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Variety effect of distinct enzyme treatments during prefermentative maceration of white winegrapes on volatile organic compounds and chromatic traits

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ABSTRACT

The impact of prefermentative addition of five single-enzyme activities (pectin lyase, polygalacturonase, pectin methyl esterase, xylanase, and arabinase) on the phenolic content, colour characteristics, and volatile composition of grape musts resulting from pellicular maceration of Chardonnay, Arneis, Greco, and Falanghina white varieties was studied with the aim of enhancing aroma composition without negatively affecting colour perception. In addition, standard physicochemical parameters were determined. Pectin lyase, polygalacturonase, and arabinase enzymes reduced the must browning, when compared with untreated control, as reported by lower total polyphenol index, absorbance at 420 nm, and a* colour coordinate for all varieties tested. Regarding the volatile composition determined by solid-phase extraction and GC-MS analysis, variety effect was observed for the enzyme treatments studied. The concentration of free vanillin decreased significantly in these last three varieties using polygalacturonase and arabinase treatments whereas 4-vinylguaiacol increased in pectin lyase-treated samples. Free terpene compounds, such as furan linalool oxide, increased in Chardonnay and Falanghina varieties using polygalacturonase, xylanase, and arabinase for the second variety whereas pectin lyase for the first one. Contrarily, glycosylated terpenes, such us (*Z*)-8-hydroxylinalool, decreased when using pectin lyase and xylanase for Arneis; however, it also occurred for Falanghina using polygalacturonase treatment. The xylanase activity influenced mostly the volatile composition of the Arneis variety whereas arabinase did for Greco and Falanghina. The differences between enzyme-treated grape musts and control samples were less evident on the Chardonnay variety. Therefore, enzyme activity can affect the volatile composition of grape must differently depending on the target variety.

1. Introduction

The consumer preference for white wines is strongly related to the aroma profile and colour features as they influence the overall acceptability (Gómez-Míguez et al., 2007). In fact, volatile organic compounds (VOCs) play an important role in high-quality white wines. Increasingly competitive markets are currently demanding wines characterised by distinctive variety sensory traits. This encourages producers to exploit the varietal VOCs of each winegrape cultivar, including aroma precursors. In aroma-neutral varieties, varietal-free VOCs are present often below the olfactive threshold, but they can also be released during winemaking from aroma precursors (odourless glycosylated compounds), mostly present in the berry skin (González-Barreiro et al., 2015; Ribéreau-Gayon et al., 2021). In white wine production, enological strategies have been investigated to facilitate the extraction of VOCs into the grape must, enhancing the varietal character, particularly in non-aromatic winegrape varieties.

Cold prefermentative maceration is a widely studied winemaking technique, which is used to increase the release of secondary metabolites from the berry skin to the grape must at low temperatures (De Santis & Frangipane, 2010; Esti & Tamborra, 2006; Selli et al., 2006; Álvarez et al., 2006). For white winegrape varieties, the contact time of the skins

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with the juice is generally reduced (not exceeding 24 h) aiming to promote the extraction of varietal-free VOCs and aroma precursors in the alcohol-free medium and, at the same time, to prevent an excessive release of phenolic compounds that are easily oxidisable and contribute strongly to colour, bitterness, and astringency sensations (Aleixandre-Tudo et al., 2015; Bestulić et al., 2022; Petrozziello et al., 2011). However, the effect of this technique on VOCs varies according to the treatment conditions and variety treated, reporting inconsistent results (Aleixandre-Tudo et al., 2015; Olejar et al., 2016; Peinado et al., 2004; Piñeiro et al., 2006; Rodriguez-Bencomo et al., 2008; Roldán et al., 2021). The rate and extent of extraction is related to the chemical nature and water solubility of the compound, its concentration and localisation in the berry, and the maceration conditions such as skin contact temperature and time (Radeka et al., 2008).

Another technological approach to promote the release of secondary metabolites from the berry skins in contact with the grape must is the prefermentative addition of enzyme preparations. Their enological use is regulated by the International Organisation of Vine and Wine (OIV). In particular, the use of skin maceration enzymes in order to release the compounds from grape solid matter is regulated by OENO 13/04 resolution and OENO 498-2013 revision (OIV, 2013). Enzymes act on the external cellular components (medium lamella and primary cell wall). They are not able to deconstruct the cuticle but can penetrate from the pulp to the epidermal cells after grape crushing (Gao et al., 2019). In particular, pectinases are the most used enzymes in winemaking. Particularly, pectin lyase acts directly on the bonds between galacturonic acid units of the pectic component of the skin cell wall, while polygalacturonase catalyses the hydrolytic cleavage of the α-1,4 linkages of pectic acid after removal of methyl groups from pectin by pectin methyl esterase (Romero-Cascales et al., 2008). In addition, non-pectolytic enzymes contribute to the cell wall disruption. The degradation of cellulose is catalysed by cellulases, including glucanase that releases glucose oligomers with β-1,4 bond (cellulose) and glucosidase that cleaves the β-D-glucoside linkage, liberating D-glucose. Arabinase breaks down the bonds between the arabinogalactan molecules, forming the branched chain of rhamnogalacturonan type I (RG-I). Instead, xylanase acts on the xylose chains that are fundamental for the stabilisation of cellulose fibrils. Finally, protease catalyses the degradation of arabinogalactan proteins (AGP) consisting of up to 25% of the grape cell wall (Hanlin et al., 2010).

Several studies showed that the use of maceration enzymes increases the concentration of VOCs in the wine (Armada et al., 2010). Nevertheless, the efficiency of maceration enzymes has been scarcely studied for white wines. Pectolytic enzymes were used after grape crushing in combination with cold prefermentative maceration of Albariño variety at 8−10 °C for 6 h (Armada et al., 2010), Pinot blanc at 4 °C for 24 h (de Matos et al., 2020), whereas pectinases with β-glucosidase activities were used during skin contact for Dimyat, Vrachanski muscat, Aligote, Muscat Ottonel, and Plevenska rosa during 12 h (Dimitrov et al., 2017), and for Viorica during 8 h at 15 °C (Vladei, 2020). Most of these studies were focused on a single variety and enzyme preparation.

Therefore, the purpose of this study was to evaluate the effect of five different enzyme preparations, each one with a single activity, on four white winegrape varieties during cold prefermentative maceration. Specifically, this study aims to determine: i) the effectiveness of exogenous pectolytic and non-pectolytic enzymes, tested singularly at the same dosage, for the release of free and glycosylated volatile compounds from the berry skin into the grape must; ii) the impact on the extraction of total phenolic compounds and the colour characteristics of the musts obtained; and iii) the possible variety effect on the efficiency of each enzyme activity. To our knowledge, the simultaneous comparison of several single enzyme activities applied on different white winegrape varieties, regarding their influence on phenolic content, colour characteristics, and volatile composition, has not been reported up to date.

2. Materials and methods

2.1. Grape samples

Four different white winegrape *Vitis vinifera* L. cultivars were studied: Chardonnay (Trento, Trento province, north-east Italy), Arneis (Alba, Cuneo province, north-west Italy), Greco (Tufo, Avellino province, south Italy), and Falanghina (Benevento, Benevento province, south Italy). Each grape cultivar was treated separately. For each grape cultivar, 15 kg of grape bunches were harvested at ripeness and transported to the laboratory, where they were manually destemmed. Three replicates of 100 grape berries were randomly selected and used for determining the technological ripeness parameters (average reducing sugars concentration of 200, 233, 211 and 229 g/L for Chardonnay, Arneis, Greco, and Falanghina, respectively; average total acidity values of 6.98, 5.28, 8.96 and 6.08 g/L as tartaric acid for Chardonnay, Arneis, Greco, and Falanghina, respectively). For each grape cultivar, the remaining grape berries were randomly distributed in 18 independent replicates of 500 g each, accurately weighed.

