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Pre-processing cooling of harvested olives: effects on oil composition and quality parameters in two different genotypes

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Abstract

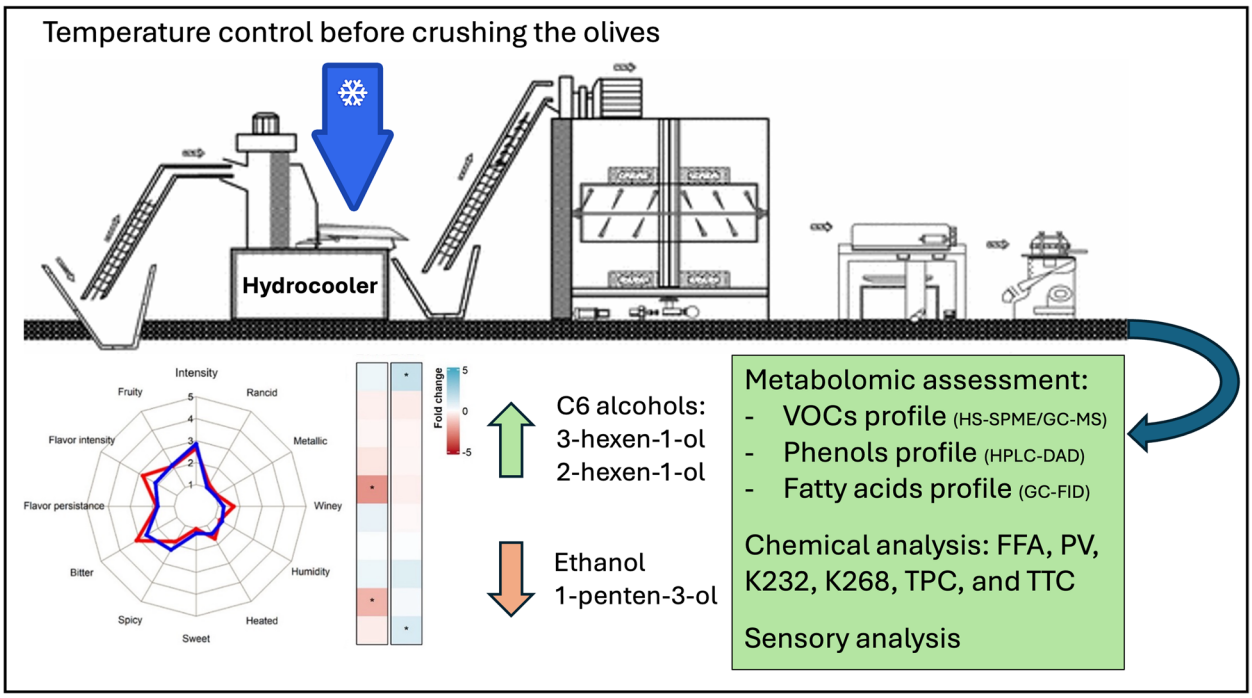
The quality of olive oil is strongly influenced by environmental conditions during olive growth. Exposure to high temperatures in the later stages of fruit development can negatively affect the fatty-acid profile, volatile organic compounds (VOCs), and polyphenol content. Harvesting and processing olives when they have high internal temperatures further degrades oil quality, particularly the aroma profile. To address this issue, trials were conducted in an industrial mill using hydrocooling as a rapid pre-processing cooling method on harvested olives from the Frantoio (2019 and 2020 seasons) and Leccino (2020 and 2021 seasons) cultivars. The aim was to investigate the effects of reducing olive mesocarp temperature on oil quality and composition. The results showed that cooling improved several chemical quality parameters in Frantoio, including reductions in free fatty acids (FFA) and peroxide values (PV). The treatment also had genotype-dependent impacts on the aroma profile, enhancing specific positive VOCs, such as 2-hexen-1-ol and 3-hexen-1-ol, in both cultivars and reducing off-flavors. Responses varied by season and genotype for other VOCs and total and specific polyphenol content. Pre-processing cooling appears to be a promising strategy for enhancing desirable aroma compounds and minimizing sensory defects.

Keywords *Olea europaea*, Hydrocooler, Temperature, Olive oil, VOCs, Off-flavors

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Graphical Abstract



Introduction

The global olive oil industry faces two interrelated challenges that demand innovative solutions. First, there is a growing demand for olive oil of improved organoleptic quality and enhanced nutraceutical properties. Consumers worldwide seek the delightful taste and aroma of extra virgin olive oil (EVOO) and are increasingly more aware and informed of its health-promoting attributes, mainly related to the monounsaturated fatty acid, polyphenol and tocopherol content (Kalua et al., 2007; Modesti et al., 2021a; Tsimidou, 2006). The EVOO secoiridoids, such as oleocanthal, oleacein, hydroxytyrosol, and squalene, show therapeutic promise against cancer, arthropathy, cardiovascular diseases, as well as antitumor activity on highly invasive breast tumor cells when acting together (Lozano-Castellón et al., 2020; Sánchez-Quesada et al., 2022). Meeting this demand requires careful cultivation and harvesting practices (early harvest), as well as innovative processing techniques that preserve and possibly enhance these valuable compounds.

The specter of climate change looms large, ushering in warmer temperatures, prolonged summer seasons, and, consequently, leading to accelerated biochemical processes that may negatively affect oil composition and quality (Fraga et al., 2020; Michalopoulos et al., 2020; Plasquy et al., 2021). The delicate balance of volatile organic compounds (VOCs) and phenolic compounds that contribute to the taste and health benefits of olive oil may be jeopardized by this acceleration. Moreover, elevated temperatures can accelerate the degradation of desirable volatiles (e.g. 3-hexen-1-ol, hexanal, or 1-octen-3-ol), or shift their biosynthesis, reducing fruity and floral notes while sometimes increasing less favorable or rancid odors (e.g. 3-penten-1-ol, 2-propanol, or benzoic acid) (Aparicio et al., 1997; Garcia-Oliveira et al., 2021; Morales et al., 1997; Servili et al., 2007). This alteration is particularly important because the aroma profile plays a crucial role in the sensory quality of olives and olive oil, and the increase in temperature can reduce market appeal and sensory acceptability. Therefore, it is imperative to adapt olive processing methods to effectively address these emerging challenges (Fraga et al., 2020).

To obtain high quality olive oils, temperature control during olive processing is applied in many olive mills. Managing the temperature of olive paste during malaxation is crucial for preserving the sensory and nutraceutical quality of olive oil. Optimal temperature ranges from 20–27 °C, where the preservation of volatile compounds and phenolics is maximized, resulting in oils with rich aromas and high antioxidant content. Moderate temperatures (28–30 °C) offer a balance between yield and quality, though with slight reductions in these beneficial

compounds. High temperatures (above 30 °C) increase oil yield but degrade VOCs and phenolics, leading to lower-quality oil with reduced aroma and health benefits (Angerosa et al., 2001; Kalua et al., 2006; Veneziani et al., 2017). In contrast, little is known about the effects of temperature control applied directly on harvested olives in the immediate pre-processing phase. In the optimal oil production chain, olives should be processed soon after harvest, avoiding any fruit storage. In fact, storing harvested olives has been recognized to decrease oil quality, even if refrigeration is applied for a better management of the mill schedule with the main goal of avoiding keeping olives at high environmental temperatures for long periods (Masella et al., 2019).

To produce high quality olive oil, nowadays early harvests of still green or partially pigmented olives are commonly performed in many olive-growing areas. This agronomical approach aims at obtaining oils with high levels of polyphenols, and with attractive aroma profiles (green, herbaceous), with hints of spicy and bitter, and, compared to oil obtained from ripe/overripe olives, reduced risks of off-flavor development. Nevertheless, harvesting early in the season could result in handling olives with high internal temperatures. When olives are delivered to the mill facilities, they are frequently kept in bins exposed to open air and direct sunlight. Under these conditions, the temperature of both the individual fruits and the bulk mass tends to rise, thereby increasing the likelihood of off-flavor formation and the initiation of fermentative processes (Kyriakidis & Dourou, 2002; Monteleone et al., 1998).

The relationship between the inner temperature of the olives before crushing and its impact on the quality of the resulting olive oil has not been extensively investigated yet. However, few studies previously reported the effect of reducing inner olive fruit temperature in the range 4–18 °C before the crushing step of the oil extraction workflow (Dourou et al., 2020; Guerrini et al., 2021; Plasquy et al., 2021). These studies, carried out at lab-scale, highlight the beneficial effects of lowering temperature management of the olives in enhancing the EVOO quality, in particular regarding the phenolic content and the volatile profile, with increases of compounds imparting green aromas. An additional study focuses on reducing the temperature during the mechanical extraction process, specifically using a cooling crusher (cooled at 19 °C) running experiments in an industrial mill (Nucciarelli et al., 2022). This approach led to improvements in the volatile profiles of EVOOs from Coratina, Peranzana, and Moresca cultivars by increasing desirable aldehydes like (*E*)-2-hexenal and reducing alcohols such as 1-penten-3-ol and 1-hexanol. Additionally, phenolic compounds were preserved, particularly in Moresca and Peranzana oils, by 17.8% and 12.1%, respectively, due to a reduction in oxidative processes (Nucciarelli et al., 2022).

Reducing inner temperatures of harvested perishable fruits (pre-cooling) is a widely practiced technique in postharvest management, particularly in refrigerated storage facilities. Among the various pre-cooling techniques, hydrocooling is commonly used for summer fruits like cherries, tomatoes, and berries. For example, hydrocooling has been shown to effectively reduce the temperature of cherries by 10–15 °C within 5–10 min, depending on the initial temperature and the water temperature used in the process (Kader, 2002). Similar results have been observed in tomatoes and berries, where rapid cooling helps to extend shelf life by reducing respiration rates and slowing down the ripening process (Cherono et al., 2018; Horvitz, 2017). Hydrocooling technique consists of a rapid cooling down of fruit inner temperature by applying a shower under cold water (~ 0 °C) for few seconds/minutes (less than 1 min for small fruits) before they enter the cold storage rooms, resulting in more efficient and immediate effects of the refrigeration.

The existing evidence on the effects of cooling olives prior to oil extraction is limited to small, short-term experiments, single-cultivar studies, or laboratory-scale cooling approaches that do not adequately reflect real-world application potential. To upscale the laboratory experiments previously conducted by our research group (Dourou et al., 2020) to an industrial level, the hydrocooling technique was implemented in a commercial mill as a replacement for the conventional olive-washing step. The trials were conducted during three harvesting years on two Italian olive cultivars, Frantoio and Leccino, and treatment effects on olive oil quality parameters and composition were investigated.

Materials and methods

Fruit material

Olive fruit of the cultivars Frantoio and Leccino were collected from a commercial orchard in Scansano municipality (Grosseto, Tuscany, Italy; 42.64501° N, 11.36086° E) during the harvest period from October to November. Sampling was carried out in 2019 and 2020 for Frantoio olives, and 2020 and 2021 for Leccino cultivar. Frantoio samples had a ripening index (Jaén Index, protocol from the International Olive Council COI/OH/Doc. No 1, November 2011) of 2.55 in 2019 and of 2.14 in 2020. For Leccino cultivar, the index was 1.71 in 2020 and 2.70 in 2021. Olive trees from the two cultivars were grown in adjacent orchards managed with identical farming practices, and plants were approximately 30 years old.



