Università degli Studi di Palermo

AREA RICERCA E TRASFERIMENTO TECNOLOGICO
SETTORE DOTTORATI E CONTRATTI PER LA RICERCA
U. O. DOTTORATI DI RICERCA

Dottorato di Ricerca in Biodiversity in Agriculture and Forestry
Dipartimento Scienze Agrarie, Alimentari e Forestali
Settore Scientifico Disciplinare AGR/15 Scienze e Tecnologie Alimentari

OENOLOGICAL POTENTIAL AND WINEMAKING TECHNOLOGIES OF WINES FROM TRADITIONAL AND ANCIENT SICILIAN CULTIVARS (*VITIS VINIFERA* L.)

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CICLO XXXVIII
ANNO CONSEGUIMENTO TITOLO 2025

The present thesis is including the following articles:

Vitaggio, C., Pollon, M., Schnitter, M., Caraci, V., Cinquanta, L., & Corona, O. (2025). Grape ripening heterogeneity in white and red cultivars (Vitis vinifera L.) grown on different calcareous soils using technological and phenolic markers. Journal of the Science of Food and Agriculture. https://doi.org/10.1002/jsfa.70231

Vitaggio, C., Pollon, M., Schnitter, M., Caraci, V., Cinquanta, L., & Corona, O. (2025). Effect of pre-fermentative cold soaking and use of different enzymes on the chemical and sensory properties of Cataratto wines. Journal of Food Composition and Analysis 143, 107560. https://doi.org/10.1016/j.jfca.2025.107560

Vitaggio, C., Schnitter, M., Caraci, V., Pollon, M., Cinquanta, L., & Corona, O. (2025). Oenological characterization and grape heterogeneity assessment of ancient minor white and red cultivars (Vitis vinifera L.) from Sicily. Submitted to Journal of European Food and Technology

Sensory characterization of wines from Sicilian ancient grape varieties. Universidade do Porto, Faculdade de Ciências (FCUP), Departamento de Química e Bioquímica, Portugal

Experiments described in this thesis were conducted at:

Department of Agricultural, Food and Forestry Sciences, University of Palermo

Laboratory of Research in Viticulture and Enology, Department Agricultural, Food and Forestry Sciences (University of Palermo), located in C.da Bosco 421, Marsala.

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Summary

ABSTRACT

This doctoral research investigates the enological potential of Sicilian grape cultivars by integrating viticultural, technological, and biodiversity-driven perspectives. Three complementary studies were carried out to address the challenges and opportunities of native germplasm within the framework of climate change and quality-driven winemaking. First, the influence of soil texture on grape ripening heterogeneity was evaluated in white (*Grillo*, *Chardonnay*) and red (*Nero d'Avola*, *Syrah*) cultivars cultivated on calcareous soils of different texture. Second, a technological approach was applied to *Catarratto* wines, a variety often affected by uneven ripening and the associated "unripe fruit" sensory perception. Finally, the oenological characterization of ancient Sicilian relic cultivars highlighted their dual role as reservoirs of genetic diversity and as adaptive resources to abiotic stress. Moreover, the wines obtained from these relic cultivars were subjected to sensory evaluation with final consumers, in order to assess their sensory profile, preference patterns, and potential market acceptance.

CHAPTER 1: INTRODUCTION

The concept of terroir, the intricate interplay of environmental conditions, soil characteristics, and grapevine genetics, is paramount in producing high-quality wines with a distinct territorial identity. Prestigious wine regions are often characterized by calcareous soils, which can enhance grape quality by shifting the vine's resource allocation from vegetative growth to reproductive development. However, achieving optimal grape maturity is complicated by grape ripening heterogeneity, a phenomenon where berries at different developmental stages coexist within the same harvest. This variability, which can be observed from veraison onwards, is increasingly exacerbated by climate change, particularly through heatwaves and prolonged drought that disrupt vine physiology. The resulting mix of underripe, ripe, and overripe berries leads to inconsistent metabolite composition, which negatively impacts the final sensory and chemical profile of the wine and presents significant technical challenges for winemakers. A key challenge is the objective quantification of grape heterogeneity. While some metrics have been developed, their relative nature makes it difficult to compare data across different datasets or vintages, highlighting the need for standardized, absolute indices.

This issue is particularly evident in our warm and arid climates, where the increase in temperature results in a decoupling between sugar accumulation and acid degradation, ultimately producing musts with elevated sugar content and low acidity. A common strategy to produce more balanced wines with moderate alcohol is to harvest grapes earlier. However, this practice often results in wines made from grapes that have not reached full phenolic or aromatic maturity, leading to undesirable sensory characteristics, most notably an "unripe fruit" mouthfeel. This unripe character is described not merely as high acidity but as a sensation of roughness or puckering on the palate, which seems to be linked to molecules that have not undergone appropriate polymerization or depolymerization during

ripening. Despite being a well-known issue for winemakers, the specific compounds responsible for this perception and the most effective oenological techniques to mitigate it remain largely unclear.

Beyond in-winery adjustments, a proactive and sustainable long-term strategy involves harnessing the region's genetic resources. Sicily, as one of the oldest centers for vine domestication, possesses a vast and varied landscape that has fostered immense biodiversity. This heritage includes a multitude of minor and ancient autochthonous cultivars that are scarcely studied and at risk of extinction. Many of these varieties were abandoned in the past for their naturally low sugar content—a trait that, in the context of climate change, is now highly desirable for producing lighter, lower-alcohol wines that align with contemporary consumer preferences. These ancient genotypes represent a valuable genetic reservoir, potentially possessing key traits such as delayed ripening and tolerance to extreme climatic conditions, which are essential for the future of Mediterranean viticulture. A comprehensive characterization is therefore critical to unlock their potential.

This thesis addresses these interconnected challenges through a multi-faceted investigation. The central hypothesis is that by developing novel metrics to quantify grape heterogeneity, applying targeted oenological interventions, and characterizing Sicily's unique genetic resources, it is possible to build resilience against climate change while enhancing wine quality and valorizing local biodiversity. The research is guided by three primary objectives:

1. To develop and apply two novel, standardized indices, the Technological Variability Index (TVI) and the Phenolic Variability Index (PVI), to quantitatively assess grape ripening heterogeneity. These indices will be used to investigate the influence of soil type (limestone vs. marlstone) and cultivar (autochthonous vs. international) on ripening uniformity.
2. To evaluate the impact of pre-fermentative cold soaking (PCS) and the addition of specific enzymes on the chemical and sensory properties of *Catarratto* wines made from early-

harvested grapes, with the goal of reducing the "unripe fruit" perception by modulating the wine's polysaccharide content, originating from both grape and yeast sources.

3. To provide the first comprehensive metabolic characterization of several minor and ancient Sicilian grape varieties, focusing on their physicochemical traits, phenolic profiles, and volatile organic compounds, in order to assess their oenological potential and suitability for modern viticulture.

This research aims to provide valuable insights for winemakers and viticulturists by offering tools for better understanding and managing vineyard heterogeneity, practical solutions for managing unripe sensory notes, and a foundation for the conservation and valorization of Sicily's viticultural heritage. Ultimately, this work seeks to contribute to the development of sustainable strategies that fortify the wine industry against the challenges of a changing climate while enhancing the production of high-quality wines with a distinct territorial identity.

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CHAPTER 2: Grape ripening heterogeneity in white and red cultivars (*Vitis vinifera* L.) grown on different calcareous soils using technological and phenolic markers

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10.1002/jsfa.70231
Journal of the Science of Food and Agriculture

Abstract

Background: Understanding grape ripening heterogeneity is crucial for optimizing vineyard practices and ensuring wine quality. In this study, the effect of calcareous soils with different textures (limestone and marlstone) on the chemical composition of both autochthonous and allochthonous grape cultivars, white (*Grillo* and *Chardonnay*) and red (*Nero d'Avola* and *Syrah*), was investigated. Grape quality in relation to soil type was assessed through key compositional parameters and the degree of ripening (in)/homogeneity at harvest, using two innovative indices: Technological Variability Index (TVI) and Phenolic Variability Index (PVI). These indices were developed to quantitatively measure grape variability at harvest based on essential technological and phenolic parameters.

Results: Soil type was the dominant factor influencing grape physicochemical and phenolic composition. Grapes from marlstone soils showed more homogeneous ripening, indicated by lower TVI and PVI values, compared to those grown on limestone. Autochthonous varieties exhibited greater adaptability and ripening uniformity across soil types than international cultivars. Multivariate analysis further revealed distinct soil–cultivar interactions for red and white grapes, underscoring genotype-dependent responses to soil properties.

Conclusion: Grape ripening behavior is strongly influenced by both cultivar and soil texture. Autochthonous cultivars, particularly *Grillo* and *Nero d'Avola*, exhibited greater ripening uniformity at harvest, suggesting a stronger adaptation to their native terroirs. Marlstone soils consistently promoted more homogeneous ripening, likely due to their superior water retention capacity. These findings highlight the importance of understanding soil effects in optimizing grape quality and support the development of more effective cultivar–soil matching strategies, especially in the face of increasing climate variability.

Keywords: grape variability, grape maturity, density sorting, wine quality, phenolic compounds, winegrapes

Introduction

The concept of terroir refers to the multifactorial interaction between environmental conditions, soil characteristics, and the genotypic traits of the cultivar. This interaction, further influenced by agronomic and enological practices, plays a crucial role in shaping the technological and sensory attributes of wine[1]. Prestigious wine-growing regions characterized by calcareous soils, such as Burgundy, Champagne, Langa, Marlborough, and parts of Sicily, are particularly well-suited for producing high-quality wines.[1] Typically, calcareous soils exhibit low levels of organic matter and a reduced cation exchange capacity, attributes that are closely linked to soil texture. Soil texture exerts a fundamental influence on key soil-plant interactions, particularly those governing water dynamics and their effects on grapevine physiology. Furthermore, calcareous soils are frequently linked to improved grape quality, as their limited availability of essential nutrients (such as phosphorus, iron, and zinc) tends to shift the vine's resource allocation from vegetative growth toward reproductive development.[1,2,3] Achieving optimal grape maturity in the vineyard is crucial to produce high-quality wines, particularly with respect to sugar accumulation, acidity, flavor development and polyphenolic evolution, including tannin maturation and anthocyanin accumulation in red grapes. Grape ripening is traditionally assessed through vineyard sampling to determine technological maturity, which corresponds to the stage at which pulp sugar accumulation is maximal, acidity is low, and the sugar-to-acidity ratio is high.[4] However, the assessment of technological maturity is generally based on a pre-harvest vineyard sampling, which provides only an average estimate of grape ripening status and does not adequately account for the inherent heterogeneity within the vineyard. As a matter of fact, grapes ripen heterogeneously, according to the vineyard location, the berry position within the cluster, cluster position in the vine, and berry size.[4] This variability in ripening can already be observed at the onset of veraison (where noticeable differences in berry coloration within the same cluster indicate varying stages of maturity) and it decreases as ripening progresses.[5] Towards the ripening process,

growth-related genes in berries that are less advanced in terms of ripening, will be overexpressed, enabling them to catch up and reduce the developmental gap present at veraison.[6,7] If the plant is unable to sustain photosynthetic activity under unfavorable conditions, such as heatwaves and prolonged drought, it may fail to compensate for developmental gaps, leading to inhomogeneous ripening. This phenomenon is becoming increasingly common in warm–arid regions as a consequence of climate change, where prolonged periods of high temperature and water stress prevail. Indeed, temperatures above 35 °C can disrupt vine physiology and berry development, while extreme events above 40–45 °C may impair photosynthesis, cause tissue damage, and reduce yields.[8] Beyond yield reduction, heat stress also alters berry composition by delaying ripening when occurring at green stages or by accelerating sugar accumulation while limiting anthocyanin and acid retention during ripening, ultimately promoting heterogeneous maturity within vineyard parcels.[9] This phenomenon can lead to the co-existence of berries with markedly different metabolite composition within the same harvest, which can negatively affect wine sensory and chemical characteristics,[10,11] thereby presenting technical challenges for the winemaker. Unripe grapes are generally characterized by low sugar content, high acidity, low concentration of anthocyanins and high concentration of flavonoids (especially from seeds).[12] The presence of unripe grapes leads to sensory notes of greenness or unripe fruit in the final wine.[13] The unripe character is a perception of puckering in the mouth noticeable from the mid-palate through the aftertaste, often mischaracterized as due to the high acidity content.[13] Additionally, these wines will result to be poor of volatile compounds which synthesis occurs in grape berries as they approach full ripeness.[14] Furthermore, in warm-arid regions, to avoid excessive sugar accumulation, and lack of freshness in wines, there is an increasing tendency to anticipate the harvest date.[14] While effective in controlling alcohol levels, this strategy may lead to a higher proportion of underripe berries within the harvest. In contrast, overripe grapes tend to result in excessively high alcohol concentrations, leading to the development of port-like aromas and a sensation of hotness in the final product.[15] Managing grape heterogeneity can therefore be considered a strategy to produce full-flavoured wines with moderate ethanol concentrations. It has been observed

that uneven ripening principally depends on soil type and depth, as these geophysical parameters are closely linked to vine vigor and yield, which in turn influence berry composition and wine quality.[16] In-field grape variability, driven by physical and environmental factors such as soil, topography, and climate has promoted the adoption of precision viticulture strategies, including staggered harvesting.[17] Grape heterogeneity represents a complex and challenging viticultural issue, not only in terms of control but also in terms of objective quantification. A measurement derived from representative vineyard sampling and replicate analyses provides a reliable estimate of the mean ripening level of a vineyard parcel. However, it cannot account for the degree of heterogeneity in berry maturation and may lead to a suboptimal decision regarding the harvest date. Armstrong et al.,[18] developed a grape heterogeneity index (GHI) as a novel metric to assess overall grape heterogeneity. The GHI was calculated as the sum of absolute residuals of seven grape maturity parameters, such as total soluble solids, pH, berry fresh weight, malic acid, 3-isobutyl-2-methoxypyrazine, total tannins, and absorbance at 520 nm, multiplied by the range of values at the bunch level, and applied on multiple dates during the two vintages to assess the effects of viticultural treatments on overall grape heterogeneity. The idea proposed in their work was that aggregating multiple measures of grape maturity variability into a composite index would enhance the ability to summarize data and facilitate straightforward comparisons. However, in the GHI, it remains difficult to compare different datasets and historical data due to its relative nature (it is relative to the dataset considered). Given the importance of capturing grape ripening heterogeneity in optimizing harvest timing and ensuring wine quality, as well as in identifying the most suitable grape variety for a given soil type, this study aimed to develop variability indices that can be easily compared with each other and with historical data. In this context, a technological parameters variability index and a phenolic variability index were constructed, each incorporating key technological and phenolic parameters deemed most relevant from an enological standpoint. These indices were applied to both traditional grape varieties cultivated in Sicily (*Grillo* and *Nero d'Avola*) and international grape varieties (*Vitis vinifera* L.), including two white cultivars (*Grillo* and *Chardonnay*) and two red cultivars (*Nero d'Avola* and

Syrah), grown on calcareous soils with different textures, limestone and marlstone, across two vintages.

Materials and methods

Vineyards

The research took place during the 2021 and 2022 vintages in four commercial vineyards situated in the hills near Menfi (AG), along the southwestern coastline of Sicily, Southern Italy. *Chardonnay* (CH), *Grillo* (GR), *Nero d'Avola* (ND) and *Syrah* (SY) were manually harvested from limestone soils (LIM) and marlstone soils (MAR). *Chardonnay* from limestone (CH LIM) vineyard is located at 37°38'31.0" N 12°56'48.1" E; *Chardonnay* marlstone (CH MAR) vineyard is located at 37°38'44.6" N 12°58'23.8" E; *Grillo* from limestone (GR LIM) vineyard is located at 37°35'27.1" N 13°00'45.4" E; *Grillo* from marlstone (GR MAR) vineyard is located at 37°38'42.2" N 12°57'59.9" E (Figure S1 and Figure S2). CH LIM is composed of 61% sand, 31% clay, 8 % silt; CH MAR is composed of 35 % sand, 36 % clay, 29 % silt; GR LIM is composed of 59 % sand, 21 % clay, 20 % silt; GR MAR is composed of 18 % sand, 51 % clay and 31 % silt (Table S1-S4). *Nero d'Avola* from limestone (ND LIM) vineyard is located at 37°38'56.6" N 12°59'45.6" E; *Nero d'Avola* from marlstone (ND MAR) vineyard is located at 37°39'18.5" N 12°59'49.7" E; *Syrah* from limestone (SY LIM) vineyard is located at 37°38'34.4" N 12°58'26.4" E (Figure S3 and Figure S4). ND LIM is of 28 %, 28.5 % clay and 43.3 % silt; ND MAR is composed of 21 % sand, 43.5 composed % clay and 35.5 % silt; SY LIM is composed of 34.4 % sand, 38.5 % clay and 27 % silt; SY MAR is composed of 34.4 % sand, 46 % clay and 20 % silt (Table S5-S8). The vineyards are distributed within a 10 km² area, with a maximum distance of 2 km between individual sites. Consequently, both macroclimatic and microclimatic conditions can be regarded as consistent across the vineyards during a specific vintage. Meteorological data were recorded using a centralized weather station positioned at 37°38'47.92" N and 12°58'03.48" E. Overall, the thermal conditions of the area align with a subtropical temperate

regime, while the precipitation pattern reflects a typical Mediterranean climate, characterized by peak rainfall occurring between October and January and a pronounced dry period lasting 5–6 months from May to September. All soil moisture regimes in the area are classified as Xeric, and soil temperature regimes are categorized as Thermic.[19] All the experimental vineyards consisted of 30-year-old *Vitis vinifera* L. vines grafted onto 140 Ruggeri (*Vitis berlandieri* × *Vitis rupestris*) rootstock. Vines were planted at a spacing of 2.50 × 1.00 m on slopes of 5–15%, with south-west to south-east exposure, at an altitude of approximately 100 m a.s.l. Training followed the Guyot system, leaving one annual cane per plant tied to the fruiting wire parallel to the ground, along with a two-bud spur for renewal. Suckering was carried out before flowering, while no leaf removal practices were applied. Irrigation was supplied via drip system with emitters spaced 0.6 m apart and delivering 4 L h^{-1}, ensuring stable soil moisture throughout the ripening period. Vineyards were managed under conventional agronomic practices, with yields at harvest averaging 110–130 quintals per hectare across both vintages.

Grapes berries sampling sorting by means of densimetric flotation and analyses of chemical-physical parameters

Grape berries of CH, GR, ND and SY cultivars (*Vitis Vinifera* L.) were collected in 2021 and in 2022 vintage when the technological maturity was achieved (white grapes at approximately 21 °Brix with a titratable acidity of 6–7 g L^{-1} of tartaric acid, while red grapes at around 23 °Brix with a titratable acidity of 5–6 g L^{-1} of tartaric acid). Approximately 2.000 berries were randomly collected from each vineyard. The berries, with pedicels attached, were sampled from the middle and lower portions of clusters, both sun-exposed and shaded, to capture the full range of ripeness levels in each vineyard. Berries were floated according to the method described by Bambina et al.[20] To sort the berries by apparent density, a flotation method using salt solutions of varying concentrations was employed. Six NaCl solutions were prepared, with a concentration increment of 20 g L^{-1} between each, ranging from 90 to 190 g L^{-1}. Berries were initially placed in the densest solution. Those that sank were determined to have a density equal to or slightly greater than the solution, while floating berries had lower

densities. The sunken berries were immediately separated and counted, and the floating berries were transferred to the next less dense solution. This process continued until all berries sank. Once sorted by density, the berries were rinsed with water, inspected visually, and any with damaged skins were discarded. The berries in each density class were then weighed and counted. For further analysis, three subsamples of 20 berries from each density class were selected, as this number provides a representative sample while ensuring sufficient material to prepare skin and seed extracts for the analysis of phenolic composition. The remaining berries were divided into three replicates, manually crushed, and centrifuged at 4000 rpm using a PK 131 centrifuge with a T516 swing-out rotor (ALC International, MI, Italy). The resulting supernatant was analyzed to determine key chemical and physical grape parameters within each density class. These parameters included reducing sugar content (g L^{-1}), pH, titratable acidity (g L^{-1}). These measurements were conducted using a Winescan™ instrument (FOSS, Hillerød, Denmark), calibrated following the EEC 2676 standard procedure.[21]

Preparation of extracts

Skins and seeds extracts were prepared following the method outlined by Squadrito et al.[22] Briefly, skins and seeds from 20 berries were manually separated from the pulp. Each component was then placed in separate plastic flasks containing 20 mL of a pH 3.2 buffer solution, prepared as follows: 5 g of tartaric acid, 22.2 mL of 1 M NaOH, 2 g of $Na_2S_2O_5$, 125 mL of 95% ethanol, and deionized water to a final volume of 1 L (23). Samples were maintained at room temperature for 4 hours before being homogenized at 5000 rpm for 1 minute using an Ultraturrax T25 high-speed homogenizer (IKA Labortechnik, Staufen, Germany). They were then subjected to an ultrasonic bath for 3 minutes and centrifuged at 4000 rpm using a PK 131 centrifuge equipped with a T516 swing-out rotor (ALC International, MI, Italy). The supernatant was transferred to a 50 mL volumetric flask. After each centrifugation, the pellet was resuspended in 5 mL of pH 3.2 buffer solution, for a total of three centrifugation cycles. All supernatants were combined and brought to a final volume of 50 mL using

the buffer solution. The inclusion of $Na_2S_2O_5$ in the extraction buffer prevented the activity of polyphenol oxidase, which is responsible for the oxidation of phenolic compounds and tissue browning. Analyses were performed in triplicate.

Analysis of phenolic compounds

Skin and seeds flavonoids and total anthocyanins

Total flavonoids in skin and seeds and total anthocyanins in skins were measured at 280 nm and 540 nm wavelenght, respectively by means of UV–Vis Spectrophotometry, by using a UV-1800 spectrophotometer (Shimazdu Scientific Instruments Inc., Columbia, MD, USA). Skin and seed samples were thawed at room temperature for 30 min and homogenized for 1 min using an Ultra-Turrax in tartaric buffer. The homogenates were subjected to ultrasound treatment (3 min, room temperature) and centrifuged at 4000 rpm for 15 min. The supernatant was collected, while the pellet was resuspended in 5 mL of tartaric buffer, vortexed, and centrifuged again. This extraction step was repeated three times, and the combined supernatants were brought to a final volume of 50 mL with tartaric buffer. Total flavonoids content and total anthocyanins content were determined after diluting extracts (the dilution factor was 50 for seeds extracts and 25 for skins extracts) in hydrochloric ethanol (ethanol:water:hydrochloric acid-37%, 70:30:1 v:v:v) according to the method proposed by Bambina et al.[20] Results were expressed as mg kg^{-1}. The analyses were performed in triplicates.

Analysis of Hydroxycinnamoyl Tartaric Acids (HCTAs) and Flavonols

To determine the hydroxycinnamoyl tartaric acids (HCTAs) and flavonols, grape skin extracts were acidified by adding 0.5 mL of 1 M phosphoric acid (H_3PO_4) to 4.5 mL of extract. The acidified solutions were filtered through a 0.45 μm nylon membrane filter and transferred to 1.5 mL HPLC vials. Chromatographic analyses were performed using a high-performance liquid chromatography (HPLC) system (Agilent 1200 Series, Milan, Italy) equipped with a diode array detector (Hewlett-

Packard 1100 DAD). Separation was carried out on a C18 reversed-phase column (Econosphere™ C18, 5 µm, 250 × 4.6 mm i.d., Lokeren, Belgium, part no. 70066). As previously described by Squadrito et al.[22], an injection volume of 20 µL was used. To analyse HCTAs and flavonols, the mobile phase was a mixture of H_3PO_4 10^{-3} M (solvent A) in CH_3OH (solvent B), changed during the analysis as follows: 5% of B for 5 min, a linear gradient from 5% to 10% of B in 5 min, from 10% to 30 % of B in 10 min, from 30% to 60% of B in 10 min and from 60% to 100% of B in 10min; then, a linear gradient from 100% to 5% of B in 5 min. The flow rate was 0.48 mL min^{-1} and the oven temperature was 40 °C. HCTAs, and flavonols were identified by comparing the retention times and absorption spectra of the pure compounds and with standards isolated in our laboratory.[23] HCTAs and flavonols were quantified by constructing calibration curves using two commercially available standard compounds, namely chlorogenic acid (for HCTAs) and quercetin (for flavonols) purchased from Sigma-Aldrich (St. Louis, MO, USA) with a purity of 95%. Both HCTAs and flavonols contents were expressed in mg kg^{-1} of berries. The analyses were carried out in triple.

Technological variability index (TVI) and the phenolic variability index (PVI)

The Technological Variability Index (TVI) and the Phenolic Variability Index (PVI), two measures of grape heterogeneity, are both based on the concept of constructing an aggregated, standardized variability index with an absolute (rather than relative) nature. To correctly combine this variability, it was necessary to standardize the data, ensuring that each metabolite or physical characteristic contributed equally to the overall variation. In developing our indices, we opted for absolute standardization rather than a relative approach, allowing for comparisons across different datasets, regardless of their units or scales. This type of standardization also facilitates the creation of variability databases. The type of metabolite standardization was the coefficient of variation (CV):

$$CV = \frac{\sigma}{\mu}$$

σ = sample standard deviation of the compound/physical-chemical parameter

μ = sample mean of the compound/physical-chemical parameter

We can define the TVI as follows:

$$TVI = CV_{Reducin(\ su(ars} + CV_{pH} + CV_{Titratable\ acidity}$$

While we can define the PVI as follows:

$$PVI = CV_{Caftaric\ acid} + CV_{coutaric\ acid} + CV_{Feftaric\ acid} + CV_{8uercetin\text{-}3\text{-}(lucoside} + CV_{skin\ NAF} + CV_{seeds\ NAF} + CV_{anthocyanins}$$

Both mean and standard deviation were determined from all subsamples collected from each density class of berries separated by flotation using six NaCl solutions ranging from 90 to 190 g L^{-1}, prepared at 20 g L^{-1} intervals.

