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RESEARCH ARTICLE

Densimetric sorting of olives to control olive oil quality

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Abstract

Extra virgin is the most valuable commercial category among olive oils, and its quality is influenced by various factors, among which the olive fruit plays a fundamental role. The olives that enter the mill exhibit significant variability in physical and chemical characteristics, potentially impacting the quality of the extracted oil. Therefore, selecting the olives in post-harvest could be a crucial step, especially for differentiating the final product and producing high-quality oil. This work aimed to conduct post-harvest densimetric sorting of the olives. For this purpose, a saline solution was used with different concentrations of salt over the 3 days of harvesting, which made it possible to divide the initial olive batch into two sub-batches with different densities. The respective oil was extracted from each sub-batch, called low- and high-density oils, respectively, and then appropriate physical–chemical analyses were performed to characterize both the olives and the oils. Although both oils were classified as extra virgin, significant differences were observed, with higher concentrations of phenolic and volatile compounds associated with positive sensory attributes in the low-density oils. Densimetric sorting of olives could represent a novel approach in the field of extra virgin olive oil production, enabling potential differentiation of the final product.

Practical Applications: The results obtained in the study could be applied at an industrial level to classify olives in the post-harvest phase. This could allow to produce extra virgin olive oils (EVOO**s**) with different chemical and sensory characteristics, particularly in terms of volatile and phenolic compounds. This would make it possible to diversify the production of EVOO, satisfy the diverse needs of consumers, allow producers to be more competitive in the market, and, in general, improve the overall quality of the final product.

KEYWORDS
physical–chemical properties, polyphenols, post-harvest sorting, volatile organic compounds

Abbreviations: 3,4-DHPEA, hydroxytyrosol; EVOO, extra virgin olive oil; LOX, lipoxygenase; MI, maturation index; p-HPEA, tyrosol; TPC, total phenolic compounds.

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1 | INTRODUCTION

Extra virgin olive oil (EVOO) represents the superior commercial grade of olive oil.[1] Derived from the fruit of the olive tree (*Olea europaea*), EVOO undergoes mechanical or physical processes, including washing, decanting, centrifugation, and filtration.[2] The denomination EVOO refers to a high-quality product that reflects superior physical, chemical, and organoleptic characteristics due to its composition. Virgin olive oil quality depends on its composition, which can be divided into two fractions, one made up of major components, including triglycerides, diglycerides, and free fatty acids (FFAs), and one made up of minor components, such as pigments, tocopherols, and phenolic compounds, both responsible for the hedonistic characteristics and health and nutritional properties.[3,4] Many factors influence the composition of EVOO, including genetic, agronomic, environmental, and technological factors. Consequently, the production of EVOO is a complex process, the quality of which is influenced by a multiple and interactive combination of these factors.[5]

Nowadays, the quality of virgin olive oil is an indispensable element to compete in the current market, and focusing studies on the factors that influence it is necessary for production. This can make it possible to obtain high-quality products that reflect certain characteristics that are increasingly demanded by consumers.

Basically, it is possible to divide the factors influencing virgin olive oil quality into three main classes. The first concerns aspects before the production process, such as olive variety, tree age, cultivation area and geographical origin, climate, and soil. The second class concerns the production process, such as harvest time and degree of ripeness, olive storage, and extraction method, whereas the third concerns the bottling and storage conditions of the final product.[6]

However, most of the quality attributes of EVOO are determined by the chemical composition and biochemical state of the raw material: olive fruit. As far as the fruit of the olive is concerned, the cultivar is undoubtedly one of the main factors responsible for the virgin olive oil “footprint.”[7] However, even within the same cultivar, there are many parameters to consider, including agronomic, harvesting, and environmental conditions. For example, even within the same plot, the soil types, the level of fertilization, the day of harvest, the stress conditions, or the thermal regimes could change.[8] Furthermore, the characteristics of individual plants can also influence fruit development and the quality of EVOO. These include not only water availability, foliage distribution, and sunlight interception but also branch pruning methods.[9] Temperature could influence the aromatic profile of virgin olive oil by decreasing the levels of volatile components.[10] Soil water availability might alter the phenolic composition.[11] Altitude may influence the fatty acid composition; oils from plants grown at higher altitudes display increased stability against oxidation.[12] Furthermore, oils derived from fruits located in the upper canopy portions showed increased stability and higher concentrations of polyphenols and saturated fatty acids in contrast to those extracted from less well-illuminated regions.[13] Moreover, the timing of the harvest plays a key role in determining the quality of EVOO.[14] In general, oils extracted from green olives are more stable due to the antioxidant effect of the high concentration of phenolic compounds, which are also responsible for the sensory characteristics of bitterness and pungency. Similarly, the concentration of volatile compounds decreases with the degree of ripeness, attenuating the aromatic notes of oil.[15]

In light of the above, it can be stated that olives harvested during the oil season may potentially present a high degree of chemical and physical variability due to the influence of many factors.

This results in a high heterogeneity of the material processed in the mill, which could potentially compromise the quality of the desired final product, downgrade the quality target, or prevent the achievement of certain standards.

In this context, the post-harvest olive sorting could be a fundamental tool for the classification of raw material to obtain oils with different chemical and sensory characteristics, allowing a potential differentiation of production.

The sorting process is in fact a crucial point in many agri-food industries and is widely applied to certain types of raw materials using different technologies. For example, in modern viticulture, grape sorting is a key step for winemaking to meet production high targets.[16] In the fresh fruit industry, sorting is a consistent method to understand fruit characteristics and meet consumer demands.[17] Generally, the use of technology has positive implications for capacity, as it replaces human inspection in assessing the quality of fruits or vegetables.[18]

Regarding the sorting of olives, especially in the context of olive table, some authors have addressed the problem using prototype technologies based mainly on sorting according to color, using image classification, or according to the surface roughness of the fruit, and again according to ripeness and the presence of external defects.[2,19]

Nevertheless, the intricate nature of the utilized equipment constitutes a substantial investment for the industries employing them, encompassing both economic costs and the workforce needed for their application and maintenance.[20]

Based on our knowledge, the selection of olives for the production of EVOO represents a new approach, for which the technologies currently available on the market may not provide adequate solutions, especially considering the high costs and the degree of specialization required, particularly for small and medium enterprises.[2]

In this context, the present study aimed primarily to classify olives based on a physical characteristic such as density, using a solution of water and salt that allowed for the densimetric sorting of the olives themselves. After sorting the olives, the oil was extracted and then subjected to chemical and sensorial analyses to characterize it and verify if olives with different densities could produce virgin olive oils with different characteristics.