Fig. 1 Presto Fresco hydrocooler equipment supplied by the company Danese Group S.r.l. (Verona, Italy)

Hydrocooler treatment and olive oil extraction

The trials were conducted at a commercial mill facility (Scansano, Grosseto, Tuscany, Italy). After harvest, 150 kg of olives were kept in plastic bins (External dimensions: 110×110×60 cm; internal dimensions: 104×104×48 cm) until hydrocooling treatment and/or regular oil extraction procedure. A maximum of 8 h elapsed between olive harvesting and the beginning of the oil extraction process, and the bins were kept in a shaded section of the mill yard during this period. For each cultivar and for each year, two sets of samples were considered: control oil (C), extracted from olives washed with water at ambient temperature; and hydrocooler oil (Hy), produced from olives subjected to hydrocooler treatment.

The olives were cooled using the commercial hydrocooling equipment Presto Fresco (Danese S.r.l., Verona, Italy) (Fig. 1). This hydrocooler boasts compact dimensions (2.5×0.8×1.5 m, length x width x height).

The equipment, which has been conceptually and technically built for treating other fruit types in small plastic boxes, has been equipped with a conveyor belt typically used in commercial olive mill plants. Applying this modification, the equipment was capable of processing about 50 kg/min of olives disposed in a single layer on the conveyor. The hydrocooler trial consisted in the application of a water shower at about 1 ± 0.5 °C for 30 s. Time and temperature have been selected after a trial performed to optimize hydrocooler treatment protocol in order to achieve specific inner temperatures in the olive fruit. Inner temperature was measured on 30 olives per batch of samples with a probe thermometer (Qimei, NY) before entering the equipment and at the exit of the hydrocooling to assess treatment efficiency. Additional measurements of the temperatures were performed on olive paste after the crushing and malaxation steps, and on the oil after the centrifugation process, employing the thermometers already installed in the mill plant.

Oil was extracted from cooled and control olives using a commercial mill produced by Mori-Tem (Model: Cultivar 1000 3GV_400), equipped with a hammer crusher, a malaxator with temperature control, and a centrifuge extractor. This system allows to process relatively small quantities of olives (10 q/h), with a required minimum volume of 150 kg per kneader. A fixed malaxation time of 20 min has been set. This relatively short duration never allowed the olive paste to reach the set temperature of 28 °C. For each set of samples, two consecutive extractions were performed for C and Hy treatments by processing 150 kg of olives every time. To ensure the collection of "clean" oil from each specific treatment, only the oils from the second extractions were collected and considered for further analysis.

After extraction, C and Hy oil samples were stored in dark glass bottles and analyzed in correspondence of: A) 2–3 weeks after production (fresh oil) and, B) after storage for one year at 15 °C in 0.75 L dark-bottles, kept in the dark with nitrogen applied to the headspace of the bottle.

Chemical parameters

Free fatty acids (FFA), peroxide value (PV), UV spectrophotometric indices (K232 and K268), total phenol content (TPC, Folin–Ciocalteau) and total tocopherol content (TTC) were analyzed at Analytical S.r.l. (Florence, Italy) in accordance with official methods described in Regulation EC 2568/91 of the Commission of the European Union (European Union Commission, 1991).

Volatile organic compounds analysis (HS-SPME/GC–MS)

Olive oil volatile organic compounds (VOCs) were analyzed using the headspace solid-phase microextraction technique (HS-SPME) followed by gas chromatography coupled to mass spectrometry (GC–MS) analysis. For the analysis, a protocol previously published by our research group (Dourou et al., 2020; Vendrell-Calatayud et al., 2022) has been applied. Briefly, 2 g of olive oil were placed

into a transparent 20 ml crimp vial. The samples were incubated at 60 °C for 30 min. VOCs were extracted at the same temperature using a divinylbenzene/carboxen/polydimethylsiloxane fiber (50/30 μm, 2 cm, Supelco Ltd., Bellefonte, PA) for 1 h. The volatiles absorbed by the fiber were desorbed in the gas chromatograph injector for 5 min. GC–MS analyses were performed using a Perkin Elmer Clarus 680 gas chromatography coupled to a Perkin Elmer Clarus 600S quadrupole mass spectrometer, equipped with a split-splitless PSSI injection port (kept at 250 °C during the analysis). Helium was used as a carrier gas at a constant flow of 1 ml/min. Compound separation was performed using a Supelcowax-10 capillary column (60 m 0.32 mm i.d., 0.5 μm film thickness, Supelco Ltd, Bellefonte, PA). The employed column temperature program was the following: 40 °C for 6 min, increased to 120 °C at a rate of 5 °C/min, then increased to 160 °C at a rate of 3 °C/min, and finally increased to 240 °C at 15 °C/min, holding this temperature for additional 5 min (46 min of total run time). Volatile compounds were identified by matching their mass spectra with the reference mass spectra from the NIST Mass Spectra library, also using the Retention index (Kovats index) and available published literature to improve identification quality. Three technical repetitions per each treatment and sample were performed. The quantification of the different identified VOCs was carried out by calculating the relative intensity, which was obtained through the division of the area of each specific compound by the sum of the areas of all the compounds identified in each specific chromatogram. This approach stands for a normalized assessment of the abundance of individual compounds within the chromatographic data, which allows for avoiding variation due to fiber decay.

Fatty acids determination by GC-FID

Fatty acids were analyzed by Analytical s.r.l. (Firenze, Italy) using a methodology in accordance with the EU standard guidelines (Appendix es II and IX of European Community Regulation EEC/2568/91). The fatty acids methyl esters (FAME) were obtained by cold alkaline transesterification using methanolic potassium hydroxide solution and extracted with n-heptane. The analysis of the fatty acid profile was conducted using a Focus GC, Thermo Scientific (Milano, Italy) chromatograph equipped with a split/splitless injector, a FID detector, and a SP-2560 fused silica capillary column (100 m × 0.25 cm i. d. × 0.2 μm film thickness, Supelco, Bellefonte, PA). Helium was used as carrier gas at an internal pressure of 110 kPa. The detector and injector were set at 275 °C and 260 °C, respectively. The oven temperature was programmed to start at 70 °C for the first 4 min, increasing to 110 °C at a rate of 8 °C/min, then increasing to 170 °C at a rate of 5 °C/min, with a 10-min hold, and finally increasing to 250 °C at a rate of 4 °C/min with a 15-min hold. The split ratio was 1:50, and the injected volume was 1 μL. The results are expressed as the relative percentage of each fatty acid. A reference sample of fatty acid methyl ester standard mixture (Supelco 37 FAME Mix, Supelco, Bellefonte, PA) was used to calibrate and identify the FAME by their retention times.

Phenolic composition determination by HPLC-DAD

For the extraction of the polar phenolic fraction, 2 g of olive oil were placed in a plastic tube and mixed with 1 mL of syringic acid solution (used as internal standard, 1.5 mg/mL in MeOH/H_2O 80:20 v/v) and 5 mL of MeOH/H_2O (80:20 v/v). The sample was vortexed for 1 min and subsequently sonicated for 15 min at room temperature (Ultrasonic Cleaner YJ5120-1, Citizen Scale, India) to improve phenolic solubilization. After centrifugation at 5000 rpm for 25 min, the hydroalcoholic phase was collected, drawn into a syringe and passed through a 0.45 μm PVDF filter. An aliquot of the extract was diluted 1:10 with water acidified to pH 3.2 with formic acid prior to injection, in order to ensure that all analytes fell within the calibration ranges and to avoid column overloading or detector saturation, and 20 μL were injected into the HPLC system for quantification. Chromatographic analyses were carried out using an HP 1100 system (Agilent Technologies, Palo Alto, CA, USA), equipped with a quaternary pump, temperature-controlled autosampler, column oven, and diode array detector (DAD). Phenolic compounds were quantified using DAD, while few runs of the oil samples have been performed on an identical HPLC system mounting electrospray ionization (ESI) mass spectrometry (MS) detector to support compound identification. On both systems phenols separation was performed using a Hypersil Gold C18 column (250 × 4.6 mm, 3 μm particle size; Thermo Electron Corporation, Austin, TX, USA) fitted with a matching guard column (10 × 4 mm), maintained at 30 °C. The same mobile phases, consisting of water acidified to pH 3.2 with formic acid (solvent A), acetonitrile (solvent B), and methanol (solvent C), and the same three-step gradient at a flow rate of 0.8 mL/min have been used in both analytical systems. The following gradient has been applied: from 2.5% to 27.5% B + C over 45 min, then increased to 50% over 10 min, followed by 5 min of isocratic elution at 50%. All solvents were HPLC grade. HPLC-DAD quantification of apigenin, methyl luteolin, luteolin, cinnamic acid, ferulic acid, o-cumaric acid, p-cumaric acid, vanillic acid, caffeic acid, vanillin, pinoresinol, tyrosol, hydroxytyrosol, hydroxytyrosol acetate and oleuropein has been achieved generating external calibration curves

using pure commercial standard references (Merck Group, Milan, Italy). For other secoiridoids and their oxidized derivatives (AHLA, AHOA, DDMOA, DDMLA, DAL, DOA, ODDMLA, ODDMOA, OAHOA, OAHLA) a calibration curve has been produced using a structurally related reference compound, namely hydroxytyrosol. Individual calibration curves were obtained by using multicomponent standard mixtures containing all phenolic standards. Eight-point calibration curves were prepared using solvent A in the range 0.08–10 µg/mL for simple phenolics (hydroxytyrosol, tyrosol, hydroxytyrosol acetate), phenolic acids (cinnamic acid, ferulic acid, o-coumaric acid, p-coumaric acid, vanillic acid, caffeic acid, vanillin), flavones (apigenin, luteolin, methyl luteolin) and lignans (pinoresinol), and 0.16–20 µg/mL for secoiridoid derivatives (AHOA, AHLA, DOA, DAL, DDMOA, DDMLA, OAHOA, OAHLA, ODDMOA, ODDMLA). Oxidized secoiridoid derivatives were assigned on the basis of their chromatographic behavior and spectroscopic features (retention time, UV spectra, and ESI–MS signals) within the routine analytical protocol (A representative chromatogram is reported in Supplementary Figure S1).

Sensory analysis

Evaluation of the olive oil was performed through quantitative descriptive analysis (QDA) from the expert panel of the University of Pisa. QDA was conducted at room temperature, in a standard sensory laboratory (ISO 8589:2010) located in the Department of Agriculture, Food, and Environment (DAFE), following the method described in the EEC/2568/91. The judges (10 assessors: 6 females and 4 males, aged between 23 and 63 years), primarily experts in oil tasting, were selected based on their availability from a larger pool of judges who regularly collaborate with the DAFE at the University of Pisa. All judges underwent standardized training to enhance their ability to recognize, describe, and quantify tastes, odors, and texture properties in accordance with ISO 8586: (2023) standards and typically worked collaboratively. Since the aim of the research was not limited to the commercial classification of the product but also included the evaluation of the treatment's effects on oil quality, a preliminary consensus panel was carried out by a subgroup of three trained assessors. A new sensory evaluation sheet was specifically developed based on the official template. To provide a more comprehensive assessment of the organoleptic profile of the experimental olive oils, the proposed sensory sheet includes additional attributes beyond those listed in the official version. A final set of 18 descriptive parameters, including both quantitative descriptors olfactory intensity, fruity (olfactory), aromatic richness, taste intensity, fruity, bitter, spicy, sweet, heating, mold, winey, sludge, metallic, rancid, and evolutionary state) and hedonic descriptors (olfactory pleasantness, taste pleasantness, and overall pleasantness), were selected. The samples were presented in a different order at each tasting session, and 5 min intervals between each sample were set. Furthermore, a oil sample was randomly replicated to verify the performance of the panel at each tasting session. For evaluation, each assessor was provided with filtered water and asked to cleanse their palate between tastings. Each attribute was evaluated on a 0-9 scale. All ratings were digitally acquired by the Input Sensory Soft 2.0 (ISS, Centro Studi Assaggiatori, Brescia, Italy). Finally, the overall hedonic index (HI) of the oils, which represents the overall acceptability of the product, was calculated based on the mean of the hedonic parameters, which were converted to a scale from 0 to 10, as previously reported (Bianchi et al., 2024). The HI was calculated at time 0 and after 1 year of storage to assess whether the acceptability of the product changed over time.