Statistical analysis

A factorial analysis of variance (ANOVA) was applied to assess each factor's individual impact. The analysis was carried out using Rstudio (RStudio, Boston, MA, USA) version [4.0.3]. The main factors were grape variety, soil type and vintage. The interaction between the grape variety and the soil type was included. Differences with p values of less than 5% ($p < 0.05$) were considered as statistically significant. In case of significant differences, a post hoc HSD Tukey's test was conducted. The results were expressed as mean values with corresponding standard error. To highlight the soil effect among white and red cultivars grown on different calcareous soils, the supervised Partial Least Squares Discriminant Analysis (PLS-DA) were carried out for each group, separately. The PLS-DA were performed by using the MetaboAnalyst web-based tool suite.[24]

Results and discussion

Density classes distribution

Flotation in salt solutions with different concentrations revealed up to six density classes (from <1075 kg m^{-3} to 1126 kg m^{-3}) as shown in Figure 1 (for white grape cultivars) and Figure 2 (for red grape cultivars), with the statistic parameters describing the distribution of the density classes for cultivars grown in different calcareous soils in 2021 and 2022 vintages. Six density classes were adopted as in the flotation study of Bambina et al.[20] The density classes distribution of grapes indicates both the ripeness heterogeneity at harvest, as it provides the measure of the ripeness levels of the berries, and the maturation stages of berries, since berry density correlates with reducing sugar content. Each density class can thus be considered as a stage of berry maturation. The berry distribution across these density classes followed a bell-shaped curve. The bell-shaped distribution can be described by the skewness, which indicates the maturation rate: positive skewness suggests delayed maturation, while negative skewness points to early maturation; and kurtosis, which measures the uniformity of maturation: low kurtosis values indicate flatter distributions, as the grapes are distributed across multiple density classes reflecting greater heterogeneity in ripeness. On the other hand, higher kurtosis values correspond to sharper distributions, indicating that berry classes with greater weight have similar ripening levels, reflecting higher uniformity in grape maturation. Analysis of berry density distributions (Table 1) revealed significant differences in ripening behavior and heterogeneity at harvest across cultivars, soils, and years. Cultivar had a strong effect on mean berry density, with GR and CH showing the highest values (1097 kg m^{-3}), followed by SY, while ND had significantly lower densities (1089.9 kg m^{-3}), suggesting genotype-specific ripening patterns. Skewness and kurtosis were not significantly influenced by cultivar, though slight negative skewness in ND and SY indicated a higher proportion of less ripe berries. Vintage did not significantly affect any distribution parameter, suggesting stability across 2021 and 2022. However, soil type significantly influenced mean, standard deviation, and median. Grapes grown on MAR soils reached higher mean densities and exhibited more homogeneous ripening (higher kurtosis), while LIM soils showed greater variability and more immature berries. Uneven ripening at harvest has substantial implications for wine composition and quality.[25,26] Tannin concentrations generally decrease during ripening, whereas

underripe berries typically show higher acidity and tannin content. Grapes that meet technological maturity criteria (sugars, pH, acidity) may still contain a large proportion of underripe berries, rich in phenolics compounds and poor in grape-derived glycosylated volatile compounds, crucial for wine quality.[14] In red wines, phenolic composition, linked to phenolic maturity, strongly affects sensory attributes like color, structure, and astringency.[12] Additionally, these underripe grapes will impart an astringent or "unripe" character to the final wines, evoking the perception of an unripe fruit on the palate.[10] Torchio et al.[4] identified the 1073 kg m^{-3} density class as responsible for green and bitter wine notes. This class was prevalent in ND from LIM soils in 2021 (23%) and MAR soils in 2022 (22%), and in SY from MAR soils in 2022 (30%). It also appeared frequently in white grapes from LIM soils across both years. Cultivar-soil interaction influenced berry density. GR on MAR soils showed the highest densities and most uniform ripening, while SY and ND on LIM soils had lower densities and flatter distributions. Notably, ND:LIM showed positive kurtosis, indicating ripening uniformity despite low density, while CH:LIM had the lowest kurtosis, reflecting high ripening variability. These findings suggest that the influence of soil characteristics on grape ripening heterogeneity is cultivar-dependent and should be considered carefully in vineyard zoning and varietal selection strategies. Although phenotypic plasticity varies among cultivars, some studies suggest that autochthonous varieties like *Aglianico* are more influenced by environmental condition than the international varieties such as *Cabernet Sauvignon*.[27]

Grape's physicochemical parameters

The main physicochemical parameters of grapes, including reducing sugars content (g L^{-1}), titratable acidity (g L^{-1}) and pH values, are listed in Table 2. Among the cultivars, GR exhibited the highest reducing sugar concentration, followed by CH, ND, and SY. Although these differences were not statistically significant, they suggest a genotypic influence on sugar accumulation potential and ripening dynamics. The titratable acidity ranged from 5.9 to 8.0 g L^{-1} among cultivars, with ND

showing the highest acidity. The effect of cultivar on pH was statistically significant. CH had the highest pH, while GR and ND had lower values. Wine quality is largely dependent on acidity and pH, due to their role in modulating microbial activity, stabilizing color in red wines, and influencing sensitivity to oxidation. They also play a crucial role in the evolution of volatile organic compounds, as esters responsible for fruity aromas undergo faster hydrolysis under low-pH conditions, while glycosides aroma precursors bound to sugars are released through acid-catalyzed hydrolysis. In addition, acidity and pH significantly affect wine taste and mouthfeel.[28] Vintage had no significant influence on reducing sugars, acidity, or pH, indicating stability of compositional traits across 2021 and 2022 seasons under the studied conditions. Soil type, however, had a marginal effect on reducing sugars ($p < 0.1$), with grapes grown in MAR soils showing higher sugar concentrations than those in LIM soils. No significant differences were observed for titratable acidity or pH between soil types. A significant interaction effect was observed for pH, with the lowest values recorded in GR LIM and the highest in CH MAR. Although reducing sugars and acidity were not significantly affected by the interaction, it is noteworthy that GR in MAR soils reached the highest sugar content, suggesting a favorable interaction between this cultivar and that soil environment in terms of sugar accumulation. Suter et al.[29] demonstrated that sugar accumulation in grape berries is strongly driven by genetic background and genotype-by-environment interactions, with berry weight and veraison timing playing major roles alongside climatic factors such as temperature, radiation, and vine water status.

Phenolic compounds

Total flavonoids, non-anthocyanic flavonoids (NAF) and total anthocyanins

The phenolic composition of grape samples, including total flavonoids, non-anthocyanin flavonoids (NAF), the ratio of NAF between skins and seeds, and anthocyanin content (only for red cultivars) are showed in Table 3. As expected, red grape cultivars exhibited significantly higher contents of total flavonoids, non-anthocyanin flavonoids (NAF), and anthocyanins compared to white cultivars

(*CH* and *GR*), in which anthocyanins were absent. The ratio of non-anthocyanin flavonoids between skins and seeds also varied by cultivar, with Syrah showing the highest values (0.7 ± 0.1), indicating a greater contribution of skin tissues to the total NAF, compared to ND (0.58 ± 0.003). No significant effects were detected for vintage or soil when considered independently, although a slight trend toward higher total flavonoid and anthocyanin content was observed in the 2022 vintage and in grapes grown on MAR soils (396 mg kg^{-1}). Notably, significant differences emerged from the interaction between cultivar and soil. Red cultivars grown on MAR soils tended to accumulate more total flavonoids and anthocyanins compared to those on LIM soils. White cultivars, by contrast, showed no such soil-related differences in flavonoid composition. These results align with Pollon et al.[1] who observed that, MAR soils induced a higher concentration of phenolic compounds in the grapes, such as anthocyanins and non-anthocyanic flavonoids, compared to LIM soils. Ferrandino et al.[30] support in their study that soils with a finer texture, such as those rich in clay and silt, enhance the accumulation of anthocyanins and non-anthocyanic flavonoids in grape berries. This effect is linked to greater abscisic acid (ABA) synthesis, triggered by early drought signals perceived in fine-textured soils. These soils initially impose more water stress than coarse ones, stimulating ABA production without inducing severe stress. ABA, in turn, enhances sugar and anthocyanin accumulation by modulating berry metabolism.[30] Nevertheless, Bambina et al.[20] found no statistically significant correlation between soil texture components (sand, silt, and clay) and the anthocyanin content in *Nero d'Avola* wines, with the sole exception of cyanidin and silt, which showed a strong inverse relationship (Pearson's $r = -0.98$).

Hydroxycinnamoyl tartaric acid (HCTAs) and flavonols

HCTAs, including caftaric acid, coutaric acid and feftaric acid, and flavonols including quercetin-3-glucuronide, quercetin-3-glucoside and quercetin aglycon were detected in all the cultivars (Table 4). Caftaric acid is important because it is involved in the browning reaction of musts and wines since it

is the substrate of choice of the grape tyrosinase turning it into reactive quinones.[31] HCTAs vary in relation to the variety and the level of maturation of the grapes.[32] Moreover, these quinones exhibit a high reactivity toward nucleophilic attacks by sulfur dioxide and sulfur-containing aromatic compounds. This interaction results in musts and future wines with diminished levels of varietal aroma and reduced effectiveness of sulfur dioxide as a protective agent.[32] The most abundant flavonol was quercetin3-glucoside in GR, CH and SY, while in ND results to be the myricetin 3-glucoside. Flavonols play important roles in terms of quality, including acting as antioxidant and as co-pigments for anthocyanins in red wines.[33,34] In white wines as the grape skins are separated earlier from the must (in most cases), the time is insufficient for significant extraction of these compounds.[35] The higher concentrations of quercetin-3-glucoside observed in the 2021 vintage compared with 2022 can be attributed to the fact that the biosynthesis of this compound is strongly influenced by abiotic stresses, particularly solar radiation and drought conditions.[36] Specifically, the 2021 season was characterized by elevated temperatures and prolonged dry periods (ISPRA). Significant differences among cultivars were observed for several compounds. *ND* and *CH* exhibited the highest concentrations of caftaric acid (414 ± 125 mg kg^{-1} and 370 ± 87 mg kg^{-1}, respectively), with a statistically significant effect ($p < 0.05$). Although *SY* also showed a high mean value (275 ± 34 mg kg^{-1}), *GR* was significantly lower (215 ± 58 mg kg^{-1}). Myricetin3-glucoside was absent in white cultivars, while red cultivars showed substantial accumulation. SY displayed the highest content of myricetin3-glucoside (83 ± 23 mg kg^{-1}) and kaempferol3-glucoside (61 ± 16 mg kg^{-1}), followed by ND. Quercetin3-glucoside also varied significantly among cultivars, with SY showing the highest value (122 ± 28 mg kg^{-1}), significantly greater than both white varieties and ND (30 ± 11 mg kg^{-1}). Vintage had a significant influence on some compounds. Caftaric acid was significantly higher in 2021 (389 ± 67 mg kg^{-1}) compared to 2022 (248 ± 42 mg kg^{-1}; $p < 0.01$). In contrast, quercetin-3-glucoside was significantly higher in 2021 (77 ± 21 mg kg^{-1} vs. 40 ± 10 mg kg^{-1} in 2022; $p < 0.05$). No significant differences were observed between LIM and MAR soils for any compound. However, significant cultivar–soil interactions were detected for caftaric acid ($p < 0.01$), myricetin-3-glucoside,

and quercetin-3-glucoside, The highest concentration of caftaric acid was found in ND MAR (610 ± 85 mg kg^{-1}), followed by CH LIM (481 ± 99 mg kg^{-1}). Myricetin3-glucoside peaked in SY LIM (108 ± 30 mg kg^{-1}), with a notable drop in the same cultivar on MAR soil (58 ± 9 mg kg^{-1}). SY LIM also showed the highest quercetin3-glucoside (142 ± 31 mg kg^{-1}), reinforcing its potential for flavonol accumulation under specific soil conditions.

Technological variability index (TVI) and phenolic variability index (PVI)

The coefficients of variation reported in Table S17 were calculated for key technological parameters of oenological importance, as well as for major phenolic compounds, including hydroxycinnamic tartaric acid derivatives (HCTAs) and flavonols, throughout the ripening process (Tables S9–S16). Reducing sugars content and pH value increased with increasing apparent density. Conversely, total acidity, tartaric acid and malic acid decreased with maturation (Table S13). Skin total flavonoids and anthocyanins tended to increase during ripening and slightly decrease in the last density class, whereas seeds flavonoids decreased with increasing ripeness (Table S14). Most flavonoids (except anthocyanins) are synthesized from early berry development until veraison,[37] after which their concentrations generally remain stable or slightly decrease due to oxidative and thermal degradation.[38] The increase in total skin flavonoids observed here may therefore reflect greater extractability linked to histochemical modifications during ripening,[39] whereas in seeds lignification reduces extractability in riper grapes.[40] The coefficient of variation for the studied variables was calculated as the ratio between the standard deviation and the weighted mean obtained from the different density classes of each sample. The coefficient of variation (CV) is a statistical measure that represents the degree of relative variability of a dataset in relation to its mean. It is commonly used to compare the dispersion of variables with different scales or units of measurement, making it a valuable indicator in comparative analyses. The technological variability index (TVI) and phenolic variability index (PVI) indices were calculated from the sum of the coefficients of variation of basic

parameters and phenolic compounds, respectively (Table S17). The TVI and PVI indices are listened in Table 5. The TVI provides critical insights into the variability of grapes at the harvest (within the same vineyard) based on basic technological parameters (reducing sugars, pH, titratable acidity). Similarly, the PVI focuses on the variability of phenolic compounds (caftaric, coutaric and fertaric acid, quercetin 3-glucoside, skin NAF, seed NAF and total anthocyanins). The development of the TVI and the PVI provided an integrated and quantitative assessment of grape ripening heterogeneity, highlighting their effectiveness in capturing differences in technological and phenolic maturity among vineyard parcels. By analyzing these indexes, it becomes possible to assess grape heterogeneity at the harvest and how consistent or stable the traits of a grape variety are when cultivated under different conditions, such as different soil types. A low TVI or PVI value indicates a higher degree of homogeneity in the grapes ripening. This is beneficial for winemaking because uniformity in grape composition often translates into wine quality.[4] For instance, if grapes exhibit low variability at the harvest in sugar content, acidity, and phenolic compounds, the chemical composition and sensory profile of the resulting wine are less likely to be negatively affected.[27] The stability of these indices across vintages is equally important. If the TVI and PVI remain stable despite climatic differences between vintages, it demonstrates the plasticity of the variety. Plasticity refers to the ability of a grape variety to adapt to changing environmental conditions, such as variations in temperature, rainfall, or soil moisture.[9] This adaptability is a valuable trait, especially in the context of climate change, where weather patterns can become increasingly unpredictable.[12] Furthermore, the TVI and PVI value can also provide information about how specific grape varieties interact with different soil types. In Table 5 significant differences in TVI were observed among the four grape cultivars ($p < 0.05$). *SY* exhibited the highest TVI value (0.25 ± 0.02), followed closely by *CH* (0.20 ± 0.1) and GR (0.21 ± 0.03), while *ND* had the lowest value (0.14 ± 0.01), indicating a lower heterogeneity of ripening at the harvest. The maximum TPVI difference observed was 0.11, which does not represent a large variation. However, it still reflects a measurable difference in technological maturity. Even relatively small deviations in grape ripening can affect must composition and

influence harvest management. Higher TVI and PVI values indicate less homogeneous grape ripening, which can result in uneven distributions of sugars, acids, phenolic and aromatic compounds among berries. Such heterogeneity may, in turn, affect the balance between sugar and acidity, the degree of phenolic maturity, and the extractability of color and tannins during winemaking. Consequently, TVI provides not only a statistical measure of variability but also a practical indicator of potential differences in wine composition, structure, and sensory expression arising from vineyard heterogeneity. No statistically significant differences were found in PVI across cultivars, which ranged between 3 and 4. Soil type significantly influenced TVI ($p < 0.05$), with grapes grown on LIM soils exhibiting higher TVI values (0.23 ± 0.03) compared to those grown on MAR soils (0.18 ± 0.02). This suggests a more pronounced heterogeneity of grape ripening in LIM soils, although PVI remained statistically unaffected. These findings agree with Bigard et al,[6] and Rolle et al,[10] whose have investigated the effect of terroir on the variability of grapes sugars concentration by comparing berry technological parameters and revealing differences in berry flotation depending on the region. No significant differences were found between 2021 and 2022 vintage. A significant interaction was observed between cultivar and soil ($p < 0.01$) for TVI. The highest value was recorded in *SY* MAR (0.281 ± 0.001), followed by *CH* LIM (0.3 ± 0.1). Conversely, ND maintained low TVI values regardless of soil type. PVI values showed no significant interaction, remaining stable across combinations. While some studies suggest that international varieties are generally more resilient,[41] the present findings indicate that autochthonous grape varieties demonstrate greater adaptability to their native growing environments compared to international cultivars. This adaptability is reflected in the greater stability of both technological and phenolic parameters across diverse pedoclimatic conditions. Phenotypic plasticity is a key trait for grapevine adaptation to climate change, requiring a multi-trait approach to account for cultivar-specific responses to environmental variability.[29] Lower TVI and PVI values, indicating greater homogeneity of grapes in terms of technological parameters and phenolic compounds should correspond to higher kurtosis values and more positive skewness values of the berry distribution. These parameters suggest that berry distributions are more

homogeneous when shifted toward higher ripening stages. This trend is more pronounced in grapes grown on MAR soils compared to LIM soils, although the values observed for LIM are not excessively high and still allow to produce high-quality wines. Based on TVI and PVI scores, grape variability decreased progressively as berries advanced toward full ripening, indicating that harvesting too early, even when technological maturity is achieved, may lead to greater heterogeneity.[20] This trend can be explained by the fact that, under favorable climatic conditions supporting effective photosynthetic activity, growth-related genes are overexpressed in less mature berries, enabling them to reach the ripening stage of more advanced berries.[7] Consequently, a more homogeneous grape maturity is achieved by harvest. In contrast, prolonged periods of high temperatures and drought can inhibit photosynthetic activity, thereby reducing the homogeneity of grape ripening. Similarly, earlier ripening, which shifts the ripening period to a time of the season when climatic conditions are less favorable for high-quality wine production, can hinder the uniform development of berries and result in increased heterogeneity at harvest.[41] Variability in sugar accumulation, acidity, and phenolic composition within the same vineyard parcel can lead to differences in the resulting wine quality and style outcomes.[9,27] Quantifying grape variability through composite indices like TVI and PVI provides an important tool for optimizing harvest timing and tailoring winemaking practices. Monitoring the stability or variability of these indices over time is therefore essential to better understand the interaction between grapevine genotype, soil, and environmental conditions. The TVI and PVI indexes, are a strategic tool not only to supports informed decision-making but also to provide a foundation for interventions aimed at improving ripening uniformity over time. Furthermore, integrating these indexes into predictive models and databases that incorporate climate conditions would enhance their applicability, enabling growers to anticipate the impact of environmental variability on grape composition. Such an approach could ultimately improve harvest timing, optimize winemaking practices, and contribute to the development of decision-support systems for viticulture under changing climate scenarios.

Soil-Grape's relationship

Soil plays a key role among the environmental factors that affect grapevine development and determine grape and wine quality.[42] Figure 3 shows the correlation heatmap between soil chemical-physical parameters and some compositional characteristics of CH, GR, ND and SY grapes grown on limestone (A) and marlstone (B) soils. The correlation heatmap A) reports the correlation patterns existing between soils's chemical-physical parameters (Table S1-S8) and the most important grapes's characteristics. In the figures, only significant correlations (*Pearson's* r values >|0.80| and $p < 0.05$) were displayed with a number. Although certain similarities exist between soil parameters and grape compositional variables, notable differences emerge in their respective correlations. In LIM soils, sand and clay exhibit positive correlations with total flavonoids and pH, whereas silt, total limestone, organic matter, and active lime are negatively correlated with these variables. A similar pattern is shown in marlstone soils concerning the correlation between soil physicochemical properties and total flavonoids and pH, except for clay and organic matter, which show opposite trends. However, Bambina et al.[20] did not observe any significant correlation between soil texture parameters, namely clay, silt, and sand, and the phenolic composition of *Nero* d'*Avola* grapes. In Ferrandino et al.[30] findings on *Nebbiolo* cultivar, increased concentrations of phenolic compounds were attributed to higher levels of abscisic acid (ABA), stimulated by finer soil particles such as clay and silt compared to coarser particles like sand. Notably, several soil parameters in marlstone soils also show significant correlations with the TVI value. Specifically, sand, and organic matter are negatively correlated with TVI, while clay, silt, total limestone, and active lime show positive correlations. Several researches have demonstrated that the physical and chemical properties of soil play a critical role in influencing vine growth, grape ripening, and the compositional attributes of the resulting wine[20,43]. The proportions of sand, silt, and clay in the soil affect its ability to retain water, exchange cations, allow root penetration, and regulate root temperature. Compared to coarser particles like sand, fine-textured soils tend to induce greater initial water stress due to their structure, while at later phenological stages,

the water stress imposed by coarse-textured soils becomes more severe, as they possess a lower capacity for water retention. This dynamic soil-plant interaction underlines the importance of soil texture in modulating stress responses and secondary metabolite accumulation in grapevines.[44] The differential progression of soil water potential across varying soil textures suggests that vines rooted in fine-textured substrates can perceive drought signals even under moderate soil moisture conditions. This perception, occurring prior to the onset of severe water stress, promotes increased accumulation of abscisic acid (ABA). This phytohormone has been shown to enhance the accumulation of sugars and phenolic compounds in grape berries by modulating key metabolic pathways.[44]

Multivariate statistical analysis

To explore the effect of soil type (LIM and MAR) on grape composition, a supervised Partial Least Squares Discriminant Analysis (PLS-DA) was performed for white and red grape cultivars, separately, allowing the visualization of spatial separation between samples based on their overall chemical composition. The PLS-DA score plot (Figure 4A) clearly distinguishes white grape samples (CH and GR) according to soil type, with distinct clustering observed for LIM and MAR groups. This separation indicates that soil type exerted a strong influence on the compositional traits of the grapes. The PLS-DA analysis reduced the number of original variables into 3 components that explained the 86.4 % of the total variance of the dataset. The corresponding VIP plot (Figure 4B) identified the most influential variables contributing to group discrimination. The colored boxes on the right of the plot indicate the relative concentrations of the metabolites in soil type. Even for the red cultivars, PLS-DA analysis reduced the number of original variables into 3 components, explaining the 97.7 % of the variance in the dataset. The Scores Plot shown in Fig. 4 A displayed two different clusters indicating the effect of soil type on the composition of ND and SY samples. According to the VIP scores in Figure 4B (white grape varieties), TVI and PVI emerged as key variables driving the separation between soil types. These indices were higher in grapes grown on LIM soils, while MAR

soils were characterized by higher values of skewness, pH, and phenolic compounds such as quercetin. In contrast, the VIP scores in Figure 5B (red grape varieties) reveal a different soil-dependent behavior. Grapes grown on LIM soils exhibited higher pH and kurtosis values, whereas those from MAR soils were associated with greater skewness, higher TVI and PVI indices, and increased concentrations of phenolic compounds including anthocyanins and quercetin. These findings underscore the strong interaction between genotype and soil type, highlighting how soil chemical-physical parameters can differentially influence ripening dynamics and phenolic composition in white and red grape cultivars, conceivably due to the its influence on water availability, nutrient dynamics, and vine vigor throughout the growing season.[20] To prevent data overfitting with PLS-DA and to ensure the reliability of the analysis, permutation test and cross validation were applied (Figure S5 and Figure S6 of the Supplementary Materials). The result of cross validation test (Figure S5 and Figure S6 A) confirmed the significance and the predictability of the model, in white cultivars (accuracy = 1; R2 = 1; Q2 = 1) and in red cultivars (accuracy = 1; R2 = 0.9; Q2 = 0.9). The empirical *p*-values yielded from the permutation tests (n = 1000) were 0.001 (Figure S5 and Figure S6 B).

Conclusion

This study highlights the predominant role of soil type in shaping the physicochemical and phenolic characteristics of white (*Chardonnay* and *Grillo)* and red (*Nero d'Avola* and *Syrah)* cultivars cultivated on different calcareous soils (limestone and marlstone) in southwestern Sicily. As a summary tool to describe overall grape heterogeneity, the technological parameter variability index (TVI) and the phenolic variability index (PVI) were proposed. The TVI is based on the sum of the coefficients of variation was calculated across different berry density classes for reducing sugars, pH, and titratable acidity, whereas the PVI aggregates the coefficients of variation for caftaric acid, coutaric acid, feftaric acid, quercetin-3-glucoside, skin NAF, seed NAF, and anthocyanins. Both

indices proved to be effective tools for quantifying grape variability across environmental and varietal factors, providing valuable insights for vineyard and winemaking management, as well as for quality prediction. Grapes from marlstone soils exhibited lower TVI values and a more uniform berry maturation, as indicated by higher kurtosis and more positive skewness indices, suggesting that variability decreased throughout ripening as berries reached higher maturity. These findings highlight that harvesting fruit too early, even if technologically mature, may result in a higher degree of grape heterogeneity. Conversely, grapes grown on limestone soils were associated with greater technological variability and higher PVI values. Sicilian varieties, both white and red, exhibited lower ripening heterogeneity compared to international cultivars across the different calcareous soils, suggesting a greater adaptability and a more uniform maturation process in response to their native pedoclimatic conditions. Multivariate statistical analyses further confirmed the dominant influence of soil in differentiating grape composition. Overall, these findings demonstrate that the TVI and PVI indices prove to be effective tools for supporting precision viticulture, optimizing harvest timing, and guiding winemaking strategies in the face of increasing climatic variability. However, the study is subject to certain limitations, particularly the relatively small number of vineyards and grape varieties sampled. Future studies should therefore extend this approach to a wider range of cultivars, and wine-growing regions in order to better assess the role of soil type in grape ripening variability, and consequently on the chemical and sensory properties of the resulting wine.

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Funding information

This study was founded by “Programma di Sviluppo Rurale Sicilia 2014-2020”, Misura16 - Sottomisura 16.1 Sostegno per la costituzione e la gestione dei gruppi operativi del PEI in materia di produttività e sostenibilità dell'agricoltura' (Bando 2018), project title: Tecniche viticole ed enologiche innovative e sostenibili per la valorizzazione dei suoli calcarei (Acronym: V.E.I.S.C.A.), CUP B93C22001440005.

Conflict of interest statement

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Acknowledgements

The authors express their gratitude to Settesoli Winery (Menfi, Agrigento), and Domenico De Gregorio, Francesco Di Carlo and Filippo Buttafuoco for their availability and valuable support.

Abbreviations

CH = *Chardonnay*

GR = *Grillo*

ND = *Nero d'Avola*

SY = *Syrah*

LIM = Limestone

MAR = Marlstone

Table 1 Statistic parameters describing the distribution of the density classes as a function of variety, soil type, and vintage.