2.2. Cold prefermentative maceration

Five single-enzyme activities were tested during pellicular maceration: Pectin lyase (*Aspergillus niger*, EC 232-894-5), polygalacturonase (*Aspergillus niger*, EC 232-885-6), pectin methyl esterase (*Aspergillus niger*, EC 232-807-0), xylanase (*Aspergillus niger*, EC 232-800-2), and arabinase (*Aspergillus niger*, EC 253-463-8). All enzyme preparations tested were supplied by AEB S.p.A. (Brescia, Italy). These are not commercially available as single-activity formulations, but they are food-grade and suitable for oenological use. For each grape variety and single enzyme activity tested, three berry replicates were treated. Each replicate was added with 10 mg/kg potassium metabisulphite and manually crushed for 40 s. For each treatment replicate (CT: control without enzymes; PL: pectin lyase; PG: polygalacturonase; PE: pectin methyl esterase; XY: xylanase; AR: arabinase), the respective enzyme preparation was first dissolved in deionised water and then added at the enzyme dosage of 10 mg/kg (0.5 mL volume increase) according to the manufacturer's guidelines for the proposed oenological objective. For control samples, the same volume (0.5 mL) of deionised water was added instead of the enzyme solution. All samples were subsequently homogenised for 20 s. Prefermentative skin contact was carried out for 13 h at 12 °C. Then, grape berries were manually pressed for 60 s, the must was separated from the solid fraction by decantation, and total must obtained was accurately weighed. Must yield was calculated and expressed as percentage with respect to the corresponding initial weight of grape berries (% w/w). An aliquot of grape must was analyzed for standard physicochemical parameters, chromatic parameters, and total phenolic compounds. The remaining grape must was frozen at −20 °C for further analysis of free and glycosylated VOCs.

2.3. Standard physicochemical parameters

After prefermentative maceration, the grape must obtained by manual pressing of crushed grape berries was centrifuged at 3000×*g* for 15 min at 20 °C using a Heittich 32R centrifuge (Tuttlingen, Germany). In the resulting supernatant, reducing sugars (sum of glucose and fructose, g/L) and organic acids (tartaric and malic acids, g/L) were quantified using a high-performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA, USA) equipped with a refractive index detector and a UV detector set to 210 nm, respectively (Giordano et al., 2009). Total acidity (g/L as tartaric acid) and pH were determined according to OIV-MA-AS313-01 and OIV-MA-AS313-15 methods (OIV, 2016) using an InoLab 730 pH meter (WTW, Weilheim, Germany).

2.4. Phenolic compounds and chromatic characteristics

At the end of cold prefermentative maceration, the grape musts were centrifuged at 3000×*g* for 15 min at 20 °C and spectrophotometric analyses were carried out using a UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) using 10 mm-optical path cuvettes. Total polyphenol index (TPI) was determined by measuring absorbance at 280 nm of the grape must diluted 20 times with ultrapure water. Visible spectra (380–780 nm) were acquired on the undiluted samples. Absorbance at 420 nm ($A_{420\ nm}$) and CIELab coordinates (L*, lightness; a*, red-green colour component; b*, blue-yellow colour component) were calculated following OIV-MA-AS2-11 method (OIV, 2016). Furthermore, the ΔE* parameter, defined as colour difference between the different grape musts obtained for each variety, was calculated from the L*, a* and b* values as follows: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

2.5. Free and glycosylated volatile compounds

After cold prefermentative maceration, the grape musts were analyzed following the method reported by Torchio et al. (2012) with some modifications. A sample volume of 100 mL, previously centrifuged at 3000×*g* for 15 min at 15 °C, was diluted three times with deionised water and spiked with 1-heptanol as an internal standard (600 μL of a 100 mg/L solution in 10% v/v ethanol). This sample solution was submitted to reverse-phase solid-phase extraction on a 5 g Sep-Pak C18 cartridge (Waters Corporation, Milford, MA, USA) previously activated with methanol and washed with deionised water. The free fraction of VOCs was eluted with 30 mL of dichloromethane, dried over anhydrous sodium sulphate, and evaporated to around 100 μL under a stream of nitrogen gas. Then, the glycosylated precursors were recovered with 25 mL of methanol, and the methanolic extract was evaporated to dryness using a vacuum rotavapor (Laborota 4010, Heidolph Instruments GmbH & Co.KG, Kelheim, Germany) set to 30 °C. The solid residue was resuspended in 10 mL of 0.2 mol/L citrate-phosphate buffer at pH 5 containing 0.1 g of polyvinylpolypyrrolidone (PVPP) and 50 mg of commercial enzyme preparation (Rapidase revelation aroma, DSM Food Specialties, Delft, The Netherlands) with glycosidase activity. The enzymatic hydrolysis occurred at 40 °C for 21 h. After adding 1-heptanol as internal standard (600 μL of 100 mg/L solution in 10% v/v ethanol), the glycosylated fraction was extracted on a 5 g Sep-Pak C18 cartridge following the method described above.

GC-MS analysis was carried out with an Agilent 7890A Series gas chromatograph equipped with a DB-WAX column (30 m × 0.25 mm i.d., 0.25 μm; Agilent Technologies, Santa Clara, CA, USA) and coupled to an Agilent 5975C mass selective detector (Agilent Technologies). A dichloromethane extract volume of 1 μL was injected in split mode (1:0.9) at 250 °C injection port temperature. The carrier gas used was helium at 1.1 mL/min flow rate. The temperature program during separation was the following: 45 °C for 2 min, then increase at a rate of 10 °C/min to 60 °C, at 2 °C/min to 160 °C, and at 3 °C/min to 230 °C for 17 min. The ion source temperature was 150 °C and the interface temperature was 230 °C. The detection was performed by electron impact mass spectrometry in SCAN mode, using an ionization energy of 70 eV. The acquisition range was *m/z* 35–350. The software used for data processing was Agilent MSD ChemStation. VOCs were identified based on the comparison of mass spectrum and retention time for each compound with the respective pure standard or with those reported in literature or NIST database (http://webbook.nist.gov/chemistry/). Regarding quantification, VOCs were determined (μg/L) using pure standards (Sigma-Aldrich, Milan, Italy) when applicable. Otherwise, semi-quantified results were reported as μg/L of the standard internal 1-heptanol, considering a response factor equal to 1.

2.6. Statistical analysis

Statistics software R (version 4.0.5, CRAN, R Foundation for Statistical Computing, Vienna, Austria) was used for data analysis. Significant differences among treatments were established by one-way analysis of variance (ANOVA) using Tukey-b test for $p < 0.05$. Volatile compounds data were standardized (*z*-scores) for each cultivar and compound form (free and glycosylated compound) and then subjected to visualisation approaches: heatmaps were created by using 'pheatmap' package, whereas for principal component analysis (PCA) 'FactoMineR', 'factoextra', 'ggpubr' and 'ggplot2' packages were used.

3. Results and discussion

3.1. Effect of enzyme treatment on grape must technological parameters

Most of the technological parameters changed significantly with the use of enzyme preparations for all varieties. From the data shown in Table 1, PL, PG, and AR enzyme treatments led to a significantly higher must yield (from +5.0% to +11.0%) than the control sample (CT) for Chardonnay, Arneis, and Falanghina varieties, whereas this increase was not able to significantly differentiate treatments for Greco variety. Other studies have reported an increase in the must yield during the pressing phase by using pectolytic enzymes (Rogerson et al., 2000; Ugliano, 2009). The concentration of juice reducing sugars was not affected by the enzymes activity. Instead, some changes were observed for the pH and total acidity values, as well as for tartaric acid concentration. The PL-treated samples showed a decreased pH value and an increased value of total acidity and tartaric acid when compared to the control sample, even though the differences were only significant for these three parameters on Arneis variety (−1.5%, +8.1%, and +9.2%, respectively), for total acidity on Falanghina (+4.5%), and for tartaric acid on Greco (+8.0%).