The sorting based on fruit density as a simple and non-destructive technique has been previously examined in other contexts, such as mulberries, yielding promising results.[20,21]

This new approach in the field of olive classification within the context of EVOO production based on densimetric sorting could prove to be an economical, fast, easy-to-use, and noninvasive method for olive classification. It allows for fruit classification in the post-harvest phase and potential differentiation in the final production of EVOO.

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2 | MATERIALS AND METHODS

2.1 | Olive harvest

Olives (*O. europaea* L.), exclusively of the Moraiolo cultivar to reduce experimental variability, were harvested by hand in 2022 in Calenzano (Tenuta San Donato, Calenzano, Florence, Italy). Harvesting was carried out on 3 different days (October 11, October 25, and November 10 2022) in October and November (called t1, t2, and t3, respectively). First, for each harvest date, the plot of land and trees free of infection or physical damage from which the olives were to be harvested were selected to obtain as homogeneous a harvest as possible. Subsequently, about 12 kg of olives per day were collected and immediately transported in special perforated boxes to the DAGRI Department (Department of Agricultural, Food, Environmental and Forestry Sciences, Faculty of Agriculture, University of Florence, Florence), where the experimental trials were conducted.

2.2 | Olive sample preparation

Olives have been suitably cleaned of any external material (leaves and sticks). For each harvest time, the whole batch was subdivided into three replicates, about 4 kg for each. In parallel, a water and salt (NaCl) solution was prepared to carry out the densimetric sorting of the olives, and different concentrations of NaCl were used. The percentage of salt used in 3 collection days was 3.49%, 0.50%, and 0.31%, respectively.

For densimetric sorting, containers (SAMLA 39 × 28 × 28 cm^3, 22 L, IKEA Systems) were filled with commercial deionized water. Subsequently, the salt (Sale marino iodato, Coop Italia) was added and manually dissolved. Before proceeding to measure the density, the temperature of the water was measured using a digital thermometer (Termometro, Delta Ohm HD2107.1, PT100, RS Components). The density values reported for the three harvest times were 1.028, 1.007, and 1.006 g/cm^3, respectively, by a laboratory floating hydrometer (Idrometro Brannan, RS Components).

This allowed the olive mass to be divided into equal parts, so that 2 kg of olives floated, called low density, and 2 kg remained at the bottom, called high density. At this point, the olives were carefully rinsed from the saline solution using a colander and tap water. Two further sub-batches were realized, so 1 kg was used to extract the olive oil and 1 kg to perform the physical and chemical analysis to characterize the olive batches.

2.3 | Olive sample analysis

Olives were characterized by physical and chemical analysis. The density value was calculated as the ratio of the mass of 20 olive samples and its volume. The density of the olives was determined in g/mL. Maturation index (MI) was determined using the method introduced by Uceda et al.,[22] which categorizes olives on an 8-point scale (ranging from 0 to 7) based on the color of the skin and flesh. The mass of olives and pits was calculated by selecting 100 randomly weighed and averaged samples.[23] Pulp and pit were measured to calculate the pulp/pit ratio. Both the measures were performed by an electronic balance (ORMA Srl, Sesto San Giovanni) with an accuracy of 0.01 g.

For the determination of water content, olive samples were heated in an oven (Thermo Scientific Heraeus Function Line Series, Thermo Fisher Scientific) at 105°C until a constant weight was achieved. Results were expressed as paste moisture content (%).

The total oil and sugar content were measured after the methods of Cherubini et al.[24] For the determination of the oil content, 10 g of olive pulp were dehydrated until a constant weight was reached. An automatic extractor with hexane was used for sample extraction (Randall 148, Velp Scientifica). Results were expressed as g/kg dry matter (DM).

The sugar content was measured by the UNI 22 608 method, with some modification reported by Cherubini et al.[24] Sugar content was determined indirectly by a potentiometric analysis. About 10 g of pulp, appropriately stoned, were used and subsequently homogenized in deionized water in a ULTRA-TURRAX (IKA). Sugar titration was performed using an automatic titrator (Compact Titrator, Crison). Sugar results were expressed as g sugar/mL solution, from which the quantity of sugars was derived, which was referred to as 1 kg of olives. The sugar content was then referred to as 1 kg of DM.

Phenolic compounds were extracted from fresh olive paste according to the method by Cecchi et al.[25] The analysis was carried out with HP 1100 liquid chromatograph, equipped with an 1100 Autosampler, a column heater module, a quaternary pump, and coupled with DAD and MS detectors, interfaced to an HP1100 MSD API-electrospray (Agilent Technologies). Furthermore, a Hypersil Gold QRP-18 column (4.6 mm, internal diameter; 250 mm, length; 3 µm, particle size) (Thermo Electron Corporation) was employed. Values were expressed as mg_{Tyr}/kg on DM for each phenolic compound.

2.4 | Olive oil extraction

From olives, the respective oil was extracted. To reduce variability due to the industrial mill and to have much more homogeneous olive samples, olive oil extraction was conducted using a micro-extraction apparatus as previously described by Masella et al.[26] Each olive sample of ≈1 kg was pressed using a laboratory-scale mill that fully reproduced a knife mill (Mori-TEM). The olive paste was blended in a cylindrical laboratory handling apparatus for 20 min at 27°C. Subsequently, it was subjected to centrifugation at 4500 rpm for 10 min to separate the oil fraction from the vegetation water and solid particles utilizing a NEYA 8 laboratory centrifuge (REMI centrifuge Neya) fitted with an S 4-175 rotor (REMI centrifuge Neya). A glass-separating funnel was used to collect the extracted oil. Finally, further clarification of the oil was conducted with an additional centrifugation (HERMLE mod. Z 206-A, Benchmark Scientific) at 6000 rpm for 10 min.

