The Use of a Cooling Crusher to Reduce the Temperature of Olive Paste and Improve EVOO Quality of Coratina, Peranzana, and Moresca Cultivars: Impact on Phenolic and Volatile Compounds

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Abstract
A new technology used to reduce the temperature of olive paste was applied to the extra virgin olive oil (EVOO) mechanical extraction process. The performance of a cooling crusher that was able to counteract the thermal increase that occurs during olive fruit grinding was analyzed to evaluate the effects on the development of volatile compounds and the concentration of hydrophilic phenols in the final product. The volatile profiles and phenolic fraction of EVOOs extracted from three different cultivars (Coratina, Peranzana, and Moresca) were positively affected by the use of lower temperatures during the crushing phase. The volatile fractions showed increases in the total aldehydes, mainly related to the concentrations of (E)-2-hexenal, and reductions in the total alcohols, mainly due to 1-penten-3-ol, 1-hexanol and (Z)-3-hexen-1-ol contents. The use of a lower temperature reduced the level of oxidative processes, protecting the phenolic compounds in the Moresca and Peranzana EVOOs by 17.8 and 12.1%, respectively.

Keywords Low temperature · Olive crushing · Volatile compounds · Hydrophilic phenols · VOO quality

Introduction
The sensory characteristics of extra virgin olive oil (EVOO) as well as its healthy properties have made it a favorite of consumers in Mediterranean countries for centuries and now throughout the world. Olive oil consumption has been constantly increasing on a global basis for the last 30 years. The final quality of EVOO is due to many factors, such as the genetic background, growing area, agronomic practices, cultivar, climatic conditions, and harvesting period of the olives (Caporaso, 2016). The improvement of EVOO quality is also influenced by mechanical extraction process and related technological parameters (Cecchi et al., 2019; Iqdiam et al., 2019; Kalogianni et al., 2019; Manganiello et al., 2021; Polari et al., 2019; Servili et al., 2015, 2019; Tamborrino et al., 2021; Taticchi et al., 2019, 2021; Veneziani et al., 2017). In particular, the study of new EVOO technologies is aimed at improving the content of phenolic compounds and the EVOO flavor improving the organoleptic and bioactive properties. A high phenol content in EVOO improves its quality due to several beneficial properties for human health and to its antioxidant effects (Hashim et al., 2005; Marx et al., 2020; Pantano et al., 2017; Servili et al., 2014; Tripoli et al., 2005). The volatile compounds responsible for EVOO flavors are generated through the lipoxygenase (LOX) pathway, whereas molecules that are responsible for EVOO off-flavors are generated from oxidation phenomena, conversion of some amino acids, fermentation process, or fatty acid metabolism. The volatile compounds responsible for positive sensory notes are mainly represented by C5 and C6 saturated and unsaturated aldehydes, alcohols, and esters, associated with green, leaf-like, and fruity sensory notes (Kalua et al., 2007).

The new challenge of the olive oil sector concerns the control of the temperature during the extraction process, mainly during crushing and malaxation steps, to extract EVOOs of higher quality. The management of olive or olive paste temperatures interferes with the different enzymatic activities, such as those of polyphenol oxidase (PPO),
peroxidase (POD), and LOX, which regulate the development and the quantitative and qualitative compositions of volatile and phenolic compounds. Temperature is one of the most important process parameters that can be adjusted accordingly to the characteristics of the different genetic origins of olives, playing a basic role in the definition of the final quality of oils (Gómez-Rico et al., 2009; Leone et al., 2018a; Lukić et al., 2017; Selvaggini et al., 2014, Veneziani et al., 2015).

Accordingly, several studies have recently focused on the reduction of processing temperature by thermal conditioning of crushed olive paste using heat exchangers (Veneziani et al., 2017). The technological innovation, carried out with the use of a tubular thermal exchanger, was used for a rapid cooling treatment of the olive paste. The rapid cooling of the raw material results in an increase in the phenolic concentrations in the EVOOs. The higher phenolic content is probably due to the inhibition of PPO activity that results from the high value of temperature for the optimal oxidation process (Taticchi et al., 2013). These results were obtained without altering the legal quality parameters of EVOO, and with modifications of the volatile profile characterized by a significant increase of the total aldehydes due to the different LOX activities that characterized different olive cultivars (Veneziani et al., 2018).