The technological parameters related to the acidity of grape must changed in the PL-treated samples probably as a result of the degradation of pulp and skin cell walls. Pectins are acidic polysaccharides with a high degree of methyl esterification, which are mainly composed of homogalacturonans in grape berry cell walls (accounting for 80%; Hanlin et al., 2010). It is well known that enzymes degrade cell wall polysaccharides through de-esterification and depolymerisation reactions (Nunan et al., 2001). PL acts by the hydrolytic cleavage of the α-1,4 linkages causing the direct pectin depolymerisation without altering the esterification level (Spagna et al., 1995; Wu et al., 2007). In fact, hydrolytic enzymes not only increase the juice yield, but also the galacturonic acid concentration and total acidity (Sharma et al., 2017). Nevertheless, the effect depends on several factors, including enzyme type and concentration, as well as pH, time, temperature, and grape variety. In addition, the enzyme treatment can facilitate the release of tartaric acid from flesh cell vacuoles, therefore increasing total acidity and decreasing the pH value (Hanlin et al., 2010).

3.2. Effect of enzyme treatment on total phenolic compounds and colour parameters

The use of the five single enzyme activities tested reduced the TPI value, expressed as absorbance at 280 nm, for all four varieties studied, with the exception of PE and XY enzymes, even though the differences were not always significant with respect to the control sample (Table 1). TPI value showed in all varieties a significant decrease with the use of the AR enzyme (decrease of 15–28% compared to the control), while PL induced a decrease in TPI only in Chardonnay, Arneis, and Greco (from −18% to −26%), and PG only in Arneis, Greco, and Falanghina (from −18% to −27%).

Regarding colour parameters, CIELab coordinates and $A_{420\ nm}$ values were in agreement with the TPI value (Table 1). The enzyme treatments reporting a significant lower TPI value than the control (PL, PG, and AR) showed also, for all varieties tested, significantly higher values of clarity (L*) and lower ones of red-green colour component (a*), blue-yellow colour component (b*) and $A_{420\ nm}$. Therefore, PL, PG, and AR-treated

Table 1
Standard physicochemical parameters, color traits, and total phenol index of grape musts obtained from Chardonnay, Arneis, Greco, and Falanghina white winegrapes after pellicular maceration with five enzymes.

	CT	PL	PG	PE	XY	AR	sign.
Chardonnay							
Must yield (%)	57.9 ± 1.3d	62.9 ± 1.4bc	68.2 ± 1.6a	59.3 ± 1.2cd	58.7 ± 1.5d	64.5 ± 1.5ab	***
Sugars (g/L)	198 ± 4	200 ± 1	200 ± 2	200 ± 9	200 ± 16	199 ± 1	ns
pH	3.57 ± 0.01ab	3.55 ± 0.01b	3.58 ± 0.01ab	3.57 ± 0.01ab	3.58 ± 0.01a	3.55 ± 0.01ab	*
Total acidity (g/L)	5.20 ± 0.04a	5.30 ± 0.04a	5.30 ± 0.04a	5.23 ± 0.04a	5.00 ± 0.04b	5.23 ± 0.11a	***
Tartaric acid (g/L)	4.12 ± 0.15	4.27 ± 0.09	4.24 ± 0.04	4.14 ± 0.25	4.06 ± 0.26	4.25 ± 0.06	ns
Malic acid (g/L)	2.92 ± 0.06	2.97 ± 0.05	3.06 ± 0.05	2.99 ± 0.06	2.92 ± 0.29	2.90 ± 0.06	ns
L*	53.4 ± 1.5d	71.4 ± 2.7b	76.3 ± 2.3ab	60.8 ± 1.1c	53.9 ± 1.8d	78.5 ± 0.3a	***
a*	10.97 ± 0.89a	4.69 ± 0.51b	3.94 ± 0.74bc	9.73 ± 0.25a	11.13 ± 0.30a	3.20 ± 0.07c	***
b*	39.29 ± 1.37a	29.06 ± 0.89b	28.95 ± 1.58b	39.49 ± 0.03a	39.71 ± 0.05a	26.51 ± 0.42b	***
$A_{420\ nm}$	1.50 ± 0.07a	0.83 ± 0.07c	0.73 ± 0.07cd	1.28 ± 0.03b	1.49 ± 0.06a	0.65 ± 0.01d	***
TPI	13.07 ± 0.32ab	10.72 ± 0.77cd	11.83 ± 0.77bc	12.24 ± 0.11ab	13.18 ± 0.13a	9.49 ± 0.20d	***
Arneis							
Must yield (%)	54.6 ± 0.6b	63.6 ± 2.1a	64.2 ± 2.7a	54.7 ± 1.7b	54.2 ± 0.7b	65.6 ± 1.1a	***
Sugars (g/L)	232 ± 3	230 ± 5	234 ± 4	232 ± 3	231 ± 2	232 ± 5	ns
pH	3.45 ± 0.01a	3.40 ± 0.01b	3.46 ± 0.01a	3.42 ± 0.03ab	3.45 ± 0.01a	3.45 ± 0.01a	**
Total acidity (g/L)	3.83 ± 0.04b	4.14 ± 0.02a	3.93 ± 0.13b	3.94 ± 0.07b	3.89 ± 0.02b	3.88 ± 0.02b	**
Tartaric acid (g/L)	4.65 ± 0.13b	5.08 ± 0.11a	4.74 ± 0.09b	4.78 ± 0.02b	4.77 ± 0.07b	4.81 ± 0.08b	**
Malic acid (g/L)	1.29 ± 0.02	1.16 ± 0.06	1.24 ± 0.09	1.23 ± 0.02	1.20 ± 0.08	1.20 ± 0.02	ns
L*	59.6 ± 0.8d	76.8 ± 1.3b	85.4 ± 0.5a	62.6 ± 1.6c	58.3 ± 0.9d	78.3 ± 1.0b	***
a*	8.83 ± 0.01ab	3.58 ± 0.27c	2.05 ± 0.13d	8.32 ± 0.24b	8.98 ± 0.32a	3.34 ± 0.16c	***
b*	36.77 ± 0.30a	26.38 ± 0.54b	23.47 ± 0.68c	36.64 ± 0.22a	36.61 ± 0.61a	26.36 ± 0.76b	***
$A_{420\ nm}$	1.22 ± 0.02a	0.66 ± 0.03c	0.47 ± 0.02d	1.14 ± 0.05b	1.25 ± 0.03a	0.63 ± 0.03c	***
TPI	10.57 ± 0.22a	7.81 ± 0.26b	7.73 ± 0.31b	10.25 ± 0.53a	10.87 ± 0.19a	7.81 ± 0.34b	***
Greco							
Must yield (%)	69.1 ± 0.9a	72.5 ± 1.5a	72.7 ± 1.4a	68.8 ± 1.6a	68.9 ± 1.9a	71.3 ± 2.0a	*
Sugars (g/L)	211 ± 1	220 ± 9	215 ± 3	217 ± 1	210 ± 2	213 ± 2	ns
pH	3.18 ± 0.03	3.17 ± 0.01	3.17 ± 0.01	3.19 ± 0.02	3.17 ± 0.01	3.19 ± 0.01	ns
Total acidity (g/L)	7.02 ± 0.31	7.27 ± 0.09	7.03 ± 0.14	6.76 ± 0.31	7.11 ± 0.16	7.05 ± 0.29	ns
Tartaric acid (g/L)	5.63 ± 0.16b	6.08 ± 0.24a	5.57 ± 0.01b	5.47 ± 0.20b	5.60 ± 0.08b	5.61 ± 0.19b	*
Malic acid (g/L)	3.10 ± 0.15	3.35 ± 0.07	3.15 ± 0.19	3.06 ± 0.15	3.18 ± 0.12	3.05 ± 0.10	ns
L*	58.0 ± 1.3c	76.7 ± 4.0b	84.0 ± 0.6a	63.3 ± 2.4c	58.4 ± 2.0c	86.0 ± 1.1a	***
a*	11.40 ± 0.47a	4.90 ± 1.47b	3.23 ± 0.15bc	9.90 ± 1.01a	10.79 ± 0.22a	2.09 ± 0.35c	***
b*	41.89 ± 0.66a	31.16 ± 3.48b	27.70 ± 0.30bc	41.04 ± 1.74a	40.80 ± 0.58a	23.71 ± 0.91c	***
$A_{420\ nm}$	1.36 ± 0.05a	0.72 ± 0.12b	0.53 ± 0.01bc	1.19 ± 0.10a	1.32 ± 0.05a	0.45 ± 0.03c	***
TPI	13.73 ± 0.43a	11.31 ± 0.35b	10.23 ± 0.21c	13.42 ± 0.46a	13.90 ± 0.12a	9.89 ± 0.43c	***
Falanghina							
Must yield (%)	64.2 ± 2.6b	71.2 ± 0.8a	71.0 ± 0.7a	64.4 ± 1.9b	67.2 ± 0.7ab	69.7 ± 1.3a	***
Sugars (g/L)	219 ± 16	219 ± 22	233 ± 7	228 ± 8	232 ± 2	230 ± 7	ns
pH	3.43 ± 0.01	3.43 ± 0.02	3.43 ± 0.01	3.43 ± 0.01	3.44 ± 0.01	3.44 ± 0.01	ns
Total acidity (g/L)	4.93 ± 0.08b	5.15 ± 0.08a	5.03 ± 0.11ab	4.93 ± 0.04b	4.86 ± 0.04b	5.00 ± 0.04ab	**
Tartaric acid (g/L)	4.26 ± 0.40	4.37 ± 0.41	4.36 ± 0.04	4.36 ± 0.18	4.35 ± 0.08	4.45 ± 0.07	ns
Malic acid (g/L)	2.38 ± 0.23	2.48 ± 0.31	2.60 ± 0.07	2.41 ± 0.09	2.54 ± 0.05	2.56 ± 0.05	ns
L*	65.6 ± 1.7c	74.7 ± 1.0b	81.6 ± 1.1a	67.5 ± 1.9c	68.9 ± 0.3c	78.2 ± 2.1ab	***
a*	7.71 ± 0.71a	4.78 ± 0.21c	2.94 ± 0.26d	6.67 ± 0.57ab	6.33 ± 0.14b	3.63 ± 0.73cd	***
b*	36.07 ± 1.47a	30.45 ± 0.56bc	26.05 ± 0.77d	33.70 ± 0.88ab	33.13 ± 0.44ab	27.51 ± 2.28cd	***
$A_{420\ nm}$	1.05 ± 0.07a	0.76 ± 0.03b	0.56 ± 0.03c	0.96 ± 0.06a	0.92 ± 0.01a	0.65 ± 0.07bc	***
TPI	11.09 ± 0.29a	10.93 ± 0.86a	9.12 ± 0.16b	10.97 ± 0.51a	10.84 ± 0.15a	9.39 ± 0.26b	***