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2.5 | Olive oil analysis

Olive oil quality parameters were carried out according to EU official methods.[27] This involved the analysis of peroxide value (PV) (meq O_2/kg oil), FFAs (% oleic acid), and UV spectroscopic indices (K232, K270, and ΔK).

The International Olive Council (IOC) official method[28] was followed for the analysis of phenolic compounds. High-performance liquid chromatography analysis was conducted using an HP 1100 coupled with both DAD and MS detectors, the latter one equipped with an HP1100 MSD API-electrospray interface (Agilent Technologies).

Furthermore, a Poroshell 120, EC-C18 column (150 × 3.0 mm^2 id, 2.7 µm particle size; Agilent Technologies) was used for separation. Elution solvents consisting of acetonitrile, H_2O, and methanol were utilized in accordance with the IOC. Syringic acid served as the internal standard, and the chromatogram was measured at 280 nm. Finally, the phenolic concentration was quantified as mg/kg of tyrosol.

Volatile organic compound (VOC) analysis was conducted following the multiple internal standard method described by Fortini et al.[29] A headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME–GC–MS) was performed using a 50/30 µm DVB/CAR/PDMS SPME fiber (Supelco) for VOC extraction and a Trace GC–MS Thermo Fisher Scientific equipped with a ZB-FFAP (Zebron) capillary column (30 m × 0.25 mm ID, 0.25 µm DF) for their identification.

2.6 | Statistical analysis

A two-way ANOVA was applied to analyze the data. Two factors (density treatment, meaning the density sorting of the olives, and harvest time) at two levels (low and high densities) for the density treatment and three levels (t1, t2, and t3) for the harvest time were tested. The significance level was set to $p < 0.05$. When applicable, the post hoc Tukey HSD test was used to evaluate variations among means. All statistical analyses were conducted using the R software package (version 3.6.2).

3 | RESULTS

3.1 | Olive sample analysis

The value of olive density was calculated. As we expected, the floating olives reported lower values of density (0.97 ± 0.03, 0.96 ± 0.04, and 1.00 ± 0.00 g/cm^3) with respect to the olives that remained on the bottom (1.10 ± 0.01, 1.04 ± 0.01, and 1.04 ± 0.00 g/cm^3) for t1, t2, and t3, respectively. Hence, the olives have reported higher values of density, called "high density," and the other ones, "low density," concerning the density value of water and salt solution.

Furthermore, olives samples were characterized by physical and chemical analyses, and all data were analyzed with ANOVA to evaluate significant differences for the density sorting (low and high densities) and the harvest time (t1, t2, and t3).

ANOVA results highlighted a significant main effect of density treatment and harvest time, but their interaction was not significant for the parameters of MI, pulp/pit ratio, water content (%), and sugar content (g/kg). Regarding olive mass (g), a significant difference was observed for harvest time but not for density treatment. In contrast, there were no significant differences reported for the measure of polyphenols (mg/kg) for the two main effects. However, the interaction between oil content (%) in dry and fresh fruits was found to be significant. All results are reported in Table 1.

MI was significantly affected by density treatment and harvest time applied. Higher values were reported for low-density olives (3.09 ± 1.14) than for the high-density olives (2.21 ± 1.28). Over time, the value of MI has increased, and higher values were registered for t3, t2, and t1 (3.83 ± 0.42, 2.98 ± 0.60, and 1.14 ± 0.57), respectively.[30]

The pulp/pit ratio was significantly influenced by both the density treatment and the harvest time. Higher values were observed for low-density olives (3.72 ± 0.30) compared to the high-density olives (3.25 ± 0.37). Over time, in t1 was obtained the lower value (3.08 ± 0.30), the higher was in t2 (3.78 ± 0.22), and a slight decrease was instead measured at t3 (3.59 ± 035). Significant differences were registered between t1 respect t2 and t3, whereas no differences were found between t2 and t3.

Olive mass was significantly affected by harvest time, but not by density treatment. No significant differences were recorded for high- and low-density olives (1.68 ± 0.19 and 1.71 ± 0.07 g, respectively), whereas over time, olive mass increased significantly from t1 and t2 (1.59 ± 0.12 and 1.79 ± 0.08 g), while the value decreased in t3 (1.70 ± 0.15 g). No significant differences were measured in t3 with respect to t1 and t2.

Regarding water content, it was significantly affected by density treatment and harvest time. A higher value was reported for high-density olives (54.01% ± 2.01%) with respect to low-density olives (53.00% ± 1.90%). Over time, water content decreased from t1 to t2 and t3 (55.18% ± 0.90%, 54.27% ± 0.94%, and 51.07% ± 0.56%), respectively.[31] The same trend was obtained for the sugar content. Also in this case, the higher value was measured for the high-density olives (43.61% ± 3.22%) with respect to low-density olives (39.23% ± 5.15%). Over time the value decreased; a higher value was reported for t1 (44.48% ± 2.14%) with respect to t2 (42.52% ± 5.09%) and t3 (37.35% ± 3.57%).[32]

There were no significant differences observed in the content of polyphenols concerning both factors studied.

Finally, we evaluated the effects of density treatment, harvest time, and their interactions for the oil content of fresh and dry fruit. A significant main effect of harvest time was found. The content of oil in fresh and dry fruit increases in time (40.48% ± 2.09%, 44.00% ± 2.46%, 50.53% ± 3.17% and 18.13% ± 1.04%, 20.13% ± 1.24%, 24.73% ± 1.44%), respectively. No significant effect was found for density treatment.

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TABLE 1 Physical and chemical parameters of olive samples.

