micro-drops, microrganisms and solid residues, which contain enzymes and other substrates such as sugars, proteins and phospholipids, make the oil an extremely instable matrix, also due to the high water activity (Ciafardini and Zullo, 2018; Koidis et al., 2008; Koidis and Boskou, 2006; Labuza et al., 1972; Lozano-Sánchez et al., 2010; Papadimitriou et al., 2013). Several Authors reported a shorter shelf life of veiled EVOOs compared to filtered EVOOs due to the more rapid decay of quality parameters and the development of off-flavors related to the activity of yeasts, i.e., *fusty*, *mouldy*, *wine-vinegary*, *muddy/ sediment* and *rancid* (Bakhouche et al., 2014; Fortini et al., 2016; Fregapane et al., 2006; Guerrini et al., 2020a; Zullo et al., 2013). Despite this, the market of veiled EVOO is widespread, since this product appears to consumer more *green* and less processed (Koidis and Boskou, 2006). It is also known that, even though filtration may reduce the amount of phenolic and volatile compounds, these fractions may remain more stable during shelf life preserving the EVOO flavor for a longer time (Bakhouche et al., 2014; Fortini et al., 2017; Guerrini et al., 2020a). On the other hand, in veiled EVOO, some sensory defects may be perceptible by the panel even in a few days after the bottling, leading to the downgrade of the *extra virgin* category (Guerrini et al., 2020a).

Both surface and depth filtration principles are used in the olive oil industry. In the surface filtration the retention of impurities occurs only as a function of the particles to be remove from the slurry. The filter acts a barrier effect only on the particles bigger than the holes of the porous medium. In the depth filtration, the particles of the slurry are absorbed along the

thickness of the filter medium as they can get inside from the surface holes with smaller diameter (Peri, 2014d). However, very often the two principles occur concomitantly since the retained particles of surface filtration tend to form a thick layer on the filter medium exerting increasingly greater depth action (Guerrini and Parenti, 2016).

Filter press and cartridge filters (surface filtration) are the most common technologies used in small and medium size olive mill companies, thus they are going to be discussed in this section. Filter press consists of plates placed together supporting the filter medium, i.e., the filter sheets, in vertical or horizontal stacks. Feeding and collecting plates are alternated trough the filtration chambers. Feeding plates allow the feed of the turbid oil to the filter sheets, while, collecting plates have the aim to collect the limpid oil flowing out from the other side of the filter sheets (Peri, 2014d). Cartridge filters are composed by a perforated cylinder cartridge of different materials (metal, cloth, canvas, paper, mesh, porous ceramic) placed inside an housing in which the turbid oil is fed to be collected from the central chamber of the cartridge (Berk, 2009b).

According to the Hagen-Poseuille equation, the filtration rate is measured as the volume of filtrate processed per unit of time, which is theoretically directly proportional to the pressure gradient across the filter medium, which is considered the driving force of filtration operation (Peri, 2014d). Always according to theoretical models, being constant the surface area of the filter medium during the operation, the resistances to the filtration are mainly due to the viscosity of the slurry and to the contribution of the solids retained on the filter medium. However, it is not true the principle the higher is the pressure gradient the greater the filtration rate, since over the filtration time the retained solid residues under pressure could compact themselves on the filter medium and obstruct the permeation of the turbid oil. Thus, the pressure gradient rises and the filtration rate falls. At this point, two practices can be chosen by the producer to stop the operation: i) continue the operation till the filtration rate reach a very low level, increasing the risk of solid residue permeation across the filter sheets, which have run out the retention capacity; ii) reduce the duration of the filtration cycle through a frequent replacement of the sheets, i.e., before the operating pressure or the filtration rate undergoes significant changes. Nevertheless, this implies higher consumption of filter sheets (Peri, 2014d).

The use of stainless steel pre-filters combined can be useful to reduce the use of filter sheets and the oil losses, prolonging the filtration cycle before the replacement of exhausted filter sheets (Guerrini et al., 2015). In general, even if the operative parameters in the filtration operation are few, the most careful practices are recommended in order to prevent the loss of filtration effectiveness or the development of off-flavor directly attributable to the operation itself. For instance, the periodical replacement of the filter sheets, may contribute to avoid the permeation of the retentate into the filtered oil and its contamination, albeit with more costs. Moreover, ensuring the cleanliness of the filtration devices, especially end of the filtering cycle, and of the filter sheets in clean, which need to be stored in adequate hygiene and environmental conditions (Peri, 2014d).

Table 9. Possible causes of quality hazards at the filtration phase. "Main effects on EVOO" are described as: alteration of quality chemical parameters - QCP alt., off-flavor development - OF dev., loss of phenolic compounds PC loss, loss of volatile organic compounds – VOC loss. "Effect on yield" codes: + increase, - decrease.

Cause of EVOO quality loss	Main effects on EVOO				Affected quality characteristics				Preventive actions	Effect on yield	References
	QCP alt.	OF dev.	PC loss	VOC loss	QRpr	QRsl	CV	OV			
- Dirty filter device - Dirt/ used/ contaminated filter sheets		X			X				- Plant leaning - Frequent replacement of filter medium after	none	Peri (2014d)
Water and solid suspended (low filter retention)	X	X	X	X	X	X	X	X	- Choice of adequate cut-off - Frequent replacement of filter medium - Use of stainless steel pre-filter	none	Ciafardini and Zullo (2018); Fortini et al. (2016); Fregapane et al. (2006); Guerrini et al. (2020a); Guerrini et al. (2015); Labuza et al. (1972); Peri (2014d); Zullo et al. (2013)

3.3. Other processing operations and factors into the oil mill

The EVOO quality may be affected by several operations and practices which are not directly attributable to a defined fundamental operation. For instance, the olive fruit, the olive paste and the olive oil undergo several handling and transfers across the processing.

The good hygiene and manufacturing practices have to be implemented since from the harvesting stage ensuring the best hygiene conditions of collecting nets, fruit containers and transport trailers (Goula et al., 2017). Moreover, the temporarily storage of the harvested olive fruit at the grove, should be carried out arranging the fruit on thin layer in shaded and ventilated areas, in order to ensure an early temperature control (Proietti, 2014).

At the processing phase, the crushed olive paste and the oil must exiting from decanter are transferred from a machine to another by means of mechanic pumps, exposing them to mechanical stress and air contact. The most suitable pumps should be chosen according to the matrix in order to avoid turbulence, shearing actions and increase of temperature (Peri, 2014e). A dissolved oxygen rise in the produced oil was observed as a function of the pumps used during the processing, reflecting in a less advanced oxidative state of volatile compounds related to the LOX pathway (Masella et al., 2021).

Sometimes the quality of olive oil may also affected by the practices of the company. For instance, the filtration operation may be scheduled at the end of the harvesting season, instead of filtering the oil in-line with the extraction processing. This, practice greatly exposes the unfiltered olive oil to the fast development of off-flavor development due to the water, solid and microbial contamination suspended in the matrix (Guerrini et al., 2020a).

The microbial contamination inside the extraction plant could be another problem for the quality of EVOO, especially for veiled EVOO. Some Authors compared the microbial populations isolated from fresh olives, olive pastes and oil at the decanter, pointing out that only a few species of yeasts which were isolated in the processing, i.e., paste and oil, were dominant in the olive fruit (Mari et al., 2016; Romo-Sánchez et al., 2010). This means that some microbial species are selected by the mill processing and they can colonize the plant and, consequently, the produced olive oil. Thus, the most effectiveness cleaning program of the mill plant have to be implemented for prevent the colonization of the plant by yeast and reduce the risk of sensory defect in the olive oil.

Another kind of contamination is linked to the batch processing of different olive batches. Sometimes the olive oil mill companies are equipped with two production lines, thus, they can keep two different batches of olive separated during their processing. However, when different olive batches are processed on the same extraction line, the mill personnel must to avoid the cross-batch contamination keeping each batch separated by time and space. Even though a discontinuous processing can be easily managed during the malaxing operation using different malaxer in series, a critical issue involves the decanter centrifuge which is designed to work in continuous, thus the most correct change point between different batches need to be identified (Guerrini et al., 2022).

4. Conclusions

The EVOO processing is very complex since many variables can affect the quality of the product at different levels and through different actions. The combination between the operative parameters, the type of plant and machines and the mill procedures determines if a fundamental operation is critical for the EVOO quality.

Several hazards for quality and key operative parameters can be associated to each fundamental operation but they may produce different effect on the characteristics of the olive oil. For instance, the same hazard may be responsible for the downgrading of olive oil commercial category in an operation and act only as modulating factor of the organoleptic profile, in another.

According to the risk analysis, olive fruit storage, crushing, malaxation and clarification with vertical centrifuge are the most critical operation in the EVOO production process. This results from the higher frequency of hazards for quality, which may occur on several quality characteristics. Furthermore, none of these operations can be corrected or controlled by subsequent operations, except for the clarification with vertical centrifuge. As for the latter, a low clarification efficiency can be corrected through the filtration stage, whilst the increased concentration of dissolved oxygen can be avoid adopting inert systems with technical gases.

To date, much is known about the effect of the olive oil plant and processing on the quality of EVOO and many phases have been studied in relation to the operative parameters. At the same time, the literature still lacks some critical issues that have not been investigates, which can be summarized as follows:

- the combination and the interaction between multiple operative parameters, also coming from contiguous fundamental operations;
- the lack of effectiveness in the control of operative parameters, deriving from the plant and machines that do not ensure the preferred setting;
- the influence of mill practices, considering the mill scenario from an integrated point of view

2. Aim of PhD thesis

The present work was motivated by the need to expand the current knowledge in the olive oil production, which is characterized for several operations and factors. The aim of thesis was to better understand the relationship between the olive oil quality and the operative parameters. In particular, the experimental trials performed on a controlled-industrial scale allowed to reproduce a real olive mill scenario, where different variables occur and may interact together, and where the mill practices can alter the environmental conditions.

The program of the Doctoral thesis developed through the investigation of three different fundamental operations at different stages of the process, which were identified as critical for the quality of EVOO and not completely studied.

The effect of the olive fruit management before milling on the olive oil quality was studied in relation to the use of a refrigerated cell for the olive fruit storage at controlled temperature and conditions (Article #1). Moreover, the contributing variable due to the previous harvesting phase, i.e., the harvesting method, was added to the experimental design (Article #2).

In the second activity block, a practical application for the temperature control during malaxation was proposed, designing a methodological approach for evaluate through a simple method the heat transfer performance of industrial malaxer (Article #3).

Finally, the cross-batch contamination at the decanter centrifuge was investigated, giving some technological and practical solutions in order to identify the most likely point of olive batch change (Article #4).

3. Pre-extraction stage management: cold storage of olive fruit

3.1. Preliminary remark - Article #1 and Article #2

The “post-harvest” term is usually considered unsuitable for the olive oil processing, since it is always recommended to crush the olives “as soon as possible”. However, the management of the harvested olive fruit before their crushing for the olive oil extraction is now considered an essential part of the olive oil production. In fact, the low working capacity of the plants and the heavy-condensed olive inflow in a short period of time make the storage unavoidable. A bad management of the olive fruit transport and storage is widely recognized as one of the main causes of sensory defects linked to microbial alterations and to bad health-conditions of the olive fruit, such as fusty, muddy sediment, musty-humid-earthy, winery-vinegary *vinegary* (Langstaff, 2014; Proietti, 2014).

However, in addition to the variables which can be set and controlled during storage, i.e., time, temperature, atmosphere composition, other several contributing causes, linked to environmental factors, company processing protocols and initial conditions of the fruit, are involved, affecting the rate of the spoilage of the matrix. Thus, they should be fully considered in the setting and management of the operations.

The use of refrigerated cells for the cooling and storage of post-harvest fruits is widely used in food supply chains. For food safety and quality issues of some productions, it may be necessary to maintain the cold chain from the field to the consumer, even for just a short period of time. Although the oil matrix is well-sheltered from food safety risk, due to the low water activity, several chemical and organoleptic standards must to be accomplished for preserve the virgin olive oil categories (European Commission, 2013). The trend to start the olive oil harvest season very early, combined to the anomalous climatic events, may result in relatively high average harvesting temperatures with real risks of fruit warming. Furthermore, the product differentiation in terms of composition and organoleptic profile is becoming one of the main goals of plant engineering innovation which aims not only to the implementation of new extraction technologies but also to improve the control of those processing parameters that allow to govern biochemical and oxidative reactions (Clodoveo et al., 2014).

For these reasons, the temperature control during fruit storage has widespread also in the olive oil field, aiming to prevent the off-flavor development and conditioning the olives before the milling operations.

However, the oil mill scenario holds several variables, many of which are out of the operator control and may interact with other environmental and processing factors, limiting the effectiveness of the adopted procedures.

The Article #1 and Article #2 of the thesis investigate the qualitative effect of the olive fruit storage temperature in relation to other factors, such as storage conditions and harvesting methods, which can favor the fermentation of the heaps reducing the effectiveness of the cooling system. These phenomena have to also be related to short-term storage, which is typical in the production of high quality olive oil where often the production specifications may set a limit of hours between olive harvesting and pressing.

In particular, the use of refrigerated cells can contrast with short storage time. This operation involves a heat transfer process which may encounter some resistances (Singh and Heldman, 2008) determining a loss of efficiency and avoiding the heaps to reach the desired temperature This would mean adopting a resource without any benefit on the quality of the product, but with negative implications in terms of energy consumption and environmental impacts. Among the limiting factors to the heat transfer there are the processing practices and protocols related to the operation itself or other aspects linked to previous operations such as olive harvesting. For example, the type of containers used for the storage and their capacity affect the heat transfer rate between the olive fruit mass and the storing environment , which have to be consistent with the duration of the storage. The harvesting methods may also responsible for the health condition of the olives at the arrival to the mill. The mechanical stress caused by the machines and tools used for harvesting may be responsible for the mechanical damage on the fruit and consequently for the microbial and oxidative spoilage of the fruit.

According to these considerations, the following research works report two storage studies in which different post-harvest variables are considered in relation to olive oil quality. In the first article (Article #1) the combined effects of storage temperature, i.e., room or refrigerated temperature, and the ratio between heat transfer surface of the containers and the volume of the olive fruit mass are studied. In the second article (Article #2) the storage temperature is related to the harvesting method, comparing the manual harvesting with the facilitated harvesting by means of hand-held electric combs. These two systems are widespread in the olive oil production in Central Tuscany, used alternatively or complementary. However, few

works in the literature investigated a comparison between these two systems, thus, a further research examination was needed.

3.2. Article #1 - Use of refrigerated cells for olive cooling and short-term storage: Qualitative effects on extra virgin olive oil

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

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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.

Keywords

Aroma volatiles, off-flavor, post-harvest temperature, refrigeration

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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; García et al., 1996b; García and Yousfi, 2006). Many factors support fruit softening (García et al., 1996a) 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, 2014b).

The literature reports several studies of the effects of cold storage on olive fruit (Ben et al., 2012; García et al., 1996a, 1996b; Hachicha Hbaieb et al., 2015, 2016; 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., 2008, 2012), 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., 2002b). 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 DAGRI, 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 x 113 x 58 cm) with SVR = 7 (SVR7-bin); half-filled (i.e., 125 kg) perforated plastic bins (113 x 113 x 29 cm) with SVR = 10 (SVR10-halfbin); or 20 kg perforated plastic boxes (51 x 35 x 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 (European Commission, 2013).

Biohenolic fractions were extracted, identified and determined following the International Olive Council (IOC) official method (International Olive Council, 2017a). 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 GC-MS identification of VOC was performed using a Trace GC-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), their interaction and the harvest day as blocking factor. Significance was set at $p < 0.05$. Tukey HSD post-hoc test was used to assess significant differences among the 3 SVRs. Three replicates were performed.

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 compound hydrolysis (Guerrini et al., 2020b; Migliorini et al., 2013b; 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., 2017c; Montedoro et al., 1992a; 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; 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.

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	125.83 ± 22.52	*ns*
Total oxidized form	75.41 ± 5.04	96.95 ± 21.99	*ns*
Total non oxidized form	206.14 ± 21.00	199.57 ± 47.71	*ns*
Nonox/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	*

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 (Figure 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.