All data are expressed as average value ± standard deviation (n = 3). Different Latin letters within the same row indicate significant differences among treatments according to Tukey test ($p < 0.05$). Sign: *, **, ***, and "ns" indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively. PL: pectin lyase, PG: polygalacturonase, PE: pectin methyl esterase, XY: xylanase, AR: arabinase, and untreated control (CT). L*: lightness, a*: red/green color coordinate, b*: yellow/blue color coordinate, TPI: total polyphenol index, A: absorbance.

musts evidenced a remarkably lower yellowish hue when compared to control samples without enzyme addition.

Fig. 1 shows the colour corresponding to control and treated grape musts on RGB colour space, calculated from CIELab coordinates (Table 1). This visual colour assessment allows to evidence the strong effect of PL, PG and AR enzymes on the yellowish hue reduction for all the varieties evaluated, even though in different extent depending on the variety and enzyme used. The differences found in the grape must colour were quantified through the ΔE* values calculated among treatments. The ΔE* parameter is strongly related to the ability of tasters to detect small colour differences. In wines, tasters can detect differences of one unit in this parameter when colour is directly observed (Pérez-Magariño & González-Sanjosé, 2003), however a ΔE* threshold of 2.3 units has been established to correctly discriminate the colour of white wines by the human eye (Sáenz Gamasa et al., 2009). Nevertheless, these ΔE* values could not be exactly adapted to white musts. For fruit juices, ΔE* values less than 0.5 units indicate not visually detectable colour differences, between 0.5 and 1.5 are slightly noticeable, from 1.5 to 3.0 are noticeable, between 3.0 and 6.0 are well visible and higher than 6.0 are great (Cserhalmi et al., 2006). In most cases, the colour differences found among the white grape musts obtained in this study were greater than 6.0 and, therefore, visually perceived. As it can be observed in Fig. 1, ΔE* values were higher than 11 for PL, PG, and AR treatments with respect to control samples, the lowest values corresponding to Falanghina variety. Moreover, the colour differences for PE-treated samples with respect to untreated musts were also visible according to ΔE* values between 3.0 and 7.5 units. AR and PG treatments reduced more strongly the yellowish hue but the colour difference between AR and PG-treated samples could be visually perceived. The higher colour reduction observed for either PG or AR samples depended on the variety

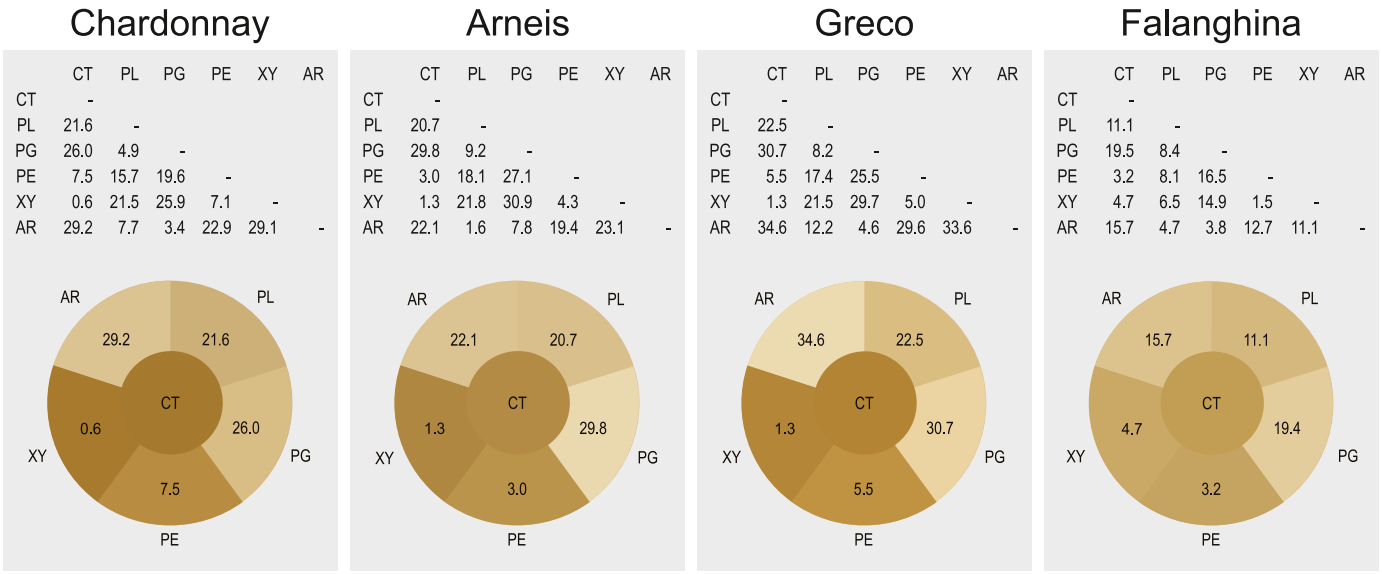

Fig. 1. Grape must colour detected at the end of the treatments for Chardonnay, Arneis, Greco, and Falanghina white winegrapes. Each grape must colour was acquired by spectrophotometry, expressed in CIELab coordinates, and then converted to RGB colour space for visualisation. For each variety, the numbers represent ΔE* among treatments while those included in the colour wheel represent the difference between each enzyme treatment (PL: pectin lyase; PG: polygalacturonase; PE: pectin methyl esterase; XY: xylanase; AR: arabinase) and the untreated control sample (CT) located in the center of the colour wheel. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

considered.