Parameter	Main effect							Interaction
	Density treatment		*p*	Harvest time			*p*	*p*
	Low density	High density		t1	t2	t3		
MI	3.09 ± 1.14 x	2.21 ± 1.28 y	***	1.14 ± 0.57 c	2.98 ± 0.60 b	3.83 ± 0.42 a	***	ns
Pulp/Pit ratio	3.72 ± 0.30 x	3.25 ± 0.37 y	***	3.08 ± 0.30 b	3.78 ± 0.22 a	3.59 ± 0.35 a	***	ns
Olive mass (g)	1.71 ± 0.07	1.68 ± 0.19	ns	1.59 ± 0.1 b	1.79 ± 0.08 a	1.70 ± 0.15 ab	*	ns
Water content (%)	53.00 ± 1.90 y	54.01 ± 2.01 x	**	55.18 ± 0.90 a	54.27 ± 0.94 a	51.07 ± 0.56 b	***	ns
Oil content dry fruit (%)	45.11 ± 2.88	44.90 ± 6.59	ns	40.48 ± 2.09 c	44.00 ± 2.46 b	50.53 ± 3.17 a	***	**
Oil content fresh fruit (%)	21.23 ± 2.11	20.77 ± 3.94	ns	18.13 ± 1.04 c	20.13 ± 1.24 b	24.73 ± 1.44 a	***	**
Sugar content (g/kg)	39.29 ± 5.15 y	43.61 ± 3.22 x	*	44.48 ± 2.14 a	42.52 ± 5.09 a	37.35 ± 3.57 b	**	ns
Polyphenols (mg/kg)	104 004.56 ± 12 092.59	99 577.78 ± 17 992.87	ns	110 332.17 ± 14 708.14	102 556.83 ± 9349.01	92 484.83 ± 16 543.62	ns	ns

Note: Means and standard deviations are reported ($n = 3$). Different letters indicate different significance levels according to the Tukey HSD post hoc test results for harvest time (a–c) and density treatment (x, y) (t1 = first-day harvest, t2 = second-day harvest, t3 = third-day harvest).
Abbreviations: MI, maturation index; ns, non-significant.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

TABLE 2 Legal parameters of olive oil samples.

Parameter	Main effect							Interaction
	Density treatment		*p*	Harvest time			*p*	*p*
	Low density	High density		t1	t2	t3		
FFA (% oleic acid)	0.27 ± 0.04	0.26 ± 0.03	ns	0.24 ± 0.03	0.27 ± 0.01	0.29 ± 0.04	ns	ns
PV (meq O_2/kg)	9.0 ± 0.9 y	9.7 ± 0.8 x	*	8.8 ± 0.6 b	9.3 ± 1.0 ab	9.9 ± 0.7 a	*	ns
K_{232}	1.87 ± 0.16	1.87 ± 0.13	ns	2.00 ± 0.05 a	1.85 ± 0.13 ab	1.77 ± 0.13 b	*	ns
K_{270}	0.15 ± 0.02	0.16 ± 0.01	ns	0.17 ± 0.01 a	0.15 ± 0.01 b	0.15 ± 0.02 b	**	ns
ΔK	0.00 ± 0.01	0.00 ± 0.01	ns	0.01 ± 0.01	0.00 ± 0.01	0.00 ± 0.00	ns	ns

Note: Means and standard deviations are reported ($n = 3$). Different letters indicate different significance levels according to the Tukey HSD post hoc test results for harvest time (a–c) and density treatment (x, y) (t1 = first-day harvest, t2 = second-day harvest, t3 = third-day harvest).
Abbreviations: FFA, free fatty acid; ns, non-significant; PV, peroxide value.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

3.2 | Olive oil analysis

The olive oil extracted was characterized by chemical analysis. All data were analyzed with ANOVA to evaluate significant differences for the density treatment (low and high densities) and the harvest time (t1, t2, and t3). In this case, the wording low and high densities referred to the oils obtained from the respective olive samples previously described. In Table 2 are reported the legal parameters for the classification of olive oils. All the oils obtained were classified as EVOO.

ANOVA results highlighted a significant main effect of density treatment and harvest time for PV; on the contrary, no significant differences were found in FFAs. Concerning UV spectrophotometric indexes (K232, K270, and ΔK), a significant main effect of density emerged for K232 and K270, whereas no significant differences were observed for ΔK. Regarding PVs, a higher value was reported for the high-density oil (9.7 ± 0.8 meq O_2/kg) with respect to the low-density oil (9.0 ± 0.9 meq O_2/kg). In the time, the value significantly increased from t1 (8.8 ± 0.6 meq O_2/kg) to t3 (9.9 ± 0.7 meq O_2/kg), whereas no

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significant differences were measured for t2 (9.3 ± 1.0 meq O_2/kg) with respect to t1 and t3.

Among UV spectrophotometric indexes, K232 and K270 were significantly affected by harvest time but not by density treatment. Over time, the values of both indexes significantly decreased from t1 (2.00 ± 0.05) to t3 (1.77 ± 0.13) for K232 and from t1 (0.17 ± 0.01) to t2 (0.15 ± 0.01) for K270.

The phenolic content of olive oil samples was analyzed with ANOVA to evaluate significant differences for the density treatment (low and high densities) and the harvest time (t1, t2, and t3). ANOVA results highlighted a significant main effect of both factors tested (density treatment and harvest time), but not their interaction. Table 3 reports the single compounds and the groups of compounds that showed significant differences.