Statistical analysis

Multivariate analyses were performed using R software (R core team, 2023, version 1.1.463–2009-2018 R-studio, Inc.). Differences between olives temperature in the different stages of the process were evaluated by t-test ($p \leq 0.05$). Metabolites and chemical parameter levels (TPC, TTC, FFA, PV, and UV spectrophotometric indices K232 and K268) were evaluated using ANOVA ($p \leq 0.05$) and Tukey HSD post hoc test ($p \leq 0.05$). Heatmap representation of the fold change (Formula 1) values were used to better visualize differences between control and treated samples in terms of polyphenols and VOCs level, where the samples were evaluated by t-test ($p \leq 0.05$).

$$\mathrm{FC} = Log_2\left(\frac{Treated\ sample}{Control\ sample}\right) \quad (1)$$

Partial Least Square Discriminant Analysis (PLS-DA) has been used for processing metabolomic (polyphenols, fatty acids, and VOCs) data. Treatment has been used as response variables, whereas polyphenols, fatty acids, and VOCs have been used as predictor variables. Variable Importance in Projection (VIP) scores were employed to filter the most important variables for the exploratory data analysis. All the analyses were conducted in triplicate. The data processing of the sensory profile has been carried out using the software Big Sensory Soft 2.0 (Centro Studi Assaggiatori, Brescia, Italy) and ANOVA interquartile two ways test ($p \leq 0.05$) has been applied choosing samples and panelists as main factors.

Table 1 Temperatures of the olives (mesocarp) from Frantoio and Leccino cultivars at harvest and following the hydrocooler treatment across the three experimental seasons. Values represent the mean of analyses conducted with their standard deviation for each year and cultivar ($n = 30$)

Year	Cultivar	Temperature at Harvest (°C)	Temperature Post-Hydrocooler (°C)	Δ Temperature—Average (°C)
2019	Frantoio	21.7 ± 1.63	12.4 ± 1.59	9.3
2020	Frantoio	24.8 ± 1.05	17.8 ± 1.13	7.0
	Leccino	23.4 ± 1.87	17.2 ± 1.95	6.2
2021	Leccino	23.5 ± 0.98	14.1 ± 1.44	9.4

Results

Effects of the hydrocooler treatment on the temperature of olives, paste and oil

During the harvesting seasons, olives from Frantoio (2019 and 2020 seasons) and Leccino (2020 and 2021 seasons) cultivars were treated using hydrocooler equipment immediately after harvest, just before starting the oil extraction. Despite the short treatment duration (approximately 30 s), the olive fruit mesocarp temperature decreased significantly with a Δ temperature ranging between 6.2 and 9.4 °C (Table 1). No significant differences were found in the extraction yield between the control and treated olives (Supplementary Table S1).

In addition to the internal temperature of the olives, the paste temperature was monitored immediately after crushing and during malaxation and centrifugation. Table 2 presents the paste and oil temperatures recorded throughout the various stages of olive processing in the different trials and for the two tested cultivars. For both cultivars and considering all years, lower paste and oil temperatures were recorded for the Hy samples, with paste temperature showing a maximum reduction of approximately 4 °C in Hy samples compared to C samples. Although there were considerable differences in the temperatures recorded at harvest and immediately after the cooling treatment across the different years (Table 1), the variations in paste temperatures after the crushing step were much less pronounced within the same sample groups (C or Hy), and showed more consistent and uniform values. It is noteworthy that malaxation temperature always showed significant reductions applying Hy treatment on olives.

Evaluation of the quality and composition parameters of the oil samples

Effect of the hydrocooler treatment on chemical parameters and fatty acid profile of fresh oil

Figures 2 and 3 show the free fatty acids (FFA), peroxide values (PVs), spectrophotometric parameters K232 and K268, total phenol content (TPC) and total tocopherol content (TTC) analyzed within 2–3 weeks after oil production in the Hy and C samples. To identify a general trend and possible significant differences induced by the treatment on oil quality, data were grouped by cultivar and separated per year of analysis.

Clear differences between Frantoio and Leccino cultivars in terms of responses to the hydrocooler treatment were present, as well as a strong effect of the harvesting year. This was potentially linked with the differences in the Jaen index recorded at harvest for the different batches, and/or the differences registered in the experimental years in terms of Δ temperature reached after hydrocooling treatment (Table 1).

As shown in Fig. 2, Hy oil samples from the Frantoio cv consistently showed significant reductions in FFA value in both 2019 and 2020. In contrast, Hy oil samples from

Table 2 Temperature of the paste after crushing and after malaxation, and oil temperature after centrifugation in different trials and for both tested cultivars. Different letters associated with each value (± standard deviation) indicate significant differences (within the same cv) between treatments and times ($p \leq 0.05$, Tukey's test). Values represent the mean of analyses conducted in triplicate for each year and cultivar

Year	Cultivar	Treatment	Paste temperature after crushing *(°C)*	Paste temperature after malaxation *(°C)*	Oil temperature after centrifugation *(°C)*
2019	Frantoio	C	26.4 ± 0.2^{a}	25.3 ± 0.2^{a}	27.8 ± 0.2^{a}
		Hy	22.3 ± 0.4^{b}	24.1 ± 0.2^{b}	25.4 ± 0.3^{b}
2020	Frantoio	C	26.1 ± 0.4^{a}	25.9 ± 0.2^{a}	26.9 ± 0.2^{a}
		Hy	23.5 ± 0.3^{b}	25.1 ± 0.3^{b}	25.7 ± 0.1^{b}
	Leccino	C	25.7 ± 0.2^{a}	25.8 ± 0.2^{a}	27.6 ± 0.1^{a}
		Hy	21.4 ± 0.1^{b}	23.7 ± 0.3^{b}	26.3 ± 0.3^{b}
2021	Leccino	C	24.8 ± 0.2^{a}	25.6 ± 0.1^{a}	27.4 ± 0.3^{a}
		Hy	20.8 ± 0.3^{b}	23.5 ± 0.2^{b}	26.1 ± 0.2^{b}

C Control, *Hy* Hydrocooler-treated

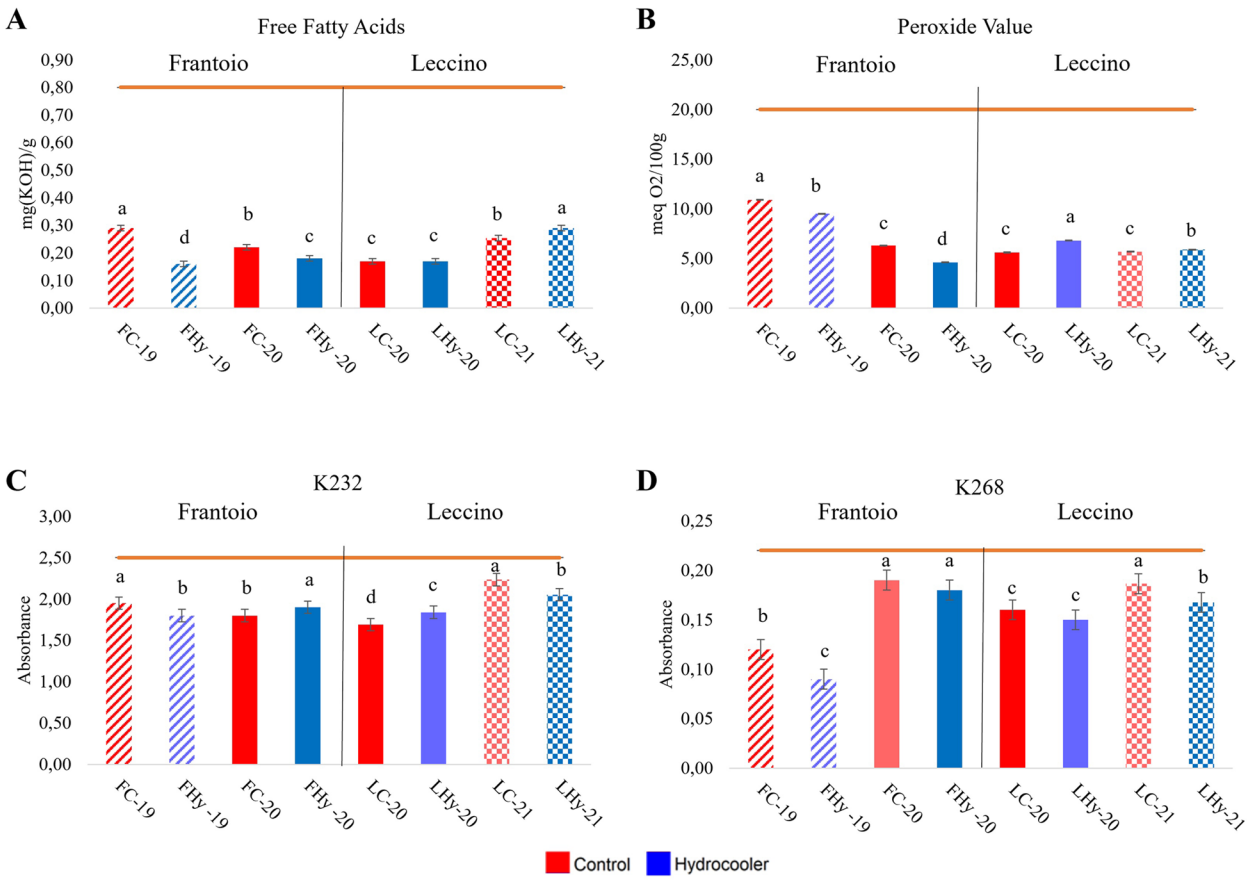


Fig. 2 **A** FFA (Free Fatty Acids; mg KOH/g), **B** PV (Peroxide Value; meq O_2/100 g), and **C** and **D** spectrophotometric indexes (absorbance at 232 and 268 nm) in different years and cultivars of the oil samples analyzed after production. Different letters associated with each column (black bars report the standard deviation) indicate significant differences (within the same cv) between treatments and times ($p \leq 0.05$, Tukey post hoc test). Values are the average of the analysis performed in triplicate each year and for each cultivar (Frantoio in 2019 and 2020; Leccino in 2020 and 2021). The orange lines represent the EU legal limits of the different parameters below which the olive oil is classified Virgin. F: Frantoio; L: Leccino; C: Control; Hy: Hydrocooler-treated samples; Bar with lines filling: Samples evaluated in 2019; Bar with solid filling: Samples evaluated in 2020; Bar with squared filling: Samples evaluated in 2021

Leccino showed no significant impact on FFA values in 2020 and a slight, but significant, increase in 2021. Considering oxidative stability, Frantoio Hy samples showed a consistent and significant decrease of PV in both 2019 and 2020. Despite displaying less intense changes, PV in Hy Leccino oils revealed an opposite trend. K232 and K268 showed different behavior in the two cultivars in the different studied years, with a consistent reduction of these parameters in the Hy samples only in 2019 for Frantoio, and in 2021 for Leccino oils (Fig. 2).

The Hy Frantoio samples showed a significant and consistent reduction in TPC (Fig. 3A). In contrast, the Hy Leccino samples presented a variable response, showing a slight but significant decrease in 2020, and a significant increase in 2021. Hy Frantoio samples showed a significant increase in TTC in 2019, but not in 2020. In comparison with the respective control oil (Fig. 3B), a reduction of TTC was shown in the Hy Leccino oil samples in 2020, but an increase was observed for this parameter in 2021.