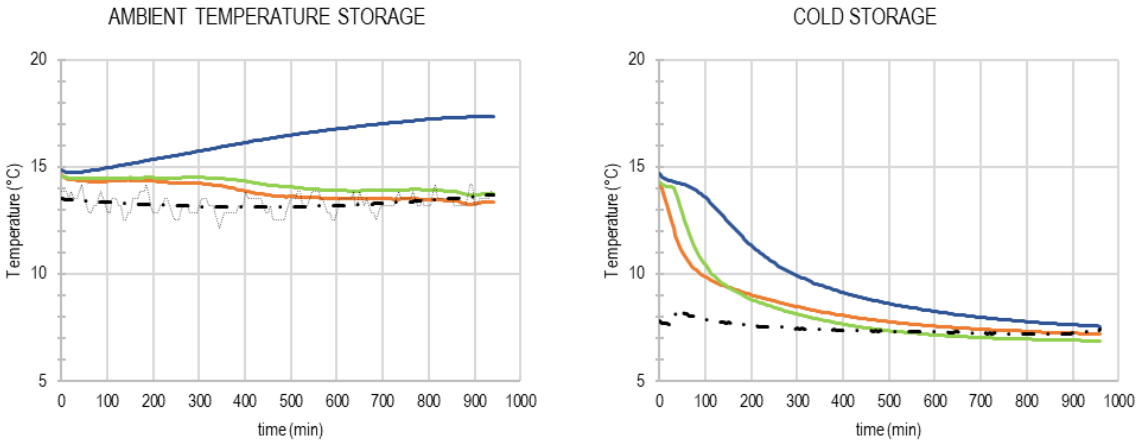

Figure 1. Tracking plots of the inner temperature (3-replicate average values) of olive fruit heaps during cold (C) and ambient temperature (A) storage at mill scale (solid lines): filled bin – SVR = 7 (blue line), half-filled bin – SVR = 10 (orange line), and plastic box – SVR = 16 (green line). Dotted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

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 ($\simeq$1%), with a simultaneous decrease in the concentration of O_2 ($\simeq$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 (European Commission, 2013), 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.

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; . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Ambient			Cold						
Biophenols (mg kg^{-1})	**SVR7-bin**	**SVR10-halfbin**	**SVR16-box**	**SVR7-bin**	**SVR10-halfbin**	**SVR16-box**	***RSE***	***p T***	***p SVR***	***p int***
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*	**

Table 3 (continued)

	Ambient			Cold						
Biophenols (mg kg^{-1})	**SVR7-bin**	**SVR10-halfbin**	**SVR16-box**	**SVR7-bin**	**SVR10-halfbin**	**SVR16-box**	***RSE***	***p T***	***p SVR***	***p int***
Sum of Compounds and Indexes										
Lignans	20.38 ^{i}	26.60 ^{hi}	20.78 ^{i}	33.98 ^{h}	25.07 ^{hi}	24.89 ^{i}	*3.58*	**	*ns*	*
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*
Nonox/Ox Ratio	2.77 ^{b}	2.15 ^{b}	2.02 ^{b}	1.53 ^{a}	1.40 ^{a}	1.54 ^{a}	*0.04*	*	*ns*	*ns*

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).

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.

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; . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Ambient			Cold						
Volatile Organic Compounds (mg kg^{-1} or µg kg^{-1})	**SVR7-bin**	**SVR10-halfbin**	**SVR16-box**	**SVR7-bin**	**SVR10-halfbin**	**SVR16-box**	***RSE***	***p T***	***p SVR***	***p int***
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*

Table 4 (continued)

	Ambient			Cold						
Volatile Organic Compounds (mg kg^{-1} or µg kg^{-1})	**SVR7-bin**	**SVR10-halfbin**	**SVR16-box**	**SVR7-bin**	**SVR10-halfbin**	**SVR16-box**	***RSE***	***p T***	***p SVR***	***p int***
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*	*ns*

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 (Figure 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 (Figure 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 (Figure 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 flavor of oil obtained from olives piled or stored in such conditions as to have

undergone an advanced stage of anaerobic fermentation" (International Olive Council, 2018b).

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 (Figure 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. Conclusions

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.

Supplementary Materials

Table S1. Previous studies of the effect of cold and/or controlled atmosphere storage on the quality of olive fruit, and the obtained olive oils. Abbreviations in table h = hours, dd=days, w= weeks, T_{amb}=ambient temperature.

References	Time (min)	Time (max)	Temperature	Controlled atmosphere	Container	Results
Kader et al. (1989)	2 w	10 w	5 °C; 7.5 °C; 10 °C	Air; 2% O_2; 5% CO_2; 2% O_2 + 5% CO_2	Plastic bags (air control); 2 l glass jars (controlled atmosphere treatments).	Storage at 5, 7.5, and 10 °C in air maintained *Manzanillo* fruit quality for up to 8, 6 and 4 weeks, respectively. Adding 2% O_2 at 5 and 7 °C extended fruit shelf life, retarded softening and black color development on the skin. Temperatures below 5 °C, and storage with 5% CO_2 should be avoided as both increase fruit chilling injuries.
Gutierrez et al. (1992)	15 dd	60 dd	5 °C; T_{amb}	Air; 3% CO_2; 5% O_2 + 3% O_2; 5% O_2 + <1% CO_2	6 kg containers (60 x 40 x 40 cm)	Storage of fruit at 5 °C prolongs the extra virgin status of the produced olive oil. No clear advantages come from the controlled atmosphere.
Castellano et al. (1993)	15 dd	60 dd	5 °C; T_{amb} (6–17 °C)	Air; 20% O_2 + 77% N_2 + 3% CO_2; 5% O_2 + 92% N_2 + 3% CO_2; 5% O_2 + 94% N_2 + <1% CO_2	6 kg containers (60 x 40 x 40 cm)	Storage at 5 °C in air decreases the incidence of physiological disorders and decay. Storage at 5 °C and 3% CO_2 + 5% O_2 for up to 30 days delays ripening and preserves green color and flesh firmness. After 30 days of storage, there is a higher incidence of chilling injury and rot.
Maestro et al. (1993)	15 dd	45 dd	5 °C; T_{amb} (12 °C)	Air; 20% O_2 + 77% N_2 + 3% CO_2; 5% O_2 + 92% N_2 + 3% CO_2; 5% O_2 + 94% N_2 + <1% CO_2	6 kg plastic containers (60 x 40 x 40 cm)	Storage at 5°C + high CO_2 (3%) + low O_2 (5%) gives the lowest polyphenol loss in olive fruit. CO_2 may act as an O_2 antagonist, decreasing polyphenol oxidase activity.

Table S1 (continued 1/4)

References	Time (min)	Time (max)	Temperature	Controlled atmosphere	Container	Results
García et al. (1994)	7 dd	60 dd	5 °C; 8 °C; T_{amb} (12 °C)	CO_2/O_2: <1/21; 3/20; 5/20; 10/19; 20/17; <1/1; <1/5	64 kg containers (5 °C, 8 °C, Tamb) 2, 6, 64, 400 kg containers (5 °C test)	5 °C storage reduces the incidence of post-harvest losses compared to 8 °C and ambient temperature storage. Controlled atmosphere does not improve olive oil quality, but at 5 °C it may increase deterioration indexes, especially with $CO_2 \geq 3\%$ or $O_2 \leq 5\%$. In general, fruit damage is directly proportional to a CO_2 increase and an O_2 decrease. Higher acidity is measured in olive oil obtained from fruit stored in 400 kg containers compared to oils from fruit stored in smaller quantities.
García et al. (1996b)	7 dd	60 dd	5 °C; 8 °C; T_{amb} (12 °C)	/	64 kg plastic containers (60 x 40 x 40 cm)	Cold storage at 5 °C is the best way to prolong olive fruit storage, and produce a good quality olive oil. The higher the storage temperature, the greater the amount of rotten fruit. Storage at 5 °C delays softening and the destruction of chlorophyll pigments. It also avoids an increase in titratable acidity and peroxide value, ensuring the extra virgin category of the oil for a longer period.
García et al. (1996a)	10 dd	60 dd	5 °C T_{amb} (12 °C)	/	14 kg plastic containers	Cold storage significantly delays olive softening, ripening and reduces decay. However, trends differ as a function of the cultivar. Cold storage at 5 °C significantly delays the increase in titratable acidity, peroxide value and other oxidative parameters.
Kiritsakis et al. (1998)	30 dd	60 dd	0 °C; 5 °C; 7.5 °C	Air; 2% O_2; 5% CO_2; 2% O_2 + 5% CO_2	2 kg pack	The best storage conditions for *Koroneiki* olives are 5 °C in air, as this maintains quality for 30 days. No advantage is detected with a controlled atmosphere, but this condition accelerates chilling injury.
Pereira et al. (2002)	7 dd	14 dd	5 °C	/	14 kg plastic containers	Storage time degrades olive oil quality and oxidative stability. The rate of deterioration differs as a function of the cultivar.

Table S1 (continued 2/4)

References	Time (min)	Time (max)	Temperature	Controlled atmosphere	Container	Results
Luaces et al. (2005)	5 dd	26 dd	5 °C 20 °C	/	2 kg batches	LOX activity is unaltered in olive fruit stored at ambient temperature, but is drastically reduced in fruit stored at 5 °C. However, HPL activity steadily decreases during storage at ambient temperature, while no change is observed in fruit stored in cold conditions.
Dourtoglou et al. (2006)	1 dd	11 dd	/	Air; CO_2	1 kg glass jar	Post-harvest storage of olives under a CO_2 atmosphere for 12 days reduces bitterness and increases color and flavor development. Total polyphenol and total flavonoid biosynthesis are reduced in olives stored under air compared with samples stored under CO_2. This is linked to lower antioxidant activity in air-stored fruit.
Clodoveo et al. (2007)	15 dd	30 dd	5 °C; T_{amb} (20 °C)	Air; 3% O_2 + 5% CO_2	1.5 kg jars	Fruit stored at 5 °C for 30 days produces better-quality olive oil than fruit stored at ambient temperature. Oxidative processes are delayed. No significant advantages are observed for the controlled atmosphere.
Kalua et al. (2008)	1 w	3 w	4 °C	/	100 kg crates	Phenolic compounds (hydroxytyrosol and luteolin rutinoside) in fruit continuously increase with cold storage, while they continuously decrease in the produced olive oil (oleuropein and ligstroside derivatives). This is due to the interaction between reactive phenolic compounds and other substrates released by the cell wall. A decrease in E-2-hexenal and hexanal during low-temperature storage, with respect to mean concentrations, is registered at weeks 1 and 3. This trend coincides with poor sensory quality olive oil and can be related to a decrease in enzymatic activity of hydroperoxide lyase.
Yousfi et al. (2008)	1 w	8 w	5 °C	/	12 kg plastic boxes	Bitterness gradually diminishes in extracted oils as a function of the amount of time fruit is stored at 5 °C.

Table S1 (continued 3/4)

References	Time (min)	Time (max)	Temperature	Controlled atmosphere	Container	Results
Yousfi et al. (2009)	/	72 h	20 °C 40 °C	Air; Closed jars; Closed jars + 30 ppm ethylene	2 kg in 3.5 L jars	Fruit respiration in closed containers causes a CO_2 accumulation and an O_2 decrease in the storage atmosphere. Heat treatment of olive fruit for 72 h at 40 °C in air significantly reduces oil bitterness, but also pigment content and oxidative stability. The use of modified atmospheres and ethylene addition induce the development of an off-flavor in olive oil.
Vichi et al. (2009)	3 dd	21 dd	5–8 °C	/	10 kg plastic bags 10 kg open boxes	Microbial growth is higher in olives stored in bags and the *musty* defect is perceived after 9 and 16 days of storage in bags and box treatments, respectively. At the same time, several volatile phenols that are present in higher percentages increase in oils from olives stored in bags, but seem to acquire their sensory significance at an advanced stage in olive alteration. Volatile phenols may be considered as analytical indices of fruit degradation.
Rinaldi et al. (2010)	15 dd	30 dd	5 °C T_{amb} (15–20 °C)	Air; 2% O_2 in N_2;	3 kg PVC containers	Storage at ambient temperature increases the respiration rate of olive fruit. Oil extracted from olives refrigerated at 5 °C had lower acidity and good resistance to oxidation. Few additional benefits are seen in the controlled atmosphere.
Inarejos-García et al. (2010)	2 dd	20 dd	10 °C; T_{amb} (20 °C)	/	50 kg containers (60 x 30 x 40 cm). Thickness: - monolayer - 10 cm - 20 cm - 60 cm	Oil obtained from *Cornicabra* olives stored at 20 and 10 °C maintains its extra virgin classification for 5 and 8 days, respectively, independent of storage conditions (monolayer, 10 and 20 cm thickness), except for 60 cm thickness. Oils from fruit stored at 20 °C develop greater off-flavor than those from fruit stored at 10 °C. Bitterness is reduced by 50% in the latter.
Yousfi et al. (2012)	4 dd	21 dd	3 °C T_{amb} (18 °C)	/	20 kg plastic boxes	Cold storage at 3 °C delays fruit deterioration and quality parameters of oils compared to ambient conditions. This effect is more apparent when olives are manually harvested. Oils from manually-harvested fruit maintained their extra virgin categorization even after 21 days of storage at 3 °C, compared to 10 days for mechanically-harvested olives.

Table S1 (continued 4/4)

References	Time (min)	Time (max)	Temperature	Controlled atmosphere	Container	Results
Ben et al. (2012)	7 dd	28 dd	5 °C T_{amb}	/	15 kg open plastic containers	Cold storage of olives at 5 °C preserves the quality parameters of produced olive oils for longer. Total phenols, oxidative stability and the radical scavenging capacity of oils decrease when fruits are stored at ambient temperature. Oil loses its extra virgin classification more rapidly for the *Chemlali* than the *Chétoui* cultivar, when olives were stored at ambient temperature.
Jabeur et al. (2015)	3 dd	25 dd	12–18 °C	/	Closed plastic bags (50 x 40 cm); open perforated plastic boxes (50 x 30 x 40 cm)	Fruit stored in open, perforated plastic boxes prevented the alteration that is produced in oils obtained from fruit stored in closed plastic bags. The latter induced hydrolytic and oxidative processes that increased the temperature, and supported fermentation in the fruit mass.
Hachicha Hbaieb et al. (2015)	1 w	4 w	4 °C T_{amb} (24 °C)	/	10 kg plastic containers	A very similar phenolic profile is found for oils extracted from fruit stored at 4 °C, and freshly-harvested fruit. However, oils obtained from fruit stored at 20 °C had the lowest phenolic content. Total phenolic compounds, orthodiphenols and secoiridoid derivatives in extracted oils decreased as fruit storage progressed. This trend was more noticeable at 20 than 4 °C. β-glucosidase activity could influence the oil phenolic profile since its activity decreases after 1 week of storage at 20 °C, which is consistent with a dramatic decrease in phenolic compounds in oils. The oxidative stability of oil decreased when olives were stored at 20 °C.
Hachicha Hbaieb et al. (2016)	1 w	4 w	4 °C T_{amb} (20 °C)	/	10 kg plastic containers	The evolution of C6 LOX compounds in oil obtained during olive ripening and storage is strongly dependent on the cultivar. C6 aldehydes and alcohols were considerably higher in *Arbequina* oils from olives stored at 25 °C than from those stored at 4 °C. On the other hand, storage of *Arbequina* fruit at 25 °C may favor microbial activity, as indicated by higher levels of volatile phenols.

Trial 1

Table S2. Quality chemical parameters of olive oil samples taken from olives stored at cold and ambient temperatures at lab scale. Letters a,b indicate significant differences ($p < 0.05$) obtained using a two-tailed student *t*-test. Significant codes: ns = not significant; . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Storage temperature	FFA [% oleic acid]	PV [meq O_2 kg^{-1}]	K_{232}	K_{270}	ΔK
Ambient (25 °C)	0.58 ± 0.03	4.32 ± 0.36	1.81 ± 0.05 [b]	0.11 ± 0.00 [b]	0.0010 ± 0.0056
Cold (6.5 °C)	0.55 ± 0.02	4.23 ± 0.19	1.61 ± 0.02 [a]	0.10 ± 0.00 [a]	0.0018 ± 0.008
p	*ns*	*ns*	**	**	*ns*

Table S3. List of phenolic compounds in olive oil samples, taken after olive storage at lab scale, that were not found to be significant after the statistical analysis ($p > 0.05$).

Trial 1 - Biophenols
Hydroxytyrosol
Vanillic acid + caffeic acid
Vanillin
Para-coumaric acid
Hydroxytyrosyl acetate
Ferulic acid
Ortho-Coumaric acid
Decarboxymethyl oleuropein aglycone, oxidized dialdehyde form
Decarboxymethyl oleuropein aglycone, dialdehyde form
Oleuropein
Oleuropein aglycone, dialdehyde form
Decarboxymethyl ligstroside aglycone, oxidized dialdehyde form
Decarboxymethyl ligstroside aglycone, dialdehyde form
Pinoresinol, 1 acetoxy-pinoresinol
Ligstroside aglycone, dialdehyde form
Oleuropein aglycone, oxidized aldehyde and hydroxylic form
Luteolin
Oleuropein aglycone, aldehyde and hydroxylic form
Apigenin

Table S4. List of volatile organic compounds (VOCs) in olive oil samples, taken after olive storage at lab scale, that were not found to be significant after the statistical analysis ($p > 0.05$) (left column). VOCs whose concentrations were below the detection limit are reported on the right column.