The first interesting result emerged for total phenolic compounds (TPCs). The density treatment significantly affected the concentration of TPC. In detail, a higher value was reported for low-density oil (767.63 ± 89.35 mg/kg) compared to the high-density oil (716.51 ± 76.82 mg/kg) for the density treatment. Similarly, the sum of secoiridoids followed the same trend. The low-density oil showed a higher value (627.78 ± 86.91 mg/kg) compared to the high-density oil (581.936 ± 77.07 mg/kg). Furthermore, the levels of oleuropein and ligstroside derivatives also showed higher values in the low-density oil (518.30 ± 57.08 and 134.05 ± 33.45 mg/kg) compared to the high-density oil (484.24 ± 44.18 and 123.51 ± 33.31 mg/kg). Finally, the sum of compounds hydroxytyrosol (3,4-DHPEA) and tyrosol (p-HPEA) resulted in higher values in the low-density oil (4.65 ± 0.87 mg/kg) compared to the high-density oil (4.04 ± 0.99 mg/kg). Regarding single-phenol compounds, the density treatment has significantly affected only decarboxymethyl liygstroside aglycone, oxidized dialdehyde form. Higher values for the low-density oil (20.30 ± 2.72 mg/kg) than the high-density oil (17.47 ± 3.37 mg/kg) have been reported.

TPC was significantly affected by harvest time. There was a noticeable decrease in TPC concentration from t1 (829.86 ± 2.72 mg/kg) to t2 (742.20 ± 50.66 mg/kg) and t3 (654.15 ± 15.73 mg/kg). Similarly, the sum of secoiridoids was significantly affected by harvest time, with values decreasing over the time from t1 to t2 and t3 (694.35 ± 2.72, 602.58 ± 35.95, and 517.63 ± 22.04 mg/kg). The same trend was observed for oleuropein (550.89 ± 49.07, 504.67 ± 24.92, and 448.24 ± 9.97 mg/kg), ligstroside derivates from t1 (168.71 ± 9.34, 123.43 ± 13.63, and 94.21 ± 3.39 mg/kg), and the sum of phenolic acid (18.10 ± 1.3, 13.38 ± 1.06, and 9.83 ± 0.81 mg/kg). In contrast, the sum of flavones increased over time from t1 (27.65 ± 1.91 mg/kg) to t3 (34.20 ± 1.62 mg/kg). Likewise, the sum of compounds hydroxytyrosol (3,4-DHPEA) and tyrosol (p-HPEA) increased over time from t1 to 2 and t3 (3.40 ± 0.56, 4.32 ± 0.71, and 5.32 ± 0.26 mg/kg).

VOCs of olive oil samples were analyzed with ANOVA to assess significant differences for the density treatment (low and high densities) and the harvest time (t1, t2, and t3). ANOVA results highlighted a significant main effect of the factors tested (density treatment and harvest time) and their interaction. After conducting the HS-SPME–GC–MS analysis, a total of 71 compounds were detected. Among these compounds, 24 single compounds were found to exhibit significant differences, and these are presented in Table 4. Moreover, the compounds were summed up and grouped into four classes according to their presumed sensory quality in the oil. In detail, the compounds with five and six carbon atoms (sum of C5 and C6) are associated with the lipoxygenase (LOX) pathway and are related to positive sensory attributes such as "fruity" and "green." The compounds with 7–10 carbon atoms (sum of C7–C10) are typically associated with the negative sensory attribute of "rancid" in the oil. Additionally, there are compounds associated with microbial activities that contribute to several negative attributes, such as "fusty," "muddy," and "vinegary" flavors in the oil. These compounds are combined and reported as the sum of microbial metabolites in the analysis.[33]

A detailed list of compounds, categorized according to the previously mentioned groups, is reported by Guerrini et al.[34]

The most interesting single compound strongly affected by density sorting was the (*E*)-2-hexenal, which is associated with positive sensory attributes like "fruity" and "green."[35,36] It turned out to be the most abundant compounds, and a higher value was reported for the low-density oil (6.11 ± 0.59 mg/kg) compared to the high-density (4.57 ± 0.79 mg/kg).

A significant effect of density sorting was observed on the different classes. For C6 compounds, a higher value was found for the low-density oil (14.67 ± 1.25 mg/kg) with respect to high-density oil (13.44 ± 1.30 mg/kg). No significant differences were found for the C5 class between the two treatments. In contrast, for the class of C7–C10 compounds, higher values were reported for high-density oil (5.88 ± 6.11 mg/kg) compared to the low-density oil (2.68 ± 2.09 mg/kg). The same trend was observed for the sum of compounds associated with microbial activities, where the high-density oil showed higher values (7.83 ± 1.81 mg/kg) with respect to the low density (6.16 ± 0.99 mg/kg).

As we expected, significant differences were observed for the single compounds and for the classes of compounds due to harvest time. Among C5 compounds, 1-penten-3-one was the most abundant, and a significant increase was registered from t1 (1.38 ± 0.24 mg/kg) to t2 (1.77 ± 0.25 mg/kg), whereas no significant differences were observed with t3 (1.66 ± 0.16 mg/kg). In contrast, a significant decrease was found for (*E*)-2-penten-1-ol and (*Z*)-2-penten-1-ol from t1 (0.09 ± 0.01 and 0.21 ± 0.03 mg/kg) to t3 (0.06 ± 0.01 and 0.15 ± 0.01 mg/kg), whereas no significant differences were found for t2. Regarding the sum of C5 compounds, a significant increase was observed from t1 (1.70 ± 0.25 mg/kg) to t2 (2.17 ± 0.22 mg/kg), whereas no significant difference was registered for t3 (1.94 ± 0.14 mg/kg). Among C6 class, two compounds (*Z*)-3-hexenal and (*E*)-2-hexenal were found to be the most abundant. Both compounds showed a significant decrease over time. Specifically, for (*E*)-2-hexenal was observed the higher value in t1 (5.85 ± 1.01 mg/kg) respect t2 (5.48 ± 0.96 mg/kg), whereas no significant differences were registered for t3 (5.48 ± 0.96 mg/kg). Regarding (*Z*)-3-hexenal, a higher value was registered for t1 (4.29 ± 0.85 mg/kg) than t3 (2.27 ± 0.33 mg/kg); no significant difference was registered for t2 (3.40 ± 0.76 mg/kg).

Instead, a significant increase was found for the following compounds (*Z*)-3-hexen-1-ol, (*E*)-2-hexenyl acetate, 1-hexanol, and

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TABLE 3 Concentration of phenolic compounds (mg/kg) in olive oil samples.