Analysis of the fatty acid profile revealed a spectrum of 12 distinct molecules, categorized into monounsaturated, polyunsaturated, and saturated groups. Monounsaturated fatty acids included palmitoleic, margaroleic, oleic, and eicosanoic acids. The polyunsaturated category comprised linoleic and linolenic acids, while the saturated fatty acids comprised palmitic, stearic, arachidic, behenic, and lignoceric acids. The isomer trans oleic + linoleic was also quantified, showing a comprehensive profile of the different fatty acid composition in the samples examined. However, no significant effects of the hydrocooler treatments were detected in terms of the identified fatty acids (data not shown).

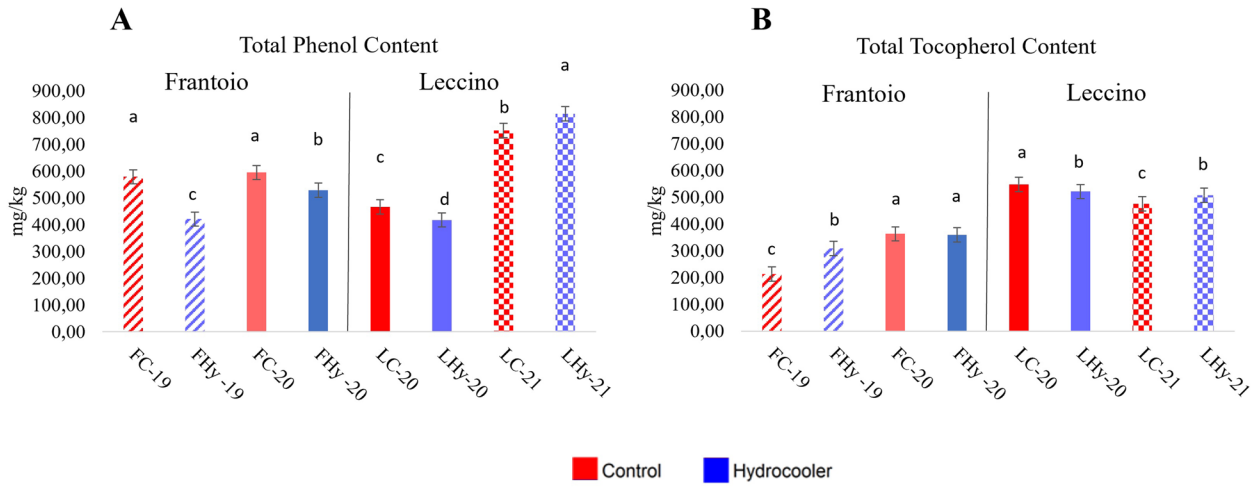


Fig. 3 Total Phenol Content (TPC) (**A**) and Tocopherol (TTC) (**B**) content of control and hydrocooler treated samples in different years and cultivars of the oil samples analyzed after production. Different letters associated with each column (black bars report the standard deviation) indicate significant differences between treatments and times ($p \leq 0.05$, Tukey post hoc test). Values are the average of the analysis performed in triplicate each year and for each cultivar (Frantoio in 2019 and 2020; Leccino in 2020 and 2021). F: Frantoio; L: Leccino; C: Control; Hy: Hydrocooler-treated samples; Bar with lines filling: Samples evaluated in 2019; Bar with solid filling: Samples evaluated in 2020; Bar with squared filling: Samples evaluated in 2021

Effects of the hydrocooler treatment on the polyphenol profile

The polyphenol analysis identified a total of 24 molecules, reported in Fig. 4 and Supplementary Table S2. This range of phenolic compounds comprised various classes, including acids, aldehydes, flavones, lignans, and secoiridoids.

The fold-change analysis results reported in the heatmap in Fig. 4 show that polyphenols level varies depending on the treatment and time. Different trends were observed for the Frantoio and Leccino cultivars, also in relation to the different trial years. Except for luteolin, the Frantoio oil samples (Fig. 4A) showed a general decrease in polyphenols in 2019 after the hydrocooler treatment, with significant decreases reported also in 2020, which, however, involved less and different compounds. The Leccino samples (Fig. 4B) instead showed a significant increase of several polyphenols in 2021, while no significant changes were recorded for oils produced in 2020 season. These results, except for Leccino samples in 2020, mirrored the recorded trends of TPC in Hy oils in both cultivars (Fig. 3), further confirmed by the total phenol content determined on the basis of the HPLC-DAD analysis (Supplementary Table S2).

In detail, the 2019 Hy Frantoio oil samples showed a significant reduction in both highly abundant polyphenols, such as pinoresinol (from 90.87 to 68.33 mg/kg), and less abundant compounds, such as hydroxytyrosol (from 2.80 to 2.33 mg/kg), apigenin (from 13.89 to 10.33 mg/kg), methyl luteolin (from 9.89 to 7.67 mg/kg), and cinnamic acid (from 12.09 to 8.84 mg/kg). The highly abundant secoiridoid derivatives, such as AHOA (aldehydic hydroxylic oleuropein aglycone; from 65.23 to 53.67 mg/kg of hydroxytyrosol equivalents) and DDMLA (dialdehyde dicarboxymethyl ligstroside aglycone; from 70.89 to 58.10 mg/kg of hydroxytyrosol equivalents), and other less abundant secoiridoid derivatives, such as AHLA (aldehydic hydroxylic ligstroside aglycone; from 5.23 to 3.67 mg/kg of hydroxytyrosol equivalents), followed the same trend and were significantly affected by the cooling treatment (Fig. 4A and Supplementary Table S2).

Positive changes in pholyphenol content were identified in Leccino oil produced in 2021. A significant increase was detected for compounds such as cinnamic acid (from 4.71 to 18.16 mg/kg), apigenin (from 2.10 to 10.80 mg/kg), methyl luteolin (from 4.62 to 9.19 mg/kg), and OAHLA (oxidized aldehydic hydroxylic ligstroside aglycone; from 15.96 to 139.23 mg/kg of hydroxytyrosol equivalents), the oxidised form of the secoiridoid derivative AHLA (Fig. 4B and Supplementary Table S2).

In addition, Frantoio oils in 2019 also revealed a significant increase in luteolin (from 5.87 to 10.67 mg/kg), whereas a significant decrease in ferulic acid (from 3.32 to 1.59 mg/kg) and AHOA (aldehydic hydroxylic oleuropein aglycone; from 121.40 to 7.88 mg/kg of hydroxytyrosol equivalents) has also been recorded in Leccino oils in 2021. Interestingly, very limited or no changes in the polyphenol profiles were detected in both Leccino and Frantoio oil samples in 2020 (Fig. 4A and B).

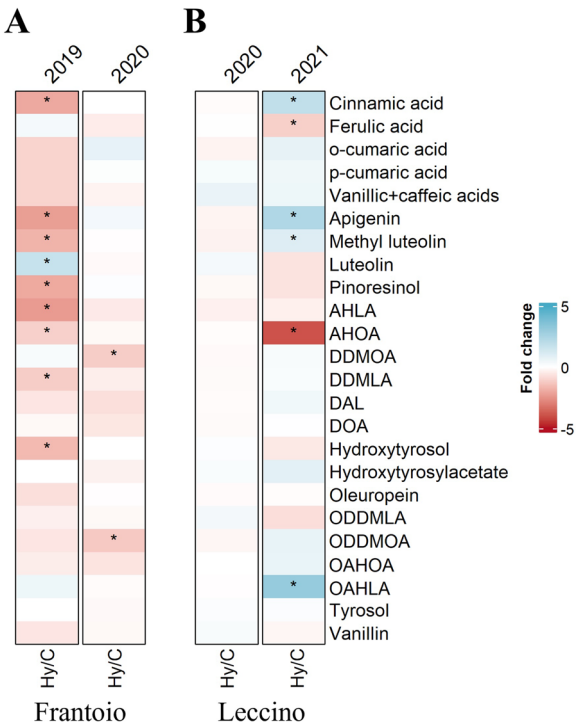


Fig. 4 Heatmap representation of the polyphenols trend after hydrocooler treatment in the oil samples after production. Panel A: Frantoio (2019 and 2020). Panel B: Leccino (2020 and 2021). Blue color: higher in Hy; red color: lower in Hy. Analysis was performed in triplicate per each year and cultivar. Fold Change (Log_2[Treatment/Control]) was used to represent modifications. Asterisks indicate significant differences between Hy and C samples (t-test $p \leq 0.05$). AHLA: Aldehydic Hydroxylic Ligstroside Aglycone; AHOA: Aldehydic Hydroxylic Oleuropein Aglycone; DDMOA: Dialdehyde Decarboxy Methyl Oleuropein Aglycone; DDMLA: Dialdehyde Decarboxy Methyl Ligstroside Aglycone; DAL: Dialdehyde Aglycone Ligstroside; DOA: Dialdehyde Oleuropein Aglycone; OAHLA: Oxidized Aldehydic Hydroxylic Ligstroside Aglycone; OAHOA: Oxidized Aldehydic Hydroxylic Oleuropein Aglycone; ODDMOA: Oxidized Dialdehyde Decarboxy Methyl Oleuropein Aglycone; ODDMLA: Oxidized Dialdehyde Decarboxy Methyl Ligstroside Aglycone; Oleuropein: Oleuropein hydrolyzed derivates

Volatile organic compound (VOC) analysis

VOCs play a crucial role in defining the olive oil aroma and flavor profile, which are key factors in its sensory quality and consumer acceptance. The analysis revealed a total of 31 VOCs, as reported in Supplementary Table S3 and Supplementary Figures S2 and S3, with different identified chemical classes, including organic acids, alcohols, aldehydes, ketones, and other compounds.

Figure 5A and C report two partial least squares discriminant analysis (PLS-DA) models built using the VOCs quantified (predictor variables) in the C and Hy fresh oil samples (treatment was used as the response variable), considering the two years together, but created separately for the two cultivars. These PLS-DA models revealed distinct VOC patterns based on the different cultivars and treatments.

Considering factors 1 and 2 together, the model built for the Frantoio oil samples explained a total of 97% of the variability within the dataset (Fig. 5A). Similarly, considering together the first two factors of the PLS-DA produced for Leccino oils, a total of 95% of the total variability was explained by the model (Fig. 5C). Both models showed an important effect of the treatment, which induced a clear clustering of Hy (right side of the plot for both models) and C samples (left side of the plot for both models). In addition, another common feature in the two models was that the impact of the year led to a separation of the samples along factor 2. The Frantoio samples from 2019 showed positive values on factor 2, while samples from 2020 were characterized by negative values. Similarly, in Leccino, the influence of the year was evident, with samples from the year 2020 reflecting positive values on factor 2 and the oils from year 2021 being associated with negative values. This distinct positioning indicates a discernible difference in VOC profile between the two years.

Figure 5B and D report the ten variables that contributed the most to the cluster formation based on their different importance in the projection score (VIPs) of the two models, together with the heatmap representation of the fold change (FC) between the C and Hy samples for the level of these variables, and the results of the univariate statistical analysis comparing the oils. The values corresponding to the relative intensity recorded for each compound can be found in Supplementary Table S3, reporting VOC results of oil samples after production.