Trial 1 - Volatile organic compounds		
Not significative VOCs	***Below detection limit VOCs***	
Heptane	Octane	EE-2,4-heptadienal
Methyl acetate	Methanol	Butanoic acid
Ethyl acetate	Methyl propionate	Pentanoic acid
Butanal, 2-methyl	Propanoic acid, ethyl ester	2,4-decadienal
Butanal, 3-methyl	Propanol	Hexanoic acid
3-pentanone	Butanoic acid, ethyl ester	Phenol, 2-methoxy-
Pentanal	Hexanal	
1-penten-3-one	2-pentanol	
1-penten-3-ol	E2-pentenal	
2-heptanone	Heptanal	
Limonene	1-pentanol	
2-heptanol	2-octanone	
E2-hexenyl-acetate	Octanal	
2-octanol	E2-heptenal	
2,4-hexadienal	6-methyl-5-hepten-2-one	
Benzaldehyde	Nonanal	
1-octanol	Z2 + E2-hexen-1-ol	
E2-decenal	E2-octenal	
Nonanol	1-octen-3-ol	
2,4-nonadienal	Acetic acid	
Phenol, 4-ethyl-2-methoxy-	ZE-2,4-heptadienal	
Phenol, 4-ethyl-	Decanal	

Table S5. Dissolved gases in olive fruit heaps at the end of storage. Initial concentrations were: 21.10 ± 0.26 % O_2; 0.03 ± 0.06 % CO_2; and 0.0070 ± 0.00 % EtOh. Letters a,b indicate a significant difference for temperature ($p < 0.05$) found at the ANOVA. RSE column reports the Residual Standard Error of the model. Significant codes: ns = not significant; . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Storage temperature									
	Ambient (25 °C)			Cold (6.5 °C)						
	SVR7-bin	SVR10-halfbin	SVR16-box	SVR7-bin	SVR10-halfbin	SVR16-box	*RSE*	*p T*	*p SVR*	*p int*
O_2 (%)	20.93 [b]	21.13 [b]	21.13 [b]	20.43 [a]	20.43 [a]	20.47 [a]	*0.36*	**	*ns*	*ns*
CO_2 (%)	0.17 [a]	0.03 [a]	0.13 [a]	0.97 [b]	1.00 [b]	0.93 [b]	*0.24*	***	*ns*	*ns*
EtOh (%)	0.0083	0.0070	0.0070	0.0073	0.0073	0.0070	*0.0003*	*ns*	*ns*	*ns*

Table S6. Quality chemical parameters of olive oil samples taken from olives stored at cold and ambient temperatures with different surface/volume ratios at mill scale. Letters a,b indicate a significant difference for temperature ($p < 0.05$) found at the ANOVA. RSE column reports the Residual Standard Error of the model. Significant codes: ns = not significant; . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Storage temperature									
	Ambient (25 °C)			Cold (6.5 °C)						
	SVR7-bin	SVR10-halfbin	SVR16-box	SVR7-bin	SVR10-halfbin	SVR16-box	*RSE*	*p T*	*p SVR*	*p int*
FFA [% oleic acid]	0.51 [b]	0.51 [b]	0.47 [b]	0.45 [a]	0.48 [a]	0.40 [a]	*0.05*	*	*ns*	*ns*
PV [meq O_2 kg^{-1}]	9.30	9.20	8.63	8.57	8.93	8.10	*0.88*	*ns*	*ns*	*ns*
K_{232}	1.78	1.78	1.77	1.76	1.77	1.77	*0.04*	*ns*	*ns*	*ns*
K_{270}	0.14	0.14	0.14	0.14	0.14	0.14	*0.01*	*ns*	*ns*	*ns*
ΔK	0.00	0.00	0.00	0.00	0.00	0.00	*0.00*	*ns*	*ns*	*ns*

Table S7. List of phenolic compounds in olive oil samples, taken after olive storage at mill scale, that were not found to be significant after the statistical analysis ($p > 0.05$).

Trial 2 - Biophenols
Hydroxytyrosol
Tyrosol
Vanillic acid + caffeic acid
Vanillin
Hydroxytyrosyl acetate
Ortho-Coumaric acid
Decarboxymethyl oleuropein aglycone, dialdehyde form
Oleuropein
Oleuropein aglycone, dialdehyde form
Decarboxymethyl ligstroside aglycone, dialdehyde form
Cinnamic acid
Ligstroside aglycone, dialdehyde form
Oleuropein aglycone, oxidized aldehyde and hydroxylic form
Luteolin
Oleuropein aglycone, aldehyde and hydroxylic form
Apigenin
Methyl-luteolin
Ligstroside aglycone, aldehyde and hydroxylic form

Table S8. List of volatile organic compounds in olive oil samples, taken after olive storage at mill scale, that were not found to be significant after the statistical analysis ($p > 0.05$) (left column). VOCs whose concentrations were below the detection limit are reported on the right column.

Trial 2 - Volatile organic compounds			
Not significative VOCs		*Below detection limit VOCs*	
Heptane	E2-hexenal	Octane	6-methyl-5-hepten-2-one
Ethyl acetate	2-heptanol	Methyl propionate	Nonanal
Methanol	Z3-hexen-1-ol	Butanal, 3-methyl	Z2 + E2-hexen-1-ol
2-butanone	2-nonanone	Propanoic acid, ethyl ester	E2-octenal
Butanal, 2-methyl	2-octanol	Propanol	1-octen-3-ol
Ethanol	Propanoic acid	Hexanal	Acetic acid
3-pentanone	Benzaldehyde	2-pentanol	ZE-2,4-heptadienal
2-butanol	1-octanol	E2-pentenal	EE-2,4-heptadienal
1-penten-3-one	E2-decenal	1-penten-3-ol	Butanoic acid
Acetic acid, butyl ester	Nonanol	Heptanal	Pentanoic acid
Butanoic acid, ethyl ester	Phenylethyl alcohol	1-pentanol	2,4-decadienal
Isobutanol	Phenol, 4-ethyl-2-methoxy-	2-octanone	Hexanoic acid
Limonene	Phenol, 4-ethyl-	Octanal	Phenol, 2-methoxy-
Z3-hexenal		E2-heptenal	E2-heptenal

3.3. Article #2 - Effect of facilitated harvesting and fruit cooling on extra virgin olive oil quality

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

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Abstract

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 quality 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 (Servili et al., 2004). Within the above category, chemical and sensory characteristics of oils are very different, especially in terms of phenolic and volatile compounds (Fiorini et al., 2018), 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 (Zanoni, 2014). 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-flavor, namely *fusty*, *musty*, *wine-vinegary* and *rancid* (Morales et al., 2005; Vichi et al., 2009). 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 (García and Yousfi, 2006) 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 (Ferguson, 2006). However, manual harvesting carries the highest costs for the companies, linked to both the labor and the reduction of productivity (Cresti et al., 2009; Mansour et al., 2018; Sperandio et al., 2017). In the last decades, mechanisation of olive harvesting accelerated powerfully leading to the introduction of several harvesting tools and machines (Vieri, 2006), which can nowadays be chosen according to the planting systems and the olive grove size (Miglietta et al., 2019). 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. (2009) 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. (2017) 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 (Gambella et al., 2013; Miglietta et al., 2019; Yousfi et al., 2012), favouring the release of cell liquids and their contact with enzymes, oxygen and microrganisms (Proietti, 2014). 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 (Gambella et al., 2013). 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 (Proietti, 2014; Segovia-Bravo et al., 2011).

Several detrimental effects on the olive oil quality, such as the increase of acidity, the increase of oxidation indexes and the off-flavor 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 (Dag et al., 2012; García et al., 1996a, 1996b, 1994; Morales-Sillero and García, 2014). 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 (Dag et al., 2012; García et al., 1996b, 1994). Moreover, the olive fruit cooling may also have a modulating effect on the composition of the volatile fraction of olive oil (Guerrini et al., 2021), even when it is limited to a pre-crushing thermal conditioning, i.e., without any storage period (Dourou et al., 2020).

However, the effectiveness of refrigeration should be linked to several factors, such as olive cultivar (Piscopo et al., 2018; Plasquy et al., 2021), ripening stage (Hachicha Hbaieb et al., 2016) and health conditions, which are affected by fly infestation rate, microbial contamination and mechanical damage of harvesting methods (Hachicha Hbaieb et al., 2016; Jiménez-Jiménez et al., 2013; Piscopo et al., 2018; Plasquy et al., 2021).

Few works have been carried out to study the combined effect of harvesting method and storage temperature (Table SI in Supplementary Materials). Among these, the work by Yousfi et al. (2012) 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 behavior 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. (2012) 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 (ISMEA, 2012). 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.

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 (Nasini and Proietti, 2014). 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 presence of fruit damage, examining a 100-unit sample. A damaged olive index (*DOI*) was assigned according to the method adopted by Famiani et al. (2020). 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:

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

The maturity index (*MI*) was calculated following the method described by Uceda and Frias (1975) that divides the olives in a 8-point-scale (range from 0 to 7), according to the color 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 (Famiani et al., 2020; Uceda and Frias, 1975) and growers often decided on an early harvest (Dag et al., 2014). 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. (2019b). 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 analyzed for quality parameters according to EU official methods (European Commission, 2013), 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 (International Organization for Standardization, 2016).

Phenolic compounds content were measured according to the International Olive Council (IOC) official method (International Olive Council, 2017b). 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. (2017). 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 (Di Giacinto et al., 2011). 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 (Table SII in Supplementary Materials). 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 (Table SII in Supplementary Materials).

The harvesting method significantly affected the total phenolic compound content (TPC), and the phenolic profile of olive oil samples (Table 1). 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 (Figure 1). The MH method favoured the best preservation of 5 derivatives of secoiridoids, namely hydroxytyrosyl acetate, decarboxymethyl oleuropein aglycone oxidized dialdehyde form, oleuropein aglycone dialdehyde form, oxidized 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 oxidized and oxidized 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. (2007); 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 (Fiorini et al., 2018) was useful to monitor the hydrolytic transformation of olive oil phenolic compounds during storage, due to the release of simple phenols from

secoiridoids compounds (Migliorini et al., 2013a). Both the highest R-Index and the highest Nonox-Ox ratio values occurred in the FH oil samples (Figure 1).

The olive fruit refrigeration improved the amounts of six phenolic compounds, namely vanillic and caffeic acids, decarboxymethyl oleuropein aglycone oxidized dialdehyde form, oleuropein aglycone dialdehyde form, lignans, cinnamic acid and oxidized 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 (Figure 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 the same time (Table 1). However, no significant interactions between the harvesting method and storage temperature were found for all measured phenolic compounds.

Table 1. 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 harvesting**	**Facilitated harvesting**	**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; . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

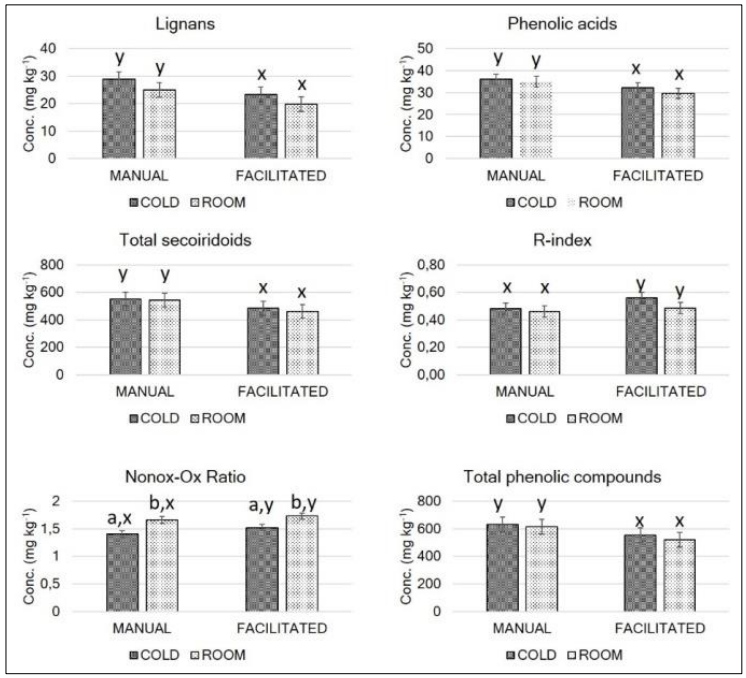

Figure 1. Group of phenolic compounds and indexes of olive oil samples taken from manual and facilitated harvesting and stored at cold (6.5°C) and room (25°C) temperatures for 24 h. Error bars represent the Residual Standard Error of the model. Letters a,b indicate a significant difference ($p < 0.05$)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

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 (Table 2 and Figure 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 (Table 2). 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 (Table 2 and Figure 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 (Figure 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 (Figure 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. (2020a). 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 (Figure 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 (Figure 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 2. 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; . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

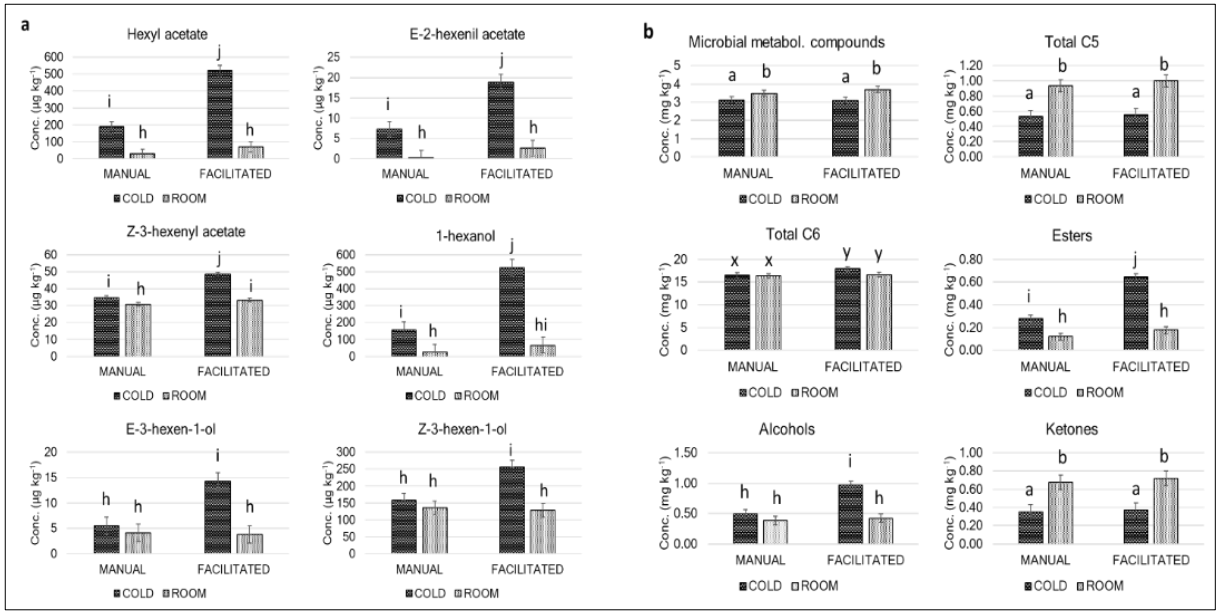

Figure 2. Volatile organic compounds in olive oil samples obtained from manual and facilitated harvesting and stored at cold (6.5°C) and room (25°C) temperatures for 24 h. Results are reported as individual molecules for C6 VOCs (a) and as group of compounds (b). Error bars represent the Residual Standard 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 (Angerosa et al., 1996). Microbial spoilage activities in the olive fruit and warming of the olive masses are the common causes of the degradation phenomena (García et al., 1996a, 1996b; Guerrini et al., 2020a; Miglietta et al., 2019; Vichi et al., 2009) and can be favoured by the fruit softening and leakage of cellular liquid (García et al., 1994; Jiménez-Jiménez et al., 2013), which can be particularly accelerated in mechanically harvested fruit (Yousfi et al., 2012). The mechanisation of the harvesting is spread, especially in the intensive or super-intensive olive orchard, in order to reduce the labor cost and increase the remuneration of companies (Mansour et al., 2018). 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 (ISMEA, 2012; Miglietta et al., 2019; Proietti, 2014; Segovia-Bravo et al., 2011). According to Famiani et al. (2020), 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 (Cresti et al., 2009), 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 quality chemical 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 (Angerosa et al., 1996; D'Imperio et al., 2010; Dag et al., 2008; Guerrini et al., 2020a; Miglietta et al., 2019; Morales-Sillero et al., 2017; Segovia-Bravo et al., 2011), 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. (2020) did not detect changes in the quality chemical 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. (2012), 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 (2014) , 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 (Angerosa et al., 1996; Guerrini et al., 2020a; ISMEA, 2012; Miglietta et al., 2019; Segovia-Bravo et al., 2011). 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 (Fernández-Bolaños et al., 1995), 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. (2017), 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. (2020) also pointed out an increase of volatile content directly proportional to olive damage, since the rupture of fruit tissues releases the LOX enzymes (Angerosa et al., 2004). This was also described in Masella et al. (2019a), 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 (Guerrini et al., 2021), 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 (Guerrini et al., 2021). 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. (2005) and Dourou et al. (2020).