Phenolic compounds	Main effect							Interaction
	Density treatment		*p*	Harvest time			*p*	*p*
	Low density	High density		t1	t2	t3		
Individual compounds								
Cinnamic acid	4.40 ± 1.42	4.60 ± 1.60	ns	5.54 ± 1.29 a	5.01 ± 0.93 a	2.95 ± 0.60 b	**	ns
p-Coumaric acid	3.07 ± 1.78	3.29 ± 1.65	ns	5.41 ± 0.27 a	2.39 ± 0.43 b	1.74 ± 0.23 c	***	ns
Decarboxymethyl ligstroside aglycone, dialdehyde form	72.34 ± 32.73	68.12 ± 32.68	ns	107.78 ± 9.99 a	68.75 ± 8.41 b	34.17 ± 3.73 c	***	ns
Decarboxymethyl ligstroside aglycone, oxidized dialdehyde form	20.30 ± 2.72 x	17.47 ± 3.37 y	*	20.91 ± 2.35	18.39 ± 4.35	17.36 ± 2.19	ns	ns
Decarboxymethyl oleuropein aglycone, dialdehyde form	298.98 ± 68.43	283.98 ± 76.19	ns	359.77 ± 31.30 a	307.43 ± 29.97 b	206.15 ± 21.15 c	***	ns
Ligstroside aglycone, aldehyde, and hydroxylic form	11.98 ± 3.51	9.92 ± 2.60	ns	13.70 ± 3.35 a	9.53 ± 1.87 b	8.72 ± 0.76 b	**	ns
Ligstroside aglycone, oxidized aldehyde, and hydroxylic form	22.62 ± 4.10	22.18 ± 4.09	ns	19.40 ± 2.19 b	20.91 ± 3.14 b	26.88 ± 1.30 a	***	ns
Oleuropein aglycone, aldehyde form	112.71 ± 20.16	97.80 ± 34.73	ns	88.85 ± 33.53 b	95.31 ± 17.45 b	131.62 ± 8.74 a	*	ns
Hydroxytyrosol acetate	1.85 ± 0.66	1.41 ± 0.48	ns	1.78 ± 0.82 ab	1.94 ± 0.29 a	1.17 ± 0.31 b	*	ns
Luteolin	24.02 ± 3.00	23.66 ± 4.31	ns	20.59 ± 2.29 b	23.23 ± 2.50 b	27.71 ± 1.35 a	***	ns
Sum of compounds								
Total phenolic compounds	767.63 ± 89.35 x	716.51 ± 76.82 y	*	829.86 ± 56.81 a	742.20 ± 50.66 b	654.15 ± 15.73 c	***	ns
Tyrosol + hydroxytyrosol	4.65 ± 0.87 x	4.04 ± 0.99 y	*	3.40 ± 0.56 c	4.32 ± 0.71 b	5.32 ± 0.26 a	***	ns
Phenolic acid	13.83 ± 3.90	13.71 ± 3.59	ns	18.10 ± 1.36 a	13.38 ± 1.06 b	9.83 ± 0.81 c	***	ns
Flavones	30.23 ± 3.05	31.27 ± 4.57	ns	27.65 ± 1.91 b	30.42 ± 4.12 ab	34.20 ± 1.62 a	**	ns
Secoiridoids	627.78 ± 86.91 x	581.93 ± 77.07 y	*	694.35 ± 54.37 a	602.58 ± 35.93 b	517.63 ± 22.04 c	***	ns
Oleuropein derivatives	518.30 ± 57.08 x	484.24 ± 44.18 y	*	550.89 ± 49.07 a	504.67 ± 24.92 b	448.24 ± 9.97 c	***	ns
Ligstroside derivatives	134.05 ± 33.45 x	123.51 ± 33.31 y	*	168.71 ± 9.34 a	123.43 ± 13.63 b	94.21 ± 3.39 c	***	ns

Note: Means and standard deviation are reported ($n = 3$). Different letters indicate different significance levels according to the Tukey HSD post hoc test results for harvest time (a–c) and density treatment (x, y) (t1 = first-day harvest, t2 = second-day harvest, t3 = third-day harvest).
Abbreviation: ns, non-significant.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

(*E*)-2-hexenol over time. These compounds reported higher values in t3 (1.68 ± 0.40, 0.09 ± 0.05, 0.14 ± 0.16, and 0.03 ± 0.03 mg/kg). For the sum of C6 compounds, a significant decrease was observed from t1 (15.09 ± 1.57 µg/kg) to t3 (13.42 ± 0.61 µg/kg), whereas no significant differences were found regarding t2 compared to t1 and t3.

Between the compounds with more than six carbon atoms (C > 6), nonanal, 2,4-hexadienal, and nonanol significantly increased over time from t1 to t2, and the higher values were registered for t2 (4.64 ± 5.41, 0.76 ± 0.07, and 0.16 ± 0.15 mg/kg), decreasing again at t3 (0.75 ± 0.07, 0.56 ± 0.06, and 0.00 ± 0.01 mg/kg). No significant differences were observed regarding the sum of C > 6 compounds.

Finally, concerning the microbial metabolite compounds, acetic acid and 3-methyl-butanal were identified as the two most abundant compounds, and both exhibited a significant decrease over time. For both

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TABLE 4 Volatile organic compounds (mg/kg) concentration in olive oil samples.

