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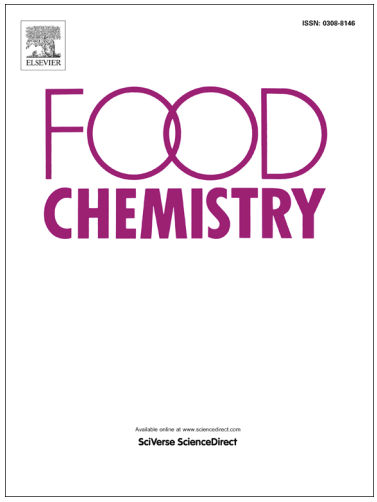

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Use of density sorting for the selection of aromatic grape berries with different volatile profile

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Running title: Berry density sorting impact on volatile profile of aromatic grapes

ABSTRACT

The aim of the study was to investigate the application of berry density sorting as a tool for the selection of grapes with different volatile and precursor profiles. The study was carried out on Moscato giallo, Malvasia di Schierano, Malvasia nera lunga, and Brachetto aromatic grape varieties. Free and glycosidically-bound volatile terpene compounds including linalool, geraniol, nerol, citronellol, and terpineol, as well as lipoxygenase activity-derived compounds, were evaluated using head space-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) in density sorted berries (1075–1119 kg m^{-3}). Total free terpenes changed with the berry density, while no significant changes were found in total glycosylated compounds, except for Malvasia nera lunga grapes where nerol, linalool, and geraniol contributed strongly to the increase of total contents with increasing berry density. Given that these variations were strongly variety-dependent, the possible use of density sorting equipment in winery for this aim may be less effective.

Keywords: free and glycosylated volatile compounds; density sorting; terpenes; green leaf volatile compounds; aromatic cultivars; winegrapes; aroma.

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1 Introduction

The olfactory aspect of a wine is one of the most important traits to satisfy the consumer requirements, in particular for wines produced from aromatic grape varieties where floral nuances given by varietal terpene molecules are the main feature. In the wine, more than 800 olfactory active volatile compounds have been identified (Ferreira, Sáenz-Navajas, Campo, Herrero, De La Fuente, & Fernández-Zurbano, 2016) with contents ranging from fractions of ng L^{-1} to several mg L^{-1} (Mozzon, Savini, Boselli, & Thorngate, 2016; Robinson, Boss, Solomon, Trengove, Heymann, & Ebeler, 2014). The pool of odorant compounds that is possible to find in wines is the result of different sources and enological treatments (Bakker & Clarke, 2012; Pisarnitskii, 2001; Urcan et al., 2017). In particular, varietal volatile compounds, or rather the odorants synthetized by the grapes, and the prefermentative volatile molecules originated by the lipoxygenase pathway after the grape cellular decompartmentalisation, play a fundamental role in the wine aroma (Ribéreau- Gayon, Glories, Maujean, & Dubourdieu, 2006).

The main classes of free varietal volatile compounds accumulated in grapes of *Vitis vinifera* L. germplasm are terpenes, benzenoids, C_{13}-norisoprenoids, and methoxypyrazines (Ribéreau- Gayon et al., 2006; Waterhouse, Sacks, & Jeffery, 2016). Grapevine berries also accumulate glycosylated aroma precursors. They are non-volatile compounds with little olfactory impact (Palomo, Pérez-Coello, Díaz-Maroto, Viñas, & Cabezudo, 2006), but their acid or enzyme hydrolysis from the sugar moiety during the process of vinification and later of wine maturation and aging causes the release of free volatiles (Dziadas & Jeleń, 2016).

Some varieties, described as “aromatic varieties”, are characterized by a higher accumulation of terpenes in the berries, both in free and glycosylated forms, due to a point mutation of the gene 1-deoxy-D-xylulose-5-phosphate synthase, which is considered one of the key genes of the methyl erythritol pathway (Schwab & Wüst, 2015).

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The terpenes accumulation in grape berries begins near véraison. Some authors describe a continue accumulation during the process of berry ripening and over-ripening (Schwab & Wüst, 2015), while others report a decrease in the terpenes content before the end of sugar accumulation (Lasanta, Caro, Gòmez, & Pèrez, 2014; Torchio et al., 2016). The variability of grape ripeness in a vineyard is usually high, and therefore the difference in metabolites composition of grape berries is not present only among plants or clusters, but also within the same cluster (Asproudi, Petrozziello, Cavalletto, & Guidoni, 2016; Pagay & Cheng, 2010). Regarding the impact of grape ripeness and its variability on aroma metabolites, Rolle, Torchio, Giacosa, and Río Segade (2015) sorted Muscat Hamburg table grape berries from a single harvest date using the densimetric flotation, and observed significant and compound-dependent relationships between density and free terpenes composition.

In wine production, the density sorting of grape berries can be a useful technique for improving the wine quality through the removal of unripe berries or those not meeting defined quality parameters. In Moscato bianco berries, it has been highlighted that the density effect is higher than the sampling date effect for both total free and glycosylated terpenes through the ripening process (Torchio et al., 2016). However, the scientific literature about this technique indicates some problematic aspects linked to the technological application, such as a grapevine cultivation environment dependence on the results (Rolle, Torchio, Giacosa, Río Segade, Cagnasso, & Gerbi, 2012) and a non-univocally variability for the different volatile compounds among berries belonging to different density classes (Torchio et al., 2016).

The aim of this study was to investigate the use of the density sorting as technique for the selection of grape berries with a certain aroma profile by potentiating target volatile compounds to obtain wines with different olfactory features, and also intends to expand the knowledge on the possible use of a berry sorting equipment in cellar. For this purpose, four aromatic varieties, namely Moscato giallo, Malvasia di Schierano, Malvasia nera lunga, and Brachetto, were chosen for their

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different varietal volatile and aroma precursor profiles (Di Stefano & Corino, 1984a; Di Stefano, Borsa, Maggiorotto, & Corino, 1995). Their grape variety dissimilarities were exploited to study the possible common trends, generated by berry density sorting, on the volatile composition.

2 Materials and methods

2.1 Grape samples

Grape samples of *Vitis vinifera* L. cv. Moscato giallo, Malvasia di Schierano, Malvasia nera lunga, and Brachetto were collected from the CNR-IPSP ampelographic collection of Grinzane Cavour (Cuneo province, north-west Italy, 44.651 N, 7.995 E). For each variety the sampling was performed by picking small groups of 3-5 berries from different parts of each cluster, for a total mass of about 15 kg of berries.

2.2 Density sorting

The grapevine berries obtained were densimetrically sorted by flotation in different saline solutions (from 80 to 180 g L^{-1} of sodium chloride, corresponding to densities between 1069 kg m^{-3} and 1119 kg m^{-3}). The sorting protocol used was provided by Fournand, Vicens, Sidhoum, Souquet, Moutounet, and Cheynier (2006) with the modifications proposed by Rolle, Río Segade et al. (2011). The sorted berries were washed with water and those damaged were discarded. For each cultivar, the berry groups obtained were weighed, and were considered all the density classes accounting for at least 3 % of berry distribution by weight. From each density class considered three replicates of 20 berries were weighted (to evaluate the average berry weight) and then were used for the evaluation of the technological parameters, while the remaining berries were used for the analysis of the volatile compounds and precursors of them.

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2.3 Technological ripeness parameters

Grape samples were manually crushed and centrifuged at 3000 × *g* for 10 min (Hettich 32R centrifuge, Tuttinglen, Germany), and the supernatants were used for the following determinations: glucose, fructose, citric acid, tartaric acid, and malic acid (g L^{-1}) were determined through an 1260 HPLC system (Agilent Technologies, Santa Clara, CA, US) equipped with a diode array detector (DAD) set to 210 nm (Rolle, Gerbi, Schneider, Spanna & Río Segade, 2011), pH was determined by potentiometry using an InoLab 730 pHmeter (WTW, Weilheim, Germany), and titratable acidity (g L^{-1} tartaric acid, as TA) was estimated following the OIV-MA-F1-05:R2011 method (OIV, 2015).

2.4 Varietal and prefermentative volatile compounds

For each variety and density class, the determination of free and glycosylated aroma compounds was performed as reported by Torchio et al. (2016), with slight modifications as described in the following subsections.