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 (Salas and Sánchez, 1999b), whereas the LOX enzyme has its optimum at 30°C (Ridolfi et al., 2002). 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 (Gardner et al., 1996; Salch et al., 1995). The above hypothesis was related to the study of Morales-Sillero et al. (2017) 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. (2021) 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 (García et al., 1994; García and Yousfi, 2006).

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 labor 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 quality chemical 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 flavor.

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.

Supplementary Materials

Table S1. Main experiments on the effect of harvesting method on olive oil quality.

Reference	Harvesting methods	Storage temperature	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 Tocopherols 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.

Table S1 (continued 1/2)

Reference	Harvesting methods	Storage temperature	Storage time (h=hours, dd=days)	Parameters tested on olive oil	Results
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 Tocopherols 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.

Table S1 (continued 2/2)

Reference	Harvesting methods	Storage temperature	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 Tocopherols 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.

Table S2. Quality chemical 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 harvesting	Facilitated harvesting	Manual harvesting	Facilitated harvesting				
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

4. Olive paste processing: temperature control during malaxation

4.1. Preliminary remark - Article #3

The crushing operation reduces the olives into the olive paste determining a significant changing of the matrix, which from solid phase becomes viscous phase. Through this process, the oil is released from the plant cell vacuole to come into contact with other fruit substrates and with atmospheric oxygen. The three olive fractions, namely solid, water and oil, are mixed, interacting between them through physicochemical and biochemical reactions. Moreover, another process of compound distribution occurs among the phases. Through the latter process, the olive oil begins to enriched of the minor components, such as polar phenolic compounds, tocopherols, chlorophylls, and to develop the aromatic molecules through the lipoxygenase pathway starting from polyunsaturated fatty acids (Angeloni et al., 2022a). On the other hand, during the following stages of the process up to the oil extraction by centrifugation, hydrolytic and oxidative phenomena occur on the olive oil, due to presence of micro-drops of vegetation water (Chaiyasit et al., 2007) and a certain amount of dissolved oxygen (Parenti et al., 2007). The management of the operative parameters has to ensure the control of the reactions, in particular to prevent hydrolytic and oxidative processes that lead to the loss of nutritional value, the development of sensory defects or the alteration of quality parameters. Conversely, if the cause-effect relationships are clear, the optimization of processing setting can lead to the modulation of the olive oil profile (Clodoveo et al., 2014).

The second operation examined in the PhD program was the malaxation, considered the core stage of the extraction process since has the main function of promoting the coalescence of oil droplets into larger drops, easier to be separated by centrifugation, through a slow kneading inside a malaxing chamber at controlled temperature. However, the coalescence process needs time making the malaxation a critical operation for the quality of the product, increasing the oil exposure to the oxygen and the risk of damaging the minor compounds. For these reasons, technological innovations in malaxation aim to improve the extraction efficiency without compromise the quality of the olive oil. Moreover, the optimization of the control of time-temperature relationship, combined to the atmosphere control, is the main goal of the latest machine development in order to manage to govern the chemical and biochemical reactions occurring during the kneading (Clodoveo et al., 2014; Peri, 2014b; Trapani et al., 2017b, 2017a).

However, the temperature parameter is still very hard to control due to the resistance to the heat transfer occurring at the heat exchanger plants, at the wall/ fluid interfaces of the malaxer and inside the olive paste, which is a very viscous fluid (Ayr et al., 2015; Clodoveo, 2012). Moreover, the current design of malaxer is into fully effective in promoting the heat transfer due to the low ratio between the heat transfer surface and volume of olive paste (Tamborrino, 2014).

The methodological study in Article #3 aims to enrich the state of the art on heat transfer during malaxation since a few studies give a clear explanation on the performance of the machines in terms of heat transfer coefficient. In particular, the work aims to make the producers capable to estimate the heat transfer rate in the malaxing plants by means of simple tools and methods, giving them a strong help in the setting and control of the operative parameters, namely the malaxing time and the temperature of olive paste and the service fluid. The more effectiveness temperature control could ensure a better management of the physicochemical reactions that occur in the matrix in order to improve the extraction efficiency and the quality of the olive oil as well.

4.2. Article #3 - A methodological approach to estimate the overall heat transfer coefficient in olive paste malaxers

A methodological approach to estimate the overall heat transfer coefficient in olive paste malaxers

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Abstract

In the olive oil industry, leading-edge technology aims to improve the quality profile of extra virgin olive oil through rigorous control of operative conditions. While effective control of time-temperature conditions appears to be particularly important during malaxation, the low ratio between the heat transfer surface area and the olive paste volume *(A/V)* is problematic, as is the viscosity of olive paste. These factors may result in a failure to reach the desired processing temperature, especially for short malaxation times. Here, we propose a methodological approach to estimate the overall heat transfer coefficient (*U*), and present the results of tests with four types of malaxers, based on heat exchange models under transient and steady state conditions (TSC and SSC models, respectively). We demonstrate similar performance for both models in estimating *U* values in the tested olive paste malaxers. The combined application of the above models is shown to be a suitable way to control heat transfer performance. The critical role of the *U* value is shown with respect to the time needed to reach the desired malaxation temperature, and we suggest that higher *A/V* values may be incorporated into the design of malaxers.

Keywords

Extra virgin olive oil, malaxation, overall heat transfer coefficient, modeling, temperature control, plant innovation

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1. Introduction

Extra virgin olive oil (EVOO) is a fundamental part of the Mediterranean diet thanks to its pleasant sensory attributes and nutritional properties; it can be considered as a functional food (EFSA, 2011a, 2011b; Stark and Madar, 2013). Plant engineering aims to determine the operative conditions (i.e., time, temperature, and oxygen exposure) at each processing step in order to manage quality characteristics (Angeloni et al., 2022a; Clodoveo et al., 2014; Guerrini et al., 2017c). The most recent improvements are focused on the oil's minor compounds, notably phenolic and volatile organic compounds from the lipoxygenase pathway (Bendini et al., 2007; Cecchi et al., 2021; Zanoni, 2014). Process settings have to be carefully chosen, and control devices have to be highly accurate. For example, if, as reported in Guerrini et al. (2021) and Jiménez et al. (2014), it is the case that the right time-temperature combination is essential to promote both positive transformation phenomena and avoid oxidative damage, then heat transfer should be fast, and temperature must be closely controlled.

A key element in EVOO processing is malaxation. This stage, which consists of the slow and continuous kneading of the olive paste, is essential, as several physical, physicochemical and biochemical time-temperature phenomena can occur (Peri, 2014b). Malaxation promotes the coalescence of olive oil droplets, which can then be more easily separated from water and solid fractions through a decrease in the viscosity of olive paste (Tamborrino, 2014). Transformation also occurs in the paste's phenolic and volatile organic compounds; time-temperature conditions and oxygen exposure cause chemical and enzymatic phenomena, which can positively or negatively affect the sensory and nutritional characteristics of the extracted oil (Clodoveo, 2012). A kinetic approach to the time-temperature relationship has been proposed in the literature, with the aim of optimizing and controlling oil yield and phenolic compounds during malaxation (Guerrini et al., 2019; Trapani et al., 2017a, 2017b; Zanoni et al., 2018). Temperature, in particular, should be carefully controlled as it affects the olive oil yield, the rate of enzymatic and non-enzymatic reactions, and the partition coefficient of chemical compounds between water and oily phases (Angerosa et al., 2001; Jiménez et al., 2014; Parenti et al., 2008; Ranalli et al., 2001; Taticchi et al., 2013).

The malaxer typically consists of a closed, horizontal- or vertical-axis tank, equipped with a rotating reel and blades that knead the olive paste. Since malaxation consists of three steps

(filling, holding and emptying), a series of malaxers is required to ensure continuous extraction. The paste temperature is usually controlled through a process fluid (water) which flows through a jacket (Clodoveo, 2012). Therefore, the malaxer can be considered as a heat exchanger under unsteady state conditions; heat is transferred from the water to the olive paste by means of combined convective-conductive-convective phenomena, and heat transfer can be described by a thermal transmittance or overall heat transfer coefficient (Balaji et al., 2020; Singh and Heldman, 2008), which is inversely related to the sum of the individual resistances to heat transfer in series (i.e., internal and external convective layer resistances, conductive resistance of the wall and internal and external fouling resistances at the wall interfaces). Unfortunately, the thermal transmittance of a malaxer is usually low; the ratio between the heat transfer surface and the olive paste volume is small, and heat transfer resistance is high, mainly due to the viscosity of the paste (Balaji et al., 2020; Clodoveo, 2012; Tamborrino, 2014).

Following an empirical approach, the overall heat transfer coefficient can be derived by dimensionless Nusselt, Prandtl and Reynolds numbers, which describe the convective heat transfer rate according to the inertial forces and the physical and thermal characteristics of fluid (Singh and Heldman, 2008). Like in a scraped-surface heat exchanger, both the design and the rotational speed of the stirring shaft affect the axial and rotational Reynolds number and the removal of boundary layer, i.e., the Prandtl number, allowing the convective action, expressed by the Nusselt number, to prevail (Rao and Hartel, 2006; Triki et al., 2021). Thus, an optimal balance between mixing, agitation and scraping of olive paste from the internal wall have to be ensured by the stirring mechanism in order to improve the convective heat transfer, along with preventing the overheating of the layer nearest to the wall, causing heat damage (Tamborrino, 2014).

Since, in recent years, mill engineers have preferred to decrease the malaxation time to improve EVOO quality, the combined effect of low thermal transmittance and short processing time has created considerable problems with respect to temperature control during malaxation.

For example, Leone et al. (2015c) showed that olive paste only reached the planned temperature at the end of the holding phase, and exceeded the desired value during the emptying step. Similarly, Ayr et al. (2015) studied fluid flow and the heat transfer performance

of a malaxer. Their findings showed the temperature distribution inside the olive paste: the lowest temperature was found in an intermediate zone between the shaft and the internal wall, while the highest temperature was found in a zone close to the horizontal axis of the shaft. Various innovations have been proposed to improve thermal control during malaxation. Examples include a dual pipe heat exchanger, placed between the crusher and the malaxer (Amirante et al., 2006; Leone et al., 2015a; Tamborrino et al., 2021b), assisted heat transfer using ultrasound, microwave or pulsed electric fields (Clodoveo, 2013; Leone et al., 2018, 2015c, 2014b; Perone et al., 2021; Tamborrino et al., 2021b, 2019) and improvements to the shape of the malaxer structure and configuration (Amirante et al., 2012; Ayr et al., 2015; Bianchi et al., 2020). However, to date, none of these technical solutions have been widely applied at industrial scale.

The above considerations suggest that measuring the overall heat transfer coefficient would improve the thermal control of malaxers. In addition, better understanding of heat transfer kinetics may aid producers in the setting of the operating parameters, namely the malaxation time and the process fluid temperature, in order to reach the desired temperature of the olive paste during the process. However, this measurement is laborious since it requires the determination of the aforementioned dimensionless convective and conductive numbers and fouling factors, which are linked to both the features of the malaxer and the paste's thermo-physical properties (Qian et al., 2021; Singh and Heldman, 2008). Likewise, the technical specifications of the machines given by the manufacturing companies, do not usually provide any information about the thermal transmittance of malaxer, due to the unwillingness to engage in laborious computations to obtain a coefficient which is highly affectable by the mill conditions. In fact, the overall heat transfer coefficient may change not only according to the mixing mode and the olive paste characteristics, but also to the machine wear over time, which contributes to increase the fouling factor (Singh and Heldman, 2008). The scientific literature never made any attempt to estimate the overall heat transfer coefficient on olive paste malaxers. To our knowledge, only Leone et al. (2015c) reported the calculated value for a prototype microwave-assisted malaxer system, even though, the comparation between coefficient on different machines was not carried out. Moreover, there is still a lack of methods and protocols for the rapid estimation of this coefficient at industrial scale. Therefore, there appears to be a need for a simple methodological approach to estimate the overall heat transfer coefficient. In this work, we propose and test two simple approaches:

(*i*) considering the malaxer as a perfect mixer in which the olive paste temperature is only time dependent (i.e., under transient conditions); and (*ii*) considering the malaxer as a heat exchanger under steady state conditions.

2. Materials and methods

2.1. Theory and governing equations

Determination of the overall heat transfer coefficient was achieved by modelling convective-conductive-convective heat transfer in the malaxer based on the following two theoretical models: (*i*) under transient conditions; and (*ii*) under steady state conditions. These two models are described below.

2.1.1. Transient conditions (the TSC model)

The malaxer was assumed to be a jacketed kettle, where temperature changes as a function of time (Toledo et al., 2018). The model was based on the following three assumptions: (*i*) the malaxer is a perfect mixer in which the olive paste temperature is only time dependent; (*ii*) the fluid temperature in the jacket (i.e., the process fluid) is constant; and (*iii*) heat losses are neglected (Johnson et al., 2016). This model is also described as *lumped parameter*, or reduced, model as it considers a single overall heat transfer coefficient and a homogeneous temperature profile inside the product. Heat transfer was modelled as follows (Singh and Heldman, 2008):

$$\ln\left(\frac{T_m - T_t}{T_m - T_0}\right) = -\frac{AU}{\rho V C p} \cdot t \qquad [1]$$

where T_0 is the initial temperature of the olive paste, T_t is the temperature of the olive paste at malaxation time t, T_m is the temperature of the process fluid ($T_m > T_t$), U is the overall heat transfer coefficient, A is the heat transfer surface area, and V, ρ, and C_p are the volume, density and specific heat of the olive paste, respectively.

Rearranging Equation [1]:

$$\ln(T_m - T_t) = \ln(T_m - T_0) - \frac{AU}{\rho V C p} \cdot t \qquad [2]$$

a linear relationship $ln\ (T_m - T_t)$ as a function of time t is obtained. Since the coefficient U is included in the slope term of Equation [2], U can be derived from the experimental measurement of the paste time-temperature profile in the malaxer, if the above proportionality condition is statistically satisfied.

2.1.2. Steady state conditions (the SSC model)

Here, the malaxer was equated to a heat exchanger under steady state conditions (i.e., a plate or tube heat exchanger) according to Leone et al. (2015c). The model was adjusted with the following three assumptions: (*i*) the product temperature variation between the inlet and the outlet of the heat exchanger was replaced with the olive paste temperature variation between the beginning and end of malaxation; (*ii*) the process fluid temperature is constant; and (*iii*) heat losses are neglected. Therefore, the space-related temperature variation in the original model, i.e., across the heat exchanger length, was replaced by the time-related temperature variation obtained during the malaxation process, assuming it within an infinitely small timeframe. This modelling approach was also previously adopted by Leone et al. (2015c). Following Singh and Heldman (2008), heat transfer in a heat exchanger under steady state conditions was modelled as:

$$\dot{q} = A \cdot U \cdot \overline{\Delta T} \qquad [3]$$

where is $\dot{q}$ the heat flow, *A* is the heat transfer surface area, *U* is the overall heat transfer coefficient, and $\overline{\Delta T}$ is the log mean temperature difference between product and process fluids at the inlet and outlets of the heat exchanger. Equation [3] was adapted to model heat transfer in the malaxer as follows:

$$\dot{q} = \dot{m} \cdot Cp \cdot (T_e - T_b) = A \cdot U \cdot \frac{(T_m - T_b) - (T_m - T_e)}{ln\left(\frac{T_m - T_b}{T_m - T_e}\right)} \qquad [4]$$

where T_b is the temperature of the olive paste at the beginning of malaxation, T_e is the temperature of the olive paste at the end of malaxation, T_m is the temperature of the process fluid, C_p is the specific heat of the olive paste, and $\dot{m}$ is the olive paste mass flow, which was equated to the ratio of the olive paste mass and the malaxation time.

Rearranging Equation [4]:

$$U = \frac{\dot{m} Cp (T_e - T_b)}{A \overline{\Delta T}} \qquad [5]$$

U can then be derived from the experimental measurement of the olive paste temperature at the beginning and end of malaxation.

2.2. Malaxers

A lab scale malaxer prototype, and three types of industrial malaxers (horizontal, vertical, and innovative) were tested.