Regarding the Frantoio oil samples, among the variables that contributed the most to the separation of oil clusters, some VOCs also revealed statistically significant differences between the C and Hy samples. Specifically, 3-hexen-1-ol and 2-hexen-1-ol (which, except for 2-hexen-1-ol in 2019, increased in the Hy oils), and 1-penten-3-one and ethyl alcohol (which, except for 1-penten-3-one in 2020, decreased in the Hy oils) (Fig. 5B). The volatile alcohols 3-hexen-ol and 2-hexen-1-ol are two highly abundant compounds and the observed increased in Hy samples is by approximately ten and two-fold, respectively. On the other hand, the observed decrease in ethyl alcohol, which is characterized by an alcoholic note and considered to be an off-flavor, is of approximately ten-fold. Moreover, 1-penten-3-one, which is another highly abundant compound, decreased by about six-fold due to the treatment. The Frantoio VIP list also shows other molecules, despite resulting non-significantly different after the univariate analysis. Of these, 2-hexenal, which is one of the most important C6

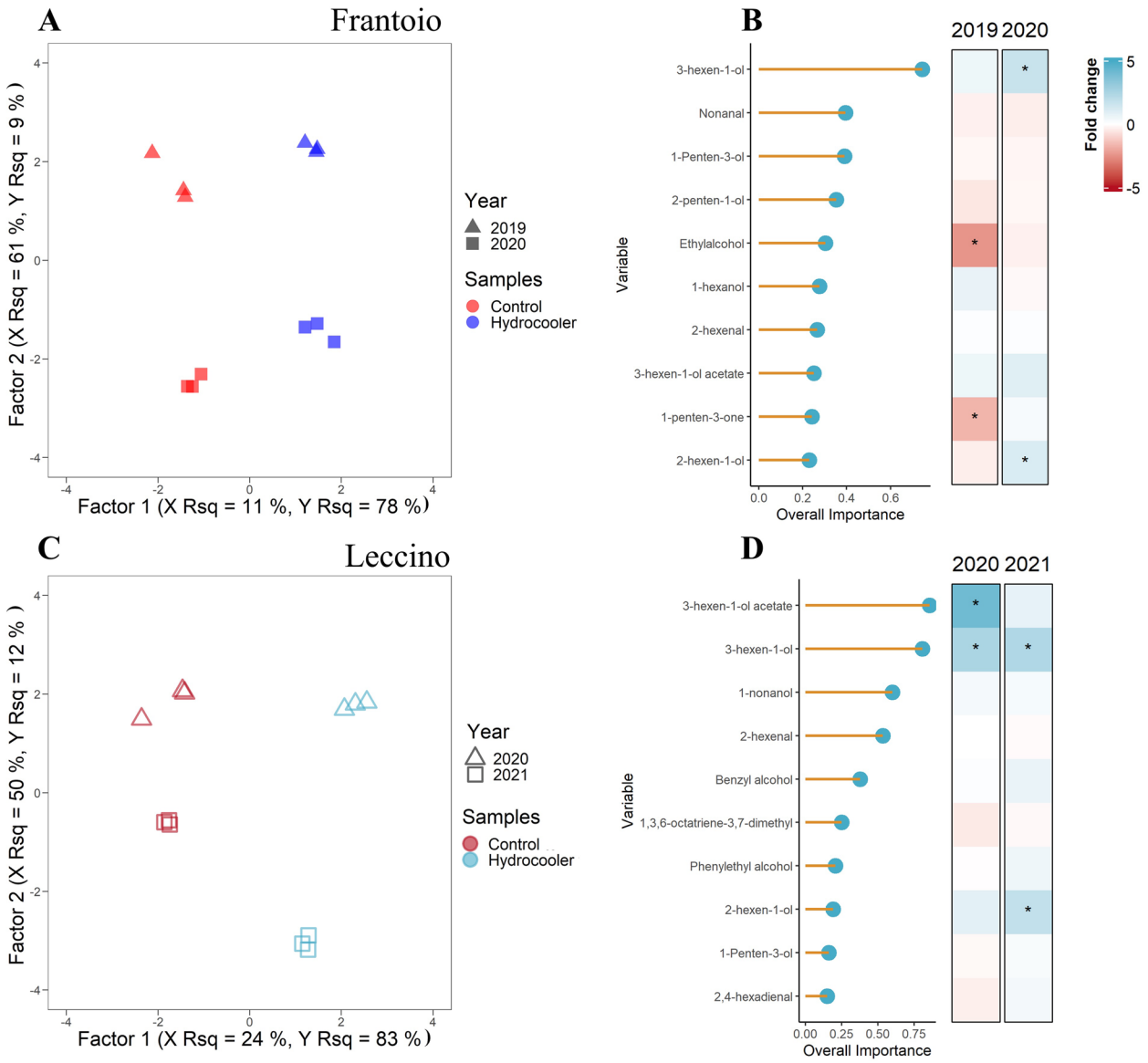


Fig. 5 Partial Least Square Discriminant Analysis (PLS-DA), Variable importance in projection (VIP), and heatmap representation of the VOCs composition of the oil samples after production. Frantoio (**A** and **B**) and Leccino (**C** and **D**). Treatment has been used as response variable, whereas all the quantified VOCs have been used as predictor variables. **A** and **C**: Score plots of the models created analysing fresh oil samples. **B** and **D**: Score of the variables that contributed the most to the grouping of the samples deriving from the different treatments with the heatmap representation (blue color: higher in hydrocooler; red color: lower in hydrocooler). Analysis was performed in triplicate per year and per cultivar; (Frantoio in 2019 and 2020; Leccino in 2020 and 2021). The asterisks indicate significant differences between the treated and control samples (t-test $p \leq 0.05$)

VOC in olive oil, and 1-penten-3-ol, which is considered an off-flavor, appeared to increase and decrease, respectively, in Hy oils (Fig. 5B).

Regarding the Leccino oil samples, 3-hexen-1-ol and 2-hexen-1-ol were included in the VIP list and showed significant differences between the C and Hy samples, with Hy oils showing higher levels in both years. These C6 alcohols are important contributors to the olive oil aroma and derive from the lipoxygenase (LOX) pathway. In addition, a volatile ester derived from 3-hexen-1-ol, namely 3-hexen-1-ol acetate, was also found to be differentially accumulated in Hy oils, showing higher levels than the C samples in both years (Fig. 5D). 3-hexen-ol acetate, 3-hexen-ol, and 2-hexen-1-ol, which are highly abundant compounds contributing to grassy and green aromas, increased by about twenty, six, and two-fold, respectively, in Leccino Hy samples. The ester 3-hexen-1-ol acetate also accumulated in the Hy samples of the

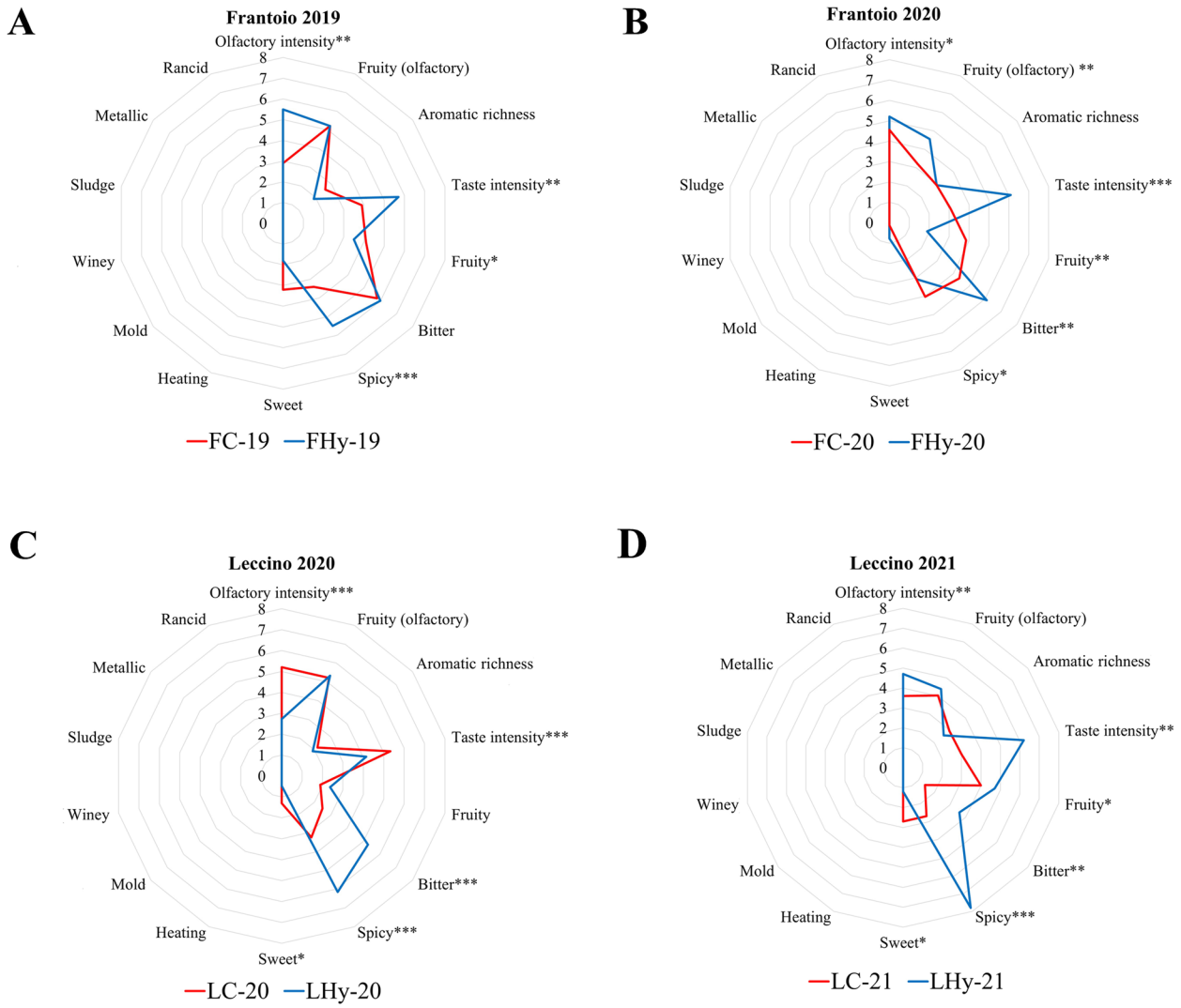


Fig. 6 Medians of quantitative descriptors obtained from sensory analysis of Frantoio oils produced in 2019 (**A**) and 2020 (**B**) and Leccino oils produced in 2020 (**C**) and 2021 (**D**) analysed immediately after the production. Significance level: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$; without asterisk = not significant ($p \geq 0.05$)

Frantoio cultivar, appearing in the VIP list. However, this result was not supported by statistical significance in the univariate analysis (Fig. 5B).

Overall, most of the modifications induced in both cultivars by the hydrocooler treatment and considering all the years of analysis regarded the class of C6 alcohols, which are associated with an herbal/green flavor (Fig. 5B and D).

Sensory analysis

To better understand how the postharvest hydrocooling treatment affected the olive oil sensory profile, a qualitative descriptive analysis was conducted to compare the sensory attributes of the fresh olive oil samples (Fig. 6). The results indicated that the hydrocooling treatment significantly enhanced the sensory attributes of olive oil from both cultivars. For the Frantoio 2019 oil samples (Fig. 6A), hydrocooling (FHy) increased olfactory intensity, taste intensity, and spiciness compared to the control (FC), although a decrease in fruity notes was observed. This trend was confirmed in the Frantoio 2020 samples (Fig. 6B), where the treated oil (FHy) showed significantly higher values for olfactory intensity, olfactory fruity, taste intensity, and bitterness. However, fruity (taste) and spiciness slightly decreased compared to the control (FC).

In the Leccino cultivar, an increase in bitterness, olfactory intensity, and spiciness was also detected in both the 2020 and 2021 samples. Specifically, in 2020 (Fig. 6C), the hydrocooling treatment (LHy) resulted in higher levels

of spiciness and bitterness compared to the control (LC), but it negatively affected taste and olfactory intensity. In contrast, in 2021 (Fig. 6D), the treated Leccino samples (LHy) showed the highest levels of taste and olfactory intensity, fruity notes, bitterness, and spiciness.

Overall, regardless of cultivar and harvest year, hydrocooling generally proved effective in producing oils with enhanced sensory profiles, particularly in terms of fruitiness, bitterness, and spiciness, a trend that aligns with findings from chemical and volatile compound analyses.