2.4.1 Free volatile compounds determination

For each subsample, 200 g of grape berries were blended (Sunbeam Products, Boca Raton, FL, US) under nitrogen atmosphere for 1 min, and then centrifuged at 3000 × *g* for 10 minutes at 4 °C. 5 mL of supernatant were diluted with a buffer solution at pH 5 (21 g L^{-1} of citric acid monohydrate, 28.4 g L^{-1} of sodium dihydrogen phosphate in ultrapure water), transferred to a 20 mL glass vial containing 2 g of sodium chloride (Sigma-Aldrich, St. Louis, MO, US) and 200 µL of internal standard (1.552 mg L^{-1} of 1-heptanol in 10% v/v ethanol). Three replicates for each variety and density class were carried out.

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2.4.2 Glycosylated compounds determination

10 mL of supernatant previously obtained were introduced on a 1-g Sep-Pak C18 reverse solid phase cartridge (Waters Corporation, Milford, MA, US) previously activated with 5 mL of methanol (Sigma-Aldrich) and washed with 10 mL of ultrapure water. After the passage of the sample, the cartridge was washed with 10 mL of ultrapure water. The free volatile compounds adsorbed on the C18 polymer were eluted with 10 mL of dichloromethane (Sigma-Aldrich) and discarded. Glycosylated compounds were eluted with 10 mL of methanol. For each passage of liquid through the C18 cartridge the flow rate was approximately 2 mL min^{-1}. The obtained methanolic extract was evaporated to dryness using a vacuum rotavapor (Buchi R-210, Flawil, Switzerland) at 35 °C and redissolved in 5 mL of the buffer solution at pH 5 previously described.

The enzymatic hydrolysis was performed by the addition of 50 mg of AR-2000 glycosidase enzyme (DSM Oenology, Delft, The Netherlands) with an incubation at 40 °C for 24 h. After the hydrolysis of glycosylated compounds, the extract was added of 200 µL of internal standard (1.552 mg L^{-1} of 1-heptanol in 10% v/v ethanol) and transferred in a 20-mL glass vial containing 2 g of sodium chloride and 5 mL of ultrapure water. Three replicates for each variety and density class were carried out.

2.4.3 HS-SPME-GC-MS conditions

The analytical determinations were performed as described by Sánchez-Palomo, Diaz-Maroto, and Perez-Coello (2005) as modified by Torchio et al. (2016). The vials were sealed using 18-mm diameter screw caps with a silicone septum (Supelco, Bellefonte, PA, US), and were then shaken for 20 min using an automated procedure (Gerstel MPS Automated SPME, Gerstel, Mülheim an der Ruhr, Germany). A 50/30 µm DVB/CAR/PDMS fibre from Supelco was exposed to the headspace of the capped vial for 20 min at 40 °C. SPME injections were performed in splitless mode at 250 °C for 5 min for the thermal desorption of analytes from the fibre. The GC–MS system used was a

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Agilent 7890C gas chromatograph coupled to an Agilent 5975 mass selective detector (Agilent Technologies, Santa Clara, CA, US).

A DB–WAX capillary column (30 m x 0.25 mm, J&W Scientific Inc., Folsom, CA, US) was used for the separation. The temperature program started at 40 °C for 5 min, increased at a rate of 2 °C min^{-1} to 200 °C for 10 min, and 5 °C min^{-1} to 220 °C, then holding at 220 °C for 5 min. The carrier gas used was helium with a flow-rate of 1 mL min^{-1}. The ion source temperature was maintained to 150 °C and the interface temperature was 280 °C. Molecules ionization took place with an energy of 70 eV. The acquisition range was 35–350 *m/z*. Peak identification and data elaboration were carried out as described by Torchio et al. (2016).

2.5 Data analysis

The R suite version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria) was used for all the statistical evaluations. Univariate analysis and their assumptions were performed thanks to the package *agricolae*. Variances homoskedasticity were checked through Bartlett's test, normal distribution of ANOVA residuals were verified thanks to the Shapiro-Wilk's test on ANOVA residuals. ANOVA null hypothesis was rejected at p value < 0.05. Tukey's HSD test was applied as *post-hoc* comparison. PCA analysis was performed thanks to the package *FactoMineR*, after standardization of the data matrix (z-scores).

3 Results and discussion

3.1 Percentage distribution of berries in density classes and related technological ripeness parameters

The berry density heterogeneity in the vineyard, assessed using the sorting method previously described, is shown in Figure 1 as berry distribution percentage in density classes. For each variety,

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we clearly observed remarkable differences in berries ripeness at harvest in the same vineyard, as already pointed out in scientific literature (Dai et al., 2011; Rolle, Río Segade et al., 2011; Torchio et al., 2016). These differences are attributable to multiple factors like exposure, soil, topography, microclimate, plant asynchronous genetic programming of the maturation process into the same bunch, position of the berry into the cluster, and plant external biotic factors (Gouthu, O'Neil¸ Di, Ansarolia, Megraw, & Deluc, 2014; Pisciotta, Di Lorenzo, Barbagallo, & Hunter, 2013; Río Segade et al., 2017).

Two types of berry distributions were observed (Figure 1): Moscato giallo and Brachetto were characterized by a distinguishable Gaussian-shaped bell curve, with the central point reaching about 39–45 % berry distribution by weight. Malvasia nera lunga and Malvasia di Schierano varieties, although evidencing a similar behaviour, presented a less narrow curve, with a higher dispersion of the berries distribution across multiple density classes. Similar distributions were previously observed for Nebbiolo, Cabernet sauvignon, and Muscat Hamburg berries sorted by densimetric flotation (Kontoudakis, Esteruelas, Fort, Canals, De Freitas, & Zamora, 2011; Rolle, Río Segade et al., 2011; Rolle et al., 2015). Regarding Malvasia di Schierano, Malvasia nera lunga, and Brachetto, the most represented berry density class was 1100 kg m^{-3}, while for Moscato giallo it was observed a greater percentage by weight of berries (45 %) in the 1094 kg m^{-3} class. This factor is undoubtedly affected by the overall ripeness in the vineyard and hence by the harvest date choice.

The technological ripeness parameters, as well as the average berry weight, are presented in Table 1. For Moscato giallo and Malvasia di Schierano, the average berry weight was not significantly different among the berry groups obtained by density sorting. On the contrary, for Malvasia nera lunga the lowest values of the average berry weight were found at the extreme density classes (1107 and 1075 kg m^{-3}), while for Brachetto the lowest value was registered at the highest berry density considered (1119 kg m^{-3}). Berry weight is an important parameter which could be related to berry size and hence to the juice/skin ratio. Density sorting studies found some

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correlations between grape density and the berry weight or berry size, with both decreasing when increasing grape density in cv. Nebbiolo sorted berries, although ripening and production area effects may limit the extent of this correlation (Rolle, Río Segade et al., 2011; Rolle et al., 2012).

As expected, the content of grape reducing sugars (glucose and fructose) is the main parameter that influenced berry density (Rolle et al., 2012). An increasing reducing sugars trend was found in all varieties when increasing berry density. When comparing the same density class among cultivars, the difference in reducing sugar contents may reach 12 g L^{-1} in some cases, and it was smaller at lower densities (less than 2 g L^{-1} in the class of 1081 kg m^{-3} for three varieties). No significant differences were found for the glucose/fructose ratio of Moscato giallo and Brachetto among different berry density classes, whilst for Malvasia di Schierano and Malvasia nera lunga this ratio increased when increasing the berry density considered. Previously, no differences were found for this parameter in Muscat Hamburg grapes among three different density classes (Rolle et al., 2015).

Moscato giallo and Brachetto showed a negative relationship between the berry density value and the malic acid content. This known fact can be explained by the physiological phenomena involved in berry ripening according to which malic acid can be used in different ways: after véraison, malic acid becomes the main source of energy for grape cells through respiration instead of glucose and fructose, and it can be also utilized in gluconeogenesis process (Etienne, Génard, Lobit, Mbeguié-A-Mbéguié, & Bugaud, 2013; Ruffner, 1982). Therefore, the riper berries, and hence those with higher densities and richer in reducing sugars, were affected more intensely by these phenomena. This behaviour was also reflected on the titratable acidity values because variations in the malic acid content may lead to a different acid equilibrium in the grape juice obtained, influencing the pH value and the dissociation reactions affecting titratable acidity. This supposition is supported by the pH differences observed and by the presence of not significantly different tartaric acid contents among the berries belonging to the density classes obtained for these

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two varieties. Furthermore, a significant difference in citric acid was observed in Moscato giallo, where the lowest content was registered for the denser berries (1100 kg m^{-3}). Although the content and contribution of citric acid to acidity are limited, as occurs for malic acid after véraison it could be partially used as a cellular energy source or for the biosynthesis of other metabolites (Etienne et al., 2013).