The use of low temperatures can represent an alternative approach to modulate volatile compounds and preserve a higher concentration of hydrophilic phenols (Plasquy et al., 2021b; Veneziani et al., 2021). Alternative techniques to the regulation of the temperature of olives, such as the use of cold rooms to stabilize the temperature of fruits during the storage period (Guerrini et al., 2021; Morales-Sillero et al., 2017; Plasquy et al., 2021a; Taluri et al., 2019; Yousfi et al., 2012), the application of dry ice to cool the olives during the crushing phase (Veneziani et al., 2017; Zinnai et al., 2016), or the reduction of fruit temperature before processing by means of cold water (Dourou et al., 2020), were also tested to assess the impacts on EVOO quality.

The cooling of olives in refrigerated cells for a short period of storage can both delay the oxidation of phenolic compounds and increase the production of LOX pathway compounds, reducing off-flavor development (Guerrini et al., 2021). For a long storage time, the best performance is obtained only with healthy fruits. The integrity of olives is usually related to the harvesting system, in particular the mechanical system, which can have a significant impact on olives and their corresponding oils (Yousfi et al., 2012). In fact, in the event that the drupes are highly damaged and it is not possible to implement a control of their temperature, to avoid unwanted fermentation, the only solution is the rapid processing of the fruits (Morales-Sillero et al., 2017). However, fruit cold storage represents a way to reduce the undesirable effects on quality characteristics and improve the oxidative stability of the product (Morales-Sillero et al., 2017; Yousfi et al., 2012).

Another method involves the use of solid carbon dioxide during the crushing step. The treatment would cause cooling of the olives, partial solidification of fruit water, and breakage of the olive cell walls, resulting in an increased extraction yield and enhancing the quality parameters related to the content of vitamin E and phenols. In addition, the significant amount of gas that is generated due to the interaction between solid CO₂ (−78 °C at atmospheric pressure) and olives/olive paste (initially at room temperature) would induce the formation of a sort of inert protective layer on the processed olives. This gaseous layer would be able to prevent oxidation and thus reduce the loss of antioxidants, such as phenols, without compromising the formation of aromas responsible for the main sensory notes of the oil (Zinnai et al., 2016).

In comparison to the use of dry ice or climatic cells, heat exchangers seem to be more efficient from an operating point of view, less costly, environmentally sustainable, and able to guarantee a high-quality standard for the final product. However, independent of the cooling method of olives or olive pastes, a key factor to achieve high-quality EVOO is temperature monitoring. In a recent lab-scale study, a slight lowering of fruit temperature just before crushing, using a bath of cold water, resulted in a decrease in oil off-flavors, whereas it enhanced the levels of green and fresh sensory notes of EVOO, highlighting the possibility of modulating the production of volatile compounds. The inner temperature showed a significant impact on the volatile profile due to the enzymes of the LOX pathway being able to produce higher amounts of the C₆ aldehydes responsible for herbal/green olfactory traits of the final product at lower temperatures (Dourou et al., 2020). The LOX pathway starts to generate the main volatile compounds immediately when the fruits are crushed and the substrates are available for the different enzymatic activities involved in EVOO flavor development. The best operative conditions to improve LOX activity during the first steps of the EVOO extraction process, including the use of low temperature, should be maintained to increase positive effects on the generation of the volatile fraction (Padilla et al., 2012; Salas & Sanchez, 1999; Veneziani et al., 2021).

Accordingly, this study aimed to evaluate the performance of a new cooling crusher that is able to reduce the temperature of olive paste just during the crushing phase. The main quality parameters related to health and sensory properties, such as volatile compounds and phenolic content of EVOO extracted from different cultivars, were analyzed to assess the impact of reducing the olive paste temperature during the first step of the EVOO mechanical extraction process with regard to the different genetic origins of the raw material.
Materials and Methods

Materials

Oleacein (3,4-DHPEA-EDA), oleocanthal (p-HPEA-EDA), oleuropein aglycone (3,4-DHPEA-EA), ligstroside aglycone (p-HPEA-EA), (+)-1-acetoxypinoresinol, and (+)-pinoresinol were obtained from VOO following the method described by Selvaggini et al. (2014). Phenyl alcohols (tyrosol and hydroxytyrosol) were purchased from Cabru S.A.S. (Arcore, Milan, Italy) and Fluka (Milan, Italy), respectively. The other solvents and chemical compounds were supplied by Merck (Merck KGaA, Darmstadt, Germany). The chemical compounds studied in this article were the following: 3,4-DHPEA-EDA (PubChem CID: 18684078); p-HPEA-EDA (PubChem CID: 16681728); 3,4-DHPEA-EA (PubChem CID: 124202093); p-HPEA (PubChem CID: 10393); 3,4-DHPEA (PubChem CID: 82755); (E)-2-hexenal (PubChem CID: 5281168); hexanal (PubChem CID: 6184); 1-penten-3-ol (PubChem CID: 12020); 1-hexanol (PubChem CID: 8103); (Z)-3-hexen-1-ol (PubChem CID: 5281167).