It is well-known that phenolic compounds are involved in oxidation reactions and browning of white musts and wines (Salacha et al., 2008). The changes observed in the TPI values and in the chromatic attributes could be related to the polysaccharide-polyphenol interactions. Some studies have reported that there is a greater interaction and affinity between polyphenols and polysaccharides of the pulp compared to those of skins (Bindon et al., 2010; Bindon & Kennedy, 2011). Several authors have demonstrated that linear or poorly branched polysaccharides (Fernandes et al., 2020), high degree of methylation (Liu et al., 2021; Watrelot et al., 2013) and homogalacturonan regions favour the interaction with polyphenols (Liu et al., 2021) since there is an increased stacking between the molecular structures. In fact, highly methylated pectins show the strongest affinity for procyanidins as a consequence of hydrophobic interactions, while highly branched pectins hinder the interaction with procyanidins, probably due to steric limitations, giving soluble complexes (Watrelot, et al., 2013, 2014). Le Bourvellec et al. (2009) highlighted that oxidised tannins with higher mean degree of polymerisation (mDP) have an increased affinity for polysaccharides.

Among pectins, homogalacturonans are particularly susceptible to enzymatic treatments, which modify their structure and, in turn, may affect their interaction with polyphenols (Castro-López et al., 2016). Therefore, PL and PG treatments could release the linear homogalacturonan fraction that interacts mostly with large and oxidised polyphenols, leading to the formation of insoluble complexes (Liu et al., 2021) and therefore to the lower TPI and $A_{420\ nm}$ values in the grape must. Furthermore, the AR enzyme acts on arabinan bond to Rhamnogalacturonan-I (RG-I), releasing polymers more flexible and available to form strong bond with tannins compared to arabinan-branched pectins (Fernandes et al., 2020). However, XY and PE treatments were less efficient in reducing total phenols probably due to a reduced capacity of xyloglucans and poorly methylated homogalacturonans to interact with oxidised polyphenols (Le Bourvellec et al., 2005; Liu et al., 2021; Watrelot et al., 2013).

3.3. Effect of enzyme treatment on free volatile composition in grape musts

For the four white winegrape varieties studied, no significant difference was found in total concentration of free volatile compounds among the different enzyme-treated musts and non-treated controls (Fig. 2, Tables S1–S4). The most abundant chemical class in the musts from Chardonnay, Arneis, Greco, and Falanghina grapes corresponded to pre-fermentative C6 compounds, accounting for at least 73%, 74%, 59%, and 46%, respectively, of total free volatile compounds. For Arneis grape musts, significant differences were not observed in total concentrations of C6 compounds among treatments. However, the PG-treated must resulted in a significantly lower concentration of (*E*)-2-hexenal than the AR-treated musts, while leading to lower concentrations of (*E, E*)-2,4-hexadienal when compared to all treatments with the exception of AR. Despite these differences, the compounds that mostly contributed to the total concentration of C6 compounds in all varieties were 1-hexanol and (*E*)-2-hexen-1-ol. In Chardonnay, Greco, and Falanghina grape musts, the enzyme treatments have not significantly influenced the concentration of this class of free volatile compounds. Therefore, a variety effect is noticeable.

These pre-fermentative compounds derive from grape polyunsaturated fatty acids, specifically linoleic and α-linolenic acids, through enzymatic reactions related to the lipoxygenase pathway. Some studies have reported that pellicular maceration at a low temperature, keeping the skins in contact with the grape must before fermentation, leads to an increased concentration of C6 compounds. This increase was reported in Pinot blanc musts macerated at 4 °C for 24 h in the presence of pectolytic enzyme (de Matos et al., 2020), but significant changes were not observed in Albariño wines obtained by adding commercial enzymatic preparations (pectinase and secondary cellulase activities) during maceration at 8–10 °C for 6 h (Armada et al., 2010). In this regard, the enzyme activity, grape variety, ripeness degree, and pre-fermentative conditions can influence the concentration of C6 aldehydes and alcohols, which are related to "herbaceous" and "leafy/grassy" aroma descriptors (de Matos et al., 2020).

Regarding free terpenes, which can contribute to the floral and fruity aroma, PL enzyme showed the greatest effect in Chardonnay and Falanghina varieties. In Chardonnay grape must, the concentration of furan linalool oxide was significantly higher in PL than CT, PG, and AR samples, while this enzyme treatment increased the concentration of geraniol in Falanghina with respect to XY and AR samples. Falanghina grape musts treated with PG, XY, and AR were richer in furan linalool