In addition, it is worth to note that a common pH trend was found for all the varieties considered, with the lowest values corresponding to the berries of the lowest density values. Since these considered varieties are often used for the production of sweet, partially fermented or *passito* wines, the sugars/acidity ratio may be of relevant importance. Given the obtained results for these parameters, berry separation by density sorting may provide berry groups with different sugar/acidity characteristics. With this aim, a possible use of a berry sorting equipment in cellar could be interesting and useful in order to selection berries with different technological ripeness parameters.

3.2 Volatile compounds and precursors of them

Twenty free volatile compounds were found in all analysed samples for the four varieties studied (Tables 2, 3, 4, 5), except for geranic acid that was found only in Malvasia di Schierano and Brachetto. Among these compounds, five of them belong to the green leaf volatile compounds group (C6 and C9 compounds derived from the lipoxygenases pathway), and hence they are considered prefermentative aroma compounds (Carlomagno, Schubert, & Ferrandino, 2016). The other fifteen compounds found are terpene compounds, these latter are responsible for the typical floral scent findable in wines obtained from aromatic cultivars (Schwab & Wüst, 2015). In addition, 18 molecules belonging to the glycosylated aroma precursor compounds were determined, fifteen of them are terpenes and the remaining three belong to the biochemical class of benzene derivatives.

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The most important terpenes commonly found in enological products, based on their low odour threshold, are (-)-*cis*-rose oxide, linalool, geraniol, citronellol, HO-trienol, α-terpineol, and nerol (Waterhouse et al., 2016). A great content in monoterpene compounds is a positive trait for the quality of wines produced from aromatic grape cultivars. However, it is worth to remember that the olfactory impact of a volatile compound is not determined only by its own olfactory threshold, but it can be modified by many chemical and physical interactions with other substances present in the matrix (Robinson, Ebeler, Heymann, Boss, Solomon, & Trengove, 2009). Glycosylated compounds in the wine, especially terpenes, represent the reserve of aroma and after the enzymatic or acidic hydrolysis (Pogorzelski & Wilkowska, 2007) these compounds become olfactory active, contributing to the perceivable aroma fraction.

The free and glycosidically bound terpene composition for each density class studied is shown in Tables 2, 3, 4, and 5 for Moscato giallo, Malvasia di Schierano, Malvasia nera lunga, and Brachetto, respectively. On one side, a differentiation of the contents of these terpene compounds is present among varieties. On the other side, as already mentioned above, the main factor affecting the grape berry density is the reducing sugar content, but the accumulation of volatile aroma compounds during ripening seems not to occur simultaneously to hexoses synthesis (Coombe & Iland, 2004; Torchio et al., 2016). In this study, it clearly emerges the different accumulation trend of hexoses and volatile compounds in the berries. This effect was not only observed at level of free and bound terpenes, but also for other chemical classes such as free C6-C9 compounds (green leaf volatile compounds) and glycosylated benzenoids (Tables S1-S4).

Regarding free terpenes sum, in Moscato giallo, Malvasia nera lunga, and Brachetto berry density classes (Tables 2, 4, 5), a similar Gaussian bell-shaped distribution was observed corresponding the highest values to intermediate berry density classes. For Malvasia di Schierano (Table 3), the highest total content of free terpenes was registered for the berries class with the lowest density value studied (1081 kg m^{-3}). This behaviour was not consistent with the glycosylated

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terpenes sum: for Moscato giallo, Malvasia di Schierano and Brachetto berries, no significant differences were observed among the different density classes obtained; while for Malvasia nera lunga a progressive increase of total content of glycosylated terpenes was found with increasing berry density (Table 4). This aspect represents a further evidence that dynamics of volatile molecules accumulation is strongly variable within the *V. vinifera* germplasm.

Linalool was the most abundant terpene in Moscato giallo white cultivar, while in the red varieties studied, such as Malvasia di Schierano, Malvasia nera lunga, and Brachetto, the prevalent compound found was geraniol. Previously, Di Stefano and Corino (1984b) noted that geraniol was the predominant terpene compound in the red aromatic grape varieties historically cultivated in Piedmont growing zone.

3.2.1 Moscato giallo

Regarding terpene compounds, ten of the fourteen identified molecules displayed significant differences among the berry density classes studied. Berries belonging to 1088 and 1094 kg m^{-3} classes showed a greater content of these ten free terpene compounds compared to the other groups, driven by the linalool content (85.0–92.6% of total free terpene compounds). Some quantitative differences between the two berry density classes were also observed for HO-trienol and *trans*-pyran linalool oxide contents, which were significantly higher for the berries with 1094 kg m^{-3} density (Table 2).

The berry density sorting was previously applied for the study of the ripening effect on another Muscat cultivar, namely Moscato bianco (Torchio et al., 2016), where free and bound volatile compounds were determined at five different sampling times in the last stages of the ripening process. The authors found that the berries sampled at the third ripening stage were those richest in free terpene compounds, and in that point the highest content of total free terpenes, as well as of free linalool, geraniol, and nerol, was found in the berries with density between 1090 and

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1100 kg m^{-3}. Taking into account that in the third sampling point of the cited study the most represented density classes by weight were 1075 and 1081 kg m^{-3}, we may deduce that in Moscato bianco the highest content of free terpene compounds was found in the underrepresented classes with density values above the average, while in this study for Moscato giallo the highest free terpene content was found in the most represented density class (Table 2 and Figure 1A). However, the behaviour of these three free terpene compounds (linalool, nerol, and geraniol), and therefore of total free terpenes, with the berry density for Moscato giallo white grape variety was quite similar to that published for Moscato bianco (Torchio et al., 2016).

Regarding the compounds derived from the action of lipoxygenase (Table S1), only two compounds [1-hexanol and *(E)*-3-hexenol] of the five detected showed significant differences among the berry groups obtained by density sorting. The highest contents of free lipoxygenases products also corresponded to the berries belonging to 1094 kg m^{-3} density class.

In Moscato giallo, no statistical differences were observed for glycosylated aroma precursors among the different berry density classes obtained, considering both bond terpenes (Table 2) and benzenoids (Table S1). Nevertheless, significant variations were observed in the contents of glycosylated terpene compounds with the berry density in Moscato bianco berries (Torchio et al., 2016). The total glycosylated terpene content ranged between 2.6 and 3.0 mg L^{-1}, with geraniol accounting for more than 1.2 mg L^{-1} in all the density classes analysed (42.6–46.3% of total glycosylated terpene compounds).

3.2.2 Malvasia di Schierano

Regarding free terpene compounds, 1081 kg m^{-3} berry density class exhibited the significantly greatest contents for some key aroma compounds such as HO-trienol, geranial, citronellol, nerol, geraniol, and geranic acid (Table 3). This class displayed also the greatest content of free terpenes sum. This particular behaviour of accumulating a higher amount of free terpene compounds in the

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berries of the lowest density was not observed in the other varieties analysed, in which the less density berry classes evidenced the lowest contents of free terpenes. This indicates that the density effect on the free terpene accumulation could be variety dependent. In fact, there were not significant differences in the content of total free terpenes, as well as of most of individual compounds, among density classes from 1088 kg m^{-3} to 1107 kg m^{-3}. Therefore, in Malvasia di Schierano, the highest contents of many free terpene compounds were achieved in berries with low representativeness according to berry distribution by weight (Table 3 and Figure 1B).

Among green leaf volatiles, free (*E*)-2-hexenal and (*E*)-3-hexen-1-ol did not show significant differences for different berry densities. From the point of view of the total free lipoxygenases compounds determined, the content was mainly conditioned by hexanal, which is the predominant C6 compound (51.0–63.0% of total free lipoxygenases products), achieving the highest content at 1088 kg m^{-3} density even though it only was significantly higher than that obtained for the berries belonging to 1081 kg m^{-3} class (Table S2).

In Malvasia di Schierano, five glycosylated terpenes of the fifteen determined varied significantly among the five density classes analysed (Table 3). Glycosylated linalool, one of the most important aroma precursor, some of its oxides, HO-trienol, and α-terpineol showed the highest contents in the densest berries (1100 and 1107 kg m^{-3}), evidencing a tangible density effect. No significant differences were found in bound geraniol, being the predominant glycosylated terpene compound (40.4–47.6%), as well as for the sum of glycosylated terpenes among the different density classes. It is important to highlight that Malvasia di Schierano has the highest contents of total glycosylated terpene compounds among all the analysed cultivars, particularly at 1100 and 1107 kg m^{-3} (4.2 and 4.4 mg L^{-1}, respectively). No glycosylated benzenoid determined was significantly different in its content among the density classes studied (Table S2).