EVOO Mechanical Extraction Process

The trials were carried out during three different olive oil production seasons: 2018/2019, 2019/2020, and 2020/2021. The tests related to 2018/2019 and 2019/2020 were performed by processing Coratina and Peranzana olives, respectively. Both cultivars were harvested in the Apulia region in the first week of November (Peranzana) and in the last week of November (Coratina). The extraction plant was located in Monte Schiavo (Ancona, Marche, Italy) and equipped with the following machines produced by Pieralisi MAIP SpA (Jesi, Ancona, Italy): a new crusher with a cooling system, “Simplex” (0.6 ton) malaxers, “DMF 4” decanter (2 ton/h), and “Bravo” vertical centrifugal separator. The trials performed in the olive oil production season of 2020/2021 were carried out with the Moresca cultivar harvested during the first week of October and processed at Frantoio Covato (Ragusa, Sicily, Italy). The Covato’s extraction plant was equipped with the following pieces of equipment produced by Pieralisi MAIP SpA (Jesi, Ancona, Italy): a new crusher with a cooling system, “Molinova ORO” malaxers (0.6 ton), “DMF 6” decanter (2.5 ton/h), and “Marte” vertical centrifuge separator.

The new cooling crusher is a hammer crusher with a cooled cover and an Archimedes screw with a cooled spiral and a cooled screw housing channel. Cold conditioning of the chilled parts of the crusher takes place by means of the circulation of cold water generated by a chiller unit, which is used as a source of cooling for the thermal group of the olive mill. Thanks to the cooling system described above, it was possible to compensate for the thermal increase during fruit grinding and to reduce the temperature of olive paste by approximately 6 ± 2 °C compared to the control tests.

The control trials were performed with the same extraction plants described above processing the olives but with the cooling system of the crusher deactivated. The malaxation phase was carried out for 20 min at 25 °C for both control and experimental tests. Two bottles of three independent extractions both for control and experimental tests were collected and rapidly analyzed. All the data are expressed as the mean value of the three independent EVOO extractions.

VOO Chemical Analysis

Legal Quality Parameters

Legal quality parameter analysis was carried out for the product classification of olive oils. Acidity, peroxide value, and spectrophotometric constants (K_{232}, K_{270}, and ΔK) were detected according to the methods presented in Regulation (UE) 2015/1830 (OJEC, 2015).

Phenolic Compounds

The phenolic fraction was extracted from the EVOOs by a liquid–liquid extraction method, mixing the EVOO sample with a methanol/water solution (80/20 v/v) as described by Taticchi et al. (2021). The quantitative concentration and qualitative composition of EVOO samples were determined using high-performance liquid chromatography (HPLC) by Agilent Technologies MOD. 1100 controlled by ChemStation (Agilent Technologies, Palo Alto, CA USA). The device was equipped with the following components: vacuum degasser system; a quaternary pump; an autosampler; a thermostated compartment for the column, and finally a diode array detector (DAD) and a fluorescence detector (FLD). Phenolic acids, tyrosol, hydroxytyrosol, and the other aglycone derivatives of oleuropein and ligstroside were detected by using the DAD with a wavelength of 278 nm, whereas the flavonoids were detected at 330 nm. The extraction methods and chromatographic tests were carried out according to the method described by Selvaggini et al. (2006). The quantitative and qualitative compositions of hydrophilic phenols, separated by using a Spherisorb ODS1 (5 μm, 4.6 mm × 250 mm, Waters, Milford, MA, USA) column, were elaborated by ChemStation software (Agilent Technologies, Palo Alto, CA, USA) and expressed in mg/kg of EVOO.
Volatile Compounds

The evaluation of EVOO volatile compounds was performed by headspace-solid phase microextraction followed by gas chromatography–mass spectrometry (HS-SPME-GC/MS). The chromatographic analysis of aldehydes, alcohols, and esters was carried out by using an Agilent Technologies GC 7890B instrument equipped with a “Multimode Injector” (MMI) 7693A (Agilent Technologies, Santa Clara, CA, USA). The operating parameters of GC–MS analysis for the identification, detection, and quantification of EVOO volatile compounds were performed according to the method reported by Taticchi et al. (2021). The volatile molecules were identified using the spectra of authentic reference compounds and with those in the NIST 2014 mass spectral library and quantified using the calibration curves for each compound by internal standard calculations. The data are expressed in µg/kg of EVOO.