Factor	mean	standard deviation	median	skewness	kurtosis
Cultivar (n=4)					
CH	1.097.31 ab	15.86 a	1100.6	0.02509589	-0.7505082
GR	1.097.78 a	14.46 a	1100.6	0.00166542	-0.7844814
ND	1.089.89 b	10.01 b	1087.9	-0.1981804	-0.2179848
SY	1.091.54 ab	11.84 ab	1091.075	-0.1346895	-0.3531417
Sign.	**	***	ns	ns	ns
Year (n=8)					
2021	1094.28632	12.8289342	1094.25	-0.0245085	-0.6474532
2022	1093.97338	13.2549825	1095.8375	-0.1285458	-0.4056049

Sign.	ns	Ns	ns	ns	ns
Soil (n=8)					
LIM	1092.15094	14.2179751	1092.6625	-0.1706201	-0.5580062
MAR	1096.10876	11.8659415	1097.425	0.01756578	-0.4950519
Sign.	*	**	**	*	ns
Cultivar:Soil (n=2)					
CH:LIM	1.097.66 ab	19.27 a	1.100.60 ab	0.05	-1.19 b
GR:LIM	1.091.43 b	17.02 ab	1.094.25 bc	-0.14	-0.90 ab
ND:LIM	1.091.22 b	10.33 c	1.087.90 c	-0.44	0.23 a
SY:LIM	1.088.30 b	10.26 c	1.087.90 c	-0.16	-0.38 ab
CH:MAR	1.096.96 ab	12.45 bc	1.100.60 ab	0.00	-0.31 ab
GR:MAR	1.104.14 a	11.89 c	1.106.95 a	0.14	-0.67 ab
ND:MAR	1.088.56 b	9.70 c	1.087.90 c	0.04	-0.67 ab
SY:MAR	1.094.77 ab	13.42 bc	1.094.25 bc	-0.11	-0.33 ab
Sign.	**	***	*	ns	*

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). CH= *Chardonnay*; GR= *Grillo*; ND= *Nero d'Avola*; SY= *Syrah*; LIM=Limestone; MAR= Marlstone.

Table 2 Wine physical chemical composition

Factor	Reducing sugars g L^{-1}	Titratable acidity g L^{-1}	pH
Cultivar (n=4)			
CH	225 ± 8	6 ± 1	3.5 ± 0.1 a
GR	241 ± 13	7.5 ± 0.4	3.2 ± 0.1 ab
ND	213 ± 6	8 ± 1	3.30 ± 0.04 bc
SY	208 ± 10	5.9 ± 0.3	3.4 ± 0.1 ab
Sign.	.	.	*
Year (n=8)			
2021	227 ± 7	6.9 ± 0.5	3.4 ± 0.1
2022	217 ± 9	6.9 ± 0.4	3.4 ± 0.1
Sign.	Ns	ns	ns
Soil (n=8)			
LIM	214 ± 6	6.5 ± 0.4	3.4 ± 0.1
MAR	230 ± 9	7.2 ± 0.5	3.4 ± 0.1

Sign.	.	ns	ns
Cultivar:Soil (n=2)			
CH:LIM	229 ± 7	6 ± 1	3.4 ± 0.1 ab
GR:LIM	219 ± 5	7.7 ± 0.2	3.100 ± 0.001 c
ND:LIM	212 ± 6	6.9 ± 0.3	3.36 ± 0.01 bc
SY:LIM	194 ± 5	5.7 ± 0.1	3.55 ± 0.02 ab
CH:MAR	221 ± 12	7 ± 1	3.67 ± 0.01 ab
GR:MAR	262 ± 5	7 ± 1	3.3 ± 0.1 a
ND:MAR	214 ± 9	8.6 ± 0.3	3.23 ± 0.02 c
SY:MAR	222 ± 10	6.0 ± 0.5	3.32 ± 0.02 c
Sign.	Ns	ns	**

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). CH= *Chardonnay*; GR= *Grillo*; ND= *Nero d'Avola*; SY= *Syrah*; LIM=Limestone; MAR= Marlstone.

Table 3 Wine polyphenols content

Factor	FT mg kg^{-1}	NAF mg kg^{-1}	NAF skin/seeds	Anthocyanins mg kg^{-1}
Cultivar (n=4)				
CH	3.919 ± 246 ab	3.919 ± 246 a	0.25 ± 0.03 b	/
GR	2.177 ± 136 b	2.177 ± 136 b	0.27 ± 0.02 b	/
ND	5.365 ± 580 a	4.471 ± 455 a	0.58 ± 0.03 ab	623 ± 102 b
SY	5.051 ± 295 a	3.785 ± 253 a	0.7 ± 0.1 a	846 ± 23 a
Sign.	**	**	**	***
Year (n=8)				
2021	3.875 ± 355	3.401 ± 269	0.5 ± 0.1	325 ± 131
s	4.381 ± 639	3.775 ± 448	0.4 ± 0.0	409 ± 158
Sign.	ns	ns	ns	ns
Soil (n=8)				
LIM	4.197 ± 475	3.687 ± 353	0.4 ± 0.1	338 ± 135
MAR	4.059 ± 570	3.488 ± 395	0.5 ± 0.1	396 ± 156

Sign.	ns	ns	ns	ns
Cultivar:Soil (n=2)				
CH:LIM	4.295 ± 192 ab	4.295 ± 192	0.236 ± 0.004	/
GR:LIM	2.208 ± 215 b	2.208 ± 215	0.25 ± 0.02	/
ND:LIM	5.147 ± 215 ab	4.374 ± 128	0.572 ± 0.004	531 ± 60 b
SY:LIM	5.139 ± 397 ab	3.874 ± 337	0.6 ± 0.1	822 ± 8 a
CH:MAR	3.544 ± 54 ab	3.544 ± 54	0.27 ± 0.04	/
GR:MAR	2.146 ± 92 b	2.146 ± 92	0.28 ± 0.03	/
ND:MAR	5.583 ± 956 a	4.569 ± 772	0.6 ± 0.1	714 ± 139 a
SY:MAR	4.963 ± 310 ab	3.695 ± 266	0.7 ± 0.2	871 ± 30 a
Sign.	*	ns	ns	*

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). CH= *Chardonnay*; GR= *Grillo*; ND= *Nero d'Avola*; SY= *Syrah*; LIM=Limestone; MAR= Marlstone. FT= total flavonoids, NAF= non-anthocyanic flavonoids.

Table 4 Hydrocinnamoyltartaric acids (HCTAs) and flavonols

Factor	Caftaric acid	Coutaric acid	Feftaric acid	Myricetin 3-glucoside	Kaempferol 3-glucoside	Quercetin 3-glucuronide	Quercetin 3-glucoside
Cultivar (n=4)							
CH	370 ± 87 ab	231 ± 76	18 ± 3	0 ± 0 b	9 ± 1 b	27 ± 15	50 ± 10 b
GR	215 ± 58 b	81 ± 21	18 ± 5	0 ± 0 b	8 ± 3 b	14 ± 9	34 ± 9 b
ND	414 ± 125 a	52 ± 16	77 ± 41	62 ± 17 a	9 ± 4 b	32 ± 9	30 ± 11 b
SY	275 ± 34 ab	151 ± 54	178 ± 92	83 ± 23 a	61 ± 16 a	56 ± 8	122 ± 28 a
Sign.	*	ns	Ns	**	**	ns	**
Year (n=8)							
2021	389 ± 67	123 ± 47	127 ± 50	43 ± 20	29 ± 13	26 ± 11	77 ± 21
2022	248 ± 42	135 ± 33	19 ± 4	29 ± 12	15 ± 6	39 ± 6	40 ± 10
Sign.	**	ns	.	ns	.	ns	*
Soil (n=8)							
LIM	320 ± 53	158 ± 52	70 ± 45	35 ± 19	24 ± 13	27 ± 9	61 ± 21
MAR	317 ± 70	100 ± 21	76 ± 37	37 ± 15	20 ± 6	38 ± 9	57 ± 13
Sign.	ns	ns	Ns	ns	ns	ns	ns
Cultivar:Soil (n=2)							

CH:LIM	481 ± 99 ab	342 ± 68	20 ± 4	0 ± 0	8.2 ± 0.4	23 ± 16	58 ± 16
GR:LIM	268 ± 84 bc	98 ± 31	21 ± 9	0 ± 0	4 ± 1	9 ± 6	30 ± 13
ND:LIM	218 ± 32 bc	36 ± 3	34 ± 17	33 ± 3	3.8 ± 0.2	17 ± 1	13 ± 2
SY:LIM	311 ± 41 bc	156 ± 74	203 ± 127	108 ± 30	78 ± 20	59 ± 4	142 ± 31
CH:MAR	259 ± 21 bc	120 ± 16	17 ± 0	0 ± 0	10.5 ± 0.4	31 ± 21	41 ± 1
GR:MAR	161 ± 18 c	65 ± 8	15 ± 2	0 ± 0	12 ± 2	19 ± 13	38 ± 9
ND:MAR	610 ± 85 a	69 ± 22	120 ± 54	91 ± 5	14 ± 4	48 ± 3	47 ± 6
SY:MAR	239 ± 19 bc	147 ± 56	153 ± 93	58 ± 9	44 ± 10	54 ± 13	101 ± 31
Sign.	**	ns	Ns	.	ns	ns	.

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). CH= *Chardonnay*; GR= *Grillo*; ND= *Nero d'Avola*; SY= *Syrah*; LIM=Limestone; MAR= Marlstone. HCTAs and flavonols are espressed in mg kg^{-1}.

Table 5 Technological variability index (TVI) and phenolic variability index (PVI)

Factor	TVI	PVI
Cultivar (n=4)		
CH	0.2 ± 0.1 ab	4 ± 1
GR	0.21 ± 0.03 bc	3 ± 1
ND	0.14 ± 0.01 c	4 ± 1
SY	0.25 ± 0.02 a	3.7 ± 0.3
Sign	*	ns
Year (n=8)		
2021	0.22 ± 0.03	4 ± 1
2022	0.19 ± 0.02	3.6 ± 0.3
Sign	ns	ns
Soil (n=8)		
LIM	0.23 ± 0.03	4.1 ± 0.3
MAR	0.18 ± 0.02	3.4 ± 0.5
Sign	*	ns

Cultivar:Soil (n=2)		
CH:LIM	0.3 ± 0.1 ab	5 ± 2
GR:LIM	0.25 ± 0.03 abc	3.9 ± 0.2
ND:LIM	0.13 ± 0.02 c	4 ± 1
SY:LIM	0.22 ± 0.05 ab	4 ± 1
CH:MAR	0.12 ± 0.01 bc	4 ± 2
GR:MAR	0.16 ± 0.03 abc	2 ± 1
ND:MAR	0.15 ± 0.04 c	4 ± 2
SY:MAR	0.281 ± 0.001 a	4 ± 1
Sign	**	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). CH= *Chardonnay*; GR= *Grillo*; ND= *Nero d'Avola*; SY= *Syrah*; LIM=Limestone; MAR= Marlstone.

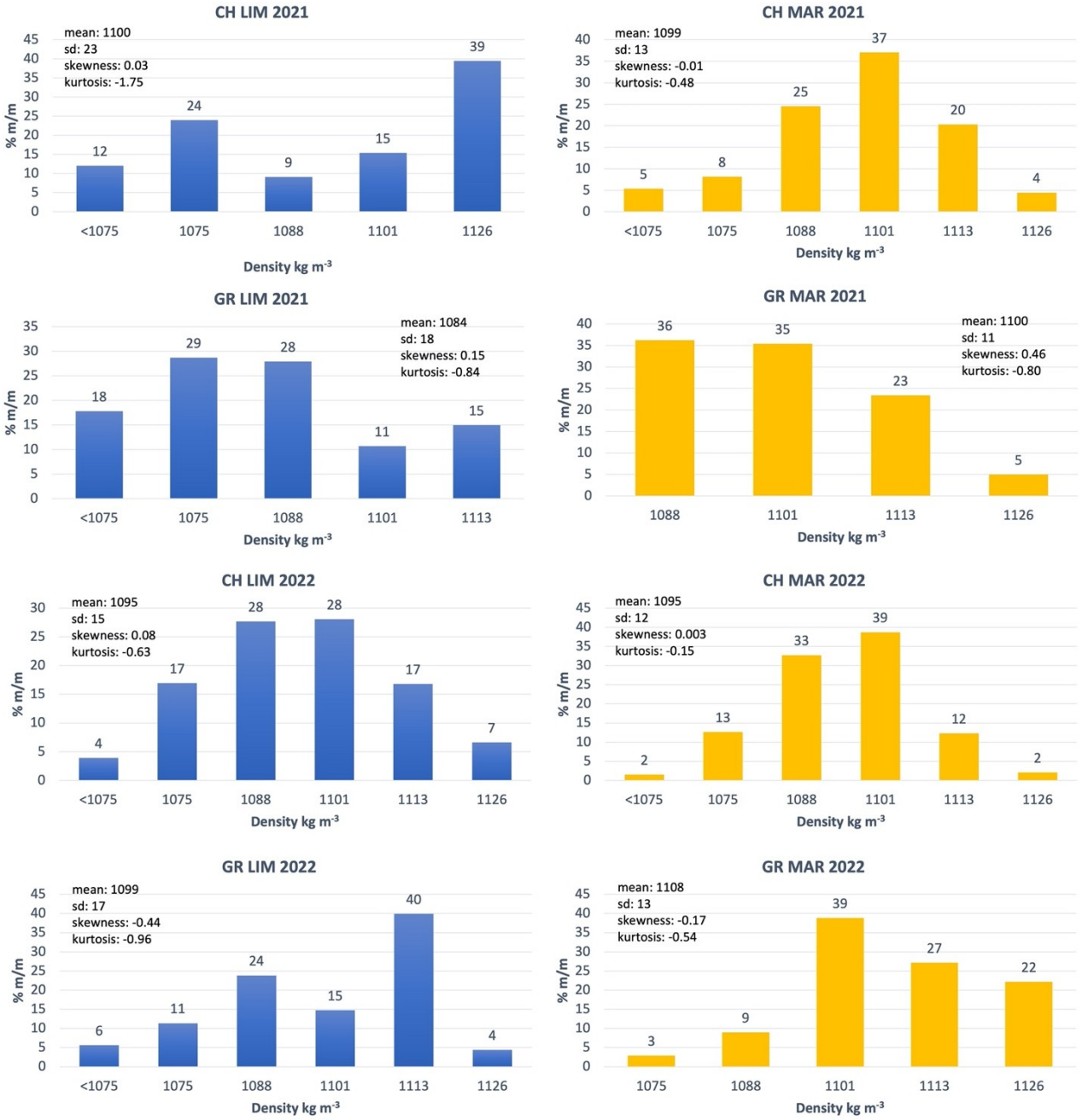

Figure 1 Density classes distributions of Chardonnay and Grillo grapes grown on different calcareous soils in 2021 and 2022 vintages. CH LIM = Chardonnay on limestone; CH MAR = Chardonnay on marlstone; GR LIM = Grillo on limestone; GR MAR = Grillo on marlstone.

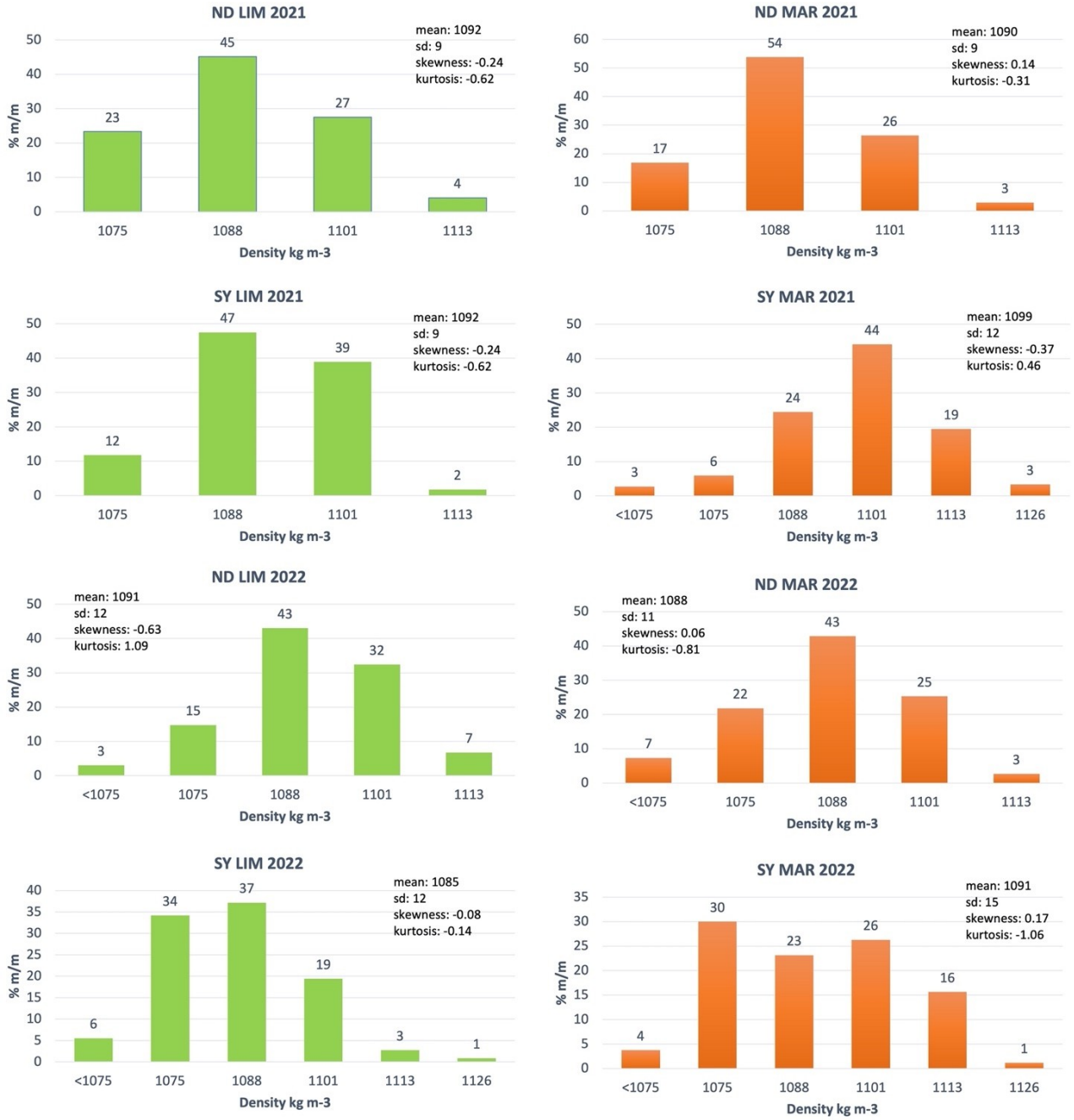

Figure 2 Density classes distributions of *Nero d'Avola* and *Syrah* grapes grown on different calcareous soils in 2021 and 2022 vintages. ND LIM = *Nero d'Avola* on limestone; ND MAR = *Nero d'Avola* on marlstone; SY LIM = *Syrah* on limestone; SY MAR = *Syrah* on marlstone.

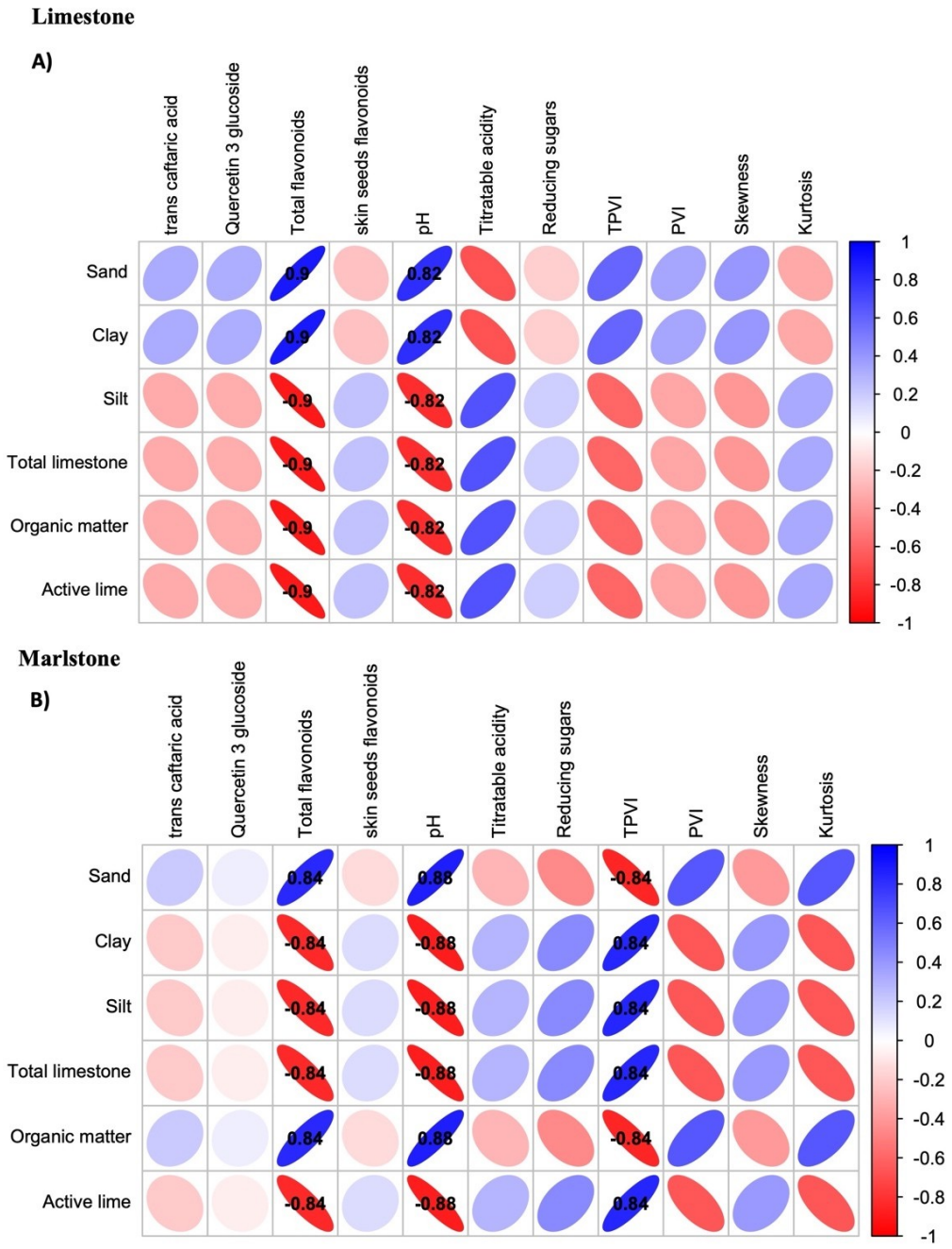

Figure 3 Correlation heatmap between soil chemical-physical parameters and the compositional characteristics of *Chardonnay, Grillo, Nero d'Avola* and *Syrah* grapes grown on limestone (A) and marlstone (B) soils. Correlations are represented using a color scale ranging from red (negative correlation) to blue (positive correlation), with color intensity proportional to the strength of the correlation. Only significant correlations with *Pearson's* r values > |0.80| and $p < 0.05$, were displayed in heatmaps.

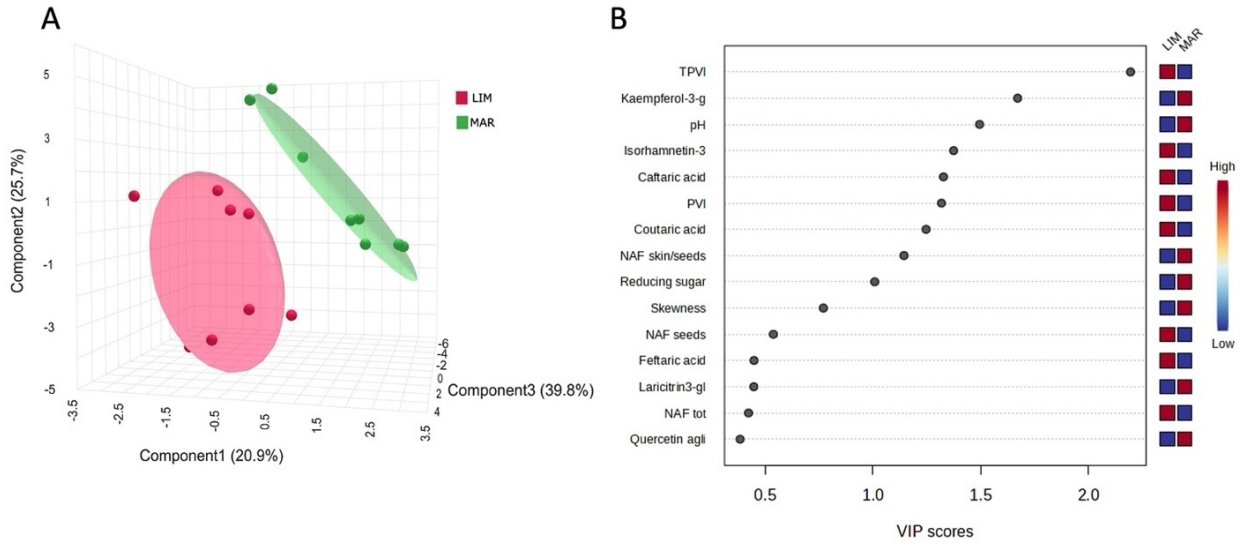

Figure 4 3D PLS-DA score plot showing the separation of grape samples based on soil type: LIM (limestone) in purple and MAR (marlstone) in green. The analysis includes the white grape cultivars (*Chardonnay* and *Grillo*). (B) Variable Importance in Projection (VIP) scores highlighting the most discriminant variables contributing to the separation between soil types

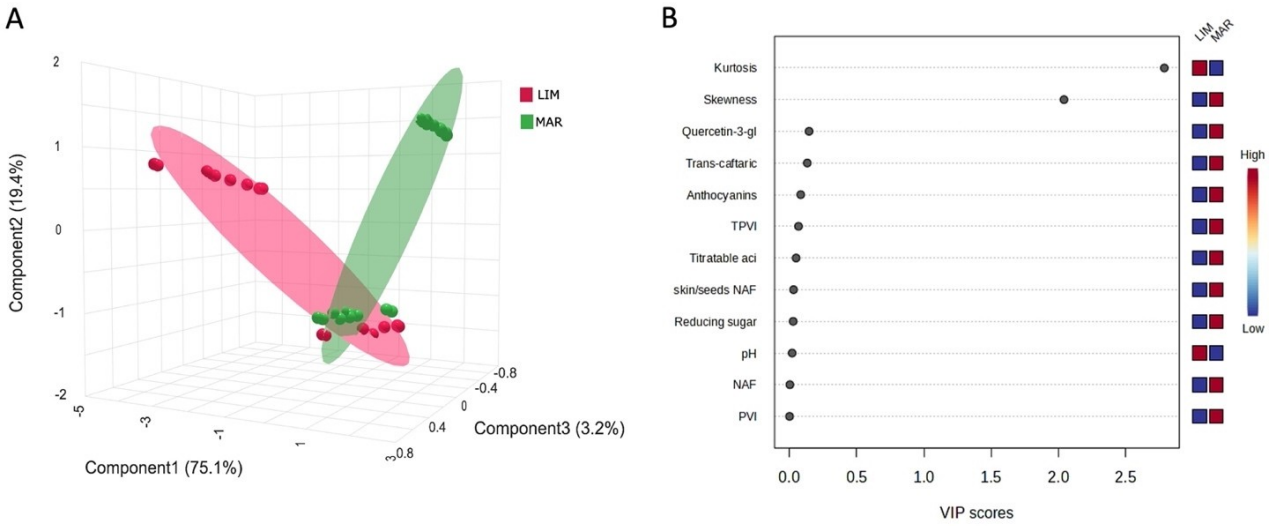

Figure 5 3D PLS-DA score plot showing the separation of grape samples based on soil type: LIM (limestone) in purple and MAR (marlstone) in green. The analysis includes the red grape cultivars (*Nero d'Avola* and *Syrah*). (B) Variable Importance in Projection (VIP) scores highlighting the most discriminant variables contributing to the separation between soil types

Supplementary materials

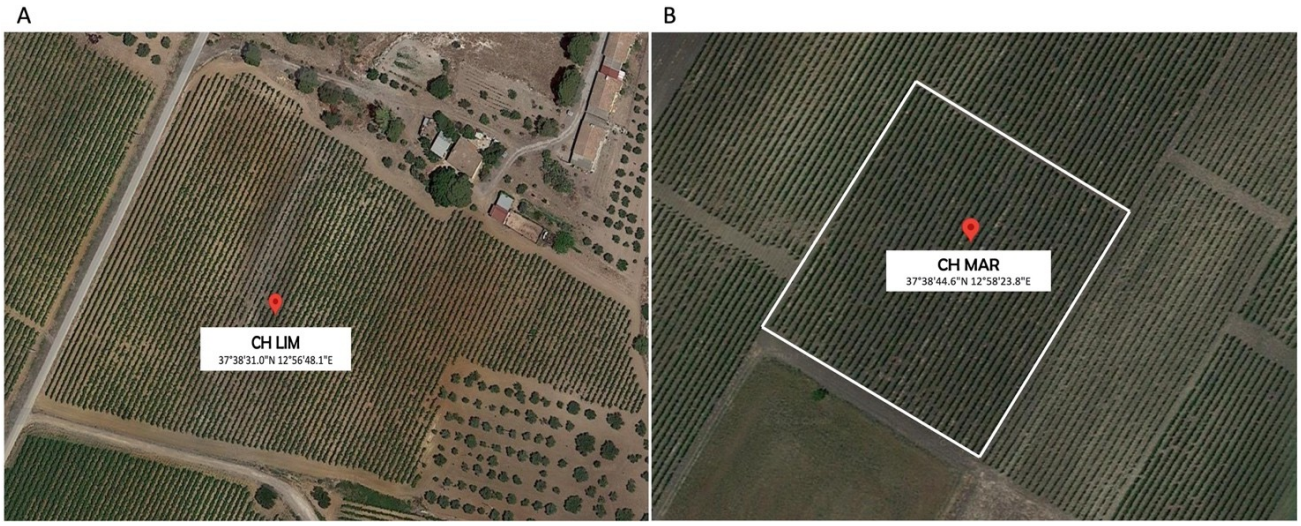

Figure S1 Satellite images of the two vineyard parcels used in the study for *Chardonnay* cultivar: limestone soil (CH LIM) (A) and marlstone soil (CH MAR) (B), both located in southwestern Sicily. The coordinates of each vineyard are indicated.