2.2.1. The lab scale prototype malaxer

A prototype cylindrical jacketed malaxing tank (Bagnoli David, Barberino Tavarnelle, Florence, Italy) was equipped with a rotating shaft that was installed on the top lid and powered by a 24 V electric motor (P205 24.64, Micro Motors, Verderio, Lecco, Italy). The bottom of the tank was closed with a Teflon lid, and the bottom and top lids were sealed using DN100 thread and a locking ferrule. The shaft was equipped with eight blades, rotating at 43 rpm, having a distance from the wall of less than 0.1 cm. The tank jacket was connected to a refrigeration unit (CILLS037PA/404, Rivacold, Vallefoglia, Pesaro e Urbino, Italy) and a thermostatic bath (Kiss 205B, Huber, Offenburg, Germany) providing cooling and heating process fluids (water), respectively. Geometric data are as follows: 0.10 m tank internal diameter, 0.20 m tank height, and 0.01 m jacket thickness. Two probe housings were provided to measure the temperature of the olive paste and the process fluid in the middle of the tank, and in the jacket outlet pipe, respectively. The housing in contact with olive paste was placed at the half of the tank body and at an intermediate depth between wall and shaft. Moreover, a third housing for temperature probe were placed on the top lid for the monitoring of the tank head space. A graphical representation of the prototype is shown in Figure 1. Hence, the plant was specifically designed to ensure a homogeneous olive paste temperature profile, avoiding colder or warmer zone through a high heat transfer surface to volume ratio and an effective mixing and scraping of the internal wall. Aiming to take a representative measurement, a preliminary test was carried out to check the matching between the temperature of the head space and olive paste under processing and right after the malaxer discharge.

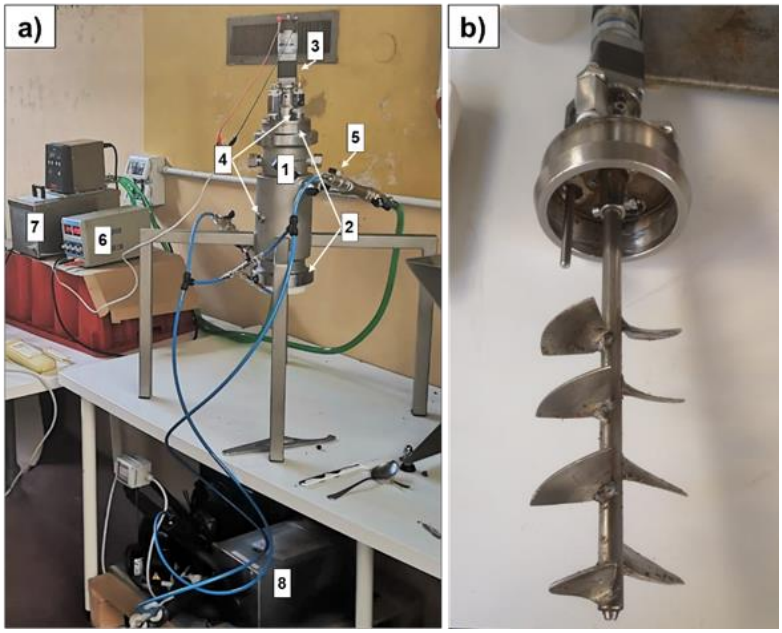

Figure 1. The lab scale prototype malaxer (a) and a close-up of the shaft with blades (b). Numbers indicate the following equipment: 1) the malaxing jacketed tank; 2) top and bottom lids with sealing rings; 3) the electric motor; 4) the olive paste temperature probe housing; 5) the process fluid temperature probe housing; 6) the power supply; 7) the thermostatic bath; 8) the cooling unit.

2.2.2. Industrial malaxers

The following conventional malaxers were used: (*i*) a horizontal malaxer (Malaxer 501, Alfa Laval, Monza, Italy), operating in the Fattoria di Maiano olive oil mill (Fiesole, Florence, Italy); (*ii*) a vertical malaxer (Giorgio Mori Project, Mori-TEM, Barberino Tavarnelle, Florence, Italy), operating in the Azienda Agricola Buonamici olive oil mill (Fiesole, Florence, Italy); and (*iii*) an innovative malaxer (Mori-TEM, Barberino Tavarnelle, Florence, Italy) (Figure 2) installed at Azienda Agricola Bianconi Sara (Bucine, Arezzo, Italy). The latter consists of five cylindrical jacketed malaxing units, which can operate both in series and parallel modes according to the setting of the feeding pump system. The device was designed to ensure continuous malaxation, avoiding the holding phase; it was also designed to facilitate cleaning through a 30° slant that makes it possible to both remove the reel shaft from the top, and to easily reach the internal surfaces with washing equipment. The narrow and elongated shape of the selected malaxing units was designed to improve heat transfer efficiency, by increasing the ratio between the heat transfer surface and the volume of olive paste, achieving a more uniform temperature distribution inside the olive paste mass. All the malaxers were equipped with temperature sensors placed on the lower half of the tank (manufacturing characteristics). This ensured that a well representative value of the average temperature could be measured during the malaxation stage, since the paste in contact with the sensor was constantly renewed by the stirring action.

The main characteristics of three malaxers are shown in Table 1.

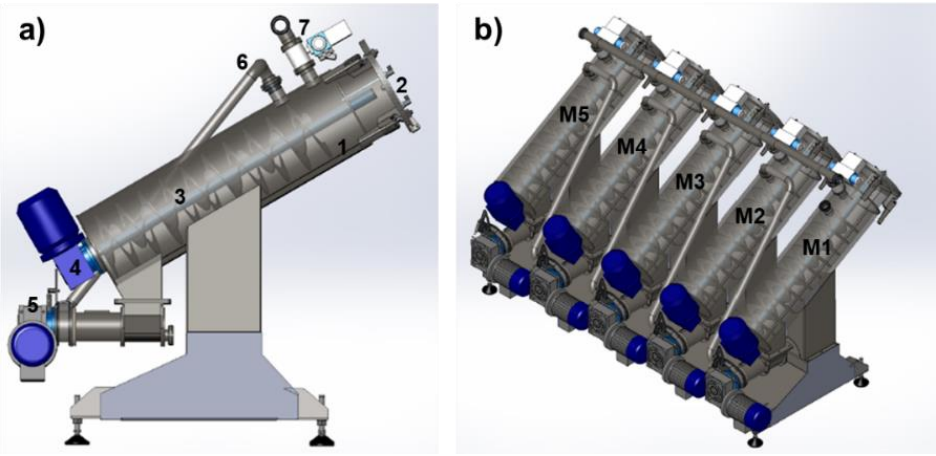

Figure 2. The innovative malaxer. A single unit (a), and the group of five malaxing units (b). M# = the number of the unit in the sequence. Numbers indicate the following details: 1) the jacketed malaxing tank; 2) the sealing lid; 3) the reel shaft; 4) the electric motor; 5) the single unit outlet pump; 6) the olive paste inlet for M2, M3, M4, and M5 in series mode; 7) the olive paste inlet for M1 in series mode and for M1, M2, M3, M4, and M5 in parallel mode.

Table 1. Main characteristics of the industrial malaxers. The heat transfer surface area of each malaxer was determined as the section where heat transfer between olive paste and process fluid occurs.

Malaxer	Malaxing units (n°)	Shape	Mixing	Size (cm)	Heat transfer surface area (m^2)	Maximum capacity (kg)
Horizontal	1	Cradle	Rotating reel	150 Length, 70 Height, 60 Width	2.1	400
Vertical	1	Cylindrical	Rotating reel	80 ID, 120 Height	3.0	500
Innovative	5	Cylindrical (30° slanted)	Rotating reel	31 ID, 137 Height	5.8*	350**

ID = Internal diameter. * 1.2 m2 for each malaxing unit. ** 70 kg for each malaxing unit.

2.3. Experimental trials

Experimental trials were carried out during the 2021 olive harvest. The aim was to apply and compare the mathematical models in Equations [2] and [5] in order to determine the overall heat transfer coefficient of each of the malaxers.

2.3.1. Lab scale trials

Trials were carried out at room temperature (25 °C) and samples were both heated and cooled using the lab scale prototype malaxer. First, 1.2 kg batches of olive oil fruit were crushed by a crusher (Mori-TEM, Barberino Tavarnelle, Florence, Italy), then 1 kg of olive paste was added to the malaxer. During malaxation, olive paste temperature was measured at 60 second intervals using a temperature probe Pt100 (HD2107.1, Delta Ohm, Caselle di Selvazzano, Padova, Italy), which was placed in the malaxer body housing, in contact with the olive paste.

2.3.2. Industrial scale trials

The following measurement criteria were applied during data collection:

- olive paste mass values were consistent with the maximum capacity of the malaxer;
- malaxation time included filling and holding steps;
- malaxation time was determined by the mill's protocol and did not exceed approximately 60 min;
- initial and final temperatures of the olive paste were measured as close as possible to the malaxer;
- to make it easier for mill workers to take temperature measurements, probe and thermal scanner thermometers or machine temperature sensors were used.

In order to measure the temperature of the fluids, i.e., water and olive paste, different strategies were adopted according to the mill abilities. Direct measurement were preferably adopted to take the olive paste temperature, alternatively, the value detected by the internal machine's sensor was taken (if available), since it was well representative of the average temperature of the whole paste volume. In this case, preliminary trials were carried out to validate the consistency between the temperature of the olive paste measured by the machine's sensor and the average temperature. The latter was obtained from multiple temperature measurements taken through probe in different zones of the paste, after

stopping the machine. On the other hand, thermal scanner thermometer was used when fluid temperature was hard to detect by probe. However, since thermal scanners were suitable only for surface measurements, the detected value had to be representative of the average temperature of the fluid. Therefore, the inlet and outlet pipes were chosen as points of measurement, since there, the fluid was concentrated in a reduced volume. Hence, the surface temperature could be assumed to be equal to the internal one. Moreover, before carrying out the measurement the thermal scanner thermometer was previously calibrated on a scale of temperature taken with probe.

Approximately 400 kg of olive paste was used in trials with the horizontal malaxer (HM trials); paste temperature was measured either with a thermal camera (FLIR E50bx, Teledyne FLIR LLC, Wilsonville, OR, USA) or a temperature probe Pt100 (HD2107.1, Delta Ohm, Caselle di Selvazzano, Padova, Italy). In HM trials, the initial temperature of the olive paste was measured at the outlet pipe of the crusher using the thermal camera; this outlet pipe was assumed to be the malaxer's inlet. The final olive paste temperature was directly measured by the temperature probe, by opening the lid at the end of the holding step (i.e., just before the unloading step) and inserting the probe into the core of the paste for a few seconds at a depth of 25 cm.

Approximately 450 kg of olive paste was used in trials with the vertical malaxer (VM trials); paste temperature was measured using the machine's temperature sensor. In VM trials, both initial and final temperatures of the paste were directly measured using the machine's temperature sensor at the beginning of the loading step and immediately before the unloading step, respectively. The above choice was due to an inability to open the machine without stopping the whole process; moreover, in this case, the outlet temperature at the crusher was not comparable to the temperature at the malaxer's inlet, as the two machines were not close to each other.

Trials with the innovative malaxer (IM trials) involved all five malaxing units in series, each with a capacity of 70 kg. Approximately 350 kg of olive paste was used in each replicate. The olive paste temperature was taken using the machine's temperature sensor, and the process fluid temperature was measured using a thermal camera (FLIR E50bx, Teledyne FLIR LLC, Wilsonville, OR, USA). Obtaining olive paste temperature measurements was difficult due to the complexity of the in-series continuous system, where filling and emptying operations

were controlled by a filling level sensor installed on each unit. Therefore, since no clear time-temperature relationship could be identified within an individual unit, the whole system was considered as a single continuous compartment (Comba et al., 2011), with a mass flow of 0.23 kg s^{-1} (i.e., 840 kg h^{-1} crusher throughput). Initial and final temperatures of the olive paste were directly measured by the machine's temperature sensor at the beginning of the loading step and immediately before the unloading step, respectively.

Process fluid temperatures in all the malaxers were measured at inlet and outlet jacket pipes during malaxation using a thermal camera (FLIR E50bx, Teledyne FLIR LLC, Wilsonville, OR, USA), and the average value was used in the application of the models.

All the above industrial scale trials were carried out at ambient temperature (19 ± 3 °C).

2.4. Olive paste specific heat and density

The mixture calorimeter method (Plester et al., 1956) was used to measure olive paste specific heat (C_p). An exactly-weighted mass of olive paste (200 kg) at ambient temperature (15–20 °C) was added to an exactly weighted mass of hot water (700 kg at 40–50 °C) in a custom-made calorimeter. C_p was determined as follows:

$$C_p = \frac{Q_{op}}{m_{op}(T_{eq} - T_i)} \quad [6]$$

where m_{op} is the mass of olive paste, T_i is the initial temperature of the olive paste, T_{eq} is the temperature of the olive paste at thermal equilibrium with water, and Q_{op} is the heat acquired by the olive paste after thermal equilibrium is reached. The latter corresponds to the equivalent heat released by the hot water, calculated through the multiplication of the specific heat of water (4.187 kJ kg^{-1} $°C^{-1}$) by the water mass and temperature gradient, i.e., the difference between the initial temperature and the final temperature at the thermal equilibrium.

Moreover, in the TSC model the mass term is expressed as density by volume, which are commonly explicitly reported in the governing equations from the literature, e.g. Equation [1]. However, since the mass of olive paste was known from its exact weighing, the calculation of the real value of olive paste density and volume was avoidable. Thus, the olive paste density was simply chosen according to values reported in the literature (Perone et al., 2021), whilst the olive paste volume was derived from the ratio between the mass and the density.

2.5. Data processing

For lab scale trials, a linear model was built to estimate the slope and intercept of the TSC model. The obtained coefficients were tested with an ANOVA. *U* values determined by TSC and SSC models were compared using a *t*-test (threshold p=0.05). In industrial scale trials, *U* values for the different malaxers were compared with a one-way ANOVA, where the independent factor was the type of malaxer. The significance threshold was set at p<0.05, and the Tukey HSD *post hoc* test was applied to compare the different type of malaxers.

3. Results and discussion

3.1. Comparison of heat exchanger models

Lab scale trials were carried out by both heating and cooling olive paste samples in the prototype malaxer. In the heating trials, olive paste samples were heated in triplicate from approx. 22 °C to approx. 38 °C, while the process fluid temperature was held at 40 °C (Figure 3a). In the cooling trials, olive paste samples were cooled twice, from approx. 39 °C to approx. 8 °C, while the process fluid temperature was held at 3 °C (Figure 3b). TSC and SSC models were compared based on the above experimental temperature profiles; fixed malaxation parameters were used, as shown in Table 2.

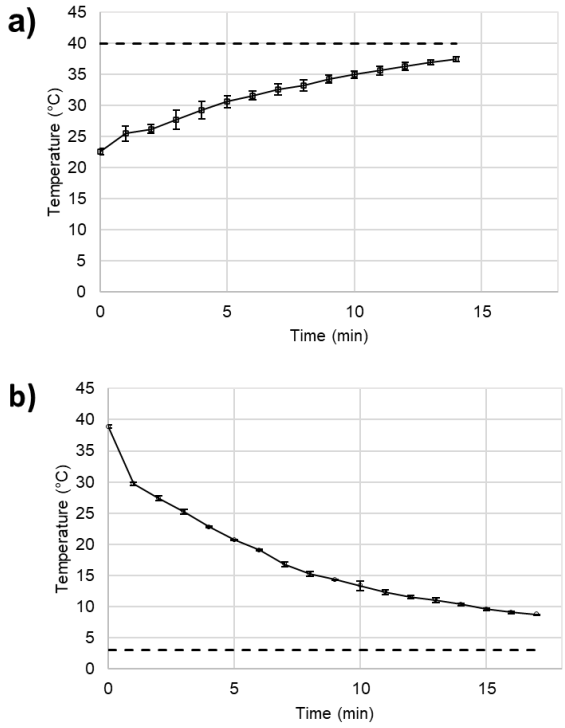

Figure 3. Temperature ramps during malaxation: a) heating mode with olive paste at room temperature; b) cooling mode with pre-heated olive paste. Solid lines describe olive paste temperatures, and dashed lines show the temperature of the process fluid. Error bars represent the standard deviation.

Table 2. Parameters used in lab scale trials.

Parameter	Value	Method/Reference
Heat transfer surface A (m^2)	0.0628	Determined from the internal cylindrical surface of the malaxing jacket (0.1 m internal diameter, 0.2 m height)
Process fluid temperature T_m (°C)	Heating trials: 40.0 Cooling trials: 3.0	Temperature probe
Olive paste mass (kg)	1	-
Olive paste density ρ (kg m^{-3})	1100	Data reported in the literature (Perone et al., 2021)
Olive paste volume V (m^3)	0.0009	The ratio of the olive paste mass and its density
Olive paste specific heat Cp (kJ kg^{-1} $°C^{-1}$)	3.15 ± 0.13	The mixture calorimeter method (Plester et al., 1956).