Statistical Analysis

The results obtained from the analysis of EVOOs were analyzed using SigmaPlot Software 12.3 (Systat Software Inc., San Jose, CA, USA) which employed t test analyses with a rejection p value of 0.05 of samples extracted with and without the use of a cooling crusher.

Results and Discussion

The main aim of this study was to evaluate the impact of the temperature reduction of olive paste during the crushing step on some of the main EVOO quality characteristics, i.e., hydrophilic phenols, volatile compounds, and quality indices. The cooling process of olive paste was recently studied, mainly with regard to the use of heat exchangers applied after the crusher (Veneziani et al., 2017, 2018) and the cooling of olive fruits before their processing using climatic chambers or refrigerated water (Dourou et al., 2020; Guerrini et al., 2021; Morales-Sillero et al., 2017; Yousfi et al., 2012). In contrast, during this study, the analysis concerned the use of an innovative crusher equipped with a rapid cooling system that was able to compensate for the thermal increase during fruit grinding and reduce the temperature of olive paste during the crushing step. The possibility of controlling the temperature during the crushing phase allows us to determine the optimal conditions for some of the enzymes of the LOX pathway that showed higher activity levels at lower temperatures. The best performance of LOX enzymes was obtained with temperatures ≤ 20 °C, mainly due to the optimal activity of hydroperoxide lyase (HPL) for the conversion of 13-hydroperoxides into C5 and C6 aldehydes (Padilla et al., 2012; Salas & Sanchez, 1999; Veneziani et al., 2021).

Regarding legal quality parameters, the data did not show any significant variation in any of the EVOOs extracted from the three different cultivars. The free acidity and peroxide values and the specific extinction coefficients (K232, K270, and ΔK) were not modified by the use of lower temperature during the crushing phase compared to the control samples (Table 1).

The new cooling crusher showed a significant impact on the phenolic fraction, probably due to the inhibition of enzymatic activities responsible for the degradation of phenolic compounds at lower temperatures, which were able to reduce the efficiencies of PPO and POD activities typically characterized by high optimal temperatures over 40 °C (Taticchi et al., 2013). The data of hydrophilic phenols showed a significant improvement in the total phenols of the Moresca and Peranzana cultivars processed with the cooling crusher, with increases of 17.8 and 12.1% when compared with the control trials, respectively (Table 2). The improvement in phenolic concentration was quantitatively and qualitatively due to an increase in

Table 1 Legal quality parameters of EVOOs extracted from different cultivars with (cold) or without (control) the use of cooling system of a new crusher

|                  | Control Coratina | Cold Coratina | Control Peranzana | Cold Peranzana | Control Moresca | Cold Moresca |
|------------------|------------------|--------------|-------------------|---------------|-----------------|--------------|
| Free fatty acid (% oleic acid)* | 0.28 ± 0.03a | 0.26 ± 0.01a | 0.28 ± 0.01a | 0.28 ± 0.01a | 0.41 ± 0.01a | 0.46 ± 0.04a |
| Peroxide values (meqO2/kg) | 2.8 ± 0.2a | 3.0 ± 0.2a | 7.1 ± 0.9a | 6.7 ± 0.6a | 9.5 ± 1.1a | 10.0 ± 1.0a |
| K232            | 1.643 ± 0.01a | 1.642 ± 0.06a | 1.984 ± 0.1a | 1.960 ± 0.02a | 1.831 ± 0.05a | 1.804 ± 0.04a |
| K270            | 0.177 ± 0.004a | 0.184 ± 0.01a | 0.148 ± 0.01a | 0.150 ± 0.01a | 0.123 ± 0.008a | 0.125 ± 0.01a |
| ΔK              | −0.004 ± 0.0003a | −0.004 ± 0.0003a | −0.004 ± 0.0004a | −0.002 ± 0.0000b | −0.006 ± 0.0000a | −0.005 ± 0.0005b |