Figure S2 Satellite images of the two vineyard parcels used in the study for *Grillo* cultivar: limestone soil (GR LIM) (A) and marlstone soil (GR MAR) (B), both located in southwestern Sicily. The coordinates of each vineyard are indicated.

Figure S3 Satellite images of the two vineyard parcels used in the study for *Nero d'Avola* cultivar: limestone soil (ND LIM) (A) and marlstone soil (ND MAR) (B), both located in southwestern Sicily. The coordinates of each vineyard are indicated.

Figure S4 Satellite images of the two vineyard parcels used in the study for *Syrah* cultivar: limestone soil (SY LIM) (A) and marlstone soil (SY MAR) (B), both located in southwestern Sicily. The coordinates of each vineyard are indicated.

Table S6 Chemical and physical parameters of the limestone soil (CH LIM) where *Chardonnay* vines are cultivated.

CH LIM						
Soil depth (cm)	Total limestone (%)	Organic matter (%)	Active lime (%)	Sand (%)	Clay (%)	Silt (%)
20	13.7	1.3	2	61	31	8
40	10.2	1.11	1.25	/	/	/
60	18	0.73	2.75	/	/	/
80	28.2	0.31	8.75	/	/	/

Table S7 Chemical and physical parameters of the marlstone soil (CH MAR) where *Chardonnay* vines are cultivated.

CH MAR						
Soil depth (cm)	Total limestone (%)	Organic matter (%)	Active lime (%)	Sand (%)	Clay (%)	Silt (%)
20	24.1	3.69	12.75	35	36	29
40	24.5	2.89	16.25	/	/	/
60	24.5	2.18	14.25	/	/	/
80	24.9	1.72	14.75	/	/	/
100	24.1	1.68	14.75	/	/	/

Table S8 Chemical and physical parameters of the limestone soil (GR LIM) where *Grillo* vines are cultivated.

GR LIM						
Soil depth (cm)	Total limestone (%)	Organic matter (%)	Active lime (%)	Sand (%)	Clay (%)	Silt (%)
20	51.1	2.02	17.25	59	21	20
40	56.9	1.52	17.25	/	/	/
60	48.6	2.28	19.75	/	/	/
80	61.9	0.61	20.15	/	/	/

Table S9 Chemical and physical parameters of the marlstone soil (GR MAR) where *Grillo* vines are cultivated.

GR MAR						
Soil depth (cm)	Total limestone (%)	Organic matter (%)	Active lime (%)	Sand (%)	Clay (%)	Silt (%)
20	38.2	1.72	10	18	51	31
40	37.4	1.3	15	/	/	/
60	40.3	0.96	15.75	/	/	/
80	41.1	0.57	16	/	/	/
100	41.5	1.72	16.2	/	/	/

Table S10 Chemical and physical parameters of the limestone soil (ND LIM) where *Nero d'Avola* vines are cultivated.

ND LIM						
Soil depth (cm)	Total limestone (%)	Organic matter (%)	Active lime (%)	Sand (%)	Clay (%)	Silt (%)
20	20.7	3.1	4	28.1	28.5	43.4
40	23.7	3.3	4.3	/	/	/
60	18	3.4	4.2	/	/	/

Table S11 Chemical and physical parameters of the marlstone soil (ND MAR) where *Nero d'Avola* vines are cultivated.

ND MAR						
Soil depth (cm)	Total limestone (%)	Organic matter (%)	Active lime (%)	Sand (%)	Clay (%)	Silt (%)
20	17.9	2.9	9.8	20.96	43.5	35..54
40	16.6	2.1	8.3	/	/	/
60	17.4	1.9	9.6	/	/	/
80	18.7	2.3	9.9	/	/	/

Table S12 Chemical and physical parameters of the limestone soil (SY LIM) where *Syrah* vines are cultivated.

SY LIM						
Soil depth (cm)	Total limestone (%)	Organic matter (%)	Active lime (%)	Sand (%)	Clay (%)	Silt (%)
20	20	4.4	3	34.4	38.5	27.1
40	21.1	3.9	7.8	/	/	/
60	22.1	3.7	5	/	/	/

Table S13 Chemical and physical parameters of the marlstone soil (SY MAR) where *Syrah* vines are cultivated.

SY MAR						
Soil depth (cm)	Total limestone (%)	Organic matter (%)	Active lime (%)	Sand (%)	Clay (%)	Silt (%)
20	56.1	2.5	14.75	34.4	46	20
40	59.8	1.37	15.25	/	/	/
60	66	0.84	18.75	/	/	/
80	69.8	0.08	21	/	/	/
100	60.2	0.42	19.75	/	/	/

Table S9 Technological parameters content during ripening of *Chardonnay* and *Grillo* cultivar grown on limestone and marlstone soils in 2021 and 2022 vintages.

Vintage 2021					
CH LIM					
	Density class	**% m/m**	**Reducing sugars g L^{-1}**	**Titratable acidity g L^{-1}**	**pH**
I	1071	12	148 ± 5	7 ± 1	3.48 ± 0.01
II	1075	24	160 ± 10	5.0 ± 0.3	3.49 ± 0.02
III	1088	9	173 ± 8	4.8 ± 0.4	3.54 ± 0.03
IV	1101	15	204 ± 7	4.3 ± 0.5	3.53 ± 0.02
V	1126	39	220 ± 4	4.4 ± 0.2	3.52 ± 0.03
CH MAR					
I	1075	8	212 ± 4	8 ± 1	3.73 ± 0.02
II	1088	25	225 ± 3	8 ± 1	3.68 ± 0.02
III	1101	37	241 ± 9	8 ± 1	3.65 ± 0.02
IV	1113	20	248 ± 7	7 ± 1	3.71 ± 0.00
V	1126	4	271 ± 8	6.9 ± 0.5	3.77 ± 0.01
GR LIM					
I	1075	23	154 ± 3	8 ± 1	2.96 ± 0.00
II	1088	28	194 ± 10	7.1 ± 0.2	3.10 ± 0.02
III	1101	11	222 ± 9	7.0 ± 0.2	3.13 ± 0.01
IV	1113	15	250 ± 4	7.8 ± 0.1	3.09 ± 0.02
V	1088	/	279 ± 4	7.4 ± 0.3	3.22 ± 0.03
GR MAR					
I	1075	/	/	/	/
II	1088	36	226 ± 5	8.4 ± 0.3	3.27 ± 0.02
III	1101	35	254 ± 6	8.8 ± 1.1	3.17 ± 0.03
IV	1113	23	287 ± 6	8.1 ± 0.3	3.31 ± 0.01
V	1126	5	331 ± 5	7.7 ± 0.3	3.35 ± 0.01

Vintage 2022					
CH LIM					
	Density class	**% m/m**	**Reducing sugars g L^{-1}**	**Titratable acidity g L^{-1}**	**pH**
I	1075	17	173 ± 7	7.1 ± 0.5	3.27 ± 0.01
II	1088	28	203 ± 4	6.9 ± 0.2	3.30 ± 0.01
III	1101	28	231 ± 5	7 ± 1	3.21 ± 0.03
IV	1113	17	257 ± 5	6.1 ± 0.2	3.30 ± 0.03
V	1126	7	303 ± 6	6 ± 1	3.46 ± 0.02
CH MAR					
I	1075	13	176 ± 8	6 ± 1	3.56 ± 0.03
II	1088	33	192 ± 4	6.2 ± 0.2	3.67 ± 0.03
III	1101	39	214 ± 6	6.2 ± 0.1	3.66 ± 0.01
IV	1113	12	233 ± 7	6 ± 1	3.70 ± 0.03
V	1126	2	261 ± 3	6 ± 1	3.73 ± 0.02
GR LIM					
I	1075	11	173 ± 7	8 ± 1	3.08 ± 0.03
II	1088	24	213 ± 4	8 ± 1	3.10 ± 0.03
III	1101	15	239 ± 3	7.9 ± 0.2	3.09 ± 0.01
IV	1113	40	246 ± 10	7.7 ± 0.3	3.12 ± 0.01
V	1126	4	294 ± 10	7.6 ± 0.2	3.18 ± 0.01
GR MAR					
I	1075	3	203 ± 5	7 ± 1	3.24 ± 0.03
II	1088	9	224 ± 3	6.4 ± 0.3	3.29 ± 0.03
III	1101	39	253 ± 8	6.4 ± 0.1	3.42 ± 0.00
IV	1113	27	277 ± 6	6.2 ± 0.3	3.45 ± 0.02
V	1126	22	311 ± 3	6.2 ± 0.3	3.43 ± 0.02

Note: Data are reported as mean plus and minus standard error of the mean. CH LIM = *Chardonnay* on limestone; CH MAR = *Chardonnay* on marlstone; GR LIM = *Grillo* on limestone; GR MAR = *Grillo* on marlstone.

Table S10 Skin and seeds total flavonoids content during ripening of *Chardonnay* and *Grillo* cultivar grown on limestone and marlstone soils in 2021 and 2022 vintages.

Vintage 2021				
CH LIM				
	Density class	**% m/m**	**Skins total flavonoids (mg 100 berries^{-1})**	**Seeds total flavonoids (mg 100 berries^{-1})**
I	1071	12	74 ± 6	331 ± 84
II	1075	24	73 ± 6	397 ± 23
III	1088	9	66 ± 6	314 ± 33
IV	1101	15	87 ± 6	312 ± 2
V	1113	39	83 ± 11	313 ± 14
VI	1126	/	/	/
CH MAR				
	Density class	**% m/m**	**Skins total flavonoids (mg 100 berries^{-1})**	**Seeds total flavonoids (mg 100 berries^{-1})**
I	1071	8	61 ± 4	334 ± 7 ab
II	1075	25	61 ± 5	379 ± 7 a
III	1088	37	56 ± 1	264 ± 10 ab
IV	1101	20	59 ± 4	222 ± 74 b
V	1113	4	63 ± 8	262 ± 32 ab
VI	1126	/	/	/
GR LIM				
	Density class	**% m/m**	**Skins total flavonoids (mg 100 berries^{-1})**	**Seeds total flavonoids (mg 100 berries^{-1})**
I	1071	18	108 ± 2	447 ± 8
II	1075	29	110 ± 14	443 ± 89
III	1088	11	99 ± 17	379 ± 52
IV	1101	15	98 ± 7	454 ± 44
V	1113	/	/	/
VI	1126	/	/	/

GR MAR				
	Density class	**% m/m**	**Skins total flavonoids (mg 100 berries^{-1})**	**Seeds total flavonoids (mg 100 berries^{-1})**
I	1071	/	/	/
II	1075	/	/	/
III	1088	36	121 ± 14	510 ± 74
IV	1101	35	109 ± 9	422 ± 100
V	1113	23	120 ± 8	435 ± 37
VI	1126	5	86 ± 7	365 ± 96

Vintage 2022				
CH LIM				
	Density class	**% m/m**	**Skins total flavonoids (mg 100 berries^{-1})**	**Seeds total flavonoids (mg 100 berries^{-1})**
I	1071	4	94 ± 8	509 ± 6
II	1075	17	91 ± 8	559 ± 4
III	1088	28	81 ± 7	416 ± 1
IV	1101	28	111 ± 5	473 ± 1
V	1113	17	122 ± 6	334 ± 51
VI	1126	7	82 ± 2	300 ± 19
CH MAR				
	Density class	**% m/m**	**Skins total flavonoids (mg 100 berries^{-1})**	**Seeds total flavonoids (mg 100 berries^{-1})**
I	1071	/	/	/
II	1075	13	143 ± 16 ab	610 ± 72 a
III	1088	33	145 ± 4 ab	445 ± 21 ab
IV	1101	39	175 ± 7 a	351 ± 87 bc
V	1113	12	119 ± 14 bc	348 ± 25 bc
VI	1126	2	81 ± 19 c	217 ± 23 c

	Density class	% m/m	Skins total flavonoids (mg 100 berries^{-1})	Seeds total flavonoids (mg 100 berries^{-1})
GR LIM				
I	1071	6	133 ± 29	625 ± 49 a
II	1075	11	131 ± 11	614 ± 21 a
III	1088	24	167 ± 22	636 ± 3 a
IV	1101	15	162 ± 45	541 ± 33 ab
V	1113	40	181 ± 7	662 ± 94 a
VI	1126	4	117 ± 7	359 ± 39 b
GR MAR				
	Density class	**% m/m**	**Skins total flavonoids (mg 100 berries^{-1})**	**Seeds total flavonoids (mg 100 berries^{-1})**
I	1071	/	/	/
II	1075	3	158 ± 8 ab	534 ± 32
III	1088	9	178 ± 3 a	510 ± 74
IV	1101	39	142 ± 14 b	464 ± 41
V	1113	27	157 ± 2 ab	435 ± 37
VI	1126	22	131 ± 2 b	447 ± 21

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA. Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). CH LIM = *Chardonnay* on limestone; CH MAR = *Chardonnay* on marlstone; GR LIM = *Grillo* on limestone; GR MAR = *Grillo* on marlstone.

Table S11 Flavonols content (g kg^{-1} of berries) during ripening of *Chardonnay* and *Grillo* cultivar grown on limestone and marlstone soils in 2021 and 2022 vintages.

Vintage 2021							
CH LIM							
Density class	**% m/m**	**Quercetin 3-glucuronide**	**Quercetin 3-glucoside**	**Laricitrin-3-glucoside**	**Kaempferol-3-glucuronide**	**Kaempferol-3-glucoside**	**Quercetin aglicon**
I	12	2.6 ± 0.3 a	194 ± 9 a	132 ± 29 ab	3 ± 1 b	15 ± 6 a	2.0 ± 0.4 a
II	24	3.0 ± 0.5 a	213 ± 45 a	161 ± 26 a	4 ± 1 a	23 ± 4 a	2.7 ± 0.2 a
III	9	0.6 ± 0.1 b	64 ± 3 b	61 ± 6 bc	3 ± 1 b	14 ± 2 ab	0.8 ± 0.1 b
IV	15	0.2 ± 0.2	2 ± 2 b	2 ± 1 c	BDL	0.2 ± 0.2 c	BDL
V	39	BDL	0.3 ± 0.1 b	0.9 ± 0.4 c	BDL	0.29 ± 0.04 bc	BDL
VI	/	/	/	/	/	/	/
CH MAR							
I	/	/	/	/	/	/	/
II	8	1.7 ± 0.4 a	107 ± 48	97 ± 28 a	5 ± 3	27 ± 13	8 ± 4
III	25	0.66 ± 0.03 b	21.8 ± 0.4	22.0 ± 0.3 b	1.25 ± 0.03	6.5 ± 0.3	0.9 ± 0.1
IV	37	0.37 ± 0.01 b	26 ± 1	25 ± 1 b	1.23 ± 0.02	5.9 ± 0.3	1.98 ± 0.01
V	20	0.6 ± 0.4 b	37 ± 3	37 ± 11 b	1.9 ± 1.1	9 ± 4	2 ± 1
VI	4	0.5 ± 0.1 b	29 ± 4	36 ± 4 b	2.4 ± 0.2	11 ± 1	1.2 ± 0.3
Vintage 2021							
GR LIM							
I	18	1 ± 1	108 ± 74	69 ± 58	4 ± 4	15 ± 18	3 ± 3
II	29	0.4 ± 0.3	29 ± 36	13 ± 18	0.4 ± 0.6	2 ± 3	1 ± 1
III	11	0.30 ± 0.01	30.1 ± 0.2	17 ± 1	0.6 ± 0.2	3 ± 1	0.8 ± 0.1
IV	15	0.3 ± 0.1	28 ± 2	21 ± 5	1 ± 1	7 ± 3	0.8 ± 0.1
V	/	/	/	/	/	/	/
VI	/	/	/	/	/	/	/
GR MAR							

I	/	/	/	/	/	/	/
II	/	/	/	/	/	/	/
III	36	0.4 ± 0.2	38 ± 14	28 ± 18	2 ± 2	9 ± 8	1.2 ± 0.3
IV	35	0.55 ± 0.01	59 ± 3	46 ± 8	4 ± 1	18 ± 6	1.80 ± 0.04
V	23	0.3 ± 0.1	60 ± 2	51 ± 5	4.6 ± 0.3	22 ± 3	1.5 ± 0.2
VI	5	0.21 ± 0.04	41 ± 5	29 ± 4	3 ± 1	14 ± 3	1.3 ± 0.2

Vintage 2022

CH LIM

Density class	% m/m	Quercetin 3-glucuronide	Quercetin 3-glucoside	Laricitrin-3-glucoside	Kaempferol-3-glucuronide	Kaempferol-3-glucoside	Quercetin aglicon
I	4	104 ± 9 a	59 ± 15 ab	/	0.3 ± 0.5 c	6 ± 2	/
II	17	100 ± 18 a	64 ± 9 a	/	1.7 ± 0.2 ab	8 ± 1	/
III	28	36 ± 2 b	28 ± 2 bc	/	1.0 ± 0.1 bc	5.8 ± 0.4	/
IV	28	33 ± 1 b	23 ± 2 c	/	0.8 ± 0.3 bc	4 ± 1	/
V	17	15 ± 0 b	13.6 ± 0.1 c	/	0.50 ± 0.01 c	2.36 ± 0.01	/
VI	7	34 ± 2 b	37 ± 9 abc	/	2.2 ± 0.3 a	12 ± 5	/
CH MAR							
I	/	64 ± 2	32 ± 1 b	0.61 ± 0.02	0.73 ± 0.03 b	6.4 ± 0.3 b	/
II	13	83 ± 35	46 ± 19 b	2 ± 1	2 ± 1 b	9 ± 4 b	/
III	33	42 ± 7	24 ± 6 b	0.6 ± 0.1	0.9 ± 0.2 b	5 ± 2 b	/
IV	39	57 ± 9	37 ± 10 b	0.9 ± 0.3	2 ± 1 b	8 ± 4 b	/
V	12	73 ± 8	65 ± 19 ab	1 ± 1	4 ± 2 b	20 ± 10 ab	/
VI	2	103 ± 12	105 ± 15 a	BDL	11 ± 2 a	45 ± 12 a	/
GR LIM							
I	6	20 ± 9 abc	6 ± 2 c	/	BDL	1.1 ± 0.3 cd	/
II	11	29.56 ± 0.02 ab	17 ± 2 b	/	0.52 ± 0.05 c	3.6 ± 0.1 b	/
III	24	8 ± 2 c	4 ± 1 c	/	0.11 ± 0.03 ab	0.7 ± 0.3 d	/
IV	15	9.8 ± 0.5 c	7 ± 3 c	/	0.2 ± 0.1 ab	1 ± 1 cd	/
V	40	13 ± 1 bc	12 ± 1 bc	/	0.5 ± 0.1 c	2.8 ± 0.3 bc	/

VI	4	35 ± 5 a	31 ± 4 a	/	1.9 ± 0.2 a	10 ± 1 a	/
GR MAR							
I	/	/	/	/	/	/	/
II	3	161.22 ± 0.01 a	123 ± 1 b	4 ± 2	5.81 ± 0.05 a	35 ± 1 a	/
III	9	33 ± 4 bc	23 ± 6 a	0.6 ± 0.2	1.1 ± 0.2 b	7 ± 2 b	/
IV	39	31 ± 3 bc	21 ± 5 b	0.38 ± 0.01	0.8 ± 0.2 b	7 ± 2 b	/
V	27	40 ± 2 b	29 ± 1 b	0.49 ± 0.01	1.37 ± 0.05 b	10 ± 1 b	/
VI	22	27 ± 3 c	18 ± 1 b	0.4 ± 0.1	1.0 ± 0.1 b	7 ± 1 b	/

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA. Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). CH LIM = *Chardonnay* on limestone; CH MAR = *Chardonnay* on marlstone; GR LIM = *Grillo* on limestone; GR MAR = *Grillo* on marlstone. Density class: I= 1071, II = 1075, III = 1088, IV = 1101, V = 1113, VI = 1126 kg m^{-3}.

Table S12 Hydrocycinnamoyltartaric acids (HCTAs) content (g kg^{-1} of berries) during ripening of *Chardonnay* and *Grillo* cultivar grown on limestone and marlstone soils in 2021 and 2022 vintages.

			Vintage 2021		
			CH LIM		
	Density class	**% m/m**	**Caftaric acid**	**Coutaric acid**	**Feftaric acid**
I	1071	12	1356 ± 155 a	1038 ± 103 a	38 ± 21 b
II	1075	24	1681 ± 158 a	1150 ± 74 a	75 ± 11 a
III	1088	9	229 ± 23 b	204 ± 13 b	11 ± 2 c
IV	1101	15	213 ± 113 b	110 ± 78 b	5 ± 3 c
V	1126	39	17 ± 9 c	13 ± 4 c	4 ± 2 c
			CH MAR		
I	1075	8	1330 ± 149 a	657 ± 33 a	74 ± 13 a
II	1088	25	290 ± 5 b	83 ± 81 b	7 ± 6 b
III	1101	37	113 ± 108 b	81 ± 77 b	11 ± 1 b
IV	1113	20	226 ± 12 b	134 ± 8 b	21 ± 3 b
V	1126	4	155 ± 13 b	100 ± 10 b	11 ± 1 b
			GR LIM		
I	1075	23	620 ± 159	219 ± 59	54 ± 20
II	1088	28	316 ± 29	111 ± 8	24 ± 2
III	1101	11	205 ± 12	74 ± 5	17 ± 1
IV	1113	15	150 ± 16	57 ± 3	16 ± 2
V	1126	/	/	/	/
			GR MAR		
I	1075	/	/	/	/
II	1088	36	164 ± 14	63 ± 9	17 ± 2
III	1101	35	211 ± 13	86 ± 7	21 ± 1
IV	1113	23	198 ± 16	84 ± 3	17 ± 5
V	1126	5	114 ± 1	48 ± 2	15 ± 5

Vintage 2022					
CH LIM					
	Density class	**% m/m**	**Caftaric acid**	**Coutaric acid**	**Feftaric acid**
II	1075	17	923 ± 37 a	629 ± 17 a	30 ± 7
III	1088	28	170 ± 9 c	142 ± 7 c	9 ± 1
IV	1101	28	249 ± 5 c	188 ± 4 c	10 ± 1
V	1113	17	133 ± 4 c	87 ± 3 b	11 ± 1
VI	1126	7	279 ± 12 b	175 ± 4 c	17 ± 1
CH MAR					
II	1075	13	282 ± 98	129 ± 44	23 ± 11
III	1088	33	175 ± 39	73 ± 13	10 ± 2
IV	1101	39	241 ± 8	99 ± 5	13 ± 1
V	1113	12	261 ± 13	105 ± 5	29 ± 6
VI	1126	2	301 ± 42	86 ± 43	84 ± 51
GR LIM					
II	1075	11	382 ± 28	136 ± 1	17 ± 1
III	1088	24	101 ± 1	36 ± 1	4 ± 1
IV	1101	15	103 ± 11	37 ± 2	5 ± 1
V	1113	40	115 ± 3	43 ± 1	6 ± 1
VI	1126	4	130 ± 3	57 ± 1	10 ± 1
GR MAR					
II	1075	3	668 ± 29	271 ± 11	48 ± 10
III	1088	9	145 ± 3	58 ± 3	12 ± 1
IV	1101	39	126 ± 14	50 ± 6	10 ± 1
V	1113	27	151 ± 7	60 ± 4	13 ± 1
VI	1126	22	83 ± 1	31 ± 1	10 ± 1

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA. Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). CH LIM =

Chardonnay on limestone; CH MAR = *Chardonnay* on marlstone; GR LIM = *Grillo* on limestone; GR MAR = *Grillo* on marlstone.