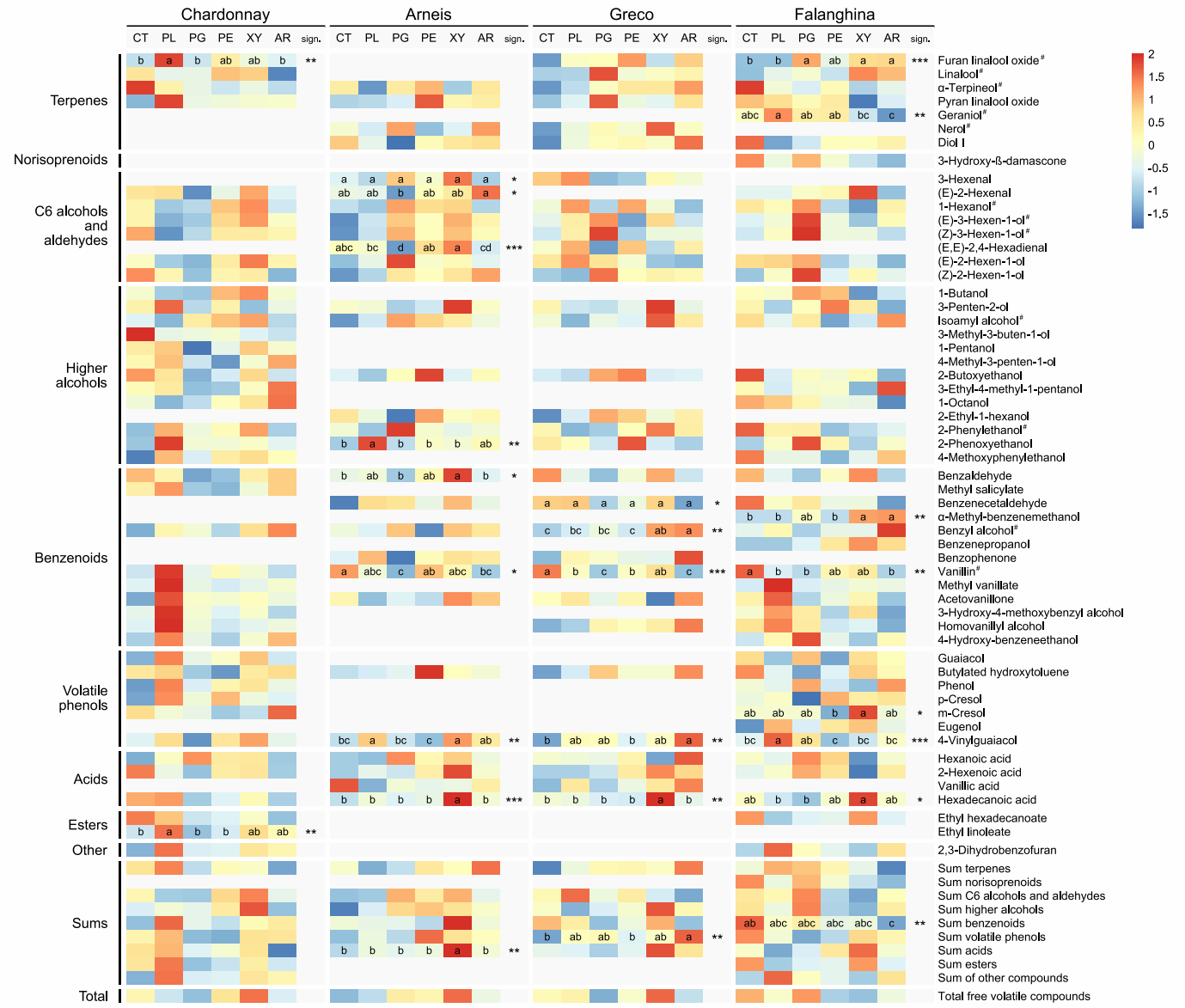

Fig. 2. Heatmap visualisation of free volatile compounds on data standardized (*z*-scores) inside each cultivar. For each variety, columns represent the treatments and the compounds are classified in the rows. Different Latin letters within the same cultivar and row indicate significant differences among treatments according to Tukey test ($p < 0.05$). Sign: *, **, *** indicate significance at $p < 0.05$, 0.01, 0.001, respectively. Compounds with a hash ("#") subscript indicates quantitative determination expressed as μg/L, all remaining compounds are expressed as μg/L of 1-heptanol equivalent.

oxide than CT and PL samples where this compound was not detected. For each variety, none of the major terpene compounds showed significant differences among the enzymatic treatments or with respect to the untreated control, and therefore the total concentration of terpenes did not either. However, the slightly increased total terpene concentrations in Chardonnay, Arneis, Greco, and Falanghina could be related to furan linalool oxide, nerol, diol I, and geraniol, respectively.

Other studies reported a strong variety effect of maceration during 12 h with the use of pectinases with β-glucosidase activity on the release of free terpene compounds, increasing in white wines from Muscat Ottonel and Plevenska rosa grapes but decreasing in those from Aligote, Dimyat, and Vrachanski muscat (Dimitrov et al., 2017). As already observed for C6 compounds, the concentrations of terpenes and C13-norisoprenoids in Albariño wines treated with commercial enzymatic preparations during maceration at 8–10 °C for 6 h were similar or significantly lower than those found in the control wines (Armada et al., 2010). Nevertheless, in this last study higher concentrations of nerol and geraniol were found in enzyme-treated musts as also occurred in the present study for nerol using PG and AR in Arneis grape musts, for nerol using all treatments in Greco, and for geraniol using PL, PG and PE in Falanghina, although the differences were not significant with respect to control samples. Also in the present study, free norisoprenoids, detected only in Falanghina musts, were not affected significantly by the enzyme treatments.

A different behaviour was observed for benzenoids, whose total concentration decreased significantly when enzyme preparations were used for the Falanghina variety, particularly in AR-treated samples when compared to the respective untreated grape must (−26%). This reduction may be due to a significantly lower concentration of vanillin, being associated with vanilla aroma descriptor. The concentration of vanillin also decreased significantly with respect to control samples when PG and AR treatments were used for Arneis and Greco. In addition, it seems that all treatments produced a decrease in the concentration of vanillin for Greco and Falanghina grapes. On the contrary, the grape musts

treated with XY and AR preparations showed a significantly higher concentration of benzyl alcohol than control samples for the Greco variety, while a significant increase of benzaldehyde was observed for the Arneis variety when the XY treatment was used. Armada et al. (2010) have also reported that the use of maceration enzymes (pectinase and secondary cellulase activities) increased the concentration of acetovanillone for cold-macerated Albariño wines with respect to control samples, while no clear tendency was observed for benzyl alcohol.

Very few significant differences were found for higher alcohols among treatments, however there are some remarkable aspects. The only significant difference was observed for the Arneis variety treated with PL enzyme where the concentration of phenoxyethanol was

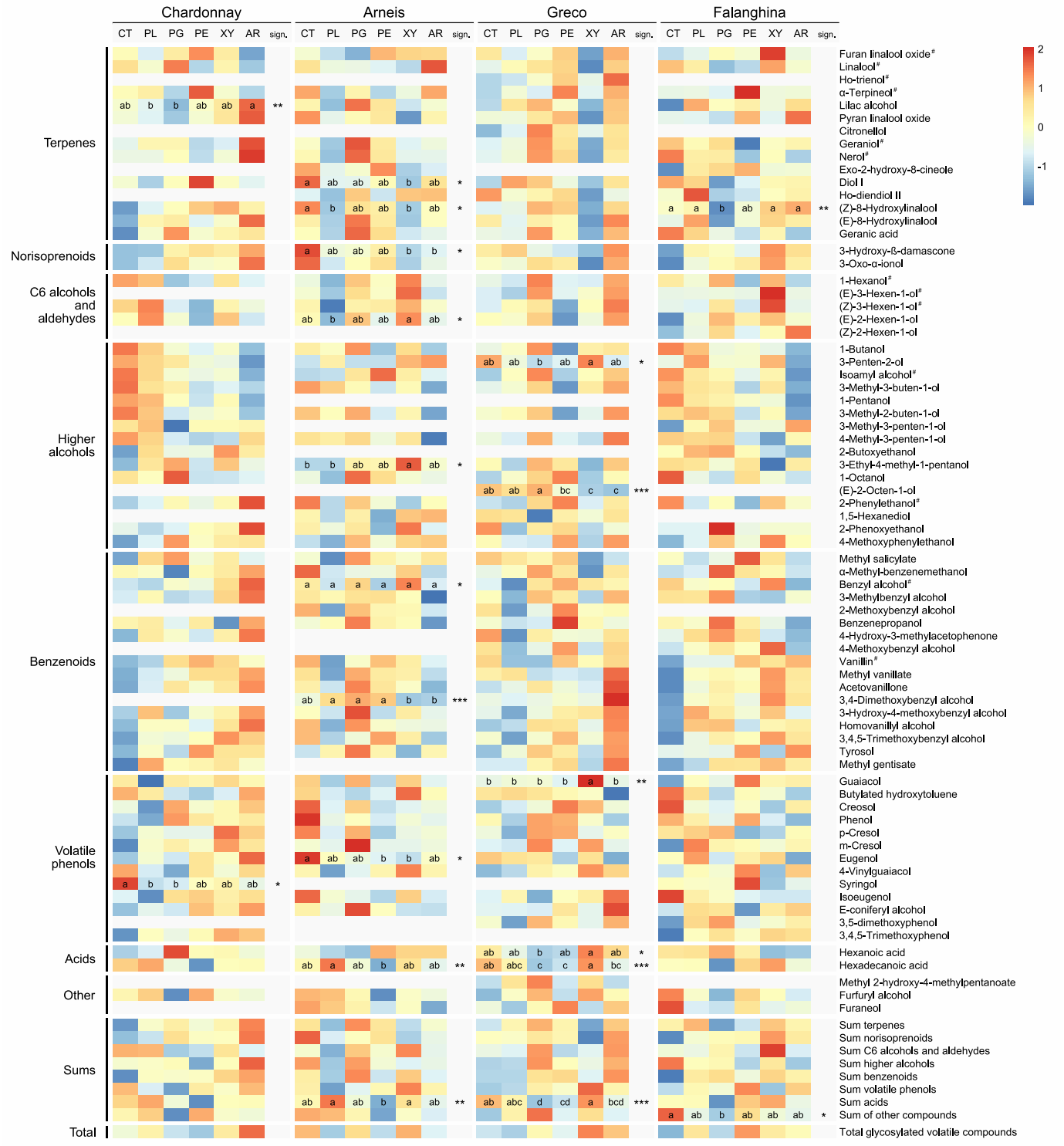

Fig. 3. Heatmap visualisation of glycosylated volatile compounds on data standardized (*z*-scores) inside each cultivar. For each variety, columns represent the treatments and the compounds are classified in the rows. Different Latin letters within the same cultivar and row indicate significant differences among treatments according to Tukey test ($p < 0.05$). Sign: *, **, *** indicate significance at $p < 0.05$, 0.01, 0.001, respectively. Compounds with a hash ("#") subscript indicates quantitative determination expressed as μg/L, all remaining compounds are expressed as μg/L of 1-heptanol equivalent.