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3.2.3 Malvasia nera lunga

Nine of the fourteen free terpene compounds determined were statistically variable among the six different berry groups obtained by density sorting: in particular, the second most abundant class by berry distribution, namely 1094 kg m^{-3} class, evidenced significantly higher contents with respect to all other classes analysed for linalool, citronellol, nerol, and geraniol (Table 4). Since these four compounds are some of the most important terpene aroma components in grapes (Waterhouse et al., 2016), their impact on the products derived from Malvasia nera lunga grapes is of great relevance. In addition, the berries belonging to this density class achieved also a significantly higher (more than double) content of the free terpenes sum compared to all the other density classes analysed.

As opposed by Malvasia di Schierano, the distribution of free terpenes sum across Malvasia nera lunga density classes displayed a Gaussian-bell shaped curve with a long tail towards the higher density classes. Indeed, the less dense class (1075 kg m^{-3}) contained the lowest total free terpenes quantity.

The berry density sorting permitted a high differentiation at level of green leaf volatile compounds (Table S3) because the content of only one compound [(*E*)-3-hexen-1-ol] of the five detected was not significantly different among the density classes obtained. The 1094 kg m^{-3} class evidenced the greatest green leaf volatile compounds content of this study. High contents of these free compounds, which give negative herbaceous notes (Matsui, 2006; Podolyan, White, Jordan, & Winefield, 2010), may indicate a major lipoxygenase enzymes activity as a result of gene expression regulation.

A high impact of density sorting was found in the glycosylated terpene composition of Malvasia nera lunga grapes (Table 4). The contents of twelve glycosylated terpene compounds of the fifteen detected resulted to be significantly different among the six density classes studied. The trend already seen in Malvasia di Schierano was also found for this variety, with the grapes belonging to the density class 1107 kg m^{-3} evidencing higher contents of glycosylated terpenes

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compared to the other density classes, in particular nerol, geraniol, β-ocimene, α-terpineol, and geranial, although other compounds were found also in high quantities such as *trans*-furan linalool oxide, linalool, HO-trienol, citronellol, *cis*-rose oxide, *trans*-rose oxide, and geranic acid. The trend of single glycosylated terpenes content to increase with increasing the berry density was confirmed for the sum of glycosylated terpenes. It was possible also to observe significantly lower contents of some important glycosylated terpenes such as nerol, geraniol, linalool, citronellol, geranial, and geranic acid in the berries belonging to the lowest density class (1075 kg m^{-3}). The increased contents of glycosylated linalool, nerol, and geraniol with increasing berry density were also observed in Malvasia moscata (Urcan et al., 2017).

Malvasia nera lunga berries distinguished themselves from the other varieties analysed for their very high contents of glycosylated *trans*- and *cis*-rose oxide, which could represent an important reserve of aroma in wines produced from this variety because of the low sensory threshold of their respective free forms, especially for *cis*-rose oxide (Guth, 1997).

Few differences were also noted for glycosylated benzenoids (Table S3), with an increasing trend with increasing density due to benzyl alcohol. Nevertheless, these differences were only significant between 1075 and 1107 kg m^{-3} density classes.

3.2.4 Brachetto

Seven free terpene compounds of the fifteen detected were significantly different among the six density groups obtained (Table 5). Geraniol was the most abundant free terpene compound in this grape variety (46.0–70.9%, lower contribution to total free terpenes with increasing the berry density), as previously found by others (Di Stefano & Corino, 1984a). The berries belonging to the 1100 kg m^{-3} density class showed the highest contents of linalool and geraniol, those of 1107 kg m^{-3} density class were the richest in HO-trienol, citronellol, and geranic acid, whereas similar contents of nerol were found in both 1100 and 1107 kg m^{-3} density classes. Regarding total free terpenes, it

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is important to take into account that the highest contents were achieved for the most abundant density class (1100 kg m^{-3}), although not significantly different quantities were also found in berries of 1094, 1107 and 1115 kg m^{-3} densities.

In Brachetto berries, all compounds detected belonging to the green leaf volatile compounds group showed significantly different contents among the density classes obtained. The 1088 kg m^{-3} berry density class recorded the lowest content of green leaf compounds sum (Table S4).

Only three glycosylated terpenes of the fifteen detected were variable in concentration among different berry density classes (Table 5): geranic acid, HO-trienol, and *trans*-pyran linalool oxide. These three compounds accounted for a very small contribution to the total glycosylated terpene content, thus evidencing a very little influence on the sensory traits. HO-trienol contents decreased with increasing density values, whereas geranic acid and *trans*-pyran linalool oxide showed a Gaussian-bell shaped curve with the maximum contents at 1107 kg m^{-3} density class. Nevertheless, the differences in total glycosylated terpene content among berries belonging to the density classes studied were not significant, as occurred for geraniol, the predominant compound, as its contribution to total glycosylated terpenes (ranging between 47.1–54.4%) decreased with increasing the berry density.

Among bound benzenoids (Table S4), only methyl salicilate evidenced significant differences among the six density classes, with the highest content at the 1094 kg m^{-3} density class. The sum of glycosylated benzenoids detected was statistically unaffected by the density sorting.

3.3 Multivariate analysis

Because the berry density effect on the volatile composition seems to be variety dependent, a Principal Component Analysis (PCA) was carried out on the free terpene composition data to better know the existence of common behaviours for the varieties studied with relation to berry density sorting, and therefore to exploit this technique to potentiate a certain target volatile compound

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and/or a certain aroma profile independently on the variety. The resulting loadings plot and the scatter plot are shown in Figures S1a and S1b, respectively. The first principal component (PC1), which accounted for about 45% of total variance, was correlated mainly with linalool and its oxide forms, while geranial, geraniol, and nerol were satisfactorily correlated with PC2 (about 24% of total variance explained). Only Moscato giallo samples were well differentiated in the right side of the graph but no clear trend with berry density was observed.

When PCA was performed using glycosylated terpene compounds (Figure S2), PC1 and PC2 explained about 45 and 25% of total variance, respectively. Malvasia nera lunga samples are located at the most positive values of PC1, which was correlated mainly with *cis*-rose oxide, *trans*-rose oxide, and citronellol (Figure S2a), Brachetto samples are located at central values of PC1 and negative values of PC2. Moscato giallo and Malvasia di Schierano are located at the negative values of PC1 but not efficiently separated from each other either by PC1 or PC2. PC2 was highly correlated with nerol, α-terpineol, and *trans*-pyran linalool oxide (Figure S2a), and it permitted the separation of Malvasia nera lunga samples according to the berry density in three groups (particularly 1075, 1081-1088-1094-1100, and 1107 kg m^{-3}) and of Malvasia di Schierano samples in three groups (1081, 1088-1094, and 1100-1107 kg m^{-3}). No clear density effect was observed in the scatter plot for Moscato giallo and Brachetto (Figure S2b).

4 Conclusions

The application of density sorting in cellar is now possible thanks to new equipment present on the market. However, in this study the density sorting technique has proven only in part to be able to separate berry groups with different volatile compounds and aroma precursor profile. Furthermore, it is important to mention that these results are linked to the grape varieties analysed. In general, the highest accumulation of free volatile terpenes has never resulted coinciding with the highest berry density and hence with the greatest sugar content. This suggests that for the

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maximization of free terpenes concentration in these varieties there is not the need to have berries with the maximum sugar concentration achievable: this aspect is important for wine production because a high hexoses concentration means possible issues during yeasts fermentation (as high osmotic pressure in must can cause high production of acetic acid by yeasts) and high ethanol contents. Higher berry densities involve also greater pH values and lower values of titratable acidity: this can be a negative aspect for obtaining aromatic sparkling wines or other special wines where a good acidity is a positive feature.

Therefore, preliminary analyses must be carried out to effectively evaluate the potential aroma of each grape batch in order to select a correct density when using density sorting equipment in winery. Although nowadays gas chromatography is quite widespread in enological laboratories, the cost of the volatile profile analyses is still generally high. A necessary condition is that the working density chosen must be able to valorise and differentiate the olfactory aspects of each of two potential groups of berries obtained by density sorting, considering in turn the actual quantity of berries belonging to each group. Given these results and considerations, the use of a density sorting equipment in winery could lose an applicative interest for this purpose when considering all varieties together, but it could have an applicative interest for Malvasia varieties. Particularly for Malvasia di Schierano and Malvasia nera lunga, the selection of 1100 kg m^{-3} density permitted to separate representative berry groups richer in aroma precursors of great relevance for the wine quality (density ≥ 1100 kg m^{-3}, berry distribution by weight ≥ 43%) but would penalize hardly free terpenes composition.