Volatile organic compounds	Main effect							Interaction
	Density treatment		*p*	Harvest time			*p*	*p*
	Low density	High density		t1	t2	t3		
1-Penten-3-one	1.65 ± 0.25	1.56 ± 0.30	ns	1.38 ± 0.24 b	1.77 ± 0.25 a	1.66 ± 0.16 ab	*	ns
2-Pentanol	0.01 ± 0.01	0.01 ± 0.01	ns	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	ns	ns
(*E*)-2-Penten-1-ol	0.07 ± 0.02	0.08 ± 0.02	ns	0.09 ± 0.01 a	0.08 ± 0.02 ab	0.06 ± 0.01 b	*	ns
(*Z*)-2-Penten-1-ol	0.18 ± 0.04	0.18 ± 0.04	ns	0.21 ± 0.03 a	0.19 ± 0.05 ab	0.15 ± 0.01 b	*	ns
Sum of C5 compounds	2.00 ± 0.25	1.88 ± 0.31	ns	1.70 ± 0.25 b	2.17 ± 0.22 a	1.94 ± 0.14 ab	**	ns
Hexanal	3.90 ± 0.52	4.32 ± 1.01	ns	4.12 ± 0.69	4.48 ± 1.11	3.72 ± 0.43	ns	ns
(*Z*)-3-Hexenal	3.46 ± 1.25	3.19 ± 0.91	ns	4.29 ± 0.85 a	3.40 ± 0.76 a	2.27 ± 0.33 b	***	ns
(*E*)-2-Hexenal	6.11 ± 0.59 x	4.57 ± 0.79 y	***	5.85 ± 1.01 a	4.69 ± 0.96 b	5.48 ± 0.96 ab	**	ns
(*Z*)-3-Hexen-1-ol	1.15 ± 0.35	1.22 ± 0.60	ns	0.81 ± 0.11 b	1.06 ± 0.36 b	1.68 ± 0.40 a	***	ns
(*E*)-2-Hexenyl acetate	0.03 ± 0.02 y	0.06 ± 0.06 x	***	0.01 ± 0.00 c	0.03 ± 0.01 b	0.09 ± 0.05 a	***	***
1-Hexanol	0.01 ± 0.02 y	0.08 ± 0.14 x	*	0.00 ± 0.00 b	0.01 ± 0.02 b	0.14 ± 0.16 a	**	*
(*E*)-2-Hexenol	0.01 ± 0.02	0.01 ± 0.02	ns	0.00 ± 0.00 b	0.00 ± 0.00 b	0.03 ± 0.03 a	*	ns
Sum of C6 compounds	14.67 ± 1.25 x	13.44 ± 1.30 y	*	15.09 ± 1.57 a	13.67 ± 1.34 ab	13.42 ± 0.61 b	*	ns
6-Methyl-5-hepten-2-one	0.01 ± 0.00	0.01 ± 0.00	ns	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	ns	ns
Nonanal	1.22 ± 1.02	3.48 ± 4.62	ns	1.66 ± 1.15 ab	4.64 ± 5.41 a	0.75 ± 0.07 b	*	ns
2.4-Hexadienal	0.66 ± 0.12	0.67 ± 0.12	ns	0.68 ± 0.10 a	0.76 ± 0.07 a	0.56 ± 0.06 b	**	ns
Nonanol	0.03 ± 0.05	0.08 ± 0.15	ns	0.00 ± 0.01 b	0.16 ± 0.15 a	0.00 ± 0.01 b	*	ns
Sum of C7–C10 compounds	3.61 ± 1.58	4.60 ± 1.87	ns	4.65 ± 1.36	4.62 ± 2.31	3.05 ± 1.11	ns	ns
Acetic acid	2.10 ± 0.72	2.55 ± 0.93	ns	2.98 ± 0.44 a	2.06 ± 0.82 b	1.94 ± 0.88 b	*	ns
3-Methyl-butanal	1.13 ± 0.54	1.45 ± 0.77	ns	1.69 ± 0.72 a	1.48 ± 0.25 ab	0.71 ± 0.54 b	*	ns
2-Methyl-3-methyl-1 butanol	0.54 ± 0.05 x	0.33 ± 0.10 b	***	0.48 ± 0.13 a	0.36 ± 0.15 b	0.48 ± 0.11 a	*	ns
Hexyl ester-acetic acid	0.01 ± 0.01 y	0.02 ± 0.03 x	*	0.00 ± 0.00 b	0.01 ± 0.01 b	0.04 ± 0.03 a	**	ns
1-Octanol	0.02 ± 0.00 y	0.03 ± 0.02 x	*	0.02 ± 0.00	0.03 ± 0.02	0.02 ± 0.00	ns	ns
Sum of microbial metabolite VOCs	6.16 ± 0.99 y	7.83 ± 1.81 x	*	7.80 ± 1.50	6.98 ± 2.06	6.22 ± 1.16	ns	ns

Note: Means and standard deviations are reported ($n = 3$). Different letters indicate different significance levels according to the Tukey HSD post hoc test results for harvest time (a–c) and density treatment (x, y) (t1 = first-day harvest, t2 = second-day harvest, t3 = third-day harvest).
Abbreviation: ns, non-significant.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

compounds, the higher values were registered at t1 (2.98 ± 0.44 and 1.69 ± 0.72 mg/kg, respectively), whereas the lower at t3 (1.94 ± 0.88 and 0.71 ± 0.54 mg/kg, respectively). A similar trend was observed for 2-methyl-3-methyl-1 butanol; the higher value was recorded at t1 (0.48 ± 0.13 mg/kg) and lower at t2 (0.36 ± 0.15 mg/kg), but the concentration increased again at t3 (0.48 ± 0.11 mg/kg). On the contrary, hexyl ester–acetic acid increases significantly over time, although the values are quite low. The higher value was registered at t3 (0.04 ± 0.03 mg/kg). In this case, the harvest time did not have a significant effect on the sum of microbial metabolite compounds.

4 | DISCUSSION

The main scope of this study was to assess the effect of olive oil sorting of olive drupes on the quality of the resulting EVOOs and to investigate the potential advantages of distinguishing the raw materials entering the olive mill. Interesting results have emerged, especially in the context of olives, as densimetric sorting is a relatively emerging approach regarding virgin olive oil production that offers abundant insight opportunities for further developments. First, it was possible to obtain two different classes of raw material with distinct values of

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density. Subsequently, physical and chemical analyses were conducted to characterize the olive classes and the respective oils obtained.