In heating trials, linear relationships (R^2 = 0.99) were obtained by applying Equation [2] to temperature profiles (Figure 4a). The TSC model predicted a value of approx. 110 ± 8 W m^{-2} $°C^{-1}$ for the overall heat transfer coefficient *U* (Table 3) using the following equation:

$$U = \frac{s\rho VCp}{A} \quad [7]$$

where *s* is the slope of the linear relationship. Then, Equation [5] was applied to determine *U* based on the SSC model. This gave a value of approx. 115 ± 11 W m^{-2} $°C^{-1}$ based on data given in Table 3. The difference between these two values was not significant (p=0.63); in both models there was a logarithmic difference between olive paste and process fluid temperatures as a function of time in the applied experimental conditions.

Similar results were found for cooling trials. Linear relationships (R^2 = 0.99) were obtained by applying Equation [2] to the temperature profiles (Figure 4b). An approximate value for *U* of 86 ± 2 W m^{-2} $°C^{-1}$ was determined by the TSC model (Table 3) and a value of 90 ± 1 W m^{-2} $°C^{-1}$ was determined by the SSC model also based on data given in Table 3. The *U* value in cooling trials was less than in heating trials due to a decrease in the olive paste internal convective heat transfer term at low temperature (Singh and Heldman, 2008).

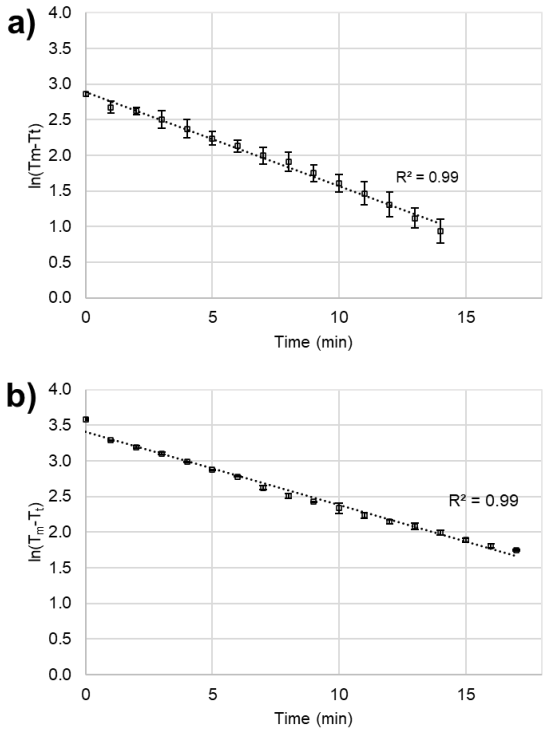

Figure 4. Linear relationship of the logarithmic difference between olive paste and process fluid temperatures applying the TSC model to temperature ramps during malaxation in heating mode a), and cooling mode b). Error bars represent the standard deviation.

Table 3. Processing parameters and the overall heat transfer value *U* of olive paste in lab scale trials, applying TSC and SSC models.

	Lab scale trials			
	Heating trials		Cooling trials	
Olive paste parameters	***TSC model***	***SSC model***	***TSC model***	***SSC model***
Measured initial temperature T_0, T_b (°C)	22.6 ± 0.5	22.4 ± 0.6	38.9 ± 0.3	38.9 ± 0.3
Measured final temperature T_e (°C)	37.4 ± 0.4	37.4 ± 0.4	8.8 ± 0.1	8.8 ± 0.1
Mass *m* (kg)	1	1	1	1
Malaxation time (min)	14	14	17	17
Log mean temperature difference $\overline{\Delta T}$ (°C)	-	7.7 ± 0.3	-	16.5 ± 0.1
Correlation coefficient of the linear relationship R^2	0.99	-	0.99	-
Overall heat transfer coefficient *U* (W m^{-2} C^{-1})	110 ± 8	115 ± 11	86 ± 2	90 ± 1

3.2. Overall heat transfer coefficient of industrial malaxers

Only the SSC model was used to estimate *U* values in industrial malaxers, as no difference was found between TSC and SSC models, and it was easier to obtain the required measurements in the SSC model than the TSC model at olive mill scale. Table 4 shows mean values of measured and calculated data in the seven trials carried out in HM and VM machines, using similar initial (20 °C) and final (24 °C) olive paste temperatures. Table 4 also shows mean values of measured and calculated data in the three trials carried out with the IM (17 °C and 27 °C for initial and final temperatures, respectively).

Mean *U* was significantly higher in the HM (104 ± 17 W m^{-2} $°C^{-1}$) than the VM machine, which was less efficient by approx. 40% (70 ± 16 W m^{-2} $°C^{-1}$). The above *U* values are consistent with theoretical principles of heat transfer in a heat exchanger (Singh and Heldman, 2008; Toledo et al., 2018). Low *U* values can be partially compensated for by a reduction in heat flow thanks to an increase in surface area; consistently, identical values of heat flow per temperature gradient unit (i.e., *U x A* values shown in Table 4) were determined for the two malaxers thanks to an increase in *A* values: from 2.1 m^2 in the HM to 3 m^2 in the VM. However, it is known that low *U* values maximize the temperature at the contact surface between liquids and solids, increasing the risk of heat damage at the malaxer wall. In principle, this can be avoided by lowering the process fluid temperature, but this decreases the log mean temperature difference, reduces heat flow, and increases residence time. Our experimental data are consistent with the above statement (Table 4); for approximately the same increase in olive paste temperature, the VM had the lowest process fluid temperature (28 °C in VM trials *vs* 38 °C in HM trials), the lowest log mean temperature difference (5 °C in VM trials *vs* 16 °C in HM trials), the lowest heat flow (1080 W in VM trials *vs* 3485 W in HM trials) and the longest residence malaxation time (53 min in VM trials *vs* 27 min in HM trials).

Mean *U* was highest for the IM (182 ± 14 W m^{-2} $°C^{-1}$); the HM was less efficient by approximately 75%. Since the overall heat transfer area of the IM was also high (5.8 m^2), there was high heat flow per temperature gradient unit (1056 W $°C^{-1}$). Therefore, heat transfer performance was better in the IM compared to the HM and VM machines in two ways: (*i*) although malaxation time was similar to that of the HM (approx. 25 min), it corresponded to the highest olive paste temperature gradient (9.6 °C in IM trials *vs* 4 °C in HM trials); and (*ii*) the above temperature gradient was obtained by process fluid temperatures that were

similar to those of the VM (approx. 30 °C), reducing the risk of heat damage at the malaxer wall.

Although a thermo-physical explanation of the above *U* values is beyond the scope of this work, they may be related to the technical characteristics of the tested malaxers. For example, *U* values may be low in the VM because the mixing apparatus is typically designed to avoid the vertical transfer of olive paste to protect it from oxidative phenomena (Migliorini et al., 2009). In contrast, the narrow, elongated shape of the units used in the IM could have improved mixing, resulting in the high *U* values reported here.

Table 4. Mean values of measured and calculated data in trials using industrial malaxers.

	Industrial scale trials		
	Horizontal malaxer	**Vertical malaxer**	**Innovative malaxer**
Fixed and measured parameters			
Heat transfer surface area (m^2)	2.1	3	5.8
Olive paste mass (kg)	413 ± 24	469 ± 28	350
Olive paste volume (m^3)	0.375	0.426	0.32
Malaxation time (min)	27 ± 3	53 ± 9	25
Olive paste specific heat (J kg^{-1} K^{-1})	3150	3150	3150
Process fluid temperature (°C)	38 ± 2	28 ± 1	30 ± 1
Initial temperature of olive paste (°C)	20.1 ± 0.8	21.5 ± 0.8	17.1 ± 0.4
Final temperature of olive paste (°C)	25 ± 1	23.9 ± 0.6	26.7 ± 0.1
Calculated parameters			
Olive paste temperature gradient (°C)	4 ± 1	2.4 ± 0.9	9.6 ± 0.5
Mass flow (kg s^{-1})	0.25 ± 0.03	0.15 ± 0.03	0.23
Heat flow (J/s)	3485 ± 693	1080 ± 285	7076 ± 375
Log mean temperature difference (°C)	16 ± 2	5 ± 1	7 ± 1
Heat transfer coefficients			
U (W m^{-2} $°C^{-1}$)	104 ± 17	70 ± 16	182 ± 14
U x A (W $°C^{-1}$)	219 ± 36	212 ± 47	1056 ± 80

3.3. Examples of application

Various applications can be derived from the above modelling approach to characterize the heat transfer performance of malaxers. First, malaxation time could be estimated based on combined SSC and TSC models. Once the *U* value has been determined by the SSC model, the TSC model can be applied to estimate the time (*t*) to reach the desired malaxation temperature (T_t) given the initial olive paste temperature (T_o), using the following relationship:

$$t = ln\ \frac{(T_m - T_o)}{(T_m - T_t)} \cdot \frac{\rho V Cp}{AU} \qquad [8]$$

The effect of *U* on time *t* can be calculated. For example, applying Equation [8] for the same *A* and *V* values (2.1 m^2 and 0.375 m^3), and the olive paste density and specific heat values given in Table 2, *U* values determined in this work indicate considerable variation in malaxation time: 18, 32 and 48 min are necessary to reach a malaxation temperature of 25 °C starting from 20 °C (T_m = 38 °C) for 182, 104 and 70 W m^{-2} $°C^{-1}$ *U* values, respectively.

A second application makes it possible to quantify the effect of combining *U* values and the heat transfer surface area with the olive paste volume ratio (*A/V*) and malaxer performance. It seems useful to evaluate the effect on the process fluid temperature, in order to minimize heat damage to olive paste at the wall. Once *U* has been determined using the SSC model, the TSC model can be applied to estimate the process fluid temperature (T_m) needed to reach the desired malaxation temperature (T_t) for a desired time (*t*), using the following relationship:

$$T_m = \frac{T_t - T_o \cdot \exp\left(-\frac{A}{V}\frac{U}{\rho Cp}t\right)}{1 - \exp\left(-\frac{A}{V}\frac{U}{\rho Cp}t\right)} \qquad [9]$$

The effect of *A/V* on the process fluid temperature can be deduced. Although low *U* values can be compensated for by an increase in the heat transfer surface area (*A*), the risk of heat damage at the wall is high, as noted in section 3.2. Instead, if the *A/V* value is considered, T_m decreases as *A/V* increases for the same malaxation time. For example, applying Equation [9] with the lowest *U* value determined in this work (70 W m^{-2} $°C^{-1}$), an increase in *A/V* from 6 to 18 m^{-1} (the lowest and highest *A/V* values applied in this work, respectively, see Table 4) causes a variation in T_m from 50 to 32 °C, assuming a malaxation time equal to 25 min, and initial and final olive paste temperatures equal to 20 and 25 °C, respectively. Consistently,

combining high U values and high A/V values gives the lowest T_m values: 182 W m^{-2} $°C^{-1}$ and 18 m^{-1}, resulting in 27 °C T_m if the above operating conditions are applied.

4. Conclusions

This work was motivated by the need to deepen the malaxation from a temperature control point of view, since the olive paste characteristics, the current machine design and the processing practices, may create considerable problems to the heat transfer. In the mill scenario, producers lack the technical tools to know the heat transfer kinetics inside the olive paste and they can't easily predict the optimal operating parameters, mainly malaxation time and process fluid temperature. In addition, plant manufacturers do not provide information on heat transfer coefficients of the malaxers as they require laborious computations. Despite several solutions for the improvement of the heat transfer on malaxation has been proposed by the research on olive oil plant engineering, only few Authors quantified the results in terms of *U* coefficient. Furthermore, a simple method to apply in the mill for the evaluation of *U* was lacking in the literature.

The lab-scale trials demonstrated that TSC and SSC models are equally effective in estimating *U* values in olive paste malaxers, and the usefulness of their combined application. Thus, we propose a relatively simple methodological approach, based on the application of the SSC model and the measurement of initial and final olive paste temperatures to determine *U* values in different types of malaxers. The above approach was shown to be suitable to control heat transfer performance in the tested malaxers (for use by both plant manufactures and olive oil mill engineers). Estimates of the *U* value may be the first step in characterizing a new industrial malaxer design.

Our work confirms data reported in the literature regarding the poor heat exchange efficiency of malaxers. It demonstrates that *U* values may be improved by changing the design of equipment, which is shown to be the cause of significant variation in the time needed to reach the desired malaxation temperature. We also show the important role of *A/V* values; malaxers could be designed to increase the *A/V* value, and minimize heat damage to olive paste at the wall. The ability to estimate *U* values also allows mill engineers to estimate operating conditions, such as the time needed to reach the desired malaxation temperature, the time-temperature kinetics of olive paste during malaxation, and the process fluid temperature needed to reach a desired malaxation temperature for a desired malaxation time.

5. Olive oil extraction through decanter centrifuge: a yield and quality issue

5.1. Preliminary remark - Article #4

The third part of PhD thesis is focused on the last stage of olive paste processing, i.e., the olive oil extraction by centrifugation. In particular, the Article #4 examines the first centrifugation stage from which the oil must is obtained. The latter needs a further clarification treatment through vertical centrifuge or filtration in order to remove the residual water and solid fractions.

The decanter machine consists in a hollow cochlea rotating inside a cylindrical conical bowl for the separation of the three olive oil fractions, i.e., vegetation water, solids and oil, by centrifugal force. The differential speed between cochlea and drum results in the expulsion of the pomace to one end, while the lighter liquid phases are recovered from the other end by means of overflow levels, which can only be set by opening the machine drum (Baccioni and Peri, 2014).

Depending on how the olive paste fractions are separated, two main types of decanter machines exist: i) three-phase decanter, from which pomace olives (about 50% of water content), vegetation water and oil must are obtained, here a water addition is needed to dilute the incoming olive paste; ii) two-phase decanter, in which the olive paste components are separated as high moisture pomace (about 60% of water content) and oil (Amirante et al., 2010).

Although the decanter is clearly a continuous-working machine, often, in the olive oil mill batch processing is adopted.

The experimental study in Article #4 aims to investigate yield and quality issues due to the batch processing.

Sometimes the olive oil mill companies are equipped with two production lines, thus, they can keep two different batches of olive separated during their processing.

When different olive batches are processed in the same extraction plant, the producers have to keep them separated by time and space, in order to avoid the cross-batch contamination.

While malaxation is a discontinuous processing, thus two different batches can be managed kneading them in two separated malaxer, placed in parallel, the centrifugal extraction is a continuous operations carried out through the same machine, i.e., the decanter.

To our knowledge, the cross-batch contamination at the decanter centrifuge was never studied in literature. Thus, it was never measured and quantified in terms of the degree of contamination of the oil batch. Moreover, monitoring technologies able to detect the batch change point at the decanter, has never been implemented.

Thus, the present work tries to give a solution to the oil contamination between different batches through the identification of the change point between two consecutive olive batches. This could help to avoid several issues linked to the processing of defective olive batches, which represent a risk for the next processed batch, or monovarietal olives, the latter have also to face authenticity issues.

5.2. Article #4 - Contamination between olive batches in a continuous horizontal centrifuge during virgin olive oil production

Cross-batch contamination in a continuous horizontal decanter centrifuge during virgin olive oil production

Lorenzo Guerrini, Ferdinando Corti *, Piernicola Masella, Luca Calamai, Giulia Angeloni, Agnese Spadi, Bruno Zanoni, Alessandro Parenti

Abstract

Cross-batch contamination in a decanter centrifuge during virgin olive oil production cannot be avoided using current technology. The extent of this contamination is investigated using industrial-scale tests, by measuring the volatile profile and color on three consecutive oil batches, collected at the decanter outlet at different extraction times. The extent of contamination varied, pointing out qualitative consequences, as defective molecules are found. The latter are often active at low concentrations, and the measured cross-batch contamination can lead both to the downgrading of large batches of virgin olive oils and to the adulteration of monovarietal and certified productions. An innovative method, based on the direct determination of the color (*L* and *a** coordinates) of oil at the outlet of the decanter is able to identify the same compositional change point indicated by gas chromatography, and could be successfully used to mitigate the effects of cross-batch contamination.

Practical applications: an in-line colorimetric system could be implemented at the decanter outlet to detect the point of change between different olive batches. Otherwise, the virgin olive oil exiting from the decanter at the beginning of one batch can be collected separately in order to avoid the contamination due to the previous batch.