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FIGURE CAPTIONS

Figure 1. Berry weight distribution among density classes obtained for the four varieties analysed: (a) Moscato giallo, (b) Malvasia di Schierano, (c) Malvasia nera lunga, (d) Brachetto. Curve points with values in black colour were samples above 3 % by total weight and hence considered for the volatile composition study.

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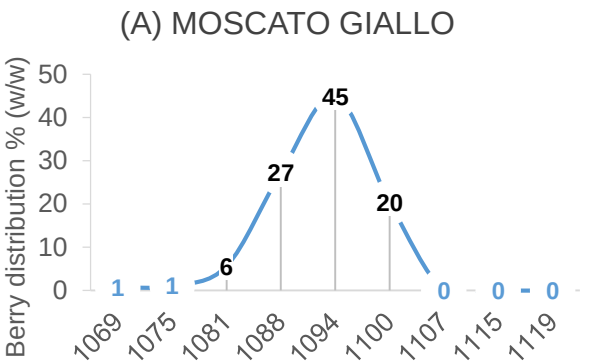
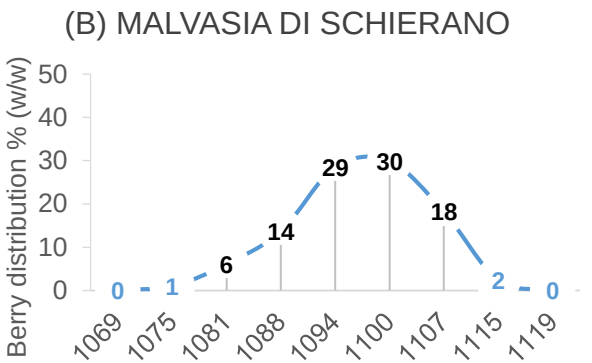
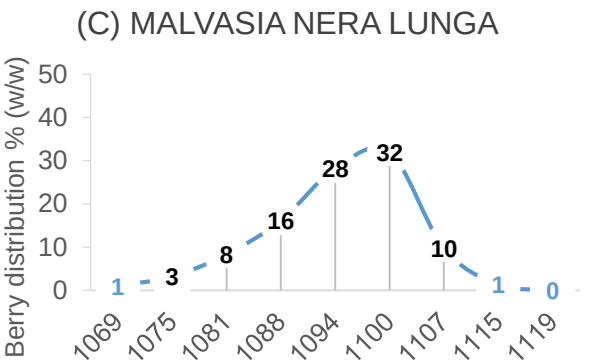

(A) MOSCATO GIALLO
Berry distribution % (w/w)
50
40
30
20
10
0
1
1
6
27
45
20
0
0
0
1069
1075
1081
1088
1094
1100
1107
1115
1119


(B) MALVASIA DI SCHIERANO
Berry distribution % (w/w)
50
40
30
20
10
0
0
1
6
14
29
30
18
2
0
1069
1075
1081
1088
1094
1100
1107
1115
1119


(C) MALVASIA NERA LUNGA
Berry distribution % (w/w)
50
40
30
20
10
0
1
3
8
16
28
32
10
1
0
1069
1075
1081
1088
1094
1100
1107
1115
1119

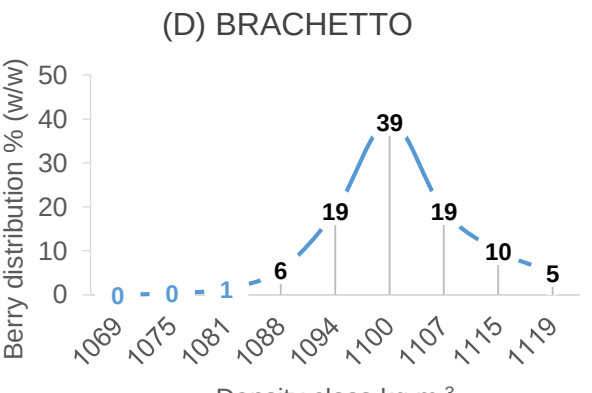

(D) BRACHETTO
Berry distribution % (w/w)
50
40
30
20
10
0
0
0
1
6
19
39
19
10
5
1069
1075
1081
1088
1094
1100
1107
1115
1119
Density class kg m-3

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Table 1. Non-volatile physicochemical parameters of the juices derived from berries sorted by density class.

Cultivar (harvest date)	Density class kg m^{-3}	Berry weight g	Reducing sugars g L^{-1}	Glucose /Fructose ratio	Citric acid g L^{-1}	Tartaric acid g L^{-1}	Malic acid g L^{-1}	pH	Titratable acidity g L^{-1} as tartaric acid
Moscato giallo (August 31st, 2012)	1081	2.28	195.00 d	0.97	0.31 a	4.28	2.37 a	3.55 c	4.31 a
	1088	2.44	208.24 c	0.97	0.30 a	4.36	2.13 a	3.59 c	4.20 b
	1094	2.49	223.03 b	0.96	0.29 a	4.17	1.90 b	3.69 b	3.77 c
	1100	2.07	239.86 a	0.95	0.26 b	4.24	1.56 c	3.76 a	3.45 d
	Sign.	ns	***	ns		ns	***	***	***
Malvasia di Schierano (September 21st, 2012)	1081	2.93	196.45 c	0.89 d	0.13	6.03	1.08	3.23 c	5.70
	1088	2.89	206.35 c	0.90 c	0.12	5.87	1.13	3.26 bc	6.15
	1094	2.90	220.20 b	0.92 b	0.12	5.91	1.18	3.29 bc	5.81
	1100	2.85	234.77 a	0.92 b	0.12	6.27	1.14	3.32 ab	6.04
	1107	2.75	243.66 a	0.93 a	0.11	6.23	1.06	3.36 a	5.91
	Sign.	ns	***	***	ns	ns	ns	***	ns
Malvasia nera lunga (September	1075	2.44 c	180.97 d	0.88 b	0.20	4.64 ab	1.26	3.44 d	3.86
	1	2	19	0.92 ab		4	1	3	3.

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13th, 2012)	081	.66 b	6.87 cd		.20	.91 a	.26	.48 cd	98	
	088	1.91 a	218.26 bc	0.93 a	.19	4.03 c	1.21	3.51 c	34	3.
	094	1.01 a	230.91 ab	0.93 a	.20	4.26 bc	1.12	3.52 c	43	3.
	100	1.66 b	240.84 a	0.94 a	.17	4.31 bc	1.08	3.59 b	23	3.
	107	1.28 c	248.41 a	0.94 a	.19	4.70 ab	0.96	3.65 a	23	3.
	Sign.	**	**	*	ns	*	ns	*		ns
Brachetto (August 29th, 2012)	088	1.87 ab	213.89 c	0.98	.30	6.67	2.75 a	3.35 e	60 ab	6.
	094	1.04 a	222.34 c	0.98	.29	6.75	2.54 ab	3.37 de	32 bc	6.
	100	1.91 ab	229.96 bc	0.98	.29	6.75	2.40 b	3.44 cd	04 cd	6.
	107	1.86 ab	247.58 abc	0.98	.31	6.96	2.61 ab	3.48 bc	93 d	5.
	115	1.74 b	257.91 ab	0.98	.33	6.64	2.42 b	3.51 ab	74 d	5.
	119	1.42 c	268.59 a	0.98	.33	6.27	2.37 b	3.57 a	36 e	5.
	Sign.	*	*	ns	ns	ns	*	*	*	**

All data are expressed as average value ± standard deviation (n=3). Sign.: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. Different Latin letters within the same column and cultivar indicate significant differences among density classes according to Tukey HSD test ($p< 0.05$).

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Table 2. Free and glycosylated terpene composition (μg L^{-1} juice) of the berries sorted by flotation for Moscato giallo cultivar.