Influence of temperature treatment on olive oil quality one year after production

To assess the impact of the hydrocooler treatment on the oil quality and composition after storage, C and Hy oil samples from both cultivars were stored for one year at 15 °C in 0.75 L dark-bottles, kept in the dark with nitrogen applied to the headspace of the bottle.

Chemical parameters

Figure 7 presents the FFA, PV, and spectrophotometric parameters K232 and K268 values recorded in oil samples after one year of storage, while Fig. 9 shows the TPC and TTC values of the same oil samples. The analysis of chemical quality parameters clearly revealed different responses of the Frantoio and Leccino cultivars. Notably, Hy Frantoio samples showed a lower FFA value than that of the control in 2019 (as observed in the fresh oil), while no difference was detected in 2020. In contrast, Leccino exhibited no change in FFA in 2020 but a reduction in 2021. The peroxide value decreased consistently in the Hy Frantoio samples for both years, while Leccino remained stable in 2020 and showed a decrease in PV only in 2021. Considering the behavior of the two spectrophotometric indexes in oils from Leccino, a significant decrease for K232 and K268 in 2021 and 2020, respectively, has been observed. The results for Frantoio oils were even more

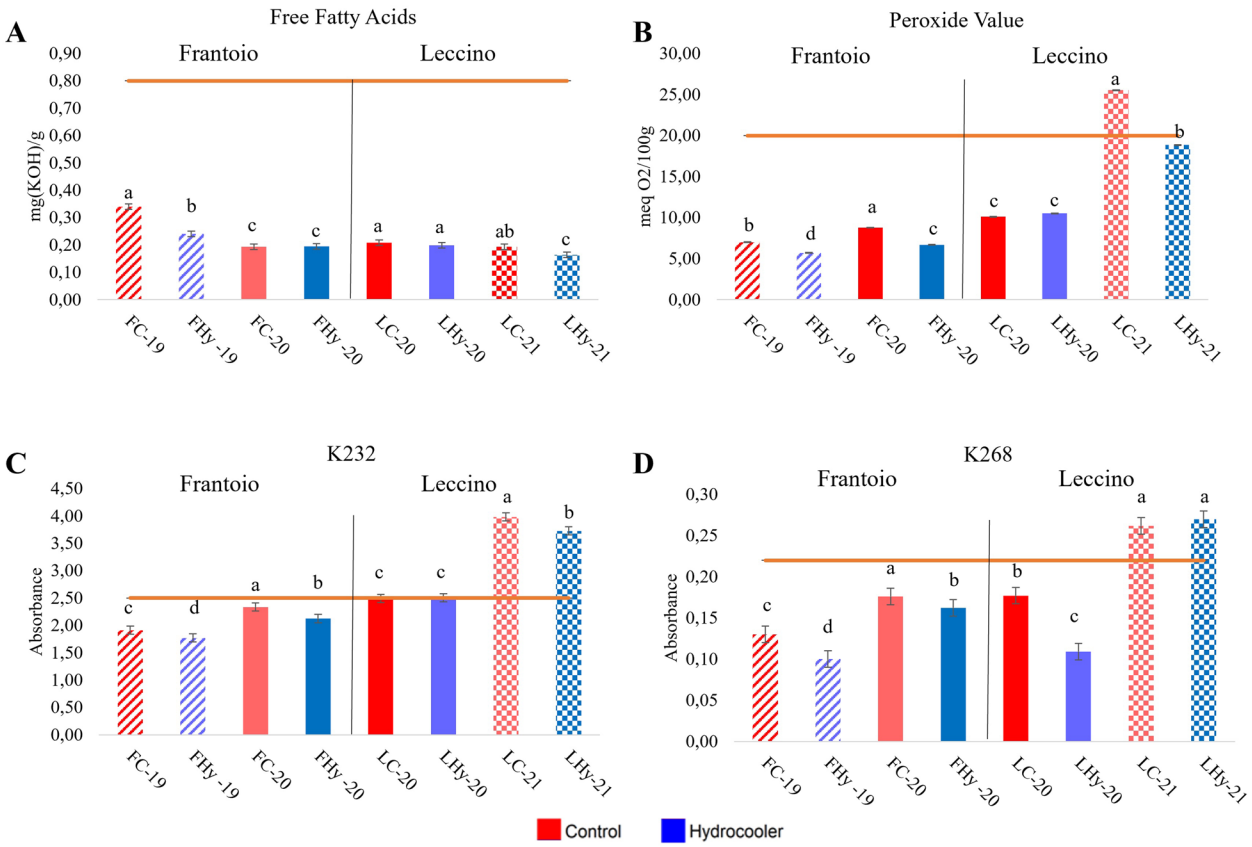


Fig. 7 **A** FFA (Free Fatty Acids; mg (KOH)/g), **B** PV (Peroxide Value; meq O2/100 g), and spectrophotometric indexes (absorbance at 232 and 268 nm) (**C** and **D**, respectively) in different years and cultivars of the oil samples analyzed after one year of storage. Different letters associated with each column (black bars report the standard deviation) indicate significant differences (within the same cv) between treatments and times ($p \leq 0.05$, Tukey's test). Values are the average of the analysis performed in triplicate per year and cultivar (Frantoio in 2019 and 2020; Leccino in 2020 and 2021). The orange lines represent the UE legal limits of the different parameters below which the olive oil is classified Virgin. F: Frantoio; L: Leccino; C: Control; Hy: Hydrocooler-treated samples; Bar with lines filling: Samples evaluated in 2019; Bar with solid filling: Samples evaluated in 2020; Bar with squared filling: Samples evaluated in 2021

consistent, with a reduction in K232 and K268 after the cooling treatment detected in both years.

After one year of storage, in both Hy Frantoio and Leccino samples the TPC was reduced by the cooling treatment in the 2019 and 2020 season, respectively, but it showed an increase in 2020 and 2021 seasons, respectively, thus demonstrating a season-specific behavior (Fig. 8). This specific trend has been confirmed also by the total phenol content determined on the basis of the HPLC-DAD analysis (Supplementary Table S4). At the level of individual phenolic compounds, a general increase in oxidized secoiridoid derivatives in both cultivars has been observed, confirming the instability of aldehydic secoiridoids during storage (Supplementary Table S4). The TTC in Frantoio exhibited an increase in 2019 but a decrease in 2020 in response to the cooling treatment, while in Leccino the opposite trend was observed, with a decrease in 2020 and an increase in 2021.

Multivariate analysis of fatty acid, polyphenol, and VOC data

PLS-DA analyses of the fatty acid, polyphenol, and VOC data of the oil samples one year after their production were performed separately for the two cultivars (Fig. 9) in order to better investigate the long-term effects on oil composition of applying cooling treatment on the olives. The model in Fig. 9A refers to the Frantoio cultivar and explained a total of 89% of the variability considering factors 1 and 2 together. On the other hand, the model in Fig. 9C refers to the Leccino oils and explains a total of 92% of the variability considering the first two factors together. The PLS-DA score plots shown in Fig. 9A and 9C shows the substantial and persistent differences between C and Hy olive oil samples from both Frantoio and Leccino cultivar. A clear distinction between Hy and C samples is shown even after one year of storage under optimal conditions, and the differences between the harvest seasons are still visible.

Considering the Frantoio oils, VIP analysis (Fig. 9B) revealed behenic acid (C22:0), methyl luteolin, 2-hexenal, and ferulic acid as primary variables contributing to the distinction between samples. Variations between years introduced inconsistency, with lower level of polyphenols such as methyl luteolin and hydroxytyrosol only in 2019, while ferulic acid was higher in Hy oils only in 2020 (phenolic concentration values and relative intensity of VOCs in stored oils are shown in Supplementary Tables S4 and S5, respectively). On the other hand, for the Leccino samples, VIP analysis (Fig. 9D) revealed important variables influencing cluster separation, with lignoceric acid (C24:0), DOA (dialdehyde oleuropein aglycone), ferulic acid, and hydroxytyrosol acetate emerging as primary contributors to the observed different clustering of the oils.

Interestingly, also after one year of storage some common variables in the top 10 VIP list were identified as being affected by the hydrocooler treatment in both cultivars, namely 2-hexenal, DOA and ferulic acid, this latter appearing increased in Hy oils from both cultivars and years.

Sensory analysis

To verify the influence of hydrocooler treatment on sample shelf life, a descriptive analysis was performed after

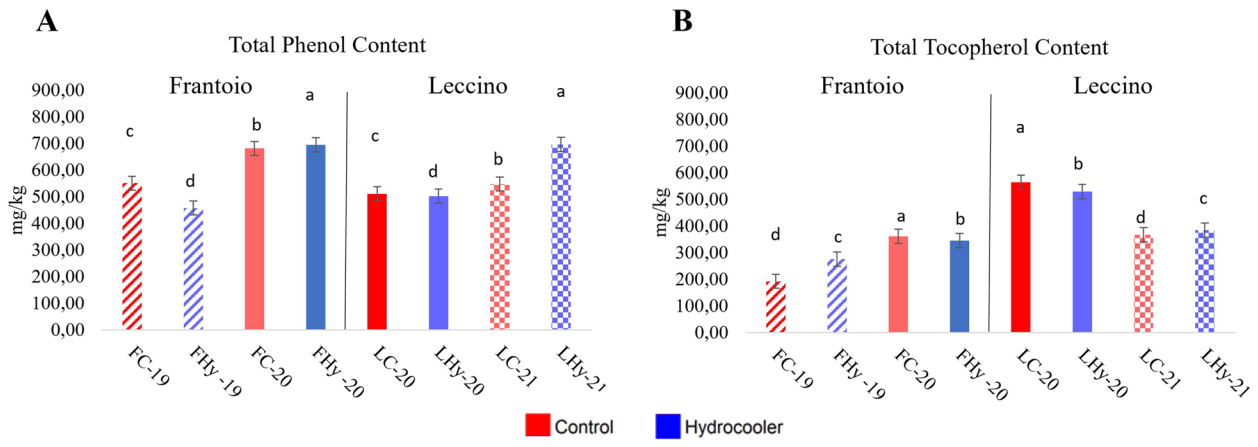


Fig. 8 Total Phenol Content (TPC) (**A**) and Tocopherol Content (TTC) (**B**) of control and hydrocooler treated samples in different years and cultivars in the oil samples after one year of storage. Different letters associated with each value (± standard deviation of the measurement) indicate significant differences between treatments and times ($p \leq 0.05$, Tukey's test). Values are the average of the analysis performed in triplicate per year and per cultivar (Frantoio in 2019 and 2020; Leccino in 2020 and 2021). F: Frantoio; L: Leccino; C: Control; Hy: Hydrocooler-treated samples; Bar with lines filling: Samples evaluated in 2019; Bar with solid filling: Samples evaluated in 2020; Bar with squared filling: Samples evaluated in 2021