Table S13 Technological parameters content during ripening of *Nero d'Avola* and *Syrah* cultivar grown on limestone and marlstone soils in 2021 and 2022 vintages.

Vintage 2021					
ND LIM					
	Density class	**% m/m**	**Reducing sugars g L^{-1}**	**Titratable acidity g L^{-1}**	**pH**
II	1075	12	176 ± 3	7 ± 1	3.38 ± 0.01
III	1088	48	186 ± 7	7.3 ± 0.3	3.37 ± 0.31
IV	1101	39	232 ± 8	7.4 ± 0.4	3.36 ± 0.44
V	1113	2	268 ± 7	6.8 ± 0.5	3.53 ± 0.46
ND MAR					
II	1075	17	178 ± 4	8 ± 1	3.19 ± 0.02
III	1088	54	227 ± 3	8 ± 1	3.20 ± 0.02
IV	1101	26	253 ± 9	8 ± 1	3.24 ± 0.02
V	1113	3	285 ± 7	7 ± 1	3.32 ± 0.01
VI	1126		/	/	/
SY LIM					
II	1075	12	178 ± 3	7 ± 1	3.40 ± 1.40
III	1088	48	197 ± 9	5.9 ± 0.2	3.61 ± 0.02
IV	1101	39	213 ± 9	4.9 ± 0.3	3.58 ± 0.01
V	1113	2	/	/	/
VI	1126	3	/	/	/
SY MAR					
II	1075	6	152 ± 4	7.1 ± 0.4	3.04 ± 0.01
III	1088	24	188 ± 5	5.2 ± 0.3	3.27 ± 0.02
IV	1101	44	213 ± 6	5.8 ± 0.6	3.40 ± 0.03

V	1113	19	231 ± 6	5.5 ± 0.3	3.37 ± 0.01
VI	1126	3	279 ± 5	4.2 ± 0.3	3.49 ± 0.01
Vintage 2022					
ND LIM					
	Density class	**% m/m**	**Reducing sugars g L^{-1}**	**Titratable acidity g L^{-1}**	**pH**
II	1075	15	197 ± 10	6.8 ± 0.3	3.31 ± 0.01
III	1088	43	214 ± 10	6.7 ± 0.2	3.34 ± 0.01
IV	1101	32	235 ± 3	6.7 ± 0.2	3.34 ± 0.01
V	1113	7	257 ± 10	6.4 ± 0.3	3.46 ± 0.01
VI	1126	7	181 ± 6	6.0 ± 0.7	3.51 ± 0.02
ND MAR					
II	1075	22	181 ± 8	9.1 ± 0.6	3.11 ± 0.03
III	1088	43	189 ± 4	9.2 ± 0.2	3.16 ± 0.03
IV	1101	25	198 ± 6	9.0 ± 0.1	3.16 ± 0.01
V	1113	3	223 ± 7	9.1 ± 0.6	3.30 ± 0.03
VI	1126	2	253 ± 3	7.8 ± 0.6	3.35 ± 0.02
SY LIM					
II	1075	12	140 ± 4	6.2 ± 0.5	3.33 ± 0.03
III	1088	48	171 ± 3	6.3 ± 0.3	3.44 ± 0.03
IV	1101	39	220 ± 7	6.4 ± 0.1	3.44 ± 0.00
V	1113	2	196 ± 6	6.0 ± 0.2	3.57 ± 0.02
VI	1126	4	234 ± 3	4.7 ± 0.3	3.98 ± 0.02
SY MAR					
II	1075	30	164 ± 6	7.3 ± 0.5	3.18 ± 0.01
III	1088	23	179 ± 5	7.4 ± 0.2	3.16 ± 0.01
IV	1101	26	205 ± 7	6.7 ± 0.8	3.24 ± 0.03
V	1113	16	230 ± 3	6.7 ± 0.3	3.29 ± 0.03
VI	1126	3	235 ± 3	5.1 ± 0.2	3.54 ± 0.01

Note: Data are reported as mean plus and minus standard error of the mean. ND LIM = *Nero d'Avola* on limestone; ND MAR = *Nero d'Avola* on marlstone; SY LIM = *Syrah* on limestone; SY MAR = *Syrah* on marlstone.

Table S14 Skin and seeds total flavonoids and anthocyanins content during ripening of *Nero d'Avola* and *Syrah* cultivar grown on limestone and marlstone soils in 2021 and 2022 vintages.

Vintage 2021				
ND LIM				
Density class	**% m/m**	**Skins total flavonoids (mg 100 berries^{-1})**	**Seeds total flavonoids (mg 100 berries^{-1})**	**Anthocyanins (mg 100 berries^{-1})**
II	/	/	/	/
III	12	304 ± 22	306 ± 4	34 ± 6
IV	48	282 ± 60	426 ± 123	57 ± 1
V	39	454 ± 10	520 ± 131	111 ± 4
VI	2	289 ± 5	340 ± 33	91 ± 3
ND MAR				
Density class	**% m/m**	**Skins total flavonoids (mg 100 berries^{-1})**	**Seeds total flavonoids (mg 100 berries^{-1})**	**Anthocyanins (mg 100 berries^{-1})**
II	/	/	/	/
III	12	260 ± 1	445 ± 2	44 ± 9
IV	48	347 ± 18	317 ± 26	83 ± 2
V	39	400 ± 60	312 ± 8	115 ± 3
VI	2	357 ± 37	486 ± 10	115 ± 7
SY LIM				
Density class	**% m/m**	**Skins total flavonoids (mg 100 berries^{-1})**	**Seeds total flavonoids (mg 100 berries^{-1})**	**Anthocyanins (mg 100 berries^{-1})**
II	/	/	/	/
III	12	294 ± 41	300 ± 24	67 ± 13
IV	48	347 ± 6	235 ± 6	105 ± 3
V	39	404 ± 29	263 ± 4	126 ± 17
VI	2	367 ± 6	198 ± 11	109 ± 0

SY MAR

Density class	% m/m	Skins total flavonoids (mg 100 berries^{-1})	Seeds total flavonoids (mg 100 berries^{-1})	Anthocyanins (mg 100 berries^{-1})
I	3	248 ± 3	282 ± 45	39 ± 2
II	6	470 ± 24	245 ± 13	94 ± 1
III	24	406 ± 9	266 ± 12	115 ± 6
IV	44	436 ± 55	257 ± 9	118 ± 1
V	19	583 ± 77	229 ± 15	213 ± 20
VI	3	536 ± 69	237 ± 47	185 ± 8

Vintage 2022

ND LIM

Density class	% m/m	Skins total flavonoids (mg 100 berries^{-1})	Seeds total flavonoids (mg 100 berries^{-1})	Anthocyanins (mg 100 berries^{-1})
I	3	275 ± 8	466 ± 49	26 ± 2
II	15	327 ± 19	495 ± 25	54 ± 6
III	43	400 ± 13	466 ± 53	108 ± 18
IV	32	463 ± 10	457 ± 23	110 ± 6
V	7	446 ± 1	380 ± 15	116 ± 1

ND MAR

Density class	% m/m	Skins total flavonoids (mg 100 berries^{-1})	Seeds total flavonoids (mg 100 berries^{-1})	Anthocyanins (mg 100 berries^{-1})
I	7	345 ± 17	540 ± 2	81 ± 8
II	22	340 ± 31	487 ± 29	76 ± 19
III	43	405 ± 20	475 ± 23	112 ± 11
IV	25	479 ± 15	460 ± 22	148 ± 5
V	3	428 ± 1	363 ± 6	123 ± 8

SY LIM

Density class	% m/m	Skins total flavonoids (mg 100 berries^{-1})	Seeds total flavonoids (mg 100 berries^{-1})	Anthocyanins (mg 100 berries^{-1})
I	6	388 ± 31	366 ± 6	105 ± 1
II	34	384 ± 10	418 ± 42	117 ± 5
III	37	409 ± 7	485 ± 4	156 ± 31

IV	19	385 ± 22	453 ± 24	137 ± 8
V	3	489 ± 6	448 ± 11	197 ± 17
VI	1	503 ± 4	416 ± 8	178 ± 8

SY MAR

Density class	% m/m	Skins total flavonoids (mg 100 berries^{-1})	Seeds total flavonoids (mg 100 berries^{-1})	Anthocyanins (mg 100 berries^{-1})
I	4	327 ± 18	515 ± 22	110 ± 1
II	30	351 ± 10	544 ± 2	108 ± 12
III	23	504 ± 46	473 ± 9	150 ± 18
IV	26	507 ± 31	500 ± 33	184 ± 8
V	16	550 ± 29	340 ± 36	215 ± 19
VI	1	439 ± 10	387 ± 10	174 ± 2

Note: Data are reported as mean plus and minus standard error of the mean. ND LIM = *Nero d'Avola* on limestone; ND MAR = *Nero d'Avola* on marlstone; SY LIM = *Syrah* on limestone; SY MAR = *Syrah* on marlstone. Density class: I= 1071, II = 1075, III = 1088, IV = 1101, V = 1113, VI = 1126 kg m^{-3}.

Table S15 Flavonols content (g kg^{-1} of berries) during ripening of *Nero d'Avola* and *Syrah* cultivar grown on limestone and marlstone soils in 2021 and 2022 vintages.

Vintage 2021

ND LIM

Density class	% m/m	Myricetin 3-glucuronide	Myricetin 3-glucoside	Quercetin 3-glucuronide	Quercetin 3-glucoside	Isorhamnetin 3-glucoside	Laricitrin 3-glucoside	Kaempferol 3-glucuronide	Kaempferol 3-glucoside	Myricetin aglycon	Syringetin 3-glucoside	Quercetin aglycon
I	12	0.00 ± 0.00	8.38 ± 0.11	68.20 ± 1.38	45.80 ± 8.55	67.41 ± 7.72	0.00 ± 0.00	2.58 ± 0.01	22.52 ± 2.88	0.50 ± 0.02	0.27 ± 0.27	27.75 ± 0.88
II	48	2.77 ± 0.08	28.77 ± 2.02	12.55 ± 1.43	11.80 ± 1.12	6.33 ± 0.25	0.00 ± 0.00	0.80 ± 0.06	1.05 ± 0.14	3.97 ± 0.14	0.00 ± 0.00	9.22 ± 1.00
III	39	2.61 ± 0.31	31.77 ± 4.48	9.63 ± 1.18	10.55 ± 1.24	7.70 ± 0.85	0.00 ± 0.00	0.89 ± 0.18	0.99 ± 0.05	4.67 ± 0.36	0.00 ± 0.00	12.32 ± 1.71

IV	2	2.98 ± 0.14	42.30 ± 6.09	19.02 ± 6.28	20.78 ± 6.43	8.71 ± 1.49	0.00 ± 0.00	0.96 ± 0.39	1.74 ± 0.48	6.77 ± 0.66	0.00 ± 0.00	15.14 ± 0.59

ND MAR

Density class	% m/m	Myricetin 3-glucuronide	Myricetin 3-glucoside	Quercetin 3-glucuronide	Quercetin 3-glucoside	Isorhamnetin 3-glucoside	Laricitrin 3-glucoside	Kaempferol 3-glucuronide	Kaempferol 3-glucoside	Myricetin aglycon	Syringetin 3-glucoside	Quercetin aglycon
I	17	14.17 ± 1.92	115.78 ± 14.76	75.84 ± 8.42	74.36 ± 12.63	0.00 ± 0.00	0.00 ± 0.00	3.66 ± 0.58	23.34 ± 4.51	0.25 ± 0.25	0.00 ± 0.00	42.28 ± 3.02
II	54	14.22 ± 0.00	120.28 ± 0.11	73.31 ± 1.38	82.74 ± 8.55	0.00 ± 7.72	0.00 ± 0.00	4.06 ± 0.01	26.02 ± 2.88	0.93 ± 0.02	0.00 ± 0.27	43.04 ± 0.88
III	54	13.68 ± 0.08	118.03 ± 2.02	52.61 ± 1.43	64.30 ± 1.12	0.00 ± 0.25	0.00 ± 0.00	4.12 ± 0.06	24.75 ± 0.14	1.21 ± 0.14	0.00 ± 0.00	54.10 ± 1.00
IV	26	4.14 ± 0.31	44.92 ± 4.48	15.72 ± 1.18	9.97 ± 1.24	8.37 ± 0.85	0.00 ± 0.00	2.09 ± 0.18	6.88 ± 0.05	0.27 ± 0.36	0.00 ± 0.00	19.21 ± 1.71
V	3	4.55 ± 0.14	56.64 ± 6.09	17.23 ± 6.28	14.11 ± 6.43	9.88 ± 1.49	0.00 ± 0.00	2.14 ± 0.39	8.31 ± 0.48	0.53 ± 0.66	0.00 ± 0.00	19.78 ± 0.59

SY LIM

Density class	% m/m	Myricetin 3-glucuronide	Myricetin 3-glucoside	Quercetin 3-glucuronide	Quercetin 3-glucoside	Isorhamnetin 3-glucoside	Laricitrin 3-glucoside	Kaempferol 3-glucuronide	Kaempferol 3-glucoside	Myricetin aglycon	Syringetin 3-glucoside	Quercetin aglycon
I	12	15.08 ± 2.37	164.03 ± 32.28	111.81 ± 22.99	326.33 ± 56.61	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	151.35 ± 21.74	2.37 ± 0.45	0.00 ± 0.00	37.56 ± 6.94
II	48	22.26 ± 2.32	226.95 ± 13.34	88.57 ± 8.16	255.68 ± 18.25	1.36 ± 1.36	0.00 ± 0.00	0.00 ± 0.00	152.34 ± 13.19	1.52 ± 0.31	0.00 ± 0.00	46.92 ± 0.01
III	39	4.38 ± 0.38	53.97 ± 1.97	22.20 ± 2.01	62.31 ± 1.43	0.36 ± 0.36	0.00 ± 0.00	0.00 ± 0.00	37.77 ± 2.33	0.42 ± 0.19	0.00 ± 0.00	12.20 ± 0.91
IV	2	4.91 ± 0.08	62.61 ± 3.36	21.55 ± 1.45	36.21 ± 2.61	24.03 ± 0.72	0.00 ± 0.00	0.00 ± 0.00	44.48 ± 1.92	0.00 ± 0.00	0.00 ± 0.00	14.95 ± 1.49

SY MAR

Density class	% m/m	Myricetin 3-glucuronide	Myricetin 3-glucoside	Quercetin 3-glucuronide	Quercetin 3-glucoside	Isorhamnetin 3-glucoside	Laricitrin 3-glucoside	Kaempferol 3-glucuronide	Kaempferol 3-glucoside	Myricetin aglycon	Syringetin 3-glucoside	Quercetin aglycon
I	3	8.78 ± 0.33	63.97 ± 3.79	137.26 ± 5.86	182.70 ± 16.94	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	60.52 ± 6.33	1.16 ± 0.03	0.00 ± 0.00	21.55 ± 2.50
II	6	10.25 ± 0.51	99.64 ± 10.73	100.38 ± 13.37	213.17 ± 39.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	82.19 ± 14.47	1.64 ± 0.13	0.00 ± 0.00	27.59 ± 0.48
III	24	11.61 ± 1.09	121.87 ± 4.27	128.94 ± 2.85	266.26 ± 4.77	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	113.37 ± 4.46	0.96 ± 0.96	0.00 ± 0.00	29.87 ± 9.83
IV	44	3.90 ± 0.19	39.10 ± 2.67	44.61 ± 6.99	93.49 ± 12.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	38.14 ± 4.58	0.50 ± 0.07	0.00 ± 0.00	6.81 ± 3.81
V	19	7.02 ± 4.06	72.47 ± 26.47	48.36 ± 24.30	89.59 ± 63.32	4.64 ± 4.64	0.00 ± 0.00	0.00 ± 0.00	33.88 ± 21.84	0.56 ± 0.56	0.00 ± 0.00	19.20 ± 5.37
VI	3	9.35 ± 0.30	72.90 ± 6.89	63.58 ± 4.61	122.37 ± 10.53	7.06 ± 7.06	0.00 ± 0.00	0.00 ± 0.00	46.28 ± 3.84	0.67 ± 0.11	0.00 ± 0.00	15.80 ± 2.77

Vintage 2022

ND LIM

Density class	% m/m	Myricetin 3-glucuronide	Myricetin 3-glucoside	Quercetin 3-glucuronide	Quercetin 3-glucoside	Isorhamnetin 3-glucoside	Laricitrin 3-glucoside	Kaempferol 3-glucuronide	Kaempferol 3-glucoside	Myricetin aglycon	Syringetin 3-glucoside	Quercetin aglycon

I	3	6.22 ± 0.18	70.33 ± 0.47	84.09 ± 2.86	42.53 ± 1.15	14.49 ± 0.47	3.23 ± 0.17	2.53 ± 0.16	11.31 ± 0.50	1.43 ± 0.23	1.00 ± 0.05	0.00 ± 0.00
II	12	2.07 ± 0.36	27.30 ± 2.66	13.85 ± 0.29	10.04 ± 0.20	5.18 ± 0.32	1.10 ± 0.18	0.56 ± 0.01	3.59 ± 0.15	0.29 ± 0.02	0.12 ± 0.12	0.00 ± 0.00
III	43	2.03 ± 0.79	31.70 ± 9.19	12.11 ± 4.19	7.55 ± 2.87	6.10 ± 1.76	1.00 ± 0.74	0.37 ± 0.20	4.26 ± 0.92	0.18 ± 0.18	0.20 ± 0.20	0.00 ± 0.00
IV	39	3.00 ± 0.05	43.07 ± 1.89	15.35 ± 0.43	10.95 ± 0.04	8.17 ± 1.24	1.61 ± 0.03	3.66 ± 3.11	3.33 ± 3.10	0.34 ± 0.11	0.14 ± 0.14	0.00 ± 0.00
V	2	4.50 ± 0.57	63.65 ± 7.60	19.75 ± 1.34	16.99 ± 0.29	13.27 ± 0.50	2.59 ± 0.17	1.02 ± 0.12	9.35 ± 0.09	0.53 ± 0.06	0.17 ± 0.17	0.00 ± 0.00

ND MAR

Density class	% m/m	Myricetin 3-glucuronide	Myricetin 3-glucoside	Quercetin 3-glucuronide	Quercetin 3-glucoside	Isorhamnetin 3-glucoside	Laricitrin 3-glucoside	Kaempferol 3-glucuronide	Kaempferol 3-glucoside	Myricetin aglycon	Syringetin 3-glucoside	Quercetin aglycon
I	7	10.46 ± 10.46	95.94 ± 95.94	92.30 ± 10.62	92.94 ± 10.24	16.86 ± 15.75	6.72 ± 0.86	24.24 ± 14.39	9.28 ± 7.59	0.71 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
II	17	20.42 ± 1.28	195.48 ± 1.18	88.53 ± 19.90	68.34 ± 4.10	28.23 ± 2.77	10.37 ± 1.14	6.71 ± 0.47	19.98 ± 2.15	1.82 ± 0.22	1.74 ± 0.78	0.00 ± 0.00
III	43	4.89 ± 0.73	48.43 ± 2.37	26.86 ± 1.34	24.64 ± 4.41	8.61 ± 0.18	1.77 ± 0.27	6.13 ± 3.39	2.69 ± 2.10	0.33 ± 0.15	0.00 ± 0.00	0.00 ± 0.00
IV	26	7.64 ± 0.02	72.72 ± 0.63	30.81 ± 1.49	28.29 ± 0.54	10.96 ± 0.15	3.20 ± 0.32	4.09 ± 0.29	5.91 ± 0.07	0.33 ± 0.05	0.15 ± 0.15	0.00 ± 0.00
V	3	6.89 ± 0.35	71.56 ± 0.89	53.33 ± 4.71	58.12 ± 14.20	12.94 ± 2.82	2.31 ± 0.35	4.65 ± 0.32	15.79 ± 10.70	0.35 ± 0.01	0.38 ± 0.38	0.00 ± 0.00

SY LIM

Density class	% m/m	Myricetin 3-glucuronide	Myricetin 3-glucoside	Quercetin 3-glucuronide	Quercetin 3-glucoside	Isorhamnetin 3-glucoside	Laricitrin 3-glucoside	Kaempferol 3-glucuronide	Kaempferol 3-glucoside	Myricetin aglycon	Syringetin 3-glucoside	Quercetin aglycon
I	6	13.98 ± 0.87	133.85 ± 9.51	152.33 ± 5.42	226.81 ± 17.28	45.83 ± 3.26	4.43 ± 0.15	8.77 ± 0.23	111.02 ± 9.10	1.73 ± 0.36	4.29 ± 0.49	0.00 ± 0.00
II	12	19.86 ± 2.97	174.77 ± 16.19	159.77 ± 27.16	300.63 ± 50.57	63.62 ± 10.73	7.98 ± 1.35	11.99 ± 3.05	140.07 ± 15.93	1.44 ± 0.60	4.23 ± 1.41	0.00 ± 0.00
III	42	4.11 ± 0.85	39.72 ± 4.54	32.47 ± 5.63	59.10 ± 7.90	13.94 ± 1.84	1.36 ± 0.36	2.66 ± 0.67	29.25 ± 3.28	0.48 ± 0.28	0.72 ± 0.08	0.00 ± 0.00
IV	39	4.91 ± 0.23	53.56 ± 1.38	33.85 ± 3.59	67.69 ± 3.47	19.61 ± 0.47	1.78 ± 0.11	3.25 ± 0.11	37.07 ± 0.31	0.28 ± 0.02	0.90 ± 0.07	0.00 ± 0.00
V	2	4.20 ± 0.71	55.44 ± 4.47	30.19 ± 7.67	59.37 ± 13.25	20.48 ± 1.24	1.68 ± 0.34	3.06 ± 0.70	39.20 ± 3.23	0.39 ± 0.07	1.02 ± 0.18	0.00 ± 0.00

SY MAR

Density class	% m/m	Myricetin 3-glucuronide	Myricetin 3-glucoside	Quercetin 3-glucuronide	Quercetin 3-glucoside	Isorhamnetin 3-glucoside	Laricitrin 3-glucoside	Kaempferol 3-glucuronide	Kaempferol 3-glucoside	Myricetin aglycon	Syringetin 3-glucoside	Quercetin aglycon
I	4	2.78 ± 0.34	21.14 ± 3.05	45.47 ± 3.45	28.03 ± 0.51	7.10 ± 1.26	0.00 ± 0.00	1.92 ± 0.37	11.55 ± 0.08	0.40 ± 0.19	0.19 ± 0.19	0.00 ± 0.00
II	30	6.05 ± 0.38	67.60 ± 0.05	61.22 ± 8.28	101.34 ± 12.51	22.87 ± 1.18	1.95 ± 0.10	2.84 ± 1.01	50.49 ± 2.03	0.91 ± 0.16	1.31 ± 0.14	0.00 ± 0.00
III	23	2.12 ± 0.70	26.59 ± 8.08	22.75 ± 4.89	37.83 ± 12.79	8.81 ± 2.58	0.36 ± 0.36	1.52 ± 0.21	17.69 ± 6.93	0.23 ± 0.11	0.28 ± 0.28	0.00 ± 0.00
IV	26	3.46 ± 0.21	39.44 ± 2.43	26.13 ± 1.72	36.25 ± 2.16	9.95 ± 0.99	1.56 ± 0.06	1.84 ± 0.14	15.79 ± 1.01	0.23 ± 0.02	0.50 ± 0.05	0.00 ± 0.00

V	16	3.69 ± 0.00	50.55 ± 4.27	20.91 ± 3.66	46.34 ± 8.74	18.10 ± 0.49	1.56 ± 0.05	1.55 ± 0.28	32.61 ± 1.67	0.30 ± 0.02	0.85 ± 0.10	0.00 ± 0.00
VI	1	3.99 ± 0.00	55.01 ± 0.00	38.09 ± 0.00	71.16 ± 0.00	17.92 ± 0.00	1.24 ± 0.00	2.60 ± 0.00	30.01 ± 0.00	0.32 ± 0.00	0.99 ± 0.00	0.00 ± 0.00

Note: Data are reported as mean plus and minus standard error of the mean. ND LIM = *Nero d'Avola* on limestone; ND MAR = *Nero d'Avola* on marlstone; SY LIM = *Syrah* on limestone; SY MAR = *Syrah* on marlstone. Density class: I= 1071, II = 1075, III = 1088, IV = 1101, V = 1113, VI = 1126 kg m^{-3}.

Table S16 Hydrocycinnamoyltartaric acids (HCTAs) content (g kg^{-1} of berries) during ripening of *Nero d'Avola* and *Syrah* cultivar grown on limestone and marlstone soils in 2021 and 2022 vintages.

			Vintage 2021		
			ND LIM		
	Density class	**% m/m**	**Caftaric acid**	**Coutaric acid**	**Feftaric acid**
II	1075	12	785 ± 31	49 ± 8	195 ± 23
III	1088	48	238 ± 18	35 ± 19	47 ± 4
IV	1101	39	144 ± 13	23 ± 12	32 ± 12
V	1113	2	241 ± 19	12 ± 1	58 ± 8
			ND MAR		
II	1075	17	1,002 ± 75	53 ± 3	274 ± 1
III	1088	54	922 ± 62	42 ± 9	239 ± 22
IV	1101	26	194 ± 6	17 ± 0	64 ± 2
V	1113	3	268 ± 5	20 ± 0	73 ± 2
			SY LIM		
II	1075	12	516 ± 58	75 ± 4	592 ± 56
III	1088	48	544 ± 14	75 ± 7	552 ± 15
IV	1101	39	118 ± 26	16 ± 3	118 ± 21
V	1113	2	108 ± 4	19 ± 0	139 ± 10
			SY MAR		
II	1075	6	527 ± 32	99 ± 5	642 ± 34
III	1088	24	509 ± 16	166 ± 79	449 ± 227
IV	1101	44	129 ± 2	26 ± 1	159 ± 5
V	1113	19	185 ± 17	33 ± 1	247 ± 15
VI	1126	3	136 ± 13	26 ± 1	178 ± 11
			Vintage 2022		
			ND LIM		
II	1075	15	159 ± 13	38 ± 2	7.1 ± 0.3
III	1088	43	140 ± 12	33 ± 3	11 ± 2

IV	1101	32	160 ± 3	37 ± 1	9.9 ± 0.4
VI	1113	7	212 ± 15	44 ± 0	14 ± 2
			ND MAR		
II	1075	22	1,141 ± 57	154 ± 109	166 ± 141
III	1088	43	240 ± 13	76 ± 11	7 ± 1
IV	1101	25	258 ± 5	61 ± 2	10 ± 1
V	1113	3	220 ± 5	107 ± 44	11 ± 1
			SY LIM		
II	1075	12	109 ± 8	108 ± 7	7 ± 2
III	1088	48	111 ± 4	100 ± 1	15 ± 1
IV	1101	39	97 ± 4	97 ± 8	26 ± 0
V	1113	2	0 ± 3	0 ± 3	0 ± 4
			SY MAR		
II	1075	30	368 ± 14	436 ± 0	41 ± 2
III	1088	23	126 ± 21	142 ± 23	13 ± 1
IV	1101	26	137 ± 8	86 ± 5	12 ± 1
V	1113	16	109 ± 27	122 ± 30	15 ± 1

Note: Data are reported as mean plus and minus standard error of the mean. ND LIM = *Nero d'Avola* on limestone; ND MAR = *Nero d'Avola* on marlstone; SY LIM = *Syrah* on limestone; SY MAR = *Syrah* on marlstone.