Keywords

Change points, decanter, olive oil quality, volatile compounds

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1. Introduction

Modern virgin olive oil production is continuous, and involves several steps. The first is the harvest, which can be mechanical, manual or facilitated (Corti et al., 2022). After harvest, olive fruit are cleaned and their leaves are removed. Then, they are crushed to produce a paste. Next, the paste is malaxed to improve the size of the oil drops, and enhance the centrifugal extraction of virgin olive oil (Trapani et al., 2017a). Malaxers can be open, closed or sealed, and vertical or horizontal. In all cases, they are made of stainless steel and equipped with a thermostatic jacket to control the temperature of the paste. During crushing and malaxation, several chemical and enzymatic phenomena occur that dramatically change the virgin olive oil quality profile (Catania et al., 2016; Trapani et al., 2017a; Veneziani et al., 2021).

After malaxation, the oil is separated from water and pomace with a horizontal continuous centrifuge, called a decanter. Decanters significantly improve the operative capacity of olive mills, and their qualitative effects have been widely studied. In general, they can be divided into two-phase (oil is separated from a water/pomace mixture) and three-phase (oil, water and pomace are all separated) systems (Baccioni and Peri, 2014). However, intermediate solutions have been proposed, and successfully introduced into olive mills (Altieri et al., 2013; Leone et al., 2015b). From a qualitative perspective, there is a widespread consensus that the two-phase decanter has less detrimental impact on the phenol fraction (Caponio et al., 2014; Di Giovacchino et al., 2001; Jiménez Marquez et al., 1995; Kalogeropoulos et al., 2014).

After decanting, the oil usually undergoes a second centrifugal separation, this time with a vertical centrifuge, to further separate it from any residual water and suspended solids. However, vertical centrifuges are known to dissolve oxygen into the oil, causing phenol depletion and a shorter shelf-life (Guerrini et al., 2017a). Two solutions have been proposed to overcome this problem: an inert gas coupled with the vertical centrifuge (Angeloni et al., 2022b); and in-line filtration with a decanter (Fortini et al., 2016).

During extraction, discontinuous malaxers and a continuous centrifuge (i.e., the decanter) are used one after the other. The decanter is continuously fed using several malaxers that work in parallel, with alternating loading, working and unloading cycles. The fact that malaxers can work in parallel has significant operational benefits for the mill, and almost all facilities have adopted this technique. Although new innovations have tried to improve the workflow, by,

for example, removing the need for the holding phase when the paste is in the malaxer (Tamborrino et al., 2011), continuous solutions are still not widely used.

A second problem for mills is that they process batches from different olive producers, and the virgin olive oil from a specific batch must be returned to the owner of the fruit. Despite efforts by mill owners to keep batches separate (in both space and time) during processing, the design of the system forces a certain degree of mixing. Depending on the capacity of the decanter and its overflow settings, a certain amount of separated oil from aqueous and solid phases remains in the machine when a new batch of paste enters to be processed.

Extraction efficiency can be improved by optimizing the balance between the mass flow rate of the paste at the decanter inlet and the differential speed between the cochlea and the drum, and/or by adjusting the overflow level (Tamborrino et al., 2015). However, the latter can only be done by stopping the machine and opening the casing that covers the drum, which wastes both time and labor. In practice, it is impossible to avoid leaving a certain amount of oil residue in the decanter at the end of each processed batch. Furthermore, there is always a certain delay between the output of paste at the end of the malaxation stage, and the output of oil from the decanter. It is therefore very difficult to determine the exact moment that processing of one batch ends and the next batch begins and keeping them separate.

This phenomenon raises both quantitative and qualitative issues: i) returning the exact amount of oil contained in a certain batch of olives to the producer is not possible; and ii) it is not possible to ensure that the qualitative features of the virgin olive oil are directly due to the characteristics of the olive fruit and the process settings adopted for a specific batch. In practice, the latter can change depending on the initial mass of olives, their oil content and the extraction yield.

Against this background, the aims of the present work are; i) to study how different olive batches contaminate each other inside the decanter; and ii) to set up a control system to detect the batch change.

2. Materials and methods

2.1. Trials

Trials were carried out in November 2020, and hosted by the cooperative olive mill at Azienda Agricola Le Mura (Bucine – AR, Italy). In order to simulate the actual working conditions of a cooperative olive mill, four olive batches were taken from three different growers (namely, Le Muricce, Le Mura, Petrolo, and Il Sole Verde), which own orchards located in different areas of the Bucine (AR - Italy) municipality.

Batches were processed as follows: 500 kg of olives were washed, and leaves were removed. Then, fruits were crushed with a hammer crusher (MORI-TEM Srl, Barberino Tavarnelle - FI, Italy). The obtained paste was malaxed at 27 °C for 25 min through a malaxer group of five units working in series (MORI-TEM Srl, Barberino Tavarnelle - FI, Italy), before feeding the horizontal centrifuge at a flow rate of 840 kg/h. The centrifuge was a two-phase decanter (TL 1000, MORI-TEM Srl, Barberino Tavarnelle – FI, Italy), with nominal productivity of 900 kg/h. The decanter worked at 3500 rpm, with a differential speed between the drum and cochlea of 19 rpm.

The first batch was taken as the starting batch, the other batches were considered as the three replicate trials. For each replicate, eleven virgin olive oil samples (50 ml each) were collected at the exit of the decanter in two separate vials. The first sample was collected at the end of the previous batch of processed olives (i.e., immediately after the 5^{th} malaxing unit was unloaded) in order to sample the oil fraction closest to the following batch. The remaining 10 samples were collected after 1, 3, 6, 10, 15, 20, 25, 30, 35 and 40 L of oil had been produced from a specific batch. One of the vials was immediately frozen, and stored at −18 °C before determination of its volatile compounds. The other vial was maintained at ambient temperature and used for color determination.

2.2. Volatile organic compound determination

Volatile organic compound (VOC) profiles were obtained through solid-phase microextraction of the headspace sampling coupled with gas chromatography and mass spectrometry (HS-SPME-GC-MS) analysis. The multiple internal standard normalization method (Fortini et al., 2017) was used for the quantification of analytes, starting from a stock standard solution of 71 analytes in refined oil, which was diluted for six calibration scale levels. Compounds and

concentration ranges were chosen according to Italian virgin olive oil characteristics (Di Giacinto et al., 2011).

According to the aforementioned method, the internal standard (ISTD) consisted of a mixture of 11 molecules dissolved in refined olive oil, with a final concentration of 75 mg kg^{-1} for each ISTD. ISTDs were chosen in order to be representative of different molecular masses and different VOC classes, mainly alcohols, aldehydes, ketones, esters, carboxylic acids, and hydrocarbons. To facilitate peak isolation, ISTD molecules were either deuterium-labelled, or found to be absent and therefore unable to interfere with other compounds (namely 3,4-dimethylphenol, 4-methyl-2-pentanol, hexanoic acid-d11, 1-butanol-d10, ethyl acetate-d8, toluene-d8, ethyl hexanoated11, acetic acid 2,2,2-d3, 6-chloro-2-hexanone, 3-octanone and trimethyl acetaldehyde).

Then, 0.1 g of ISTD mix was added to a 4.3 g sample up to the calibration level, in a 20 ml vial, which was sealed with an open hole screw cap and a PTFE/silicone septa. The HS-SPME-GC-MS analysis was carried out using a 50/30 µm DVB/CAR/PDMS SPME fiber (Supelco, St. Louis, USA) for the extraction of VOCs, and a Trace GC-MS Thermo Fisher Scientific equipped with a ZB-FFAP capillary column (Zebron) (30 m × 0.25 mm ID, 0.25 µm DF) for their identification.

After 5 min equilibrium at 60 °C, the fiber was exposed for 20 min in the vial headspace under orbital shaking at 500 rpm. Then it was immediately desorbed for 2 min in the GC injection port operating in splitless mode at 260 °C. Compounds were identified by comparing their mass spectra and retention times with those of the ISTD mix analytes. Compound quantification was carried out by plotting the compound peak over the relative ISTD peak (area ratio) against the ratio of compound concentrations (amount ratio), in order to normalize analyte calibration concentrations with respect to the concentration of their respective ISTD, as described in the official method.

2.3. Color measurement

All of the samples obtained from the processing of the three olive batches were analyzed for CIELab coordinates using the following method. A transparent plastic cuvette (polystyrol/ polystyrene, 10 x 10 x 45 mm, Sarstedt, Nümbrecht, Germany) filled with the sample was placed against a white background. Then, a picture was taken in the presence of light, keeping a fixed distance (approx. 30 cm) between the camera and the cuvette. ImageJ software (Version 1.53e) was used to analyze the picture for L^*, a^* and b^* values using the ColorInspector 3D Plugin.

2.4. Data processing

Data processing was performed with the aim of identifying the time of change between 2 consecutive batches. The change point was consequently defined as the number of L of virgin olive oil eluted from decanter with the highest probability of being the bound between 2 contiguous batches. Cross-contamination of batches was assessed with a Bayesian analysis using a Markov chain Monte Carlo method to find change points in a data series, as described by Barry and Hartigan (1993). In order to apply this method, we made two hypotheses; i) we assumed the existence of an unknown partition between data in two blocks, and ii) that the mean was constant within each block. Our first hypothesis (the search for a change point between two batches of olives) was considered to be verified if there was a change in at least one of the measured parameters (otherwise the two batches would be identical with respect to their VOC composition and color). Our second hypothesis seemed reasonable since our two blocks corresponded to two different batches.

Thus, we assumed that for each measured parameter the change in the mean would be lower within the same batch than between two different batches. Finally, the algorithm assessed, for each sampling point and for each measured parameter, the probability of it being a change point between the two means. Overall, the aim of the analysis was to identify, for each replicate, the moment when the composition changed between one batch and the following batch.

3. Results and discussion

Measurements at the outlet of the decanter showed that the composition of the virgin olive oil changed between the start and the end of centrifugal extraction in all three replicates. Beginning with replicates 2 and 3, a statistically significant change point was identified for 15 of the measured VOC. This was also true for 16 VOC in replicate 1. For the remaining VOC, similar concentrations were found in the processed and preceding batches, and no point of change was detected.

3.1. Change in concentration of a "model" compound between batches

In order to illustrate what happens inside the decanter as the batch changes, Figure 1 reports the change in (*E*)-2-hexenal found in replicate 1. It should be noted that the curve is representative of all of the significant reported VOC, but (*E*)-2-hexenal was chosen as it is recognised as making an important contribution to the fruity flavor in virgin olive oil (Guerrini et al., 2017c). The curve starts when paste from the first batch exits the malaxer, and the decanter begins to be fed with paste from the following batch. In this replicate, in this condition, the concentration of (*E*)-2-hexenal is around 12 mg/kg, and the centrifuge is full of paste from the first batch of olives. After 10 L of oil has been produced, the (*E*)-2-hexenal concentration starts to increase almost linearly, reaching a value of around 16 mg/kg after 30 L, and remaining stable until the end of the extraction. The increase in (*E*)-2-hexenal between 10 L and 30 L reveals contamination between the two batches, as the oil has an intermediate composition. The transition begins when there is about 20 L of oil in the decanter.

These changes in the chemical composition of virgin olive oil during centrifugal extraction make it possible to quantify the extent of cross-contamination between batches. Three points on the graph are of particular interest, the lower knee (where the (*E*)-2-hexenal concentration begins to increase), the upper knee (where its concentration stops increasing), and the middle of the rising slope (the compositional change point estimated using the chosen statistical method). In the following, we refer to the middle separation point as the change point, and the difference between the two knees as the duration of the transition between batches. Finally, we explore different strategies to target these three phenomena.

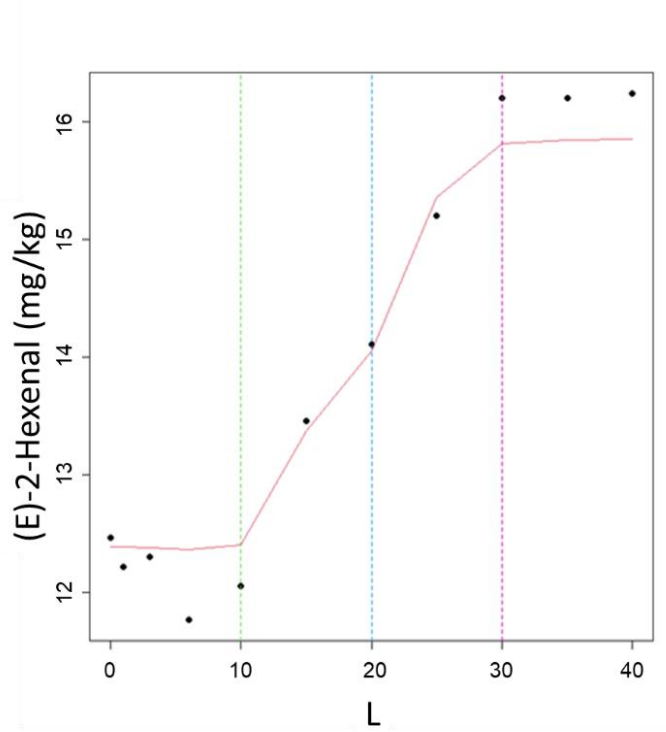

Figure 1. Concentration of (*E*)-2-hexenal found in replicate 1 as function of the number of liters of virgin olive oil produced. The red line reports the *a posteriori* mean predicted by the model.

3.2. Variability in the change point for different compounds

We examined patterns for all VOC detected with HS-SPME-GC-MS. Obviously, we discarded from the analysis those VOC where no significant differences were found between the considered olive batch and the previous batch. By plotting the estimated point of change for all VOC, a frequency distribution for each replicate was obtained (Figure 2). In replicates 2 and 3, the most frequent point of change was after 15 L, while in replicate 1 it was after 20 L. Replicate 1 had the narrowest range, as the change point was between 15 L and 25 L for all of the significantly different compounds, and the point of change was different for different compounds (Figure 2). The range of the distribution was wider for replicates 2 and 3. Here, virgin olive oil composition changed between 10 L and 25 L; moreover, two compounds (2-butanol and methyl acetate) changed after 2 L in replicate 3. The Supplementary Materials (Table S1) reports the change point for each compound.

This variability in the change point among the three replicates, using the same decanter settings, could be due to the different composition of the olive batch in terms of solids, oil and water (Guerrini et al., 2017b). The latter may cause variation in paste consistency and, in turn, a change in the oil droplet settling time (Kalogianni et al., 2019; Trapani et al., 2017b).

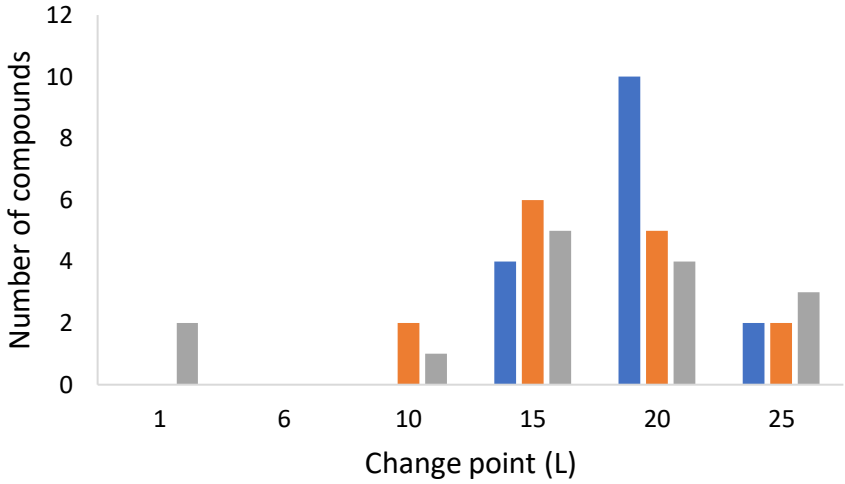

Figure 2. Frequency distribution (number of compounds) of the detected compositional change point. Blue bars represent replicate 1, orange bars represent replicate 2, and grey bars replicate 3.

3.3. Duration of cross-contamination

The duration of the transition in VOC composition was different for different compounds and replicates. Beginning with replicate 1, on average, 20 L of oil was required to change VOC concentrations in the virgin olive oil in the previous batch to concentrations in the virgin olive oil in the following batch. However, in replicates 2 and 3, this amount was roughly half (10 L) (Figure 3). In replicate 2, the distribution of the duration of contamination was quite narrow—for 13 of the 15 compounds of interest it was between 5 L and 10 L. The duration was longest for replicate 3, while replicate 1 showed intermediate behavior.