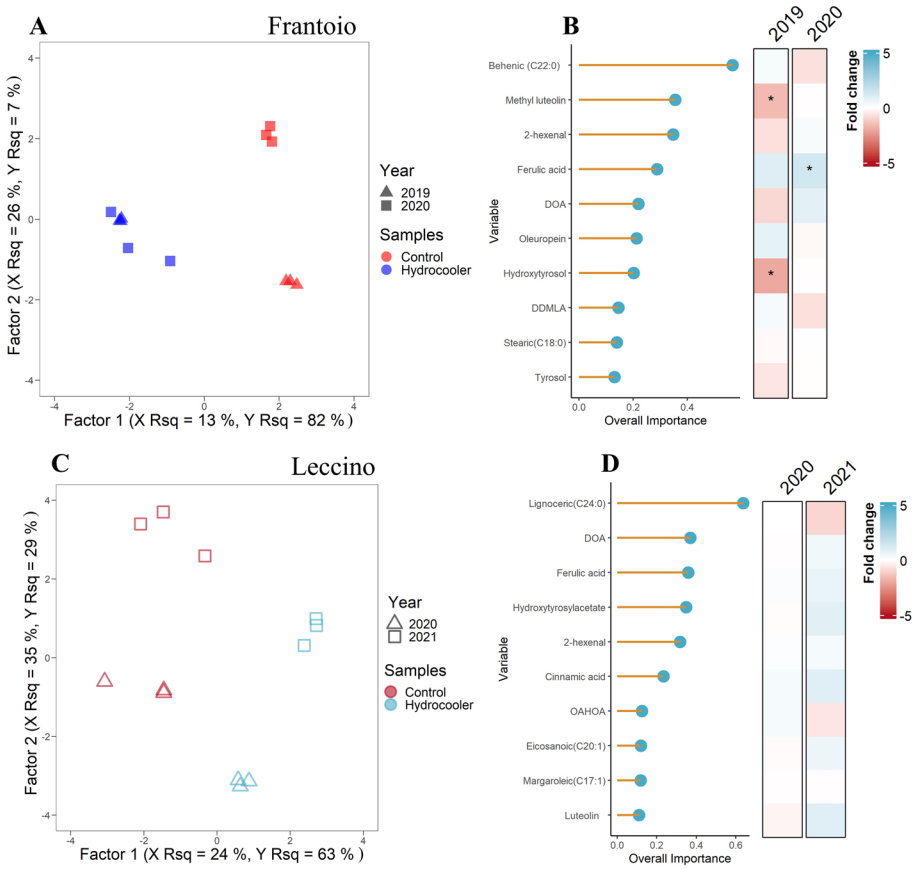


Fig. 9 Partial Least Square Discriminant Analysis (PLS-DA), Variable importance in projection (VIP), and heatmap representation of the fatty acids, polyphenols, and VOCs composition in oil samples after one year of storage. Frantoio (**A** and **B**) and Leccino (**C** and **D**). Treatment has been used as response variable, whereas fatty acids, polyphenols, and VOCs have been used as predictor variables. **A** and **C** Score plot of the model of analysed oil samples. B and D: Score of the variables that contribute most to the grouping of the samples deriving from the different treatments with the heatmap representation (blue color: higher in hydrocooler; red color: lower in hydrocooler). Analysis was performed in triplicate per year and per cultivar (Frantoio in 2019 and 2020; Leccino in 2020 and 2021). The asterisks indicate the significant differences between the treatment and control (t-test $p \leq 0.05$). DOA: Dialdehyde Oleuropein Aglycone; DDMLA: Dialdehyde Decarboxy Methyl Ligstroside Aglycone; OAHOA: Oxidized Aldehydic Hydroxylic Oleuropein Aglycone; Oleuropein: Oleuropein hydrolyzed derivate

one year of storage only for the 2020 samples of Frantoio and Leccino (Fig. 10).

After one year of storage, the control oils (FC and LC) developed sensory defects, regardless of the cultivar. Specifically, the presence of rancid and winey notes negatively impacted the overall sensory evaluation, with the presence of these notes resulting in a non-eligibility for the EVOO classification. In contrast, the Hy oils maintained higher sensory quality throughout the observation period, regardless of the cultivar. More specifically, Hy Frantoio oils (FHy) showed significant improvements in olfactory and taste intensity, bitterness, fruitiness, and spiciness compared to the control oils (Fig. 10A). A similar trend was observed in the Leccino 2020 oils (Fig. 10B).

As previously noted, the hedonic quality of a product is a key factor in determining consumer acceptance. Figure 11 presents the Hedonic Index (HI) calculated for all analyzed oils, with a threshold value of six set for acceptability. All oils -except LC-21, FC-20 (after 1 year), and LC-20 (after 1 year)-scored above this threshold. In general, oils produced with the hydrocooling treatment were consistently more appreciated than control ones. This trend was maintained after one year of storage: both LHy-20 and FHy-20 samples scored above the acceptability threshold, while the control oils (LC-20 and FC-20) were rated below it, consistent with the presence of sensory defects such as rancid and winey notes.

Discussion

The olive oil industry has increasingly recognized the significant impact on oil quality of temperature control during the malaxation process. Studies have shown it can enhance the oil's nutraceutical properties by preserving key volatile compounds and antioxidants (Angerosa et al.,

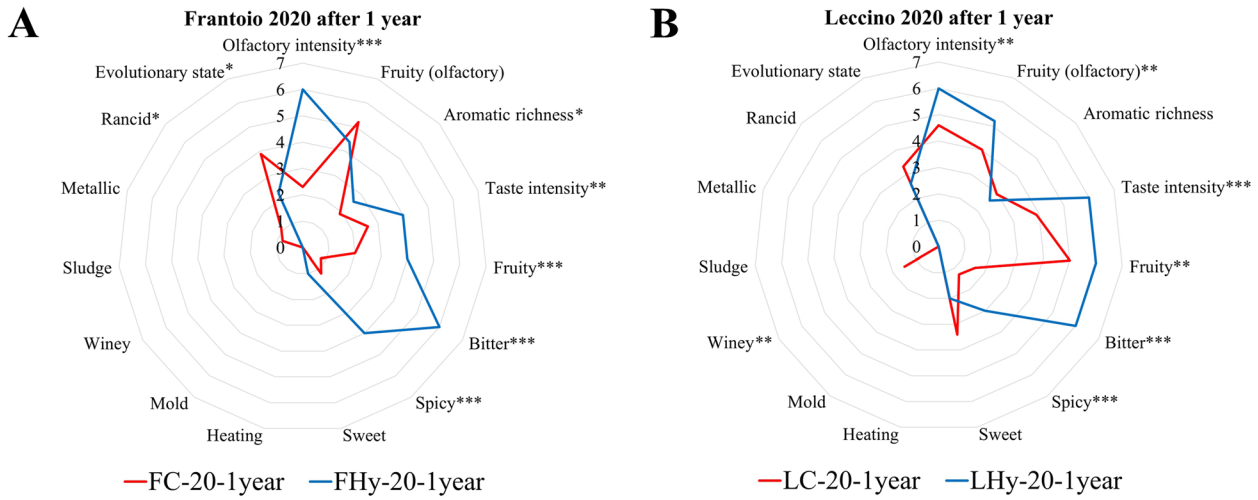


Fig. 10 Medians of quantitative descriptors obtained from sensory analysis of Frantoio oils produced in 2020 (**A**) and Leccino oils produced in 2020 (**B**) after one year of storage. Significance level: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$; without asterisk = not significant ($p \geq 0.05$). (for technical reasons the organoleptic analysis of the Frantoio samples produced in 2019 and Leccino samples produced in 2021 was not performed)

2001; Kalua et al., 2006; Veneziani et al., 2017, 2018). Temperature reduction has been successfully applied in other sectors of the fruit industry, such as in viticulture where cooling harvested grapes (*Vitis vinifera*) for 24 h before vinification resulted in changes in the aroma of the resulting wines (Modesti et al., 2021b). This indicated that the management of fruit temperature before processing could have an impact on the composition and aroma of the final produce. The previously published lab-scale research enabled the application of these principles to olive oil production (Dourou et al., 2020; Guerrini et al., 2021), leading to the trials discussed here and carried out at the commercial mill level. These trials showed the strict relation between lowering the temperature of

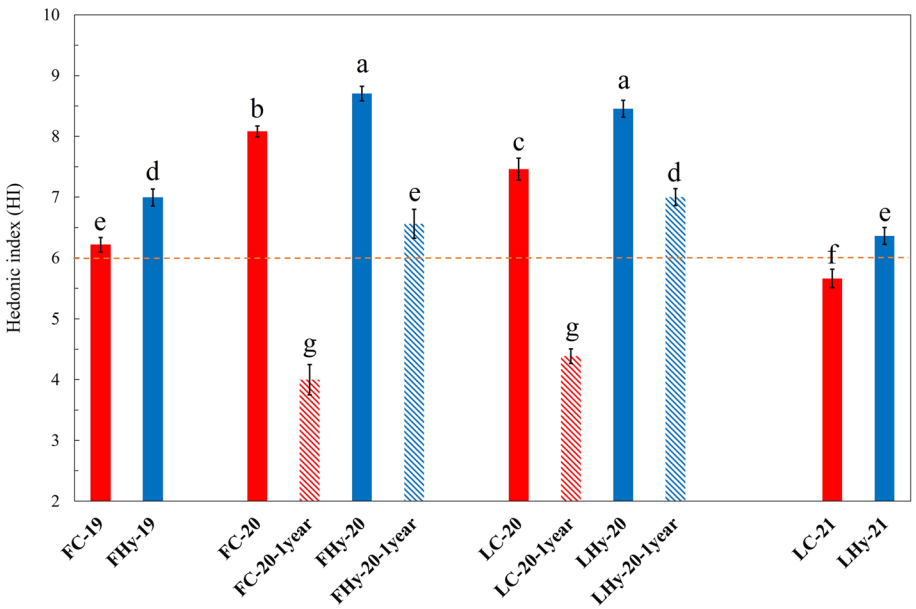


Fig. 11 Hedonic index (HI) of all the oil produced in the two years from Frantoio and Leccino cultivars. Different letters indicate significant difference among values ($p < 0.05$). FC-19/FC-20: Frantoio control oils after production in 2019 or 2020; FHy-19/FHy-20: Frantoio Hy oils after production in 2019 or 2020; LC-20/LC-21: Leccino control oils after production in 2020 or 2021; LHy-20/LHy-21: Leccino Hy oils after production in 2020 or 2021; FC-20-1 year/FHy-20-1 year: Frantoio control and Hy samples produced in 2020 after one year of storage; LC-20-1 year/LHy-20-1 year: Frantoio control and Hy samples produced in 2020 after one year of storage

the olive and the increase in the green aromas of the olive oil, thus highlighting the potential interest in this treatment for the olive oil industry. This work demonstrates that hydrocooler technology can be directly used in the mill plant without delaying the olive oil processing, since the treatment could replace the washing step used in traditional mills.

Based on the preliminary results obtained during the initial mill experiments (2019), the objective of the treatment, in terms of the optimal quality of the final oil, was to cool the olives until the fruit reached an internal temperature of approximately 17–18 °C. The internal temperature of the mesocarp varies over the years, most probably due to the climate and weather at harvest time, as well as to cultivar-related differences. The analysis of temperature data demonstrated a consistent and significant reduction in temperature induced by the treatment on both cultivars in each year of analysis. These differences were observed at each step evaluated, highlighting the treatment efficiency in decreasing the temperatures of the olive, paste and oil throughout the extraction process (Tables 1 and 2). At the same time, the differences in terms of temperature at harvest and the different cooling treatments of the internal olive tissue appeared to change in the different years (Table 1). However, these differences were greatly reduced after crushing, when the temperature of the samples from different years and cultivars appeared more homogeneous within each treatment type (C and Hy, Table 2). This effect is most likely attributable to the thermal inertia of the crusher, considering that the trial was carried out in a commercial mill operating almost continuously throughout the harvesting season. Such operating conditions likely contributed to smoothing or homogenizing the impact of the cooling treatment across different seasons. Overall, the considerably lower malaxation temperatures reached by olive paste in Hy samples likely played the most important role in the observed effects on oil composition and technological parameters.