*The data are the mean values of three independent EVOO extractions, ± standard deviation. For each cultivar, the values in each row having different letters (a, b) are significantly different from one other (p < 0.05)
flavor due to the increase in total aldehydes and reduction of the cultivars showed a significant improvement in EVOO volatile fraction. The oils extracted from all the new cooling crusher had a positive influence on the reduction of the oxidative process of phenols. The concentrations of Coratina phenolic compounds, with values often very high (over 1000 mg/kg) and probably close to the upper limits of solubilization into the oily phase, confirmed the results of previous analysis (Veneziani et al., 2017), showing a moderate increase in the hydrophilic phenols of oils extracted from different cultivars that is usually more moderate in the phenolic composition of EVOOs. The oils extracted from all cultivars showed a significant improvement in EVOO flavor due to the increase in total aldehydes and reduction of the thermal increase of olive paste. The reduction in the thermal increase of olive paste during the traditional crushing process, due to the use of the new cooling crusher, had a positive influence on the volatile fraction of EVOOs. The oils extracted from all the cultivars showed a significant improvement in EVOO flavor due to the increase in total aldehydes and reduction of the thermal increase of olive paste during the traditional crushing process, due to the use of the new cooling crusher, had a positive influence on the volatile fraction of EVOOs. The oils extracted from all the cultivars showed a significant improvement in EVOO flavor due to the increase in total aldehydes and reduction of the thermal increase of olive paste during the traditional crushing process, due to the use of the new cooling crusher, had a positive influence on the volatile fraction of EVOOs. 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on the volatile fraction was not influenced by the different genetic origins of the olive fruits, showing the same impact on the activities of the enzymes of the LOX pathway with a common trend resulting in the development of volatile compounds in all the EVOOs. The reduction in temperature directly during the crushing step has had a uniform effect on the regulation of LOX activities in different cultivars. This effect is not confirmed when low temperatures are used immediately after the crushing phase (Veneziani et al., 2017, 2018, 2021), showing a higher influence of the cultivars and their specific enzyme activity load (Sánchez-Ortiz et al., 2012; Tomé-Rodríguez et al., 2021).

The effects on EVOO phenolic and volatile compounds of different cultivars can be very different from both quantitative and qualitative points of view, as evaluated by several studies related to new extraction technologies and the control of process parameters (Kalogianni et al., 2019; Pérez et al., 2021; Selvaggini et al., 2014; Taticchi et al., 2019, 2021; Veneziani et al., 2021). The influence on the final concentration of health and sensory molecules was always more uniform for the phenolic fraction and less stable for the volatile concentration, which was highly influenced by the genetic origin of the raw material due to a different level of overall enzymatic activity for the LOX pathway that induced a differentiated response on the production of aldehydes, alcohols, and esters. For the first time, a new technology seems to be able to modulate, in the same way, the production of different classes of volatile compounds of different cultivars, showing variability only quantitatively, not qualitatively. However, further studies on different cultivars are required to confirm the improvement of aldehyde content and the decrease in the concentration of alcohols able to enhance the flavor of the final product.

### Conclusions

The use of a new cooling technology was able to compensate for the thermal impact of the crushing step on olive paste, reducing its temperature. The processing of olives at lower temperatures had significant effects on EVOO quality compared to the control samples. The improvement of...
quality characteristics is associated with the concentration of hydrophilic phenols and volatile compounds involved in the main health and sensory properties of the final product. As supposed during the design of the cooling crusher, the new technology mainly had a significant effect on the enzymes belonging to the LOX pathway, showing a common positive impact independent of the cultivar. The reduction in temperature during the crushing phase highlighted a uniform trend in the development of the volatile molecules. Total aldehydes increased mainly due to the improvement of the concentration of (E)-2-hexenal, and total alcohols decreased mainly due to the reduction of 1-penten-3-ol, 1-hexanol, and (Z)-3-hexen-1-ol content without any significant alterations in the amount of esters. In contrast, the impact of the cultivars was significant from a quantitative point of view, showing increases and decreases ranging from +9.4 to +29.6% for the concentration of aldehydes and from −4.0 to −31.0% for the alcohol content, respectively. The increase in aldehydes, linked to green sensory notes, and the reduction in alcohols, responsible for ripe fruit sensory notes, were more significant for the cultivars characterized by the lowest concentrations of total volatile compounds. The new technology also had a positive impact on the EVOO phenolic fractions of the Moresca and Peranzana cultivars, whereas Coratina EVOOs did not show any significant increase of total phenols. The cooling crusher represents a new technology that is able to modulate the development of volatile compounds and protect the phenolic fraction from oxidative processes to improve the quality standard of the final product.

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Author Contribution Gianluca Veneziani: conceptualization, investigation, writing—original draft, writing—reviewing and editing; Davide Nucciarelli: conceptualization, investigation, writing—original draft; Sonia Esposto: conceptualization, investigation; Luigi Daidone: methodology, validation, formal analysis, investigation, writing—original draft; Stefania Urbani: methodology, validation, formal analysis, investigation; Roberto Selvaggi: methodology, validation, formal analysis, investigation; Agnese Taticchi: conceptualization, visualization; Maurizio Servili: supervision, project administration, funding acquisition.

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Availability of Data and Material The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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