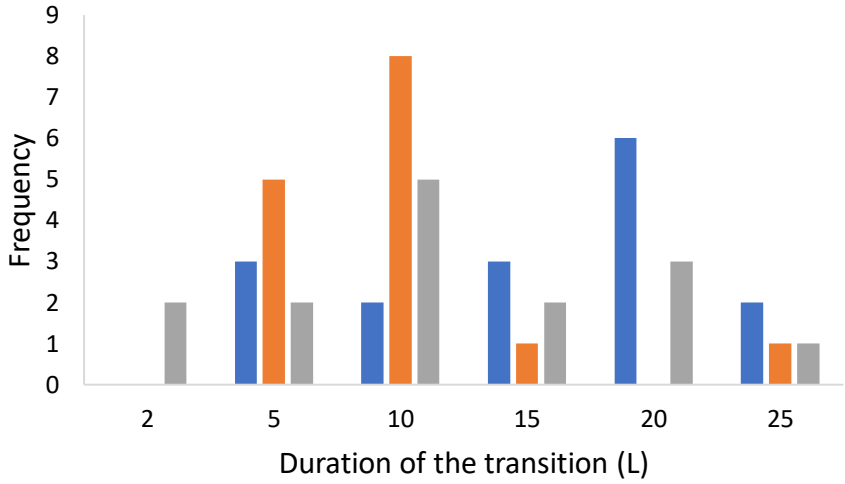

Figure 3. Frequency distribution of the duration of the transition between batches. The y-axis reports the number of compounds, while the x-axis shows the liters of virgin olive oil that exited the decanter outlet between the two knees. Blue bars represent replicate 1, orange bars represent replicate 2, and grey bars replicate 3.

3.4. Quantitative, qualitative, and experimental implications

From a quantitative perspective, the correct determination of the change point is of primary importance in order to be able to return the right virgin olive oil to the right producer. The 5 L of variable quality oil observed among replicates cannot be considered negligible. Moreover, in the 2020 season, average virgin olive oil yield in Arezzo was 14.83% (SIAN, 2022), while our trials used an average batch size of 500 kg (personal communication from the mill owner). Thus, 5 L of oil is equivalent to about 31 kg of paste (density = 917 kg/m^3), which represents roughly 6% of the average batch. Furthermore, if two consecutive batches are different with respect to total oil and extractable oil, the duration of the contamination phase will play a role in the amount of virgin olive oil returned to each producer, as yield is intermediate between the yields of the two original batches. The latter, quantitative drawback is directly related to the use of a continuous centrifuge coupled with discontinuous malaxers, and cannot be avoided by the correct determination of the change point between batches.

Qualitative drawbacks could be more dramatic. In our trials, several compounds were found to change between batches. Table 1 summarizes them, their sensorial description, and their odor thresholds (OT). In particular, it shows that they have different sensory characteristics, and very low OT. Hence, cross-contamination between a small number of compounds could change the sensory properties of a large amount of virgin olive oil. This change could, of course, be positive, if the compound is considered to have a positive attribute; however, if it is related to a sensory defect, the contaminated batch could be downgraded in terms of its commercial category. The latter point is particularly important as, under EU law (European Commission, 2001), a virgin olive oil with a recognizable sensory defect cannot be commercialized as *extra virgin*, whilst, it is classified either as *virgin* or *lampante* if the median of defect is below or above 3.5, respectively. In this case, even a small degree of contamination could have a negative impact on quality, as off-flavors can be perceived as soon as they exceed the OT, which for some molecules is very low (Morales et al., 1997). Finally, blending may compromise the sensory profile of the virgin olive oil, as chemical interactions can modify the expected flavor of a given substance (Chambers IV and Koppel, 2013).

For example, during our trials, concentrations of 2-heptanol and (*E*)-2-decenal were found to significantly change between batches. (*E*)-2-decenal has been described as “painty, fishy,

fatty" (Garcia-Oliveira et al., 2021), while 2-heptanol as "mushroom, chemical" (Aparicio-Ruiz et al., 2018). According to their respective references, both compounds have an OT of 10 µg/kg. Consequently, their occurrence in small quantities can contaminate a very large quantity of virgin olive oil in the next batch. Hence, a sensory defect and product downgrading can occur if the point of separation between batches is not carefully chosen. These considerations regarding organoleptic quality can be extended to several other situations, notably the need to avoid contamination between organic and non-organic batches, in the case of protected designation of origin (PDO)/ protected geographical indication (PGI) production, and when a virgin olive oil from a specific cultivar is produced, as monocultivar virgin olive oils have both a specific chemical composition, and aromatic and sensory profile (Aparicio and Luna, 2002; Cecchi and Alfei, 2013). In the latter scenario, olive batches may be very small, which increases the risk of obtaining a product that contains a significant percentage of oil from a cultivar other than that declared on the label.

Finally, several studies of virgin olive oil production have been carried out in real or pilot mills using a decanter centrifuge (Angeloni et al., 2022b; Caponio et al., 2018; Tamborrino et al., 2017; Vallone et al., 2022; Veneziani et al., 2022), and they typically measure VOC as in the present work. While conducting trials at industrial scale can improve the reliability of the obtained results, it creates several other problems, notably with respect to obtaining and randomizing oil samples, controlling process variables, running a large number of replicates, etc. In addition to these difficulties, contamination between batches should also be considered when industrial-scale trials are run. In particular, the virgin olive oil that exits the decanter during the transition between batches should be kept separate from the analyzed oil.

Table 1. Name, sensory description, and odor threshold of compounds found to significantly change between consecutive batches in the trials.

Compound name	Description	Odor threshold (μg/kg)	Reference
2-heptanol	Mushroom, chemical	10	Aparicio-Ruiz et al. (2018)
2,4-decadienal	Strong, fatty	2150	Garcia-Oliveira et al. (2021)
2-nonanone	Hot milk, soap, fusty	N.A.	Guerrini et al., 2020a; Zhou et al. (2019)
Methyl acetate	Ethereal, sweet	200	Tena et al. (2007)
(*E*)-2-decenal	Painty, fishy, fatty Soapy, fatty	10 563	Garcia-Oliveira et al. (2021)
Propanoic acid	Pungent, sour	720	Garcia-Oliveira et al. (2021)
Nonanol	Fatty Rancid	280 13500	Garcia-Oliveira et al. (2021)
1-penten-3-one	Green Green, pungent	50 0.73	Aparicio and Luna (2002); Reiners and Grosch (1998)
(*Z*)-3-hexenyl acetate	Green Banana-like	750 200	Aparicio and Luna (2002); Reiners and Grosch (1998)
(*Z*)-2-penten-1-ol	Olive fruit, sweet	250	Brkić Bubola et al. (2019)
(*E*)-3-hexen-1-ol	Green	1500	Morales et al. (2005)
Limonene	Light, floral	1150	Genovese et al. (2018)
(*E*)-2-hexenal	Green, apple like, bitter almond	424	Genovese et al. (2018)
1-pentanol	Fruity	470	Reboredo-Rodríguez et al. (2012)
Acetic acid hexyl ester	Green, fruity, sweet	1040	Aparicio and Luna (2002)
1-hexanol	Fruit, banana, soft	400	Reboredo-Rodríguez et al. (2012)
(*Z*)-3-hexen-1-ol	Green	6000	Aparicio and Luna (2002)
2,4-hexadienal	Fresh, green, floral, citric	2000	Reboredo-Rodríguez et al. (2012)
1-propanol	Pungent, pineapple	-	Magagna et al. (2016)
(*Z*)-3-hexenal	Green Leaf like	3 1.7	Garcia-Oliveira et al. (2021)

3.5. A simple method to determine the change point

In theory, the change point could be calculated from the following: the decanter geometry; the rotation speed; the bowl-screw differential speed; the feed rate; the paste composition, consistency and density; and the distribution of the diameter of oil droplets (Records and Sutherland, 2001). However, this method is both difficult and uncertain. Obviously, chromatography is incompatible with production requirements, given both the time and cost of an analysis. Moreover, the task of the recognition of the change point between batches should be carried out by a sensor with a fast response, a high precision, a high sensitivity, a certain degree of ruggedness ensuring durability in the olive mill environment, and available at affordable costs. The technologies best matching with these requirements, nowadays available, are those based on optical sensors working in the visible range. Simple sensor systems for the continuous monitoring of the physicochemical parameters of the virgin olive oil could be easily implemented on the processing plant for an online quality control. The virgin olive oil color is mainly related to the content of chlorophylls and carotenoid pigments and it can change according to the variety and stage of ripeness (Moyano et al., 2008). Therefore, a different chemical composition in the minor components of the virgin olive oil most likely correspond to a different color. Recently, the color measurement has been used, coupled with phenolic content, for the classification of Spanish PDO extra virgin olive oil (Becerra-Herrera et al., 2018). Hence, the continuous monitoring of CIELab coordinates appeared to be a good candidate for our task.

We monitored the change between continuous batches using CIELab coordinates. Our aim was to understand if a colorimeter, combined with data processing at the exit of the decanter, could be used as process controls. The results are reported in Table 2. Parameters *L* and *b** were found to significantly change between batches in all three replicates, while parameter *a** only changed in replicate 1. Hence, here we focus on *L* and *b**. Beginning with *b*,* this indicates a change point between batches at 20 L, 15 L and 15 L in replicates 1, 2 and 3, respectively. These change points are consistent with the most frequent change points found for compounds analyzed using HS-SPME-GC-MS. Results for *L* are consistent with *b** in replicates 1 and 2, while in replicate 3 they indicate 15 L rather than 20 L.

Thus, the results of our colorimetric determination could be a promising avenue for the development of an online sensor to identify the separation point between two subsequent

decanter batches. From a quantitative perspective, the determination of the change point with the colorimeter could help to ensure that the right producer receives the right virgin olive oil. From a qualitative perspective, virgin olive oil exiting the decanter during the transition between batches should be collected separately from the two “pure” batches. Here again, the colorimetric measure could help to find the two knees representing the beginning and end of the transition, and determine when to begin collecting a new batch.

Table 2. Change point (CP – L) and duration of the transition (DOT - L) of CIELab parameters found with the colorimeter (ns = not significant).

Parameter	Replicate 1		Replicate 2		Replicate 3	
	CP	*DOT*	*CP*	*DOT*	*CP*	*DOT*
L	20	16	15	10	20	20
*a**	ns	-	15	19	ns	-
*b**	20	20	15	5	15	5

4. Conclusions

Cross-contamination is a problem when two batches of different olives are processed in a decanter, and cannot be avoided with devices currently in use. However, it is possible to determine when the shift between batches occurs. Our trials used two methods. First, we examined the concentration of several VOC. Here, the aim was to better-understand the qualitative risks of contamination, and develop a robust technique to identify the moment of change. Second, we monitored the change between batches using a color measurement. Although this method is less accurate than chromatography, it is fast, non-destructive, cost-effective and could give results in real time. Thus, it could be easily and successfully implemented in online decanter systems. Color monitoring could detect the correct compositional point of change; virgin olive oil eluted from the decanter at the beginning of one batch could be collected separately, and the owners of the two contiguous batches could decide its fate.

The correct management of olive batches has important quantitative and qualitative consequences. It could ensure that the right virgin olive oil is returned to the owner of the right batch, reduce the risk of sensory defects, and better-protect the consumer, notably with respect to the characterization of monocultivar, POD and PGI extra virgin olive oils.

Supplementary Materials

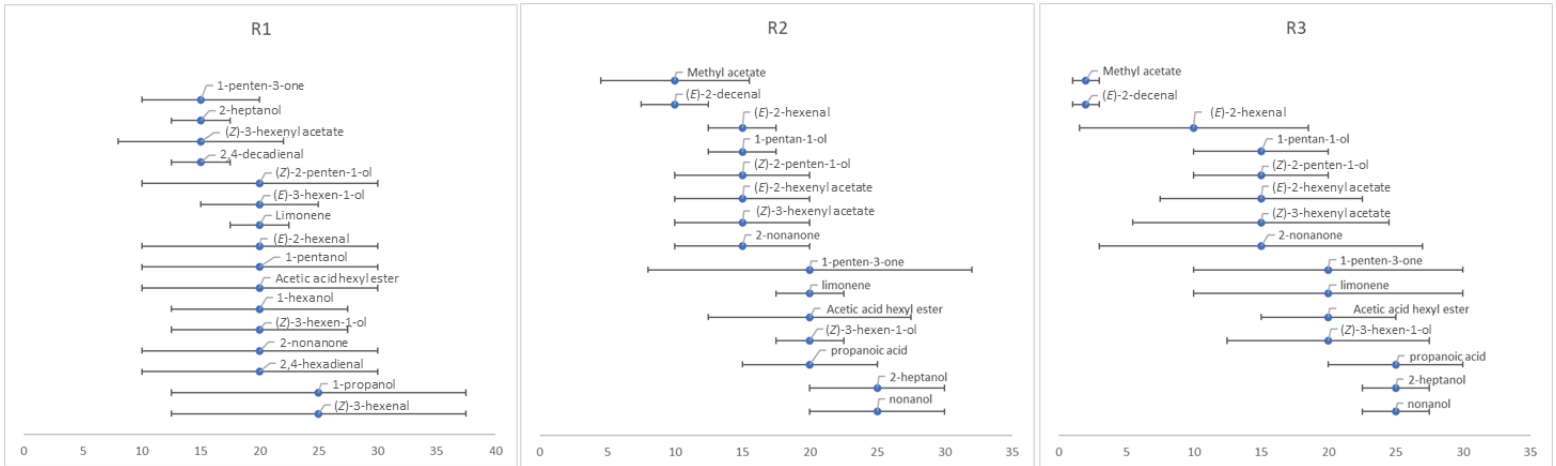

Figure S1. Detected change point (blue) and duration of the transition between batches (bars) for each statistically significant compound in the three replicates. The *x*-axis reports the L of virgin olive oil eluted from the decanter beginning at the time when processing of paste from the first batch in the malaxer ended.

6. General conclusions

The present Doctoral thesis was motivated by the need to enrich the current knowledge in the extra virgin olive oil (EVOO) processing. In particular, the topics discussed in the work deeply examined from a plant and engineering point of view some phases of the production process, considered critical for the quality of EVOO. The innovative features of the research articles concerned the adopted plant and machines, the methodological approaches and the study of relationships between the operative parameters/ procedures and the quality that were never investigated before.

A preliminary literature review was carried out to summarize the current technologies and key operational parameters involved in the fundamental processing stages. From here, some operations were identified as critical for the quality of the extra virgin olive oil (EVOO) at different levels and some literature gaps were pointed out.

After the literature background, the first two studies evaluated the effect on EVOO quality, of the use of refrigerated cells for olive fruit storage at controlled temperature before milling. The latter system it is still not very widespread but, recently, the interest from the olive oil companies is growing, even though the main discouraging factors remains linked to the plant installation and operative costs.

The obtained results showed that even for short storage time (less than 24 hours), the use of a refrigerated cell allowed to avoid the fermentation and warming of the olive heaps and to prevent the development of volatile compounds related to olive oil off-flavors, such as *fusty*. The latter could lead to the loss of the extra virgin category. In addition, a modulating effect on the phenolic and volatile profile of the oils was detected, also as a function of the harvesting methods. This means that the limiting factor to the use of refrigerated cells linked to energy costs can be considered of minor relevance, compared to quality issues related to the possible lowering of the commercial category.

In the third research study, a simple methodological approach for the estimation of overall heat transfer coefficient (*U)* in industrial malaxer was proposed, aiming to improve the temperature control of the olive paste during the malaxation stage. The results confirmed the poor heat exchange efficiency of malaxers reported in the literature but demonstrated that *U* values could be improved changing the design of machines and equipment, especially from

a heat transfer surface/ volume perspective. This naïve approach could also represent a very useful tool for mill engineers and producers in order to choice the most adequate operative conditions, i.e., malaxing time and process fluid temperature, knowing the time-temperature kinetics of olive paste during malaxation.

Finally, the fourth work investigated the effect on EVOO quality mainly linked to the processing practices of the oil mill, rather than to the operative parameters, i.e., the cross-batch contamination at the decanter centrifuge. The batch processing aims to process different batches of olive keeping them separate (in both space and time) in order to avoid contamination between batches from different producers along the extraction line. Contrarily to the malaxing stage, this phenomena cannot properly be avoided at the decanter machine, which was designed to work in continuous. Thus, it avoids to return the exact amount of oil contained in a specific batch of olives to the producer and to ensure the qualitative features directly related to characteristics of the olive fruit and the process settings adopted.

The results obtained from the analysis of the volatile fraction and color of the oils from three consecutive olive batches, made it possible to identify a point of change of the batch. The consistent matching between these two parameters suggested that a colorimeter instrument could be implemented in-line at the decanter outlet to detect the point of change between different olive batches.

The overall results obtained in this Doctoral thesis indicate that the innovation trend in olive oil plant and engineering has not to consider the fundamental operations and operative parameters in a unitary and separate manner, but in an integrated system capable to affect the quality of EVOO in terms of different features. The latter are the result of several interactions between different operative conditions, fundamental operations, machines and processing practices. The use of advanced monitoring and control systems of operative parameters through sensors and AI systems capable of collect and processing a huge amount of data may help in the choice of plant settings and in the quality control, returning feedback and feedforward responses according to the information coming from the production line.

References

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