Table S17 Coefficient variation of some technological parameters and phenolic compounds

Factor	CV Reducing sugars	CV Titratable acidity	CV pH	CV_Caftaric acid	CV_Coutaric acid	CV_Feftaric acid	CV_Quercetin 3-glucoside	CV NAF skin	CV NAF seeds	CV Anthocyanins
Cultivar (n=4)										
CH	0.14 ± 0.04	0.07 ± 0.03 ab	0.011 ± 0.001	0.86 ± 0.21	0.89 ± 0.19	0.95 ± 0.13	0.73 ± 0.17	0.12 ± 0.02	0.18 ± 0.02	-
GR	0.13 ± 0.02	0.06 ± 0.01 bc	0.021 ± 0.001	0.52 ± 0.12	0.52 ± 0.11	0.68 ± 0.23	0.62 ± 0.15	0.12 ± 0.01	0.14 ± 0.02	-
ND	0.10 ± 0.01	0.021 ± 0.001 c	0.02 ± 0.01	0.66 ± 0.07	0.59 ± 0.07	0.93 ± 0.41	0.62 ± 0.05	0.16 ± 0.03	0.20 ± 0.06	0.25 ± 0.03
SY	0.11 ± 0.02	0.111 ± 0.001 a	0.03 ± 0.01	0.64 ± 0.04	0.78 ± 0.10	0.65 ± 0.04	0.63 ± 0.07	0.17 ± 0.03	0.15 ± 0.07	0.28 ± 0.06
Sign.	ns	**	ns	ns	ns	ns	ns	ns	ns	ns
Year (n=8)										
2021	0.13 ± 0.02	0.07 ± 0.02	0.021 ± 0.001	0.70 ± 0.12	0.72 ± 0.13	0.73 ± 0.12	0.70 ± 0.10	0.14 ± 0.02	0.21 ± 0.04	0.14 ± 0.06
2022	0.11 ± 0.01	0.06 ± 0.01	0.021 ± 0.001	0.64 ± 0.06	0.67 ± 0.05	0.87 ± 0.20	0.60 ± 0.04	0.14 ± 0.02	0.13 ± 0.01	0.12 ± 0.05
Sign.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Soil (n=8)										
LIM	0.14 ± 0.02	0.08 ± 0.02	0.02 ± 0.00	0.75 ± 0.07	0.73 ± 0.07	0.79 ± 0.11	0.76 ± 0.09	0.14 ± 0.01	0.14 ± 0.03	0.11 ± 0.04
MAR	0.10 ± 0.01	0.06 ± 0.01	0.02 ± 0.00	0.59 ± 0.11	0.66 ± 0.12	0.81 ± 0.21	0.54 ± 0.05	0.14 ± 0.02	0.19 ± 0.03	0.15 ± 0.06
Sign.	*	.	ns	ns	ns	ns	ns	ns	ns	*
Cultivar:Soil. (n=2)										
CH:LIM	0.20 ± 0.04 a	0.11 ± 0.05 ab	0.01 ± 0.01	1.01 ± 0.22	0.99 ± 0.26	0.91 ± 0.42	0.85 ± 0.52	0.15 ± 0.05	0.15 ± 0.01	-
GR:LIM	0.16 ± 0.03 ab	0.07 ± 0.01 abc	0.02 ± 0.01	0.661 ± 0.001	0.63 ± 0.02	1.02 ± 0.34	0.79 ± 0.23	0.11 ± 0.01	0.15 ± 0.02	-
ND:LIM	0.10 ± 0.02 ab	0.01 ± 0.01 c	0.01 ± 0.00	0.68 ± 0.08	0.63 ± 0.10	0.59 ± 0.41	0.68 ± 0.10	0.19 ± 0.01	0.21 ± 0.13	0.27 ± 0.01 ab
SY:LIM	0.08 ± 0.03 b	0.11 ± 0.01 ab	0.03 ± 0.01	0.64 ± 0.11	0.68 ± 0.15	0.66 ± 0.12	0.71 ± 0.19	0.12 ± 0.03	0.08 ± 0.07	0.18 ± 0.03 b
CH:MAR	0.08 ± 0.03 b	0.04 ± 0.02 bc	0.01 ± 0.001	0.71 ± 0.64	0.79 ± 0.59	0.99 ± 0.14	0.62 ± 0.18	0.09 ± 0.02	0.21 ± 0.03	-
GR:MAR	0.111 ± 0.001 ab	0.04 ± 0.02	0.02 ± 0.001	0.4 ± 0.3	0.40 ± 0.31	0.33 ± 0.19	0.44 ± 0.29	0.13 ± 0.01	0.13 ± 0.05	-
ND:MAR	0.09 ± 0.02 ab	0.03 ± 0.01 c	0.03 ± 0.01	0.63 ± 0.23	0.56 ± 0.22	1.26 ± 1.18	0.56 ± 0.04	0.12 ± 0.08	0.20 ± 0.15	0.24 ± 0.08 b
SY:MAR	0.13 ± 0.02 ab	0.12 ± 0.01 a	0.03 ± 0.02	0.63 ± 0.06	0.89 ± 0.24	0.64 ± 0.06	0.55 ± 0.03	0.22 ± 0.01	0.23 ± 0.16	0.37 ± 0.03 a
Sign.	*	*	ns	ns	ns	ns	ns	*	ns	*

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA. Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance).

Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). CH LIM = *Chardonnay* on limestone; CH MAR = *Chardonnay* on marlstone; GR LIM = *Grillo* on limestone; GR MAR = *Grillo* on marlstone; ND LIM = *Nero d'Avola* on limestone; ND MAR = *Nero d'Avola* on marlstone; SY LIM = *Syrah* on limestone; SY MAR = *Syrah* on marlstone.

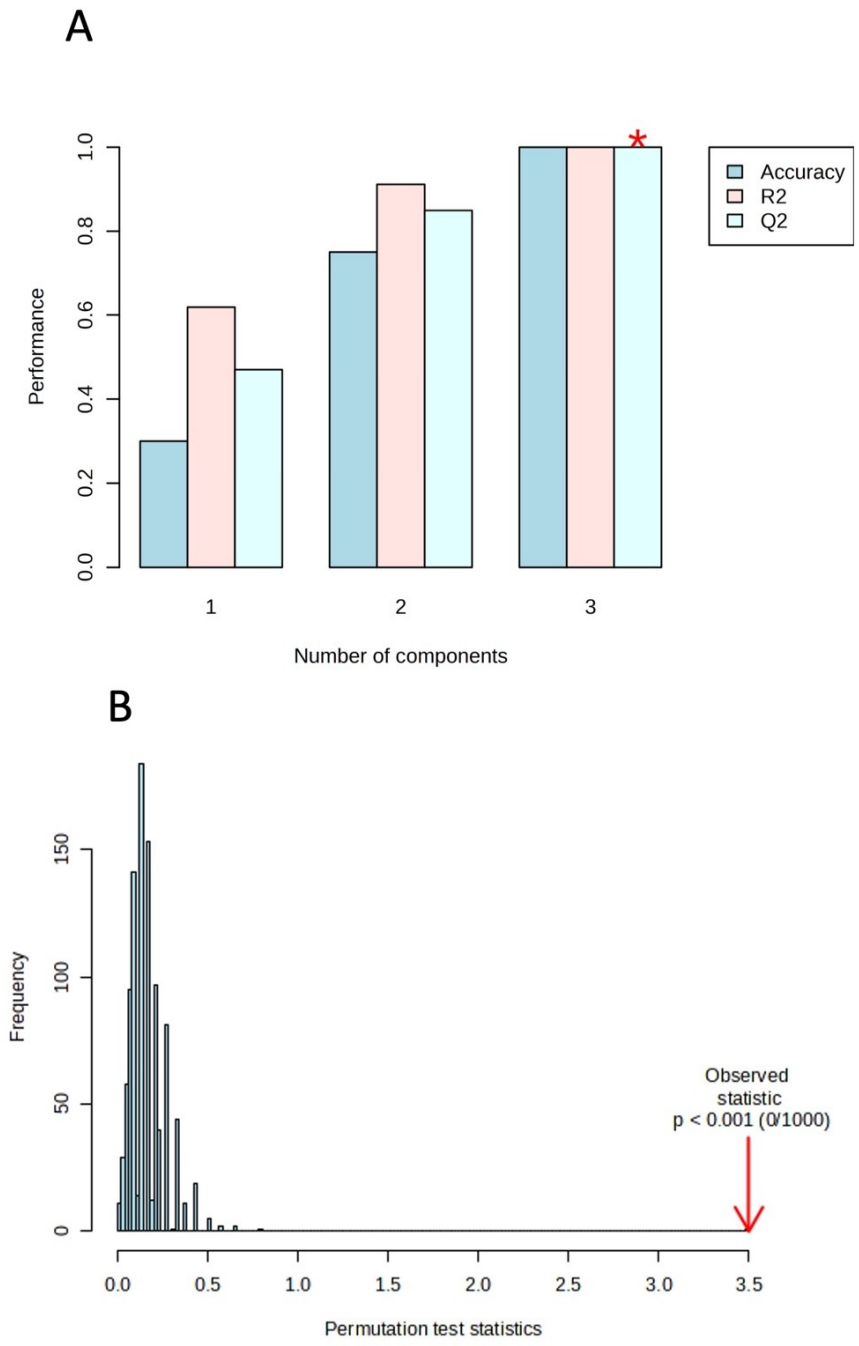

Figure S5 Permutation test (B) and cross validation (A) (n = 1000) for the PLS-DA analysis carried out on white grapes (*Chardonnay* and *Grillo*) composition.

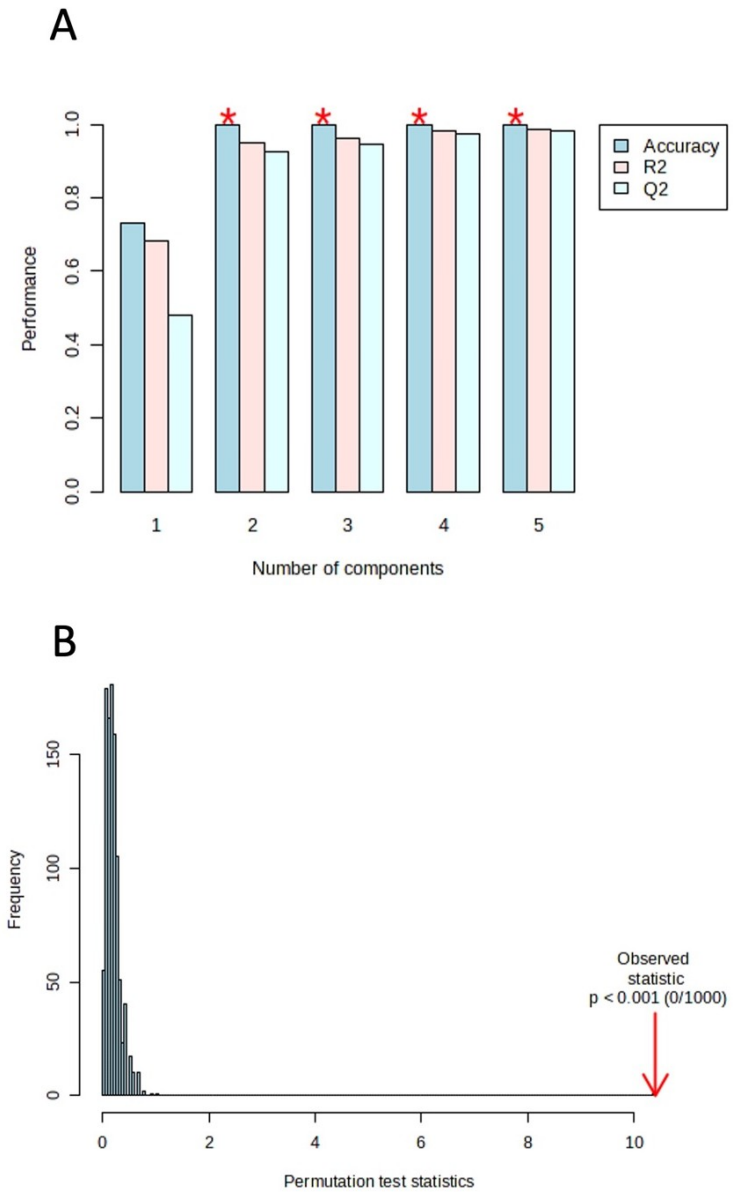

Figure S6 Permutation test (B) and cross validation (A) (n = 1000) for the PLS-DA analysis carried out on red grapes (*Nero d'Avola* and *Syrah*) composition.

CHAPTER 3: Effect of pre-fermentative cold soaking and use of different enzymes on the chemical and sensory properties of Catarratto wines

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10.1016/j.jfca.2025.107560
Journal of Food Composition and Analysis, *143*, 107560.

Abstract

The wine industry acknowledges that early harvested grapes or those with uneven ripeness often result in wines with an “unripe fruit” mouthfeel. However, the compounds causing these sensory flaws and the best winemaking techniques to address them remain unclear. This study examines the effects of pre-fermentative cold soaking (PCS) and enzyme addition during fermentation on the chemical composition and sensory properties of *Catarratto* wine, a variety commonly linked to these issues. The hypothesis suggests that grape polysaccharides released during PCS and yeast polysaccharides from fermentation contribute to producing smoother wines. Two winemaking approaches were tested: traditional non-PCS (NPCS) and PCS with 48-hour skin contact at 4°C. Each group included a control and four enzyme treatments: three pectolytic enzymes and a β-glucanase enzyme. Results showed PCS significantly increased grape polysaccharide release, doubling total colloids and enhancing the wine’s aromatic complexity. Enzyme treatments increased yeast-derived polysaccharides, with β-glucanase having the greatest impact, raising mannose levels. The addition of enzymes at the beginning of the alcoholic fermentation had no impact on fermentation kinetics but boosted yeast polysaccharide levels. Sensory analysis revealed enzyme-treated wines reduced the “unripe fruit” perception and improved smoothness. This research demonstrates for the first time the potential of PCS and enzymes to enhance the quality of *Catarratto* wines made from early harvested grapes.

Keywords: grape maturity, polysaccharides, mannose, unripe fruit mouthfeel, wine quality, sensory analsysis

Introduction

Catarratto, the most cultivated white grape in Sicily and the second in Italy, plays a key role in Italian viticulture (Carimi et al., 2010). Its wines are noted for moderate alcohol, high acidity, and pH levels influenced by vineyard elevation, with hillside grapes yielding higher acidity and malic acid. Aromatically, they feature orange blossom and citrus notes (Leder, 2020). On the palate, they are savory, salty, and have a persistent finish. However, grape heterogeneity ripening at the harvest, lead to presence of underripe, ripe and overripe fruit, simultaneously (Bambina et al., 2024a). The varying proportions of fruit contribute to differences in metabolite composition, which can adversely affect the quality characteristics and the sensory attributes of the wine (Kontoudakis et al., 2011), thereby presenting technical challenges for the winemaker. Nowadays, increasing temperatures due to climate changing condition, are leading to high accumulation of sugars level in the grapes and low total acidity, leading to wines that are too alcoholic and lack freshness. One approach to produce lower-alcohol wines with high acidity level and low pH value involves early grape harvesting (Schelezki et al., 2018). However in white wines from grapes harvested at this stage, or heterogeneous grapes ripening at harvest (where unripe berries predominate), often exhibit unripe sensory notes (Armstrong et al., 2021) often mischaracterized as bitterness. *The term "unripe" refers to a perception perceived in the mouth, a sensation of roughness that lingers even after tasting the wine.* The high acidity content of the berries does not appear to be directly related to the perception of unripeness. Several wines with naturally high total acidity due to varietal characteristics do not exhibit unripe notes. This perception instead seems to be linked to molecules that have not undergone the appropriate degree of polymerization or depolymerization typically achieved in fully ripe grapes. These compounds may be responsible for the perception of the unripe fruit character and roughness in the mouth, particularly noticeable from the mid-palate through the aftertaste. In fully ripe grapes, this phenomenon does not occur or its very low. For instance, Moscato grapes (*V. Vinifera* L.) grown in Aosta Valley or in Sicily (Pantelleria), which are harvested at high ripeness levels, do not exhibit the unripe flavor. In

contrast, Moscato from the Asti region, where early harvesting is practiced, often displays this unripe taste. This is the reason why Asti Spumante is traditionally produced with residual sugar. This issue is fundamentally tied to the ripeness level of the grapes. It is also true, that the bitterness of Muscat grape varieties can be also due to their high terpenoids content (Jones-Moore et al., 2021). Additionally, the synthesis of volatile compounds in grape berries occurs as they approach full ripeness (Kalua & Boss, 2009). *Catarratto* wines, as wines from grapes grown in hot regions, can sometimes reveals these unripe sensory notes on the palate (Pollon et al., 2024). Even though these characteristics are often observed and well known to winemakers, it is still unknown which compounds they are caused by, and which technique is best suited to solve this problem. During grape ripening, these compounds are transformed and no longer contribute to this sensory unripe perception. One hypothesis suggests that the only compounds undergoing hydrolytic transformations during grape ripening are polysaccharides, which remain intact in unripe grapes. Additionally, polysaccharides can help balance the drying sensation caused by acidity reducing the puckering taste perception. To gain a deeper understanding of this phenomenon and explore potential solutions, various enological techniques have been evaluated, including prefermentative cold soaking (PCS) and the use of different enzymes during the alcoholic fermentation. PCS is widely used in white wine production after crushing and before pressing to enhance aromatic complexity, varietal character, and color stability, while reducing oxidation (Luan et al., 2018). This technique involves keeping the must in contact with grape skins at low temperatures (5–15°C) for a few hours up to days. PCS facilitates the diffusion of polysaccharides and volatile compounds, improving sensory attributes and fermentation efficiency by releasing nitrogen, vitamins, and fatty acids (Gawel et al., 2014). However, it may also increase bitterness and astringency due to the extraction of phenolic compounds like tannins and monomeric flavan-3-ols (Sokolowsky et al., 2015). The two main sources of polysaccharides in wine are grapes and microorganisms. Wine colloids are grape-derived such as type II arabinogalactan-proteins, and rhamnogalacturonans, and yeast-derived such as mannoproteins. Mannoproteins account for

approximately 35% of the total polysaccharides in wine (Vidal et al., 2003). These polysaccharides are glycoproteins found in the outer layer of the yeast cell wall and are composed of 80% d-mannose and 10–20% protein covalently linked with d-glucose and N-acetylglucosamine residues (Rodriguez-Nogales et al., 2012). Mannoproteins, released by *Saccharomyces cerevisiae* during alcoholic fermentation, are excreted primarily during the yeast's exponential growth phase and later through enzymatic activity during wine contact with lees (Charpentier et al., 2004). These compounds have significant commercial value and have been extensively studied for their roles in wine stabilization and sensory improvement (Canalejo et al., 2024). Mannoproteins contribute to tartrate and protein stabilization, enhance sensory properties such as mouthfeel and fullness, add complexity and aromatic persistence, increase viscosity and roundness, and reduce astringency and bitterness (Martínez-Lapuente et al., 2019). Their impact depends on concentration, structural features, size, and type (Brandão et al., 2017) Commercial enzymes are used to modify the structure and levels of wine polysaccharides (Doco et al., 2007). Pectolytic enzymes, often added to must, improve juice yield, aroma, color extraction, and remove colloidal particles and pectin (Aroca et al., 2022). These enzymes also facilitate the release of polysaccharides and other compounds from grape skins (Ducasse et al., 2010). Enzyme preparations often include pectinases, β-glucanases, and glycosidases, which accelerate yeast lysis and release mannoproteins during *sur lies* élevage, positively influencing wine texture and stability (Rodriguez-Nogales et al., 2012). In this paper we investigate different enological treatments aimed at reducing or eliminating the unripe character often associated with *Catarratto* wine, obtained from early harvested grapes, thereby enhancing its sensory qualities. To better study the sensory characteristics (unripe attribute) of wines obtained from early-harvested grapes, aimed at achieving low pH values and high total acidity, the grapes typically harvested at 22-23° Brix, were instead picked at 20° Brix. The study evaluates techniques such as PCS and the addition of pectolytic and β-glucanase enzymes with secondary enzymatic activity during the alcoholic fermentation. Additionally, we assess whether a greater enrichment of grape and yeast

polysaccharides in wine by the end of alcoholic fermentation can produce softer wines without "unripe" notes.

Materials and methods

Winemaking process

A total of 550 kg of *Catarratto* grapes (*Vitis Vinifera* L.) grown in Menfi (Sicily), were manually harvested in 2022 at 20° Brix. Grapes were collected in optimal sanitary state and processed in the experimental winery of Settesoli (Menfi, Sicily). Traditional white winemaking was employed for NPCS wines, while PCS wines underwent skin contact at 4°C for 48 hours, before being pressed. Each trial was elaborated in triplicate in 5 L stainless steel tanks.

Both PCS and NPCS musts obtained were added with 5 g hL^{-1} of SO^{4+} in SO_2 eq., pectolytic enzymes (3 g hL^{-1} Hzym® Extractive FCE G, HTS enologia) and stored in a cold room (8°C) for 12-18 h to allow settling. On the clear must, five trials were conducted for each group (PCS and NPCS), including three pectolytic enzymes featuring secondary activities (Pectolytic 1; Pectolytic 2; Pectolytic 3), a glucanase enzyme (β-glucanase), and a control without the enzyme (Control) (Table 1). Diammonium phosphate (DAP) containing yeast cell walls and thiamine (0.25%) (Hnutrix® Dhizote F, HTS Enologia) was added to all the tanks to achieve 200 mg L^{-1} of readily assimilable nitrogen. The must was inoculated with a *pied de cuvée* in full fermentative activity, containing *S. cerevisiae* yeast strain (10 g hL^{-1} K1, Lallemand Wine), alongside enzyme. The alcoholic fermentation occurred at 16-18°C. Based on yeasts needs during alcoholic fermentation, open racking of 25% of the total mass and additions of organic and inorganic nitrogen (5–10 g hL^{-1}) were carried out, to promote the completion of the alcoholic fermentation and to avoid the occurrence of sulphury off-flavors. The alcoholic fermentation was finished when the concentration of reducing sugars was less than 2 g L^{-1} and white wines were dry. Then, the wines were racked off the coarse lees derived

from grapes and SO_2 was added to achieve a free SO^{4+} in SO_2 eq. of 30 mg L^{-1}. Subsequently, a portion of the wine from each trial was filtered at 1 μm (bright filtration) and bottled.

Commercial enzyme trials

Pectolytic 1: pectolytic enzyme (*Aspergillus niger)*; Pectolytic 2: pectolytic enzyme featuring β-glucosidase secondary activity (*Aspergillus niger*); β-glucanase: enzyme with pectinase and β-glucanase activity (*Aspergillus niger* and *Trichoderma harzianum*); Pectolytic 3: pectolytic enzyme featuring hemicellulose secondary activity (*Aspergillus niger).*

Wine analysis

Chemical-physical parameters

Alcohol content (%v/v), reducing sugars (g L^{-1}), titratable acidity (g L^{-1}), volatile acidity (g L^{-1}), and pH and potassium (%) were determined by means of Fourier-transform infrared (FTIR) technology through a WinescanTM FT 120 Fa. Instrument (FOSS, Hillerød, Denmark) calibrated by applying the EEC 2676 standard procedure (EEC, 1990). All the analysis were carried out in triple.

Total polyphenols and *p*-DACA (p-Dimethylaminocinnamaldehyde) reactive flavanols

Total polyphenols and *p*-DACA assay were analyzed by means of UV–Vis spectrophotometry (UV-1800 spectrophotometer, Shimazdu Scientific Instruments Inc., Columbia, MD, USA) as being (+)-catechin equivalent. Total polyphenols were determined after the dilution of the wines in hydrochloric ethanol (ethanol:water:hydrochloric acid-37 %, 70:30:1 v:v:v) (Bambina et al. 2024a). To determine the reactivity of flavanols to *p*-dimethylaminocinnamaldehyde (*p*-DACA assay), 1 mL of wine was added to 5 mL of the reagent (prepared by dissolving 100 mg of *p*-DACA in 70 mL of methanol and

25 mL of concentrated HCl, cooling the solution, and bringing the volume to 100 mL with methanol) (Corona, 2010). Total polyphenols and *p*-DACA analysis were performed in triplicate.

Total Colloids and Mannose analysis

To analyze total colloids, the total fraction was isolated from 20 mL of wine, following the method of Usseglio-Tomasset (1976). Polysaccharides were hydrolyzed by adding 2.5 mL of 1 N HCl to the sample, followed by incubation at 100°C for 1 h. Subsequently, the sample was neutralized by the addition of 4 N NaOH until reaching a pH of 7. Mannose was determined by means of UV–Vis spectrophotometry (UV-1800 spectrophotometer, Shimazdu Scientific Instruments Inc., Columbia, MD, USA) using an enzymatic assay kit (Megazyme K-MANGL 04/20, Bray Business Park, Bray, Co. Wicklow, A98 YV29, Ireland), according to the procedure outlined by Gawehn (1988). The procedure involves the phosphorylation of D-glucose, D-fructose, and D-mannose by hexokinase (HK) using ATP, resulting in glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P), and mannose-6-phosphate (M-6-P). G-6-P is then oxidized to gluconate-6-phosphate by glucose-6-phosphate dehydrogenase (G6P-DH) with NADP+, producing NADPH, which is measured at 340 nm. F-6-P is converted to G-6-P by phosphoglucose isomerase (PGI), and M-6-P is converted to F-6-P by phosphomannose isomerase (PMI) and subsequently to G-6-P. The absorbance is analyzed sequentially after each reaction step, allowing the quantification of D-glucose, D-fructose, and D-mannose based on NADPH production. D-Mannose is the major sugar component of the so-called “mannantype” polysaccharides, and in wine, it originates from the hydrolysis of polymeric molecules containing monomeric mannose, such as mannoproteins. Total colloids and mannose content analysis were carried out in triple.

Protein Stability

To assess protein stability, 10 mL of wine were filtered using a 0.45 μm membrane filter. The turbidity of the filtered sample was measured both before and after a heat treatment (30 minutes at 80°C) using a Turbidity meter and a Bentocheck instrument (Wine Time, Hi83749-02). The post-treatment turbidity measurement was taken 40 minutes after the heat treatment had concluded. The absolute difference in turbidity, expressed in NTU (Nephelometric Turbidity Units), represents the wine's stability level. The criteria for protein stability were defined as follows: < 2 NTU: Stable
> 2 NTU: Unstable. The analysis was performed in triplicate.