The low-density olives, which floated in the salt solutions during the densimetric sorting process, reported higher values of MI and the pulp/pit ratio compared to the high-density olives. Conversely, high-density olives showed opposite values for these two indexes, which indicate a general state of earlier maturation of this last class.

Although the oil content values were not significantly different for the density treatment, slightly higher values were observed for the lower density olives compared to the higher density ones.

These values agree with the previously described pulp/pit values. Indeed, in the olives, the pulp (mesocarp) and pit (endocarp) are the predominant tissues. In particular, the mesocarp is the edible portion of olives, and it is the tissue where oil is accumulated. Both tissues show almost parallel development during the initial weeks of growth, but subsequently, the mesocarp's growth predominates, leading to oil accumulation.[37]

Similar behavior to what occurs in grape sorting,[16] the lower sugar content observed in low-density olives compared to high-density olives agrees with previous values such as MI. A further interesting result is given by the average value of the mass of the olives, which in this case is not significant between the two classes of olives for the treatment. The results obtained suggest that these parameters are among the most relevant for separating olives based on different density values.

Even more interesting results have been found in the oils obtained from olives with different densities. This is an important outcome as oil is the main final product. First, the oils obtained were classified as extra virgin, based on the legal parameters for the classification of olive oils. Between these, the most interesting results are sure the PVs, the most representative parameter considered to measure oxidation in virgin olive oil. PV results significantly different for the high-density oil with respect to the low-density oil, in agreement with the phenolic compounds found in both the oil samples. This result could suggest that this class of olives could also include fruits with an oxidative degradation state due to different mechanical and biological factors, for example, mechanical damage, parasitic attacks, and microbial activity.

Among the phenolic groups, the values of the sum of secoiridoids were found to be higher in low-density oil compared to high-density oil. This class presents the highest transfer rate from olives to oil, and their content is usually proportionally to the intensity of bitterness and pungency, in particular oleuropein, ligstroside, and their derivates.[34]

Especially, the sum of hydroxytyrosol and tyrosol result significantly different for the density treatment, suggesting an advanced stage of maturation, in agreement with the values recorded for the MI.

Concerning volatile compounds, low-density oils showed higher values of the sum of C6 compounds that are typically connected to the LOX pathway and contribute to the positive sensory attributes of the oils. An interesting result was found for the (*E*)-2-hexenal attributable to "fruity" and "green" positive scents. An opposite trend was reported for the high-density oil. The lower values of C6 compounds and (*E*)-2-hexenal were found in this class.

Finally, as regards the sum of microbial metabolites, higher values have been reported for high-density oils compared to low-density ones. This result could be related to the presence of olives with a non-perfect health status, in which the oil can encounter oxygen, microorganisms, and substrates present in the olive, such as water and sugars. The metabolite microorganisms are responsible for the "fusty" defect, and this is congruent with the values found in the oil samples.

In the present study, the treatment of the harvesting period was also evaluated on the characteristics of the olives and the oils extracted.

The MI significantly increased in olive fruits during the time. The same trend was found also for the pulp/pit ratio and for average mass values. Regarding these two last indices, a significant increase was measured for the first two harvest times but not for the third. In agreement with the current literature, the oil content significantly increased as a function of the ripening time.[13] On the other hand, the request of sugars to synthesize lipids by olive cells involves a decrease in the sugar content. Many authors reported a linear relationship between decreasing sugar content and increasing oil accumulation during olive ripening.[25]

The results obtained above suggest that during the first two moments of harvesting, the olives undergo a general increase in fruit size, which is attributed to the development of the main drupe tissues. Afterward, the growth of the drupe ceases, but the accumulation of oil in the pulp continues. From the characterization of the oils obtained in the three harvest times, it emerged that the oxidative stability of the oil, based on the measured PV, decreases over time. In fact, earlier harvest times correspond to lower PVs. In contrast, a significant increase of the phenolic compounds was registered over time. The positive correlation between phenol content and oxidative stability has been described in virgin olive oil.[38]

A general decrease was also recorded in the secoiridoid compounds as the harvest time progressed. At the end, an increase in the sum of tyrosol + hydroxytyrosol compounds was registered due to the hydrolytic degradation of secoiridoids. Concerning the volatile compounds, a significant increase of C5 compounds was registered over time, in contrast to C6 compounds, according to Kalua et al.[39] The same trend was found for (*E*)-2-hexenal, a common marker of the LOX pathway and consequently, the fruitiness attribute.

5 | CONCLUSIONS

Densimetric sorting of olives during post-harvest could represent an opportunity to obtain oils with different quality characteristics, thus enabling potential differentiation of production. Initial laboratory-scale results revealed that it was indeed possible to sort the olives based on their density, which showed different values. The respective oil produced reported greater oxidative stability and a higher content of polyphenols, which could contribute to greater sensations of bitterness and spiciness. Oil showed a higher content of C6 compounds, which are usually linked to positive fruity attributes, and a lower content of microbial metabolite compounds, responsible for some sensory defects. The results obtained from this first study concerning the densimetric sorting of EVOO suggested the best qualitative characteristics

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of the oil obtained from the low-density olives compared to the high-density olives. Therefore, the densimetric sorting of olives could be an efficient and relatively straightforward method for use in the post-harvest phase. This technique allows for the classification of fruits and the production of oils with distinct characteristics. However, further research is needed to extend the study to include different olive varieties and different harvest years. Furthermore, technical studies and appropriate engineering evaluations are needed to refine the methodologies used and to improve the understanding of the results obtained.

AUTHOR CONTRIBUTIONS

Agnese Spadi: Data curation; formal analysis; investigation; methodology; software; writing—original draft; writing—review and editing. **Ferdinando Corti**: Data curation; investigation; methodology; visualization; writing—original draft; writing—review and editing. **Giulia Angeloni**: Data curation; investigation. **Luca Calamai**: Data curation; investigation. **Piernicola Masella**: Data curation; investigation; supervision. **Alessandro Parenti**: Conceptualization; data curation; project administration; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available within the article.

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