significantly higher with respect to the other enzymatic treatments and the untreated control. The largest contribution to the total concentration of these compounds in the varieties studied corresponded to isoamyl alcohol (fusel aroma descriptor) and 2-phenylethanol (rose aroma scent). In fact, the greatest differences with respect to the control musts were associated with the XY treatment for Chardonnay and Greco varieties (+10% and +14%, respectively), as a consequence of the highest concentration of these two compounds. As well, it occurred also for Arneis grape musts using PG, PE, and XY treatments (from +15% to +17%) due to the increased concentrations of isoamyl alcohol. Petropulos et al. (2014) reported that the use of a commercial pectinase enzyme preparation during maceration at ambient temperature did not influence the higher alcohols concentration, even in red wines made from Vranec variety. In previous studies, the use of maceration enzymes with mixed pectinase and secondary cellulose activities increased the concentration of isoamyl alcohol and 2-phenylethanol in cold macerated Albariño wines with respect to the control wines (Armada et al., 2010). Our results confirm that enzyme activities other than pectolytic ones can increase the concentration of these compounds in the must/wine.

Regarding volatile phenols, a significant effect of enzyme treatments was observed for 4-vinylguaiacol (spice aroma descriptor) with respect to control musts, leading to a higher concentration in the grape musts obtained from Arneis with PL and XY treatments (+43% and +53%, respectively), Greco with AR enzyme (+178%), and Falanghina using PL enzyme preparation (+54%). However, the differences were not significant for Chardonnay among treatments. Therefore, a variety effect was again evidenced in agreement with previous studies where 4-vinylguaiacol concentration increased in Airén wines with the use of maceration enzymes but decreased in Muscat à petits grains wines (Castro Vázquez et al., 2002). Finally, a significantly higher concentration of fatty acids, in particular hexadecanoic acid, was found in Arneis, Greco, and Falanghina grape musts treated with XY enzyme.

From a variety point of view, grape polysaccharides from the cell walls, such as arabinogalactan proteins (AGPs), arabinans, homorhamnogalacturonans (HG), and rhamnogalacturonans type RG-I and RG–II, can interact directly at the molecular level with volatile compounds. However, these interactions are greatly dependent on the structure and physico-chemical properties of both the polysaccharides and volatile compounds, in particular the higher hydrophobic nature results in a greater binding capacity (Villamor & Ross, 2013). Taking into account that these cell wall structural characteristics depend on the variety (Gao et al., 2019; Ortega-Regules et al., 2008), it strongly influences the diffusion of volatile compounds. In addition, these features are also affected by the grape maturity that can have played a role in the differences found among varieties in the present study (Zietsman et al., 2015).

3.4. *Effect of enzyme treatment on glycosylated volatile composition in grape musts*

Regarding glycosylated forms of volatile compounds (Fig. 3, Tables S5–S8), although the total concentration of terpenes was not significantly different among treatments, some individual terpenes have shown significant differences for Chardonnay, Arneis, and Falanghina musts with the pre-fermentative addition of enzymes. For the Chardonnay variety, the AR treatment favoured the release of lilac alcohol into the must. However, the concentrations of diol I and (*Z*)-8-hydroxylinalool decreased significantly for some treatments such as PL and XY with respect to control for the Arneis variety. Concerning Falanghina, a lower concentration of (*Z*)-8-hydroxylinalool was observed with PG treatment. Another study has highlighted that the effect was variety- and compound-dependent. In detail, α- and β-glycosidic enzymes caused, on one hand, the decrease of α-terpineol in Macabeo wines while increasing in Airén, Chardonnay, and Muscat à petits grains wines, and on the other hand the decrease of (*E*)-linalool oxide, diol I, and diol II concentrations while increasing linalool, α-terpineol, citronellol, nerol, geraniol, hydroxylinalool, and hotrienol in the wines made from the Muscat à petits grains grapes (Castro Vázquez et al., 2002).

The differences found in total glycosylated norisoprenoids were not significant among treatments for the four varieties studied. Nevertheless, a significant decrease in the concentration of 3-hydroxy-β-damascone was observed in the Arneis variety using XY and AR enzymes with respect to non-treated grape musts.

The enzyme treatments also did not have the same effect for C6 alcohols in all the four varieties studied. The only significant difference was observed for (*E*)-2-hexen-1-ol in Arneis grape musts, where the concentration in XY-treated musts was higher than in the musts treated with PL enzyme, not corresponding to statistical differences in total concentration of C6 compounds. In agreement with this effect observed for xylanase activity, previous studies based on the use of enzyme activities other than pectolytic, such as α- and β-glycosidic, have reported an increase of C6 alcohols in the resulting wines from different winegrape varieties (Castro Vázquez et al., 2002).

The different enzyme preparations studied with single activity have not significantly influenced the concentration of total glycosylated higher alcohols and the variations observed were lower than 5% with respect to control samples. Only the concentration of 3-ethyl-4-methyl-1-pentanol was significantly higher for XY treatment in the Arneis variety when compared to control and PL treatment, whereas that of (*E*)-2-octen-1-ol was lower for XY and AR in the Greco variety. Regarding benzenoids, no significant effect was observed for the enzyme treatments studied. Considering that higher alcohols and benzenoids were the mostly represented chemical classes as glycosylated form in the musts obtained, no significant differences were also observed for total glycosylated volatile compounds (as the sum of all glycosylated compounds identified). As observed in the present study for XY and AR treatments, one study reported higher concentrations of 2-phenylethanol and benzyl alcohol in Chardonnay wines obtained with the use of non-pectolytic maceration enzymes, namely α- and β-glycosidic activities, with respect to untreated wines (Castro Vázquez et al., 2002).

Variety effects were also observed for glycosylated volatile phenols. Particularly, when compared to control samples, the concentration of syringol decreased significantly in Chardonnay grape musts by using PL and PG treatments, whereas eugenol did for Arneis with PE and XY treatments. On the other hand, in Greco grape musts treated with XY activity, guaiacol was found in significantly higher concentrations with respect to control. For Falanghina, single compounds did not change significantly among grape musts subjected to different treatments. Nevertheless, the differences were not significant in total concentrations of volatile phenols among treatments for the four varieties studied.

Finally, some enzyme treatments influenced significantly the total concentration of fatty acids, mainly hexadecanoic acid. For the Greco variety, lower concentrations of hexadecanoic acid were found with respect to control samples when PG and PE treatments were used. Similar enzyme behaviour was also observed for PE activity in Arneis.

The use of exogenous enzymes involved in the skin cell wall degradation may contribute to the release of glycosylated precursors from the skin and pulp, leading to varietal aroma enhancement during winemaking. These precursors are mainly disaccharide glycosides, such as 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside, 6-*O*-α-L-rhamnosyl-β-D-glucopyranoside, and 6-*O*-β-D-apiosyl-β-D-glucopyranoside. Therefore, enzyme activities aiming to cleave the terminal sugar and, subsequently, release rhamnose, arabinose, and apiose, are required before the aglycone liberation by β-D-glucosidases (Ribéreau-Gayon et al., 2021). It confirms our findings on the potential of enzyme activities other than pectolytic ones to increase the concentration of volatile compounds in the must/wine. Despite this positive effect and some common trends highlighted, the results are influenced by the winegrape variety and the grape maturity (Zietsman et al., 2015).