Potassium bitartrate stability

The stability of potassium bitartrate in wines was assessed using a conductivity drop test with the CheckStab Alfa2016Life device (Delta Acque, Florence, Italy). In brief, 100 mL of wine, centrifuged at 3000 rpm for 15 minutes, was mixed with 2 g of micronized potassium bitartrate for white wines, both at a temperature of -0.5°C. The initial electrical conductivity of the sample at -0.5 °C (denoted as χI) was measured, followed by a second reading after 15 minutes, post-addition of potassium bitartrate (denoted as χF). The change in conductivity ($\Delta\chi$) was determined by subtracting χI from χF. Wines were considered stable if the $\Delta\chi$ was less than 30 μS cm^{-1}. The analysis was carried out in triple.

Buffer capacity

50 mL of wine were placed in a 150 mL beaker, and the pH was measured to two decimal places (pH_0). If $pH_0 < 3.30$, 5 mL of 0.1 N NaOH were added, and the new pH value was recorded. Conversely, if $pH_0 > 3.30$, 5 mL of 0.1 N HCl or 0.1 N H_2SO_4 were added, and the pH value was recorded. The analysis was performed in triplicate.

Volatile organic composition

Volatile organic compounds (VOCs) were analyzed by means of Gas Chromatography (Agilent 6890 Series GC system, Milan, Italy) coupled with Mass Spectrometry (Agilent 5973 NetWork Mass Selective Detector, Milan, Italy) following the method described by Bambina et al., (2024b). In brief, 25 mL of wine was diluted to 75 mL using deionized water and supplemented with 1-heptanol standard 98 % purchased from Thermofisher (Milan, Italy) (0.25 mL of a 40 mg L^{-1} hydroalcoholic solution). The diluted sample was then processed through a 1 g C18 cartridge (Isolute, SPE Columns, Uppsala, Sweden, part no. 221-0100-C) that had been preconditioned with 3 mL of methanol followed by 4 mL of deionized water. After washing the cartridge with 30 mL of deionized water, the volatile compounds were extracted using 12 mL of dichloromethane, dehydrated, and concentrated to a final volume of 0.5 mL. Gas chromatography was performed using a DB-WAX column (Agilent Technologies, 30 m, 0.250 mm i.d., film thickness 0.25 μm, part no. 122-7032). The oven temperature program was as follows: 40 °C for 2 minutes, ramping from 40 to 60 °C at 3 °C/min, holding at 60 °C for 2 minutes, then increasing to 190 °C at 2 °C/min, holding for 10 minutes, followed by an increase to 230 °C at 5 °C/min, and a final hold at 230 °C for 15 minutes. The injection mode was splitless, with the injector set at 250 °C and the transfer line at 230 °C. Helium served as the carrier gas, with a column flow rate of 1 mL/min. Volatile organic compounds (VOCs) were identified by comparing their mass spectra and retention indices of pure commercial standards (Table S1). When no standards were available, the NIST/EPA/NIH Mass Spectral Library (Version 2.0d, 2015 build) was used. The linear retention indices (LRI) used for compound identification are provided in Table S2. VOCs were semi-quantified relative to the peak area of 1-heptanol as the internal standard, with results expressed in μg L^{-1}.

Sensory analysis

Duo-trio test

The sensory evaluation of wines by duo-trio test (UNI ISO 10399 [45]) and the paired comparison test (UNI ISO 5495 [46] were carried out. It's been followed the ISO guidelines (UNI ISO 8589 [47]) with a panel of 18 or 14 taster. The panel was composed of technicians and students of the Degree Course in Viticulture and Oenology of the University of Palermo, whose have experience with the evaluation of wines. Test significance was evaluated according to Roessler et al., (1978).

Sorting test

The wines were also evaluated using a sorting test, following the method described by Corona et al., (2021), in which panelists ranked each attribute from the weakest to the strongest sensation. The panel consisted of 17-19 trained evaluators, who considered two variables: "Unripe fruit" and "Overall preference."

Statistical analysis

A factorial analysis of variance (ANOVA) was applied to assess each treatment's individual impact. The analysis was carried out using Rstudio (RStudio Team, Boston, MA, USA) version [4.0.3]. The main factors were prefermentative cold soaking and enzyme addition treatments. Differences with *p* values of less than 5% ($p < 0.05$) were considered as statistically significant. In case of significant differences, a *post hoc* HSD Tukey's test was conducted. Analysis was in triplicate for each fermentation replicate. The results were expressed as mean values with corresponding standard error. To point out the differences of the wines with and without the prefermentative cold soaking treatment, the Orthogonal Partial Least Squares-Discriminant Analysis (O-PLS-DA) was conducted. Additionally, a O-PLS-DA was carried out to differentiate between the Control and each enzyme addition, both analyses performed using the MetaboAnalyst web-based tool suite (Pang et al., 2021). The permutation test in O-PLS-DA assesses whether the model's classification is statistically

significant or due to random chance. It randomly shuffles class labels to generate a distribution of model performance (R^2Y, Q^2) under the null hypothesis. If the original model significantly outperforms the permuted models, it confirms its validity. The PCA of sensory attributes and chemical composition of wines was performed using XLSTAT software (Addinsoft, Paris, France), version [2024.4.0].

Results

Alcoholic fermentation and wines physical-chemical composition

The sugar fermentation kinetics of *S. cerevisiae* in PCS and NPCS *Catarratto* wines supplemented with various enzymes followed a consistent pattern (Figure 1). Alcohol content increased more rapidly after nitrogen additions and partial open racking of the must. In PCS wines, enzyme-supplemented trials exhibited slightly enhanced fermentation kinetics compared to the control and the pectolytic enzyme 2 trial, with final alcohol content ranging from 11.65% to 11.79% vol (Table 2). In NPCS wines, fermentation was more favorable with pectolytic enzyme 2 up to day 8, while other trials showed similar final alcohol content (12.05 –12.5 % vol), except for pectolytic enzyme 3 (11.19 % vol). All trials completed fermentation in 13–14 days. As the ethanol production was similar in all the trials, the addition of pectolytic and β-glucanase enzymes did not influence sugar fermentation kinetics or the performance of *S. cerevisiae*, as enzymatic activity targeted non-viable yeast cells, causing their lysis during fermentation (Garcia-Moruno et al., 2001). Table 2 highlights that enzyme treatments did not significantly affect the wines' physical-chemical properties or phenolic composition, with no notable differences in alcohol content, residual sugars, pH, acidity, or polyphenols. Consistent with our findings, a previous study examining the effect of five commercial enzyme preparations on white wine composition reported that enzymes did not significantly impact the basic physical-chemical properties (Scutarașu et al., 2022). However, the PCS treatment had a notable influence on specific parameters. This process significantly decreased total acidity, with a

corresponding increase in pH, as well as an impact on *p*-DACA reactive flavanols and total polyphenols. This results are in agreement with Alti-Palacios et al. (2023).

Potassium bitartrate data, protein stability and buffer capacity

NPCS treatment significantly increased protein stability (ΔNTU < 2) and decreased buffer capacity compared to PCS wines, while the conductivity drop was not affected (Table 3). Enzyme treatments had no significant impact on conductivity drop, protein stability and buffer capacity. These findings suggest that the increase of yeast-polysaccharide during must fermentation is insufficient for effective protein and tartaric stabilization. Similarly, the extraction of grape polysaccharides did not demonstrate a significant effect on tartrate stabilization. As expected, conductivity drop showed no significant difference ($\Delta\chi > 30$ μS cm^{-1}), as the wines were not subjected to tartaric stabilization. However, the release of mannoproteins MP40 and MP32 from yeast lysis exhibited, in various instances, a beneficial impact on the conductivity drop and protein stability in white wines (Pollon et al., 2024). Likewise, RG-II, released from grapes, have also been found to contribute to tartrate stabilization (Doco et al., 2007).

Total colloids and Mannose content

Polysaccharides are the main macromolecules in wine with colloidal properties, derived either from pectic polysaccharides found in grape cell walls or mannoproteins originating from yeast cell walls. They play a pivotal role in the chemical composition and sensory profile of wine, affecting characteristics such as bitterness, astringency, and viscosity, key elements in high-quality wines (Vidal et al., 2004). Table 4 presents the results of the colloids content and mannose concentration at the end of the alcoholic fermentation for PCS and NPCS trials, with the addition of pectolytic and β-glucanase enzymes. There is two-fold more amounts of total colloids in PCS trials with respect to NPCS trials. The release of polysaccharides in PCS trials, indicates that enhanced extraction of pectins had occurred from the grape solids. This result is in agreement with Gil et al., (2015) and

Kassara et al., (2019), whose works show that increased maceration length resulted in increased grape nd P, Pellerin by the enfpolysaccharide content of the final wines, while Kassara et al., (2019) discovered that polysaccharide-associated rhamnose (RG-II) was elevated by pectinase treatment. Pectolytic-treated wines show significant higher levels of colloids compared to the control, with Pectolytic 2 enzyme exhibiting the highest concentration. The amount of polysaccharides from grape berry cell walls released in wine depends on different parameters that include the grape variety, terroir, maturity stage, vintage, the wine-making techniques, and they can be modified to a great extent by enzyme treatments (Cejudo-Bastante et al., 2018). The concentration of mannose increased in all enzyme-treated samples compared to the control, although the differences are not statistically significant. Mannoproteins are located in the outermost layer of the yeast cell wall and can constitute up to 50% of the dry mass of *S. cerevisiae*. Their release is influenced by the yeast strain, as well as winemaking and aging conditions, and they exhibit a broad molecular mass distribution with multiple populations, characterized by a high mannose residue content compared to other sugars (Gawel et al., 2014). A steadily increase of mannose in concentration into the must during fermentation and ageing processes occurs, to become one of the most prevalent polysaccharides in wine (Guadalupe et al., 2007). Mannose is present in higher concentrations in the sample treated with the β-glucanase enzyme due to its direct action on yeast cell walls. This suggests that β-glucanase enzyme acts directly on yeast cell walls, incrementing the release of yeast-derived molecules into the medium by the end of the alcoholic fermentation. Meanwhile pectolytic enzymes, act on *S.cereviasie* cell walls, likely due to a glycosidase activity present in the commercial enzyme formulation, as a side activity (Garcia-Moruno et al., 2001). Glucans from *S. cerevisiae* are mainly β-D-1,3-linked glucose units with β-D-1,6-linked lateral glucose chains and some branched β-D-1,6-glucans with some β-D-1,3-links are also present (Pollon et al., 2024). It can be confirmed that pectolytic enzymes are able to hydrolyze glucans chains, thereby indirectly releasing yeast mannans into the must. This finding was previously reported by Garcia-Moruno et al., (2001) in their study on Barbera wine. The wine treated with pectolytic enzyme was richer in grape polysaccharides and mannose content with respect to control,

indicating that pectolytic enzyme work on both grape and yeast cell walls. The greatest release of yeast-polysaccharides occurs during the exponential growth. Gil et al., (2015) reported that yeast polysaccharides (mannoproteins) were mainly released during the first week of the alcoholic fermentation. Moreover, the increase in mannose content in wine submitted to the PCS technique is due to the higher presence of grape solids, which provide greater nutrition for yeasts in the must, leading to increased production and release of mannoproteins into wine (Gawel et al., 2014).

Volatile organic compounds (VOCs)

The investigation into volatile organic compounds (VOCs) led to the identification and quantification of 46 distinct compounds in *Catarratto* wines. These compounds were categorized into seven chemical families: acids, benzenoids, acetate esters, ethyl esters, C_{13}-norisoprenoids, alcohols, and monoterpenes shown in Tables S3 – S8 of the Supplementary Information. Table S3 provides the data of the influence of the cold soak treatments and use of enzymes during alcoholic fermentation on the fatty acids composition of the wines. Acids are generated by yeast during the breakdown of fatty acids (Molina et al., 2007). This group of chemicals in wine is related to unpleasant odors, when they exceed their odor thresholds (Molina et al., 2007). However, when they are present in low concentration, these compounds add complexity to the wine's bouquet. Observing the prefermentative factor, it is possible to note that cold soak enhances the concentration of fatty acids in wines, particularly hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, and hexadecanoic acid. In contrast, the use of enzymes during fermentation did not result in significant variations in the content of these compounds across the different experimental trials. Esters, which include ethyl esters and acetates, represent a group of chemicals in wine that play a crucial role in enhancing the complexity of its aroma. The formation of esters is influenced by several factors, including yeast strain, must aeration, fermentation technology, and fruit maturity. Key nutrients include nitrogen compounds and must solids, play a particularly important role (Alti-Palacios et al., 2023). Table S4 provides the data of the ethyl esters evaluated for the factor cold soak

treatment and enzyme. It was possible to observe that cold soak treatment has a positively influence on the concentration of these compounds, especially ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate. This finding is in agreement with Alti-Palacios et al., (2023). The factor enzyme substantially exhibits very slight differences at level of ethyl octanoate and ethyl hexadecanoate (10% significance). Alcohols are secondary products of yeast alcoholic fermentation, and based on their concentration, this chemical family can have either a positive or negative influence on wine aroma (Cai et al., 2014) . Table S5 reports the data of alcohols evaluated on the two factors studied. The enzyme factor did not show any significative effect on the concentration of these latter compounds, while cold soak treatment led to an increase in the concentrations of isoamyl alcohol, 3-ethoxypropanol and methionol. Acetate esters consist of an acid group (acetate) and an alcohol group, which can either be ethanol or a more complex alcohol derived from the metabolism of amino acids (Zhang et al., 2015). Table S6 presents the effect of the factor cold soak and enzyme on the acetate esters of the related wines. The enzyme factor did not show any significative effect also on the concentration of these compounds except in tryptophol content, while cold soak treatment led to an increase in the concentrations of hexyl acetate, phenylethyl acetate, and isoamyl acetate (10% significance). Benzyl alcohol, 2-phenylethanol, acetovanillone, 4-vinylguiacol were identified in the related *Catarratto* wines (Table S7). The factor enzyme did not show any significant effect on the concentration of this class of compounds. The cold soak treatment resulted in significantly higher concentrations of benzyl alcohol. Norisoprenoids in wine originate primarily from the oxidative degradation of carotenoids, such as beta-carotene and lutein, present in grapes. During grape maturation and vinification processes, carotenoids undergo chemical cleavage, leading to the formation of aromatic compounds (Mendes-Pinto, 2009). Monoterpenes are regarded as key odorants in aromatic grape varieties of *Vitis vinifera* L.*,* to which they impart their characteristic floral aromas. Monoterpenes, such as linalool, geraniol, nerol, citronellol, and α-terpineol generally carrying floral and/or citrus odours, are the most abundant forms of terpenes (Panighel & Flamini, 2014). Table S8 represents the effect of the factor cold soak and enzyme on the concentration of C_{13}-norisoprenoids

and monoterpenes. The factor enzyme did not show any significative difference on these two chemical families. The cold soak treatment, on the other hand, increased the concentrations of C_{13}-norisoprenoids, specifically blumenol C and vomifoliol. In contrast, the treatment resulted in a decrease in the concentration of monoterpenes, although this change was not statistically significant. However, in model systems, polysaccharides typically reduce aroma release, either by altering the viscosity of the medium or through direct molecular interactions with aromatic compounds (Jones-Moore et al., 2021). Several studies have investigated the influence of polysaccharides on wine aroma compounds, with most concluding that the reduction in aroma volatility is influenced by the hydrophobicity of both the aroma compounds and the polysaccharides (Rinaldi et al., 2019). This fact implies a longer aromatic perception because the volatile compounds retained by polysaccharides will be slowly released (Del Barrio-Galán et al., 2011). The impact of some pectic polysaccharides on some volatile compounds on wine model solution was studied (Jones-Moore et al., 2021). It was observed that uronic-rich polysaccharides could have two opposite effect: a suppressing effect, decreasing the amount of aroma in the headspace, or a "salting out" effect causing an increase in the headspace concentration of a volatile compound because of the increase in the ionic strength of the solution. Pérez-Magariño et al., (2022) found that grape and yeast derived polysaccharides increased the concentration of most of the chemical families of positive aroma compounds in white wine, such as ethyl esters of straight-chain fatty acids, alcohol acetates and terpenes associated with the fresh, fruity, and floral notes. These results suggest that polysaccharides can influence the hydrolysis/esterification balance between ethyl esters and alcohols acetate during aging or storage, favoring the maintenance of the volatile compounds associated with fruity and floral notes over time (Pérez-Magariño et al., 2022).

Sensory analysis

The sensory analysis of *Catarratto* wines was carried out, by comparison of the experimental wines, performing duo-trio test and preference test. In Table 5 the results show the outcomes of the duo-trio

test sensory test regarding the "unripe fruit" character. In both NPCS and PCS wines, the control wines exhibited a higher perception of unripe fruit compared to enzyme-treated wines. Specially, in NPCS wines, the addition of the enzyme Pectolytic 1 and Pectolytic 2 showed significant differences, with 14 panelists perceiving the control having a stronger unripe fruit perception versus 4 ($p < 0.04$). In PCS wines, the addition of Pectolytic 1, Pectolytic 2, and β-glucanase enzymes also showed significant differences, with *p*-values of $p < 0.03$ and $p < 0.02$, respectively. This finding supports the hypothesis that the PCS technique, along with the addition of pectolytic and β-glucanase enzymes during must fermentation, which facilitates the release of grape and yeast-derived polysaccharides, positively impacts wine mouthfeel, contributing to reducing the perception of unripe fruit. When the judges were asked to express a preference (Table 6), NPCS wines treated with Pectolytic 1 and Pectolytic 2 enzyme were preferred over the control, with 14 answers out of 18 ($p < 0.04$). Similarly, in PCS wines, those treated with Pectolytic 1 and Pectolytic 2 showed to be preferred over the control ($p < 0.02$), while other enzyme treatments did not reach significance. Judges described these enzyme-treated wines as less astringent, smoother, and better balanced (Table 6). These results align with some studies find in literature. Pérez-Magariño et al., (2022) in his work, found that polysaccharides reduced the perception of acidity and bitterness in white wines, giving rise to sweeter wines, while Del Barrio-Galán et al., (2011) observed a reduction in bitterness due to the addition of commercial yeast derivatives primarily composed of mannoproteins. Quijada-Morin et al., (2014) and Gawel et al., (2014), in their studies reported that polysaccharide concentrations enhanced mouthfeel properties such as fullness and viscosity reducing the perceived astringency in red wines. Quijada-Morin et al., (2014) found that among all the polysaccharides families, RG-II and mannoproteins were showing the stronger effect to reduce the astringency. Mannoproteins released by yeasts, may interact with polyphenols aggregates as steric stabilizers (Poncet-Legrand et al., 2007). This feature is related to the ability of mannoproteins to interact with polyphenols found in wine, forming hydrogen bonded complexes with them. In this way, bitter polyphenols would not be able to interact with taste receptors, developing the astringency perception (Poncet-Legrand et al., 2007). Polysaccharides are

also capable of inhibiting salivary protein-polymerized polyphenols interactions by forming ternary complexes with proteins and polyphenols through hydrophobic interactions (Gawel et al., 2016). In white wines, the reduction in unripe fruit perception may also be due to the masking effect of the sapid peptide (Hsp12p), a heat-shock protein from mannoprotein released during the autolysis of *S. cerevisiae*, which exhibits a sweet taste and thereby contributes to the reduction of wine astringency (Rinaldi et al., 2019). The group of grape polysaccharides RG-II have also been described to reduce the overall wine astringency and indirectly influence sweetness (Quijada-Morin et al., 2014). RG-II is the most grape polysaccharide to be effective to reduce the interaction between salivary proteins and wine polyphenols. The ability to smooth the perception of astringency is likely related to the branched structure and the presence of unusual sugars (which can contribute to a high-sugar characteristic), as well as the linkages between them (Quijada-Morin et al., 2014). In red wines, the interaction of polysaccharides with other compounds increases with the greater presence of the glucose moiety in the compounds they bind with, as it promotes a high number of binding sites for interactions with polysaccharides, such as gallotannins (Brandão et al., 2024). Thus, the interaction of all the structures containing glucose residues, will be highly reduced. However, in white wines, specially made from grapes not fully ripped, the total content of phenolic compounds is higher, such as condensed tannins and their role in the astringency is less clear (Simoes Costa et al., 2015). Polysaccharides can also interact with other polysaccharides molecules. The interactions of polysaccharides with other polysaccharides molecules were already described, although it was reported that they had lower affinity than the interactions of polysaccharides with other ligands (Won et al., 2018). White wines made from grapes that have not reached full ripeness are low in polyphenolic content and polysaccharide-polysaccharide binding could occur. Polysaccharides, such as RG-II and mannoproteins, may interact with other grape polysaccharides molecules that have not gone through the hydrolyzing transformation yet. One hypothesis suggests that non-hydrolyzed polysaccharides (from unripe grape berry cell walls) are responsible for the unripe character. If this hypothesis is true, the binding between polysaccharides themselves could mitigate the perception of

unripe flavor by reducing mouth puckering sensation. Furthermore, the high acidity and the low alcohol content of the resulting wine, could be involved on the astringency perception (Brandão et al., 2024) contributing to the unripe character. As these astringent attributes are localized on the mouth surfaces, the presence of polysaccharides with viscous properties may compensate for the decrease in mouth lubrication (Brandão et al., 2024). However, the astringency should not be confused with the drying sensation caused by organic acids in white wines (Gawel et al., 2016). RG-II is the most abundant grape polysaccharide in juice, and its presence is largely due to its ease of enzymatic solubilization from the cell wall and its resistance to fragmentation by pectinases used in must production (Vidal, 2001). This group of polysaccharides will be more abundant in red wines, where fermentation occurs in the presence of the grape skins, compared to white wines, which are produced by fermenting the juice of crushed and pressed grapes (Gawel et al., 2016). Therefore, we expect the impact of RG-II on the perception of astringency in traditionally vinified white wines (without skin contact) to be lower than in white wines subjected to a pre-fermentative cold soaking treatment, and even lower than in red wines. Red wines are expected to contain 100 to 150 mg of RG-II L^{-1}, while white wines should contain 30 to 50 mg of RG-II L^{-1} (Doco et al., 1997). Thus, in the vinification of white grape varieties that do not undergo maceration for enological purposes, the focus should be on extracting polysaccharides from yeast cell walls during alcoholic fermentation to mitigate the perception of unripe flavors in the mouth. Additionally, Vidal et al., (2004), observed that in a wine model solution RG-II had no effect on bitterness in absence of mannoproteins, but enhanced it in its presence. However, Quijada-Morin et al., (2014) found that an increase in mannose and rhamnose in the oligosaccharide fraction, resulting from the degradation of mannoproteins and rhamnogalacturonans, was positively correlated with astringency. This finding supports the idea that the complete polysaccharide molecule is essential for effective interaction with other astringent ligands, highlighting the need for careful evaluation of enzymatic preparations in enology to prevent undesirable polysaccharide degradation. Although polysaccharides seem to play important roles in the wine's astringency, some characteristics and composition of the initial wine are also affecting the

sensory properties of the wine. Rinaldi et al., (2012) found that in a red wine, salivary protein precipitation, a key factor in astringency perception, is influenced by ethanol, tartaric acid, fructose, and mannoproteins. Tartaric acid significantly increased salivary protein precipitation and thus the perceived astringency, while ethanol, fructose and mannoproteins reduced them. Moreover, as the concentration of each influencing factor increase, its effect on salivary protein precipitation became more pronounced (Rinaldi et al., 2012).

Multivariate statistical analysis

O-PLS-DA based on the overall wine composition

To further understand the difference between control and PCS technique and the impact of each enzyme added during the alcoholic fermentation on the chemical properties of *Catarratto* wines a supervisioned Orthogonal Partial Least Squares Discriminant Analysis (O-PLS-DA) was carried out. A preselective O-PLSDA was performed to select the variables with a VIP score greater than 1. Figure 2 (a) presents the O-PLS-DA Score Plot for PCS and NPCS wines. The Score Plot shows that the two groups, PCS and NPCS wines, are distinctly clustered. The most important 15 variable importance in projections (VIPs) are shown in Panel (b) of Figure 2. The colored boxes on the right represent the direction of association of each variable with the two experimental groups. Specifically, each color indicates whether a variable is more closely associated with one group over the other. It can be observed that the features mostly contributing to discrimination were *p*-DACA, total colloids, buffer capacity, diendiol I, linalool, hexyl acetate, and mannose. PCS treatment increased key volatile compounds such as esters (hexyl acetate, phenylethyl acetate) and alcohols ((E)-hexenol, (Z)-3-hexenol) which contribute to aroma complexity. Previous studies identified hexyl acetate and phenylethyl acetate as some of the aroma compound markers that discriminated PCS from the NPCS wines. Their corresponding odor sensory descriptors are green and fruity for hexyl acetate and rose and floral for phenylethyl acetate (Alti-Palacios et al. 2023). In addition, previous studies showed that

maceration of the crushed and destemmed grapes before fermentation led to a higher abundance of low molecular weight phenolic compounds (Cai et al., 2014). The pre-fermentative macerations increases also the content of C6 alcohols extracted in the must, as earlier reported (Cejudo-Bastante et al., 2011). Meanwhile, terpenes compounds such as diendiol I, linalool, α-terpineol, endiol were found in higher concentration in wines that were not submitted to the PCS technique. In general, Alti-Palacios et al., (2023) observed that the concentration of the aroma compounds was higher in the wines submitted to the PCS technique, except for terpenes compounds, which showed different trends between the control and PCS wines trials. PCS also enhanced structural components such as colloids and mannose which play a crucial role in mouthfeel properties of a white wine, such as reduce astringency and bitterness (Gawel et al., 2016). Additionally buffer capacity enhances the perception of acidity on the palate and improves the wine's taste persistence (Obreque-Slier et al., 2016). The permutation test results confirmed the statistical significance of the O-PLS-DA model, with empirical p-values of R^2Y: $p = 0.001$ (Supplementary Information, Figure S1 (a,b). In Figure 3 the O-PLS-DA Scores Plot in each panel (A, C, E, G) show clear clustering of the control wines (red, left side) and enzyme-treated wines (green, right side). This separation indicates a distinct impact of enzymatic treatments (Pectolytic 1, Pectolytic 2, Pectolytic 3, and β-glucanase) on the volatile organic composition and the chemical-physical parameters of the wines compared to the untreated controls. VIPs (panels B, D, F, H) of Figure 3 identify the most influential variables driving the separation. The increase in volatile compounds varies among the enzymatic treatments, reflecting the specificities of each enzyme. However, it can be observed that the addition of Pectolytic_1, Pectolytic_2, and β-glucanase to the fermenting must leads to an increase in terpenoid molecules. In most cases, terpenes are more abundant in their glycosylated form rather than in their free (unglycosylated) form. Their increase in wine can be explained by the β-glucosidase activity as a side function of certain enzymes (Rodríguez-Nogales et al., 2024). It has been stated that the increase of some aroma compounds could reduce astringency and bitterness of wines (Ferrer-Gallego et al., 2014). Volatile compounds may play a role in modulating astringency, likely due to a cognitive association between smell, taste, and

mouthfeel, as flavor perception involves olfaction, gustation, and chemesthesis (Ferrer-Gallego et al., 2014). Colloids appear prominently in the VIP scores across the enzyme treatments highlighting their impact by enzyme activity. Mannose is specifically highlighted in the β-glucanase treatment (Panel F). This results align with the findings of Pellerin & Tessarolo (2001) where the addition of commercial enzyme preparations to the fermenting must optimized and accelerated the autolysis of yeast cell walls, releasing yeast-derived polysaccharides into the medium.