Compound	Density class				
	1081 kg m^{-3}	**1088 kg m^{-3}**	**1094 kg m^{-3}**	**1100 kg m^{-3}**	**ign.**
Moscato giallo – Free terpenes					
β-Ocimene	3.86 ± 0.80 c	19.94 ± 5.67 ab	22.30 ± 1.76 a	9.54 ± 1.58 bc	
cis-Furan linalool oxide	1.67 ± 0.15	8.53 ± 5.58	13.50 ± 0.25	6.92 ± 0.89	s
trans-Furan linalool oxide	0.73 ± 0.21	5.60 ± 4.06	8.28 ± 0.40	3.70 ± 1.28	s
Linalool	592.66 ± 64.39 b	1662.01 ± 196.29 a	1805.78 ± 26.40 a	663.22 ± 54.24 b	**
HO-trienol	0.12 ± 0.14 c	1.89 ± 0.46 b	4.61 ± 0.29 a	0.68 ± 0.14 c	**
α-Terpineol	0.48 ± 0.01	2.54 ± 1.06	3.49 ± 0.75	2.59 ± 0.59	s
Geranial	0.18 ± 0.22 b	3.06 ± 1.72 ab	6.02 ± 0.32 a	2.06 ± 0.05 b	
trans-Pyran linalool oxide	2.03 ± 0.67 c	5.86 ± 1.06 bc	12.41 ± 0.21 a	7.49 ± 1.87 b	*
cis-Pyran linalool oxide	4.24 ± 0.88 b	15.65 ± 1.76 a	16.49 ± 1.26 a	11.96 ± 3.16 ab	
Citronellol	0.04 ± 0.01	0.59 ± 0.82	0.77 ± 0.04	0.15 ± 0.20	s
Nerol	9.11 ± 1.17 c	25.82 ± 7.50 ab	31.86 ± 1.14 a	12.92 ± 2.14 bc	
Geraniol	24.41 ± 1.51 b	85.83 ± 15.23 a	81.81 ± 1.01 a	56.69 ± 12.74 ab	
trans-Rose oxide	0.50 ± 0.05 c	2.55 ± 0.60 ab	3.26 ± 0.19 a	1.58 ± 0.14 bc	*
cis-Rose oxide	0.14 ± 0.01 c	0.71 ± 0.14 ab	0.92 ± 0.01 a	0.49 ± 0.05 b	*
Geranic acid	nd	nd	nd	nd	
Free terpenes sum	**640.16 ± 68.42 b**	**1840.58 ± 224.83 a**	**2011.50 ± 23.77 a**	**780.00 ± 72.24 b**	**
Moscato giallo – Glycosylated terpenes					
β-Ocimene	27.01 ± 2.50	28.57 ± 0.26	23.73 ± 8.40	23.54 ± 2.88	s
cis-Furan linalool oxide	11.61 ± 1.57	14.52 ± 2.35	16.32 ± 0.54	16.18 ± 1.46	s
trans-Furan	0.38 ±	0.45 ±	0.48 ±	0.26 ±	

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linalool oxide	0.17	0.25	0.17	0.08	s
Linalool	812.03 ± 83.35	792.67 ± 29.07	883.90 ± 88.07	753.93 ± 1.54	s
HO-trienol	13.33 ± 8.48	13.58 ± 3.67	8.59 ± 3.35	5.57 ± 1.76	s
α-Terpineol	16.94 ± 1.81	15.88 ± 2.01	14.63 ± 0.14	9.27 ± 3.27	s
Geranial	25.21 ± 14.92	37.55 ± 1.28	38.17 ± 0.03	35.27 ± 3.52	s
trans-Pyran linalool oxide	1.64 ± 0.06	1.82 ± 0.60	1.04 ± 0.60	1.43 ± 0.57	s
cis-Pyran linalool oxide	0.89 ± 0.05	1.55 ± 0.48	2.50 ± 0.21	1.58 ± 0.52	s
Citronellol	24.26 ± 1.67	24.14 ± 1.35	24.99 ± 2.22	23.93 ± 3.81	s
Nerol	664.13 ± 44.65	675.35 ± 53.51	600.81 ± 52.41	490.87 ± 51.94	s
Geraniol	1210.31 ± 59.62	1275.77 ± 103.52	1320.49 ± 124.08	1221.38 ± 141.00	s
trans-Rose oxide	5.58 ± 1.22	6.82 ± 3.57	7.35 ± 1.44	9.24 ± 0.09	s
cis-Rose oxide	1.90 ± 0.27	1.57 ± 0.04	3.77 ± 0.69	2.34 ± 1.31	s
Geranic acid	27.85 ± 3.39	34.49 ± 7.78	33.02 ± 5.94	45.64 ± 6.03	s
Glycosylated terpenes sum	**2843.05 ± 196.19**	**2924.73 ± 199.33**	**2979.79 ± 74.90**	**2640.44 ± 197.05**	**s**

All data are expressed as average value ± standard deviation (n=3). Sign.: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. Different Latin letters within the same row indicate significant differences among density classes according to Tukey HSD test ($p< 0.05$). nd: not detected.

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Table 3. Free and glycosylated terpene composition (µg L^{-1} juice) of the berries sorted by flotation for Malvasia di Schierano cultivar.

Compound	Density class					
	1081 kg m^{-3}	**1088 kg m^{-3}**	**1094 kg m^{-3}**	**1100 kg m^{-3}**	**1107 kg m^{-3}**	**ign.**
Malvasia di Schierano – Free terpenes						
β-Ocimene	3.03 ± 1.60	2.16 ± 0.24	2.87 ± 1.15	3.61 ± 3.19	0.26 ± 0.14	s
cis-Furan linalool oxide	0.18 ± 0.08	0.11 ± 0.05	0.05 ± 0.06	0.22 ± 0.16	0.01 ± 0.00	s
trans-Furan linalool oxide	0.04 ± 0.01	0.04 ± 0.01	0.07 ± 0.03	0.09 ± 0.03	0.08 ± 0.07	s
Linalool	120.15 ± 8.45 ab	96.92 ± 7.00 bc	84.93 ± 1.58 c	143.07 ± 12.71 a	139.68 ± 2.70 a	*
HO-trienol	0.49 ± 0.09 a	0.06 ± 0.05 b	0.05 ± 0.02 b	0.13 ± 0.13 b	0.12 ± 0.03 b	*
α-Terpineol	4.81 ± 2.35	1.63 ± 0.45	1.00 ± 0.77	0.67 ± 0.93	2.52 ± 0.60	s
Geranial	9.26± 0.52 a	4.43 ± 2.17 b	3.72 ± 0.53 b	4.13 ± 0.51 b	3.33 ± 1.18 b	
trans-Pyran linalool oxide	0.11 ± 0.05	0.16 ± 0.07	0.11 ± 0.06	0.17 ± 0.08	0.28 ± 0.13	s
cis-Pyran linalool oxide	4.44 ± 1.08	15.39 ± 6.26	12.61 ± 6.32	18.59 ± 0.72	14.72 ± 1.41	s
Citronellol	12.47 ± 4.56 a	3.27 ± 0.58 b	1.59 ± 0.39 b	0.67 ± 0.04 b	1.37 ± 0.42 b	
Nerol	87.76 ± 1.31 a	60.32 ± 4.16 b	62.91 ± 1.25 b	68.94 ± 0.90 b	62.57 ± 6.45 b	*
Geraniol	234.85 ± 3.07 a	162.95 ± 8.45 b	158.52 ± 9.58 b	174.97 ± 3.18 b	169.76 ± 15.35 b	*
trans-Rose oxide	2.82 ± 0.38 a	2.16 ± 0.42 ab	1.34 ± 0.01 b	1.77 ± 0.42 ab	1.47 ± 0.02 b	
cis-Rose oxide	0.94 ± 0.14 a	0.69 ± 0.09 ab	0.49 ± 0.02 b	0.61 ± 0.07 b	0.54 ± 0.04 b	
Geranic acid	37.00 ± 2.26 a	17.06 ± 7.59 b	2.21 ± 0.49 c	5.42 ± 1.05 bc	2.04 ± 0.52 c	**
Free terpenes sum	**518.34 ± 4.15 a**	**367.34 ± 37.36 bc**	**332.49 ± 8.37 c**	**423.05 ± 15.72 b**	**398.73 ± 23.90 bc**	*****
Malvasia di Schierano – Glycosylated terpenes						
β-Ocimene	25.98 ± 4.12	35.14 ± 0.24	37.99 ± 3.27	39.84 ± 6.96	39.36 ± 1.28	s
cis-Furan linalool oxide	5.49 ± 0.56 c	6.45 ± 0.28 c	8.07 ± 0.16 bc	11.45 ± 1.11 a	10.48 ± 0.69 ab	*