The effects of the treatment appeared to be strongly influenced by the different cultivars used. While Frantoio showed several improvements in chemical parameters, as indicated by decreased FFA levels in both years, Leccino displayed some negative trends. Similarly, while in Frantoio an improved oxidative stability with consistent decreases in PV across both years was detected, Leccino showed a worsening trend with increased values. Regarding K232, no consistent pattern was observed across cultivars or years. However, an improvement was noted in K268 levels for Frantoio in 2019 and Leccino in 2021, as both showed decreases (Fig. 2), thus suggesting positive changes in these parameters. Additionally, olive cooling had an impact on the TPC and TTC of the oils. Two studies performed on several cultivars (Frantoio, Leccino, Gentile, Ogliarola Garganica, Moraiolo, San Felice, Coratina, Peranzana, and Ottobratica) by cooling the paste after crushing showed no impact on the peroxide, free fatty acid, and spectrophotometric values (Veneziani et al., 2017, 2018). However, another study regarding the cooled storage of Frantoio and Moraiolo olives using refrigerated cells before oil extraction showed higher spectrophotometric values, but no differences in FFA or PV were detected in the oil (Guerrini et al., 2021). This aligns with the findings of our study and suggests a strong effect of the genotype in response to the cooling treatment. Another study, regarding the effects of applying a cooling crusher on the quality of the final olive oil quality, showed no significant impact on chemical parameters (Nucciarelli et al., 2022). On the other hand, our results demonstrated several notable modifications, as outlined in the results section. This discrepancy may be attributed to the different responses of the olive genotypes used in each study and/or to the different cooling strategies, which in our case were applied on olives before crushing differently form the approach reported by Nucciarelli et al. (2022). In general, our results indicate that the cooling treatment is more beneficial, in terms of FFA, PV, and spectrophotometric indexes, in Frantoio than in Leccino samples, in both fresh and stored oil samples.

No significant effects of the treatments on the fatty acid profile were observed in our study. This contrasts with the results of another study (Guerrini et al., 2021) where the internal temperature of the olive fruit was maintained at a lower level (7 °C for 16 h) before crushing compared to our hydrocooler experiments, which were conducted at temperatures ranging between 12.4 and 17.8 °C and applied for a very short time. Applying the lower temperatures, the previously cited work highlighted a strong effect on fatty acids (Guerrini et al., 2021). Lowering storage temperatures could induce modifications in cell membrane lipid composition, favouring polyunsaturated fatty acids such as linolenic acid (C18:3) to maintain fluidity, as observed in other plant studies and prolonged storage conditions (Lee et al., 2005; Xu & Siegenthaler, 1997). However, such alterations were not detected in our hydrocooler experiments, possibly due to the short duration of the treatments and the more limited temperature reduction.

One of the main goals of our approach at the industrial level was to evaluate the effects of reducing the temperature of the olives in terms of the polyphenol and VOC profiles of the resulting oils. These two parameters are of paramount importance in terms of overall quality of the oils and have been highly affected in lab-scale trials

carried out on Leccino olives (Dourou et al., 2020). Our results in part confirm these findings in terms of polyphenol content, highlighting that different genotypes react differently to the treatment imposed. In fact, the Leccino oil samples showed, as a general trend, better responses after the hydrocooler treatments in terms of total polyphenol content, with an increase in cinnamic and ferulic acid along with apigenin and methyl luteolin. Moreover, a significant increase in OAHLA in 2021 Leccino Hy oils has been recorded, flanked by the decrease of another secoiridoid derivative, namely AHOA.

The oxidized secoiridoid derivatives detected in this study were putatively assigned based on their retention time and UV-visible and MS spectral features. However, due to the lack of purified reference standards, full structural confirmation could not be performed and is acknowledged as a study limitation. Oxidized secoiridoids generally exhibit reduced, despite still significant, antioxidant capacity due to structural modifications in their catecholic moiety (Cuffaro et al., 2023). The much higher abundance of OAHLA compared to AHLA (often two to four-fold higher in both fresh and stored Hy samples; Supplementary Tables S2 and S4) can be explained by the intrinsic chemical instability of AHLA. Its aldehydic and catecholic functionalities make it extremely prone to autoxidation, especially during storage, promoting the formation of its corresponding oxidized form. The differences observed between cultivars and harvesting years further highlight the strong influence of genotype and environmental conditions on secoiridoid metabolism and oxidation kinetics. However, with the exception of AHOA, cooling treatment appeared to be effective in mitigating the oxidation of other detected secoiridoid derivatives, that showed increases in Hy Leccino samples in 2021, despite differences were not supported by statistical significance (Fig. 4, Supplementary Tables S2 and S4).

When evaluating the effect of storage on polyphenol concentration, different seasonal patterns were observed. Compared to fresh oil, Frantoio 2019 and Leccino 2021 stored oil samples showed a decrease, while both Frantoio and Leccino produced in 2020 showed similar values or an increasing trend in TPC after storage. This behaviour was consistent in both control and Hy oils and might be related to differences in the initial levels of specific secoiridoids, such as DDMOA and DDMLA (Supplementary Table S2), as well as to the subsequent formation of OAHLA and other oxidized secoiridoids during storage (Supplementary Table S4). These findings would indicate that seasonal conditions, by affecting olive phenol content and profile, exert a major influence on phenolic evolution in oil during storage. The observed compositional modification in terms of polyphenols may be related to the more pronounced spiciness reported by the trained panellist for the Hy Leccino oil samples, both immediately after their production and following one year of optimal storage. Moreover, these results were also partly supported by the observed significant increase in TPC for this cultivar (Fig. 3), despite the Folin-Ciocalteu assay, which is the protocol used for TPC determination, is known to be non-specific for polyphenols. This reagent reacts with all reducing compounds, including organic acids, reducing sugars, and some amino acids, potentially leading to a misestimation of the actual phenolic content measured chromatographically (Tsitsipas et al., 2025).

Considering the VOC profile, previous studies have indicated a reduction in total VOCs in olive oil with elevated temperatures, potentially leading to the development of off-flavors (Boselli et al., 2009; Boskou, 2006). Temperature control is thus crucial for ensuring a superior olive oil aroma. Cooling olive paste during the extraction process has been proposed as a method for enhancing oil quality, as in the application of a thermal exchanger for rapid olive paste cooling post-crushing (Veneziani et al., 2018). The enzyme activities in the LOX pathway, including lipoxygenase (LOX), hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH), and alcohol acyltransferase (AAT) enzymes, are highly sensitive to temperature. The optimal temperature range for these enzymes varies, typically between 15 to 35 °C, depending on the specific enzyme (Kiritsakis & Markakis, 1988). ADH and AAT exhibit optimal activity around 30–35 °C, with lower temperatures resulting in decreased alcohol and ester production (Olias et al., 1993). Conversely, HPL, the enzyme responsible for converting 13-hydroperoxide into aldehydes, operates optimally at 15 °C, potentially leading to increased aldehyde synthesis and reduced conversion to alcohols and esters (Anthon & Barrett, 2003). This may partially explain the changes in the aldehyde/alcohol ratio observed in oils from cooled fruit, with higher levels of this ratio which are considered beneficial in terms of the sensory impact (Cerretani et al., 2008).

Considering the Leccino cultivar, in oils obtained from olives cooled (at 19 °C) before crushing, an increase in VOCs from the LOX pathway, such as 2-hexen-1-ol, 3-hexen-1-ol, and 2-hexenal, has been reported, which also aligns with our findings (Dourou et al., 2020). Additionally, an increase in polyphenol content at 15 °C in Leccino has also been reported, which is also consistent with our results. An increase in C6 VOCs has also been reported in oil produced from olives undergoing cold storage before the extraction process, which suggests a positive correlation between the flavor and lowering olive temperatures (Guerrini et al., 2021). However, while in the latter study the polyphenol profile of the oil appeared to be preserved from oxidation under cold conditions,

our results in the Frantoio cultivar indicate a significant decrease in these compounds.

Still considering VOCs, the hydrocooler treatment led to a reduction in ethanol (off-flavor reduction) in the Frantoio cultivar (Supplementary Figure S3) and, also in line with previously reported results (Dourou et al., 2020; Karagoz et al., 2017), a general decreasing trend in C5 compounds such as 1-penten-3-ol and 1-penten-3-one, the latter being a derivative of 1-penten-3-ol metabolism. This highlights the importance of considering the interplay between 1-penten-3-ol and 1-penten-3-one compounds in terms of olive oil quality. This is also important considering that in the literature there is contrasting evidence of their impact, and in general of C5 aroma compounds, on the sensory attributes. Additionally, unlike our findings regarding oil chemical parameters, our VOC results are in line with other previously reported data, with an increase in (E)-2-hexenal across all cultivars after the application of cold treatments, along with a decrease in 1-penten-3-ol (Nucciarelli et al., 2022). In addition, in our study a significant increase in C6 alcohols was found, especially 3-hexen-1-ol (green aroma). This highlights the significance of using the postharvest cooling of olives to increase desirable volatile compounds, while reducing off-flavors. Studies have highlighted the significant influence of cultivar on the VOC profile of olive oil (Genovese et al., 2015; Hbaieb et al., 2016; Serrano et al., 2021). Additionally, the hydrocooling treatment has the advantage of modifying the volatile profile of the resulting oil. This is corroborated by previous research reporting that high temperatures during malaxation (25–35 °C) altered the composition of metabolites from the LOX pathway, thus reducing pleasant volatile compounds and increasing less attractive ones (Boskou, 2006; Servili et al., 2003). With respect to the reduction of off-flavours compounds detected by GC-MS, it should be noted that trained panellists did not perceive any defects in the control oils, possibly indicating that off-flavour concentrations were below their odour detection thresholds (ODH).

Considering the sensory profile, our findings reveal a particularly interesting scenario. In several cases, the differences between oils obtained from the control (C) and Hydrocooler-treated (Hy) samples were evident both in the fresh oils and after one year of storage, regardless of the cultivar. Overall, the treatment led to an improvement in both Leccino and Frantoio cultivars, although a clear influence of the harvest year was also observed. While the differences immediately after production were significant, albeit relatively modest, they became more pronounced during storage, ultimately leading to the loss of Virgin Olive Oil (VOO) classification in the control samples. Notably, oils produced with the traditional method showed a decline in quality over time, eventually being rated as unacceptable (HI < 6). This trend highlights the effectiveness of the Hydrocooler treatment in preserving oil quality over time. Regardless of cultivar or harvest year, oils from hydrocooler-treated olives consistently showed better taste and olfactory intensity, characterized by higher levels of perceived fruitiness, bitterness, and spiciness.

Conclusions

The hydrocooler treatment appeared to be suitable for industrial application in the olive oil extraction process in terms of cooling efficacy and timing and thus it can be potentially used in substitution of the olive washing step. The results, especially considering the effects of the treatment on the olive VOC profile and sensory attributes, confirmed the positive findings of previous lab-scale research. The observed improvement in specific aroma compounds, and the reduction in off-flavors appeared to be consistent considering the different seasons and cultivars. However, different outcomes in terms of TPC (as well as specific polyphenols), TTC and other important technological parameters of the oils were noted based on the season and genotype. In conclusion, pre-processing cooling of olives seems very promising for the improvement of oil quality and sensory profile, especially considering southern Mediterranean countries, which are subjected to very high temperatures during the harvesting period.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

Conceptualization, S.B. and P.T.; methodology, F.V., M.V.C. and G.M.; formal analysis, M.V.C., A.B., I.T. and G.M.; data curation, A.B., I.T., M.V.C. and S.B.; investigation, M.V.C.; resources, P.T. and C.L.; writing-original draft preparation, M.V.C., S.B., A.B., F.V., I.T. and P.T.; writing-review and editing, V.C.M., G.M, L.T., C.L., P.T., A.B., F.V. and S.B.; supervision, S.B. and P.T. All authors have read and agreed to the published version of the manuscript.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The research obtained the approval of the bioethical committee of the University of Pisa (protocol n. 0088081/2020) and was conducted according to the ethical guidelines, and informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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