Principal component analysis (PCA) of sensory attributes and chemical composition

To further explore the impact of PCS technique and the studied enzymes, as well as the relationship between the chemical composition and the examined sensory attributes an unsupervised PCA was performed. PCA was conducted for the two groups (NPCS and PCS) using wine chemical composition data, the sum of the chemical classes of volatile organic compounds, and the two key sensory attributes, including the unripe fruit attribute and overall satisfaction, as variables (Figure 4 (a) and (b), respectively). Data related to the sorting test can be found in Figure S3 of the Supplementary Information. In Figure 4a, for NPCS wines, the PCA biplot visualizes the first two principal components, PC1, which explains 49.98% of the variance, and PC2, which accounts for 25.87% of the variance, together explaining 75.85% of the total variance. For PC1, the variables with strong positive correlations (Pearson's coefficient > 0.7) include benzenoids, acetate esters, ethyl esters, C_{13}-norisoprenoids, alcohols, mannose, esters of organic acids, and colloids. Acids exhibit a weaker positive correlation. The unripe fruit attribute has a weak negative correlation with PC1. For PC2, the variables with strong positive correlations (Pearson's coefficient > 0.7) are buffer capacity and total polyphenols. On the negative side, *p*-DACA shows a strong negative correlation, while lactones and acids are negatively correlated, though less strongly. The overall satisfaction score is also correlated with PC2. NPCS_Control and NPCS_Pectolytic 1 are separated on the negative side of PC1; NPCS_Pectolytic 2 is separated on the positive sides of both PC1 and PC2; NPCS_Pectolytic 3 is separated on the negative sides of both PC1 and PC2, while NPCS_β-glucanase is located on the

positive side of PC1 and negative side of PC2. In Figure 4b, for PCS wines, the PCA biplot illustrates that PC1 explains the 50.26% of the variance, the PC2 accounts for 27.73% of the variance, together explaining the 78% of the total variance. For PC1 variables with strong positive correlations (Pearson's coefficient > 0.7) include acids, esters of organic acids and benzenoids, which contribute to the separation of samples in the positive PC1 region. On the negative side, variables with strong negative correlations (Pearson's coefficient < -0.7) include *p*-DACA, total polyphenols, acetate esters, ethyl esters, alcohols, and lactones, driving the separation of samples in the negative PC1 region. For PC2, variables with strong positive correlations include mannose, overall satisfaction, and colloids, which contribute to the separation of samples in the positive PC2 region. On the negative side, the variable unripe fruit shows a strong negative correlation, driving the separation of samples in the negative PC2 region. PCS_Control is located on the negative side of both PC1 and PC2. PCS_Pectolytic 3 is located in the negative side of PC1. PCS_Pectolytic 1 aligns in the positive side of PC1. PCS_β-glucanase and PCS_Pectoltyic 2 are located on the positive side of PC2 and negative side of PC1. It can be observed that in both groups (NPCS and PCS), the enzymes Pectolytic 2 and Pectolytic 3 often display similar behavior to the β-glucanase enzyme. They show increased concentrations of colloids and mannose, which result in higher scores for overall satisfaction. Additionally, these enzymes are consistently positioned on the opposite side of the unripe fruit attribute, exhibiting an opposite behavior compared to the control. An increase of grape and yeast derived polysaccharides in white wines at the end of the alcoholic fermentation helps to improve the mouthfeel attributes, reducing the unripe fruit character, saving time and storage space by avoiding the need for an extended maturation period of the wine on the lees. There is a difference in behavior among the enzymes, but there is also a distinction between all the enzymes trials and the control. These latter analyses suggested there is a marked impact of the addition of commercial enzymatic preparation featuring secondary activity along with fermenting yeasts on wine chemical and sensory profiles (Chong et al., 2019) from early harvested grapes.

Conclusion

The results of this study provide compelling evidence that PCS treatment and enzyme addition during alcoholic fermentation, significantly improve the chemical and sensory properties of *Catarratto* wines. PCS enhanced the release of grape polysaccharides, doubling the total colloidal fraction, while enzymes, particularly β-glucanase, increased mannose concentration. Sensory analysis confirmed reduced "unripe fruit" perception and overall preference of the resulting wines. These findings highlight, for the first time, the potential of these techniques to optimize the quality of wines made from *Catarratto* grapes obtained from early harvested grapes. These results suggest that these techniques may be applicable to all *Vitis vinifera* L. grape varieties that are harvested early for technological purposes or display berry ripening heterogeneity at harvest, conditions that often result in wines with ‘unripe’ sensory notes on the palate. This technique will be further investigated in other grape varieties, including red grapes, to assess its potential effects on phenolic compounds (such as anthocyanins and polyphenols) extracted during fermentative maceration, as well as its impact on unripe sensory perceptions.

Acknowledgements

The authors express their gratitude to Settesoli Winery (Menfi, Agrigento), as well as to the oenologist and agronomist, for their availability and valuable support.

Funding information

This study was founded by “Programma di Sviluppo Rurale Sicilia 2014-2020” project GO V.E.I.S.CA beneficiary Cantine Settesoli s.c.a.

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Table 1 Experimental setup of prefermentative treatments applied to Catarratto wines. The table includes control samples and samples treated with Prefermentative Cold Soaking (PCS) or Non-Prefermentative Cold Soaking (NPCS) in combination with different enzymatic preparations (Pectolytic 1, Pectolytic 2, β-glucanase, and Pectolytic 3). Sample names indicate the treatment and enzyme used.

Catarratto wines	Prefermentative treatment	Enzyme	Sample name
Group 1	Control	-	PCS_Control
	PCS	Pectolytic 1	PCS_Pectolytic 1
	PCS	Pectolytic 2	PCS_Pectolytic 2
	PCS	β-glucanase	PCS_β-glucanase
	PCS	Pectolytic 3	PCS_Pectolytic 3
Group 2	Control	-	NPCS_Control
	NPCS	Pectolytic 1	NPCS_Pectolytic 1
	NPCS	Pectolytic 2	NPCS_Pectolytic 2
	NPCS	β-glucanase	NPCS_β-glucanase
	NPCS	Pectolytic 3	NPCS_Pectolytic 3

Table 2 Wines physical-chemical composition and polyphenols

Factor Prefermentative (n = 5)	Alcohol strenght (% vol)	Residual sugars ($g\ L^{-1}$)	pH	Total acidity ($g\ L^{-1}$)	Volatil acidity ($g\ L^{-1}$)	K (%)	*p*-DACA ($mg\ L^{-1}$)	Total polyohenols ($mg\ L^{-1}$)
PCS	11.7 ± 0.1	1.59 ± 0.04	3.6 ± 0.1	4.3 ± 0.5	0.27 ± 0.01	1.2 ± 0.3	4.6 ± 0.1	60 ± 1
NPCS	12.0 ± 0.3	1.6 ± 0.2	3.31 ± 0.03	6.2 ± 0.1	0.22 ± 0.02	0.95 ± 0.04	3.1 ± 0.3	15 ± 2
Sign.	Ns	ns	**	**	*	ns	***	***
Enzyme (n= 2)								
Control	11.7 ± 0.3	1.6 ± 0.1	3.5 ± 0.3	5 ± 1	0.25 ± 0.01	1.0 ± 0.1	4 ± 1	40 ± 24
Pectolytic 1	12.0 ± 0.5	1.7 ± 0.1	3.5 ± 0.2	5 ± 1	0.3 ± 0.0	1.04 ± 0.04	4 ± 1	36 ± 21
Pectolytic 2	12.0 ± 0.3	1.7 ± 0.1	3.4 ± 0.1	6 ± 1	0.2 ± 0.0	0.96 ± 0.01	4 ± 1	35 ± 26
β-Glucanase	12.0 ± 0.1	1.60 ± 0.01	3.5 ± 0.3	5 ± 2	0.3 ± 0.0	1.0 ± 0.1	4 ± 1	40 ± 20
Pectolytic 3	11.6 ± 0.2	1.5 ± 0.1	3.5 ± 0.3	5 ± 2	0.2 ± 0.1	1 ± 1	4 ± 1	38 ± 22
Sign.	ns	ns	ns	ns	ns	ns	ns	Ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, . = 90% of significance, ** = 99% of significance, *** = 99 % of significance. PCS= prefermentative cold soaked wines NPCS = non-prefermentative cold soaked wines, with the addition of different pectolytic and β-glucanase enzymes.

Table 3 Conductivity drop, protein stability and buffer capacity at the end of alcoholic fermentation

Factor	Δχ Conductivity drop ($\mu S\ cm^{-1}$)	Δ NTU	Buffer capacity ($meq\ L^{-1}$)
Prefermentative (n = 5)			
PCS	149 ± 8	2.6 ± 0.5	48 ± 1
NPCS	138 ± 16	0.9 ± 0.3	32 ± 3
Sign.	ns	**	***
Enzyme (n= 2)			
Control	141 ± 24	1.7 ± 0.5	40 ± 6
Pectolytic 1	147 ± 14	1 ± 1	40 ± 5
Pectolytic 2	124 ± 25	1.2 ± 0.4	41 ± 6
β-Glucanase	139 ± 12	2 ± 1	38 ± 6
Pectolytic 3	167 ± 26	2 ± 1	41 ± 7
Sign.	ns	Ns	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, . = 90% of significance, ** = 99% of significance, *** = 99 % of significance. PCS= prefermentative cold soaked wines NPCS = non-prefermentative cold soaked wines, with the addition of different pectolytic and β-glucanase enzymes.

Table 4 Mannose content and total colloids at the end of the alcoholic fermentation in prefermentative cold soaked (PCS) and non-prefermentative cold soaked (NPCS) *Catarratto* wines, with the addition of different pectolytic and β-glucanase enzymes.

Factor	Colloids (g L^{-1})	Mannose (mg L^{-1})
Prefermentative (n = 5)		
PCS	0.5 ± 0.1	105 ± 9
NPCS	0.25 ± 0.03	55 ± 3
Sign.	**	.
Enzyme (n = 2)		
Control	0.2 ± 0.1 d	52 ± 6
Pectolytic 1	0.4 ± 0.1 c	81 ± 15
Pectolytic 2	0.5 ± 0.1. a	83 ± 11
β-Glucanase	0.4 ± 0.1 bc	106 ± 25
Pectolytic 3	0.5 ± 0.1 ab	79 ± 17
Sign.	**	Ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, . = 90% of significance, ** = 99% of significance, *** = 99 % of significance. Different letters mean different averages for the Tukey's *post hoc* test with α value of 0.05. PCS= prefermentative cold soaked wines NPCS = non-prefermentative cold soaked wines, with the addition of different pectolytic and β-glucanase enzymes

Table 5 Results of duo-trio test for the "unripe fruit" perception: performed on different pectolytic and β-glucanase enzyme-treated *Catarratto* wines, both prefermentative cold-soaked (bottom) and non-prefermentative cold soaked (top).

	Pairs of Wines	Number of Judges	Number of Correct answers	Numbers of Correct answers required for a $p < a$	Significance
Catarratto NPCS	Control vs. Pectolytic 1	18	14 vs. 4	14 for a $p < 0.04$	$p < 0.04$
	Control vs. Pectolytic 2	18	14 vs. 4	14 for a $p < 0.04$	$p < 0.04$
	Control vs. β-Glucanase	18	12 vs. 6	14 for a $p < 0.04$	ns.
	Control vs. Pectolytic 3	14	8 vs. 6	12 for a $p < 0.02$	ns.
Catarratto PCS	Control vs.Pectolytic 1	14	11 vs. 3	11 for a $p < 0.03$	$p < 0.03$
	Control vs. Pectolytic 2	14	11 vs. 3	11 for a $p < 0.03$	$p < 0.03$
	Control vs. β-Glucanase	14	12 vs. 2	12 for a $p < 0.02$	$p < 0.02$
	Control vs. Pectolytic 3	14	10 vs. 4	12 for a $p < 0.02$	ns.

Note: p: p-value; a: significance level; ns.: not significant.

Table 6 Results of preference test: performed comparing different pectolytic and β-glucanase enzyme-treated *Catarratto* wines, both prefermentative cold soaked (bottom) and non-prefermentative cold soaked (top) to control.

	Pairs of Wines		Number of Correct answers	Numbers of correct answers required for a $p < a$	Significance	Less astringent	Smoother	More balanced
Catarratto NPCS	Control vs. Pectolytic 1	18	4 vs.14	14 for a $p < 0.04$	$p < 0.04$	35%	35%	30%
	Control vs. Pectolytic 2	18	4 vs.14	14 for a $p < 0.04$	$p < 0.04$	27%	44%	30%
	Control vs. β-Glucanase	18	6 vs.12	14 for a $p < 0.04$	ns.	-	-	-
	Control vs. Pectolytic 3	14	7 vs.7	12 for a $p < 0.02$	ns.	-	-	-
Catarratto PCS	Control vs.Pectolytic 1	14	2 vs.12	12 for a $p < 0.02$	$p < 0.02$	40%	40%	20%
	Control vs. Pectolytic 2	14	2 vs.12	12 for a $p < 0.02$	$p < 0.02$	34%	42%	24%
	Control vs. β-Glucanase	14	3 vs.11	12 for a $p < 0.02$	ns.	-	-	-
	Control vs. Pectolytic 3	14	8 vs.6	12 for a $p < 0.02$	ns.	-	-	-

Note: p: p-value; a: significance level; ns.: not significant.

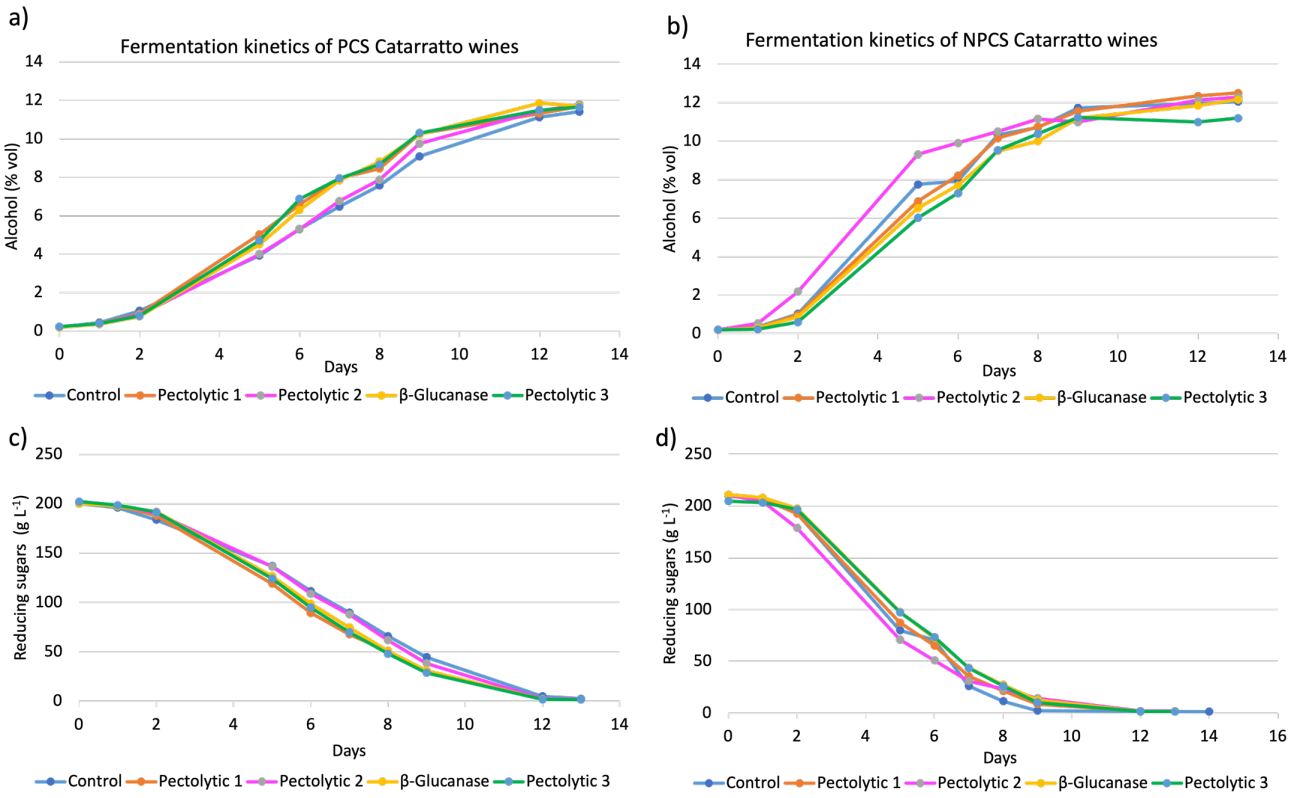

Figure 1: Fermentation kinetics of prefermentative cold soaked (PCS) (a,c) and non-prefermentative cold soaked (NPCS) (b, d) Catarratto wines with the addition of different pectolytic and β-glucanase enzymes.

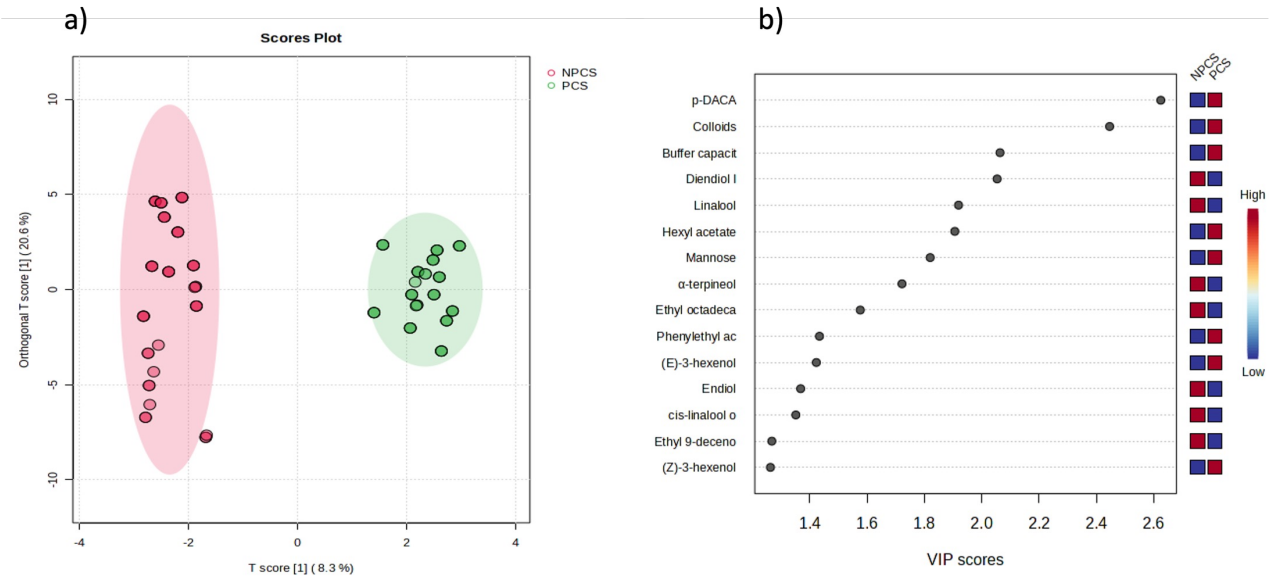

Figure 2: O-PLS-DA Score Plot (a) showing the separation between *Catarratto* wines with prefermentative cold soak (PCS) and non-prefermentative cold soak (NPCS) as based on the volatile organic composition and the chemical-physical parameters. Important variables in projections (VIPs) are reported in (b). The colored boxes on the right of the figure indicate the relative concentrations of the corresponding metabolites.

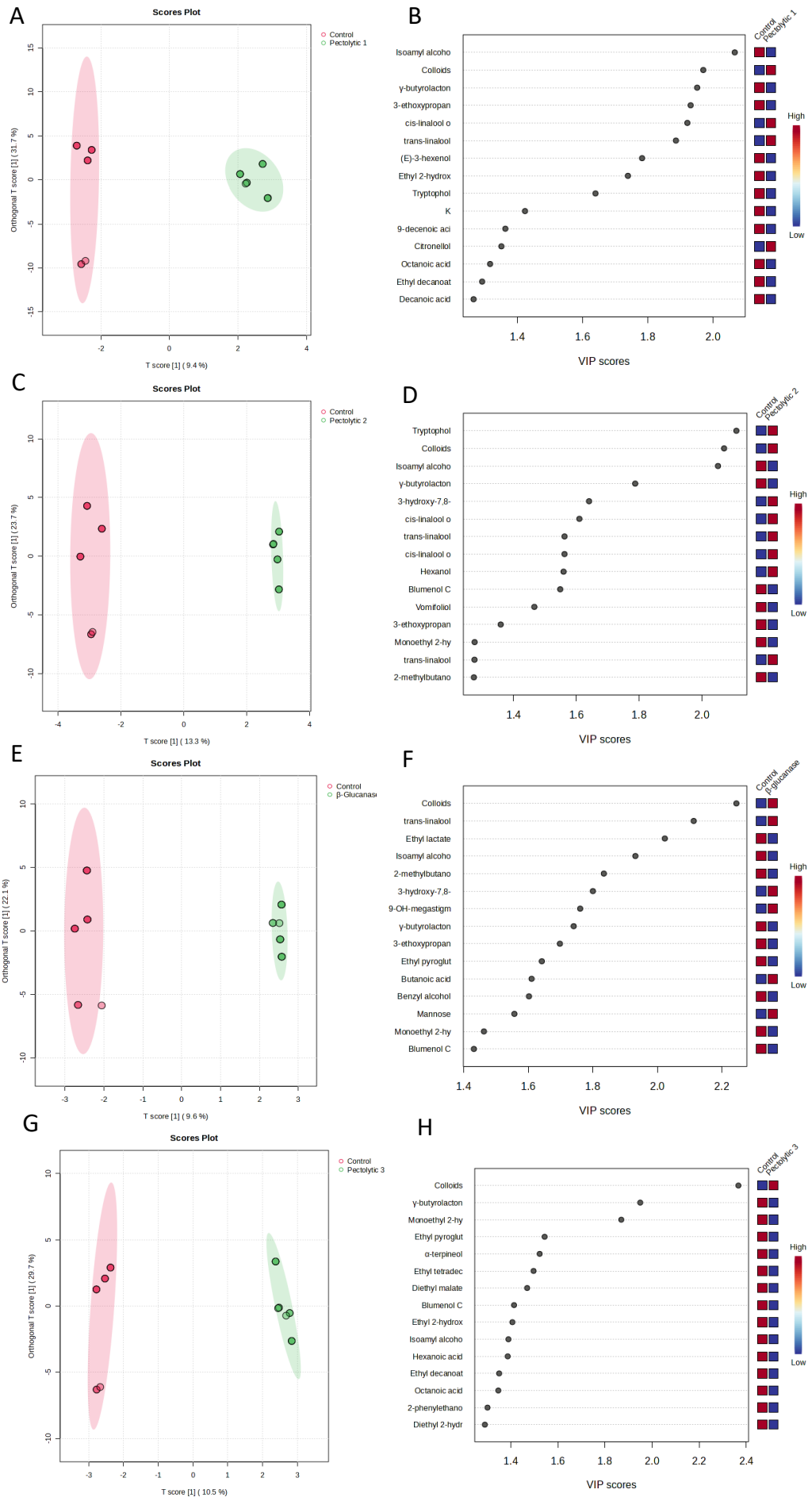
A
Scores Plot
Control
Pectolytic 1
Orthogonal T score [1] (31.7 %)
T score [1] (9.4 %)
B
Control
Pectolytic 1
Isoamyl alcoho
Colloids
γ-butyrolacton
3-ethoxypropan
cis-linalool o
trans-linalool
(E)-3-hexenol
Ethyl 2-hydrox
Tryptophol
K
9-decenoic aci
Citronellol
Octanoic acid
Ethyl decanoat
Decanoic acid
High
Low
VIP scores
C
Scores Plot
Control
Pectolytic 2
Orthogonal T score [1] (23.7 %)
T score [1] (13.3 %)
D
Control
Pectolytic 2
Tryptophol
Colloids
Isoamyl alcoho
γ-butyrolacton
3-hydroxy-7,8-
cis-linalool o
trans-linalool
cis-linalool o
Hexanol
Blumenol C
Vomifoliol
3-ethoxypropan
Monoethyl 2-hy
trans-linalool
2-methylbutano
High
Low
VIP scores
E
Scores Plot
Control
β-Glucanasi
Orthogonal T score [1] (22.1 %)
T score [1] (9.6 %)
F
Control
β-glucanase
Colloids
trans-linalool
Ethyl lactate
Isoamyl alcoho
2-methylbutano
3-hydroxy-7,8-
9-OH-megastigm
γ-butyrolacton
3-ethoxypropan
Ethyl pyroglut
Butanoic acid
Benzyl alcohol
Mannose
Monoethyl 2-hy
Blumenol C
High
Low
VIP scores
G
Scores Plot
Control
Pectolytic 3
Orthogonal T score [1] (29.7 %)
T score [1] (10.5 %)
H
Control
Pectolytic 3
Colloids
γ-butyrolacton
Monoethyl 2-hy
Ethyl pyroglut
α-terpineol
Ethyl tetradec
Diethyl malate
Blumenol C
Ethyl 2-hydrox
Isoamyl alcoho
Hexanoic acid
Ethyl decanoat
Octanoic acid
2-phenylethano
Diethyl 2-hydr
High
Low
VIP scores

Figure 3: O-PLS-DA scores plots (panels A, C, E, G) illustrating clear clustering between control wines (red, left) and enzyme-treated wines (green, right), demonstrating the distinct impact of enzymatic treatments (Pectolytic 1, Pectolytic 2, β-glucanase and Pectolytic 3) on the volatile organic composition and chemical-physical parameters. Panels B, D, F, and H show the corresponding Variable Importance in Projection (VIP) scores, highlighting the key variables driving these differences.