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trans-Furan linalool oxide	0.51 ± 0.40	0.29 ± 0.16	0.72 ± 0.01	1.96 ± 0.03	1.25 ± 0.89	s
Linalool	838.89 ± 84.74 b	873.61 ± 14.60 b	1146.27 ± 62.47 b	1554.29 ± 111.95 a	1635.37 ± 128.80 a	**
HO-trienol	1.31 ± 0.29 b	1.75 ± 1.38 b	2.23 ± 0.38 ab	4.17 ± 0.76 ab	4.77 ± 0.13 a	
α-Terpineol	13.52 ± 1.38 b	16.18 ± 1.17 ab	19.80 ± 0.64 ab	26.53 ± 6.88 ab	29.38 ± 2.60 a	
Geranial	40.40 ± 0.60	42.00 ± 1.00	46.71 ± 4.92	45.41 ± 3.33	43.40 ± 3.23	s
trans-Pyran linalool oxide	0.82 ± 0.30	0.77 ± 0.28	0.80 ± 0.04	1.68 ± 0.97	2.02 ± 0.25	s
cis-Pyran linalool oxide	0.24 ± 0.18 b	0.45 ± 0.32 ab	0.69 ± 0.12 ab	1.40 ± 0.24 a	0.62 ± 0.33 ab	
Citronellol	22.22 ± 2.32	25.38 ± 1.21	25.47 ± 2.34	25.94 ± 3.35	26.65 ± 3.11	s
Nerol	598.73 ± 68.07	766.20 ± 33.47	793.74 ± 60.21	764.62 ± 186.21	810.25 ± 51.09	s
Geraniol	1183.42 ± 119.20	1626.59 ± 65.07	1768.38 ± 176.70	1710.18 ± 421.54	1777.44 ± 126.61	s
trans-Rose oxide	10.42 ± 2.16	10.39 ± 0.42	12.22 ± 1.24	11.76 ± 1.72	11.77 ± 0.92	s
cis-Rose oxide	3.46 ± 0.77	3.66 ± 0.16	4.24 ± 0.49	3.97 ± 0.45	4.00 ± 0.14	s
Geranic acid	7.44 ± 5.48	5.39 ± 0.51	4.33 ± 1.08	5.91 ± 0.83	6.44 ± 2.30	s
Glycosylated terpenes sum	**2752.86 ± 289.06**	**3414.25 ± 117.91**	**3871.67 ± 313.82**	**4209.13 ± 744.14**	**4403.20 ± 321.46**	**s**

All data are expressed as average value ± standard deviation (n=3). Sign.: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. Different Latin letters within the same row indicate significant differences among density classes according to Tukey HSD test ($p< 0.05$).

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Table 4. Free and glycosylated terpene composition (µg L^{-1} juice) of the berries sorted by flotation for Malvasia nera lunga cultivar.

Compound	Density class 1075 kg m^{-3}	1081 kg m^{-3}	1088 kg m^{-3}	1094 kg m^{-3}	1100 kg m^{-3}	1107 kg m^{-3}	sign.
Malvasia nera lunga – Free terpenes							
β-Ocimene	0.11 ± 0.05	0.30 ± 0.12	1.1 ± 1.50	2.48 ± 0.37	0.61 ± 0.85	0.61 ± 0.06	s
cis-Furan linalool oxide	0.02 ± 0.01 b	0.07 ± 0.04 b	0.13 ± 0.03 b	0.07 ± 0.01 b	0.33 ± 0.07 a	0.38 ± 0.07 a	*
trans-Furan linalool oxide	0.04 ± 0.03 b	0.11 ± 0.08 b	0.13 ± 0.02 b	0.28 ± 0.23 ab	0.38 ± 0.01 ab	0.67 ± 0.03 a	*
Linalool	11.18 ± 1.34 b	16.05 ± 8.41 b	33.42 ± 1.84 b	73.53 ± 2.19 a	33.09 ± 11.47 b	17.08 ± 0.14 b	**
HO-trienol	0.09 ± 0.01	0.15 ± 0.02	1.08 ± 0.14	1.55 ± 0.61	0.82 ± 0.26	0.62 ± 0.87	s
α-Terpineol	0.10 ± 0.01	0.32 ± 0.10	0.24 ± 0.00	0.82 ± 0.41	0.24 ± 0.05	0.22 ± 0.10	s
Geranial	0.90 ± 0.08 a	2.36 ± 0.44 a	1.30 ± 0.42 a	4.35 ± 1.15 a	3.17 ± 1.59 a	5.41 ± 1.96 a	
trans-Pyran linalool oxide	0.03 ± 0.01	0.07 ± 0.01	0.08 ± 0.04	0.08 ± 0.10	0.18 ± 0.07	0.09 ± 0.07	s
cis-Pyran linalool oxide	3.50 ± 1.15 c	5.17 ± 0.30 bc	7.30 ± 0.39 abc	10.55 ± 1.00 a	8.41 ± 2.47 ab	2.76 ± 0.18 c	*
Citronellol	8.37 ± 1.28 b	9.39 ± 0.58 b	18.58 ± 1.07 b	37.43 ± 0.05 a	18.60 ± 7.38 b	19.67 ± 1.52 b	**
Nerol	10.88 ± 4.03 c	44.11 ± 3.53 b	43.02 ± 2.88 b	101.36 ± 1.01 a	60.14 ± 14.97 b	60.09 ± 0.52 b	**
Geraniol	25.87 ± 15.80 c	104.59 ± 5.66 b	87.79 ± 6.74 b	218.12 ± 1.36 a	87.31 ± 21.80 b	92.68 ± 0.90 b	**
trans-Rose oxide	3.02 ± 0.55	8.98 ± 0.80	8.52 ± 3.46	13.82 ± 2.88	16.35 ± 14.90	9.71 ± 4.28	s
cis-Rose oxide	0.69 ± 0.38 b	1.56 ± 0.84 b	1.69 ± 0.19 b	6.47 ± 0.60 a	3.43 ± 0.22 ab	2.26 ± 1.54 b	*
Geranic acid	nd	nd	nd	nd	nd	nd	
Free terpenes sum	**64.81 ± 14.10 c**	**193.24 ± 0.15 b**	**204.37 ± 17.15 b**	**470.92 ± 0.83 a**	**233.09 ± 70.65 b**	**212.27 ± 2.66 b**	**
Malvasia nera lunga – Glycosylated terpenes							

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β-Ocimene	15.67 ± 1.12 d	16.42 ± 0.45 cd	20.99 ± 0.73 bc	20.07 ± 1.09 bcd	22.23 ± 0.17 b	47.17 ± 2.53 a	**
cis-Furan linalooloxide	0.55 ± 0.24	3.42 ± 1.61	1.92 ± 0.15	4.13 ± 1.48	5.20 ± 0.59	4.86 ± 2.45	s
trans-Furan linalool oxide	2.52 ± 0.60 c	8.60 ± 0.49 ab	5.00 ± 2.64 bc	7.76 ± 0.08 ab	7.47 ± 0.62 ab	9.71 ± 0.03 a	*
Linalool	46.45 ± 3.47 c	127.77 ± 4.12 b	124.55 ± 1.66 b	152.65 ± 10.55 a	161.55 ± 5.32 a	158.21 ± 2.18 a	**
HO-trienol	4.70 ± 1.03 ab	5.66 ± 1.47 ab	3.50 ± 0.06 b	5.24 ± 2.96 ab	4.53 ± 0.61 ab	10.80 ± 1.84 a	
α-Terpineol	7.27 ± 0.67 b	15.41 ± 0.18 b	13.82 ± 0.97 b	14.26 ± 0.29 b	11.20 ± 5.26 b	27.42 ± 3.14 a	*
Geranial	14.87 ± 0.74 c	27.41 ± 1.36 b	26.91 ± 0.25 b	26.76 ± 0.37 b	27.82 ± 2.72 b	38.07 ± 2.40 a	**
trans-Pyran linalool oxide	0.48 ± 0.30	1.72 ± 0.88	1.48 ± 1.06	1.31 ± 0.18	1.96 ± 1.15	3.10 ± 0.66	s
cis-Pyran linalool oxide	0.70 ± 0.23	0.08 ± 0.01	0.43 ± 0.24	0.47 ± 0.19	0.78 ± 0.40	0.45 ± 0.13	s
Citronellol	66.61 ± 4.85 b	158.42 ± 11.83 a	160.43 ± 1.99 a	148.24 ± 4.65 a	154.09 ± 3.99 a	171.08 ± 3.04 a	**
Nerol	488.86 ± 34.56 d	1171.53 ± 54.18 c	1224.88 ± 43.69 bc	1288.37 ± 7.96 bc	1386.20 ± 76.28 b	1826.25 ± 15.33 a	**
Geraniol	329.78 ± 23.48 d	686.24 ± 32.86 bc	646.62 ± 39.03 c	709.38 ± 13.08 bc	751.86 ± 14.90 b	955.05 ± 0.02 a	**
trans-Rose oxide	71.65 ± 1.33 c	112.35 ± 2.17 ab	119.52 ± 10.43 ab	95.04 ± 13.79 bc	117.61 ± 5.14 ab	137.51 ± 9.79 a	*
cis-Rose oxide	24.06 ± 1.41 b	35.83 ± 8.69 ab	41.73 ± 3.95 ab	32.83 ± 5.26 ab	39.66 ± 3.49 ab	46.00 ± 3.80 a	
Geranic acid	3.60 ± 0.61 b	23.83 ± 2.67 a	23.41 ± 2.69 a	23.05 ± 6.09 a	27.52 ± 0.15 a	32.32 ± 8.19 a	*
Glycosylated terpenes sum	**1077.75 ± 67.98 d**	**2394.69 ± 94.36 c**	**2415.20 ± 75.37 c**	**2529.57 ± 33.28 bc**	**2719.70 ± 102.44 b**	**3468.01 ± 39.23 a**	****

All data are expressed as average value ± standard deviation (n=3). Sign.: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. Different Latin letters within the same row indicate significant differences among density classes according to Tukey HSD test ($p< 0.05$). nd: not detected.