3.5. Principal component analysis

To better understand if the influence of single enzyme activities on the volatile composition of grape musts was similar for the four different varieties studied, principal component analysis (PCA) was carried out on free and glycosylated compounds that showed a significant change among enzyme treatments. With the aim of minimising the varietal effect (different volatile profile and maturity), data was standardized (*z*-scores) inside each cultivar prior to PCA.

Regarding free volatile compounds, Fig. 4a explains a total variance of 43.2%. PC1 accounts for a variance of 25.7% and it is mostly correlated with benzaldehyde, hexadecanoic acid, and vanillin (r = 0.572, 0.483, and 0.418, respectively; $p < 0.001$), while PC2 (17.5% of total variance explained) is mostly correlated with 4-vinylguaiacol and benzyl alcohol (r = 0.528 and 0.509, respectively; $p < 0.001$). The samples distribution according to these two extracted principal components highlights interesting patterns: all XY-treated samples are placed in the first quadrant, all AR-treated samples in the second quadrant, while PG and PE-treated samples form distinct groups mostly in the third quadrant. CT samples are positioned in the fourth quadrant, excluding untreated Arneis sample, and also PL-treated samples show close placement among them with the exception of Chardonnay sample. This placement seems to hypothesise a specific tendency of XY to increase selected compounds such as hexadecanoic acid, benzaldehyde, and (*E*)-2-hexenal (particularly for Arneis and Falanghina samples), and a closeness of AR to 4-vinylguaiacol, benzyl alcohol, and furan linalool oxide contents (although a lower correlation for Chardonnay sample was found). However, given the loose grouping of points even belonging to the same enzyme treatment, and the limited number of compounds subjected to the analysis (only significant compounds were used), a grape variety effect is still perceivable. Another important aspect to highlight is that probably the combined use of pectolytic and non-pectolytic enzyme activities can greatly influence the aroma profile. In fact, most of the samples treated with pectolytic enzymes and arabinase are found in the second and third quadrants.

Considering glycosylated volatile compounds, Fig. 4b shows that the total variance explained was 40.1%, with PC1 (25.5%) mostly correlated with (*Z*)-8-hydroxylinalool, 3-penten-2-ol, lilac alcohol, and 3-hydroxy-β-damascone (r = 0.467, −0.416, 0.406 and 0.401, respectively; $p <$ 0.001), and component PC2 (14.6%) with 3-ethyl-4-methyl-1-pentanol, eugenol (r = −0.576 and 0.539, respectively; $p < 0.001$), and hexadecanoic acid (r = 0.401; $p < 0.01$). In this case, the pattern emerged with the free compounds elaboration was not confirmed, and a rather sparse distribution of samples (interaction between variety and treatment) according to the two selected principal components was observed. Therefore, single enzyme activities can significantly influence the aroma profile, but the varietal imprint plays an important role on glycosylated compounds, modifying the impact of enzyme treatments.

4. Conclusion

The use of single-activity enzymes during cold prefermentative maceration of white winegrapes has led to the production of grape musts with different technological, phenolic, colour, and aroma characteristics for the four varieties studied. In fact, the use of PL, PG, and AR enzymes has allowed not only to achieve a higher must yield with respect to other treatments for all varieties tested, but also the grape musts obtained were characterised by lower TPI and $A_{420\ nm}$ values than the control samples indicating reduced phenolic browning. Furthermore, the PL enzyme released a greater amount of tartaric acid into the grape must, increasing in turn total acidity values, particularly in those varieties showing the lowest values. Regarding volatile compounds, there is no single trend for all four varieties because the variety effect prevails over the enzyme influence. This variety-dependent behaviour is presumably due to interactions of these compounds with cell wall polysaccharides released from enzyme activity. However, similar patterns were observed for free volatile compounds in the must treated with arabinase, xylanase, and polygalacturonase for the four white grape varieties studied. The results obtained provide valuable information for manufacturers and producers because the effectiveness of enzyme preparations containing single or combined activities can be influenced by the target variety. Particularly, the knowledge of polysaccharide composition of each grape variety and the formulation of enzyme preparations on the basis of specific activities could help to improve the treatment efficiency in winery. Moreover, the use of specific enzymes with non-pectolytic activity can contribute to enhance the aromatic complexity and, therefore, the combination of both pectolytic and non-pectolytic activities could represent an added value in the diversification of white wines

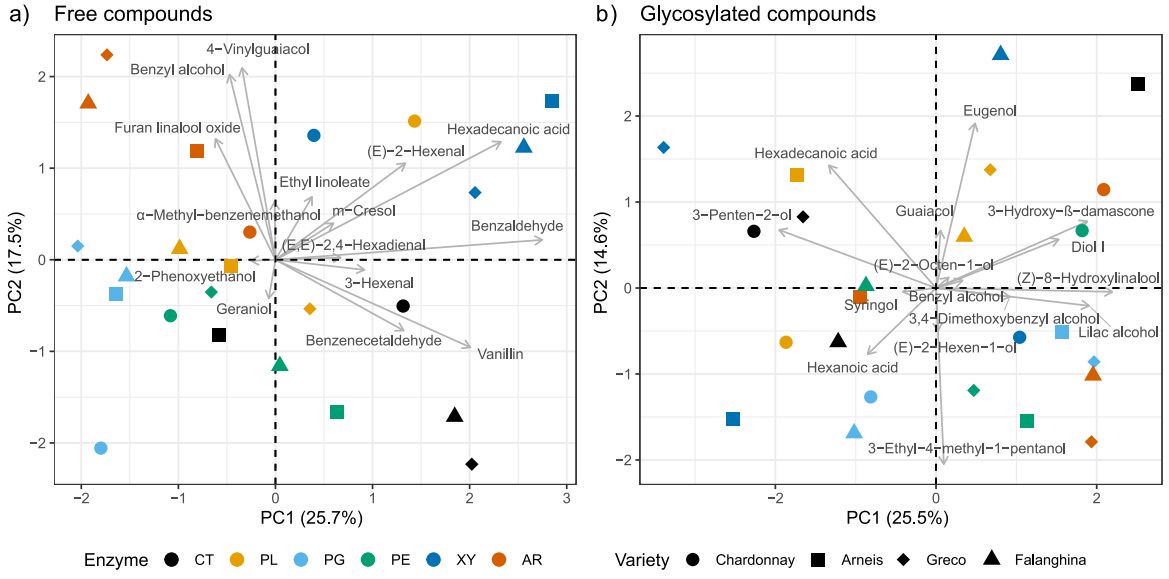

Fig. 4. Principal component analysis (PCA) of free (a) and glycosylated (b) volatile compounds of prefermentative macerated grape musts treated with different enzymes (PL: pectin lyase; PG: polygalacturonase; PE: pectin methyl esterase; XY: xylanase; AR: arabinase; CT: untreated-control) for Chardonnay, Arneis, Greco, and Falanghina varieties. PCA was performed on data standardized (*z*-scores) inside each cultivar and considering only the compounds reporting significant differences among treatments for free (a) or glycosylated compounds (b).

production with different aroma profiles.

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CRediT authorship contribution statement

Susana Río Segade: Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Mattia Malabaila:** Writing – original draft, Visualization, Investigation, Formal analysis. **Domen Škrab:** Writing – review & editing, Data curation. **Maria Alessandra Paissoni:** Writing – review & editing, Investigation. **Simone Giacosa:** Writing – review & editing, Data curation. **Carlo Montanini:** Writing – review & editing, Resources. **Luca Rolle:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.103915.

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