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Table 5. Free and glycosylated terpene composition (μg L^{-1} juice) of the berries sorted by flotation for Brachetto cultivar.

Compound	Density class						
	1088 kg m^{-3}	**1094 kg m^{-3}**	**1100 kg m^{-3}**	**1107 kg m^{-3}**	**1115 kg m^{-3}**	**1119 kg m^{-3}**	**ign.**
Brachetto – Free terpenes							
β-Ocimene	0.35 ± 0.12	2.64 ± 1.28	0.91 ± 1.25	0.31 ± 0.04	1.49 ± 0.76	0.46 ± 0.15	s
cis-Furan linalool oxide	0.01 ± 0.01 c	0.06 ± 0.01 a	0.09 ± 0.01 a	0.02 ± 0.01 b	0.06 ± 0.00 a	0.02 ± 0.01 b	**
trans-Furan linalool oxide	0.03 ± 0.02	0.06 ± 0.02	0.09 ± 0.08	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	s
Linalool	0.56 ± 0.75 c	4.81 ± 1.17 b	11.55 ± 0.54 a	5.15 ± 1.72 b	3.75 ± 0.23 bc	1.93 ± 0.87 bc	**
HO-trienol	0.05 ± 0.01 c	0.18 ± 0.23 bc	0.96 ± 0.14 ab	1.12 ± 0.18 a	0.72 ± 0.42 abc	0.06 ± 0.01 c	*
α-Terpineol	0.06 ± 0.01	0.10 ± 0.12	0.22 ± 0.27	0.30 ± 0.08	0.05 ± 0.05	0.09 ± 0.04	s
Geranial	0.62 ± 0.39	3.17 ± 0.74	5.39 ± 2.19	7.52 ± 1.78	5.09 ± 3.42	3.22 ± 1.08	s
trans-Pyran linalool oxide	0.05 ± 0.04	0.18 ± 0.11	0.10 ± 0.05	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.03	s
cis-Pyran linalool oxide	2.50 ± 1.14	9.59 ± 6.24	10.58 ± 0.53	16.75 ± 3.51	13.33 ± 0.23	10.69 ± 4.21	s
Citronellol	1.86 ± 0.59 c	7.52 ± 1.28 bc	25.04 ± 1.25 abc	36.38 ± 4.05 a	34.28 ± 14.47 ab	23.12 ± 8.31 abc	
Nerol	11.34 ± 4.84 d	49.90 ± 5.14 b	71.08 ± 4.61 a	68.62 ± 3.37 a	54.74 ± 3.37 ab	31.00 ± 5.66 c	**
Geraniol	65.97 ± 8.68 c	205.37 ± 19.63 ab	281.11 ± 21.31 a	231.97 ± 40.14 ab	179.19 ± 16.19 b	92.50 ± 4.95 c	**
trans-Rose oxide	1.31 ± 1.45	5.43 ± 0.67	7.61 ± 2.10	6.09 ± 1.57	4.39 ± 4.09	0.53 ± 0.24	s
cis-Rose oxide	0.26 ± 0.05	1.26 ± 0.98	1.23 ± 0.18	0.99 ± 0.05	1.48 ± 0.09	1.35 ± 0.92	s
Geranic acid	8.06 ± 4.89 b	36.58 ± 12.73 ab	53.11 ± 20.30 ab	64.04 ± 10.93 a	51.74 ± 13.45 ab	36.00 ± 5.66 ab	
Free terpenes sum	**93.02 ± 11.75 c**	**326.85 ± 45.14 ab**	**469.07 ± 43.62 a**	**439.34 ± 41.77 a**	**350.37 ± 41.26 a**	**201.02 ± 16.46 bc**	**
Brachetto – Glycosylated terpenes							

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β-Ocimene	28.25 ± 2.35	31.30 ± 0.31	36.34 ± 5.08	20.67 ± 0.94	22.11 ± 10.60	14.36 ± 6.90	s
cis-Furan linalool oxide	1.53 ± 0.20	2.95 ± 1.64	2.39 ± 1.65	4.50 ± 0.07	4.59 ± 1.74	3.77 ± 0.20	s
trans-Furan linalool oxide	0.15 ± 0.11	0.50 ± 0.01	0.22 ± 0.10	0.73 ± 0.52	0.71 ± 0.54	0.30 ± 0.18	s
Linalool	59.91 ± 13.89	55.46 ± 3.58	54.40 ± 10.22	55.77 ± 0.12	70.15 ± 12.78	46.28 ± 0.14	s
HO-trienol	3.65 ± 0.98 a	3.77 ± 0.37 a	1.92 ± 0.77 ab	1.06 ± 0.25 b	1.30 ± 0.34 b	0.39 ± 0.41 b	*
α-Terpineol	14.15 ± 2.99	15.44 ± 1.41	15.61 ± 2.59	13.36 ± 0.28	16.50 ± 11.86	12.71 ± 0.05	s
Geranial	23.68 ± 14.95	24.89 ± 11.11	32.72 ± 2.86	32.91 ± 2.52	32.55 ± 1.96	26.76 ± 0.29	s
trans-Pyran linalool oxide	0.02 ± 0.01 c	0.03 ± 0.01 c	0.73 ± 0.36 bc	2.45 ± 0.01 a	1.06 ± 0.17 b	0.93 ± 0.23 b	**
cis-Pyran linalool oxide	0.35 ± 0.06	0.47 ± 0.38	0.42 ± 0.19	0.89 ± 0.15	0.67 ± 0.53	0.30 ± 0.22	s
Citronellol	63.06 ± 3.58	70.28 ± 2.72	73.67 ± 5.49	74.04 ± 3.70	64.80 ± 3.86	68.56 ± 1.27	s
Nerol	627.14 ± 38.80	708.46 ± 44.89	720.34 ± 104.35	814.98 ± 14.50	819.68 ± 102.47	781.15 ± 10.43	s
Geraniol	1056.12 ± 70.08	1146.92 ± 64.77	1122.38 ± 158.79	1113.64 ± 12.35	1089.44 ± 152.23	909.19 ± 21.04	s
trans-Rose oxide	29.09 ± 0.29	34.74 ± 2.60	35.38 ± 5.65	40.53 ± 1.50	41.28 ± 11.12	31.78 ± 2.58	s
cis-Rose oxide	11.63 ± 1.76	9.21 ± 4.57	8.39 ± 2.51	13.92 ± 0.63	10.51 ± 6.84	10.72 ± 0.75	s
Geranic acid	24.07 ± 6.58 b	37.06 ± 0.33 ab	37.13 ± 2.84 ab	53.45 ± 1.52 a	45.00 ± 9.20 ab	21.86 ± 9.32 b	
Glycosylated terpenes sum	**1942.81 ± 151.80**	**2141.49 ± 115.10**	**2142.04 ± 285.52**	**2242.89 ± 35.01**	**2220.34 ± 267.39**	**1929.08 ± 44.95**	**s**

All data are expressed as average value ± standard deviation (n=3). Sign.: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. Different Latin letters within the same row indicate significant differences among density classes according to Tukey HSD test ($p< 0.05$).

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Highlights

- Four grape varieties were analyzed for their volatile profile after density sorting
- Changes in free volatile compounds were variety-dependent, with few common trends
- Little differences in total glycosylated content among berries sorted by flotation
- Limited effectiveness of density sorting to obtain different aroma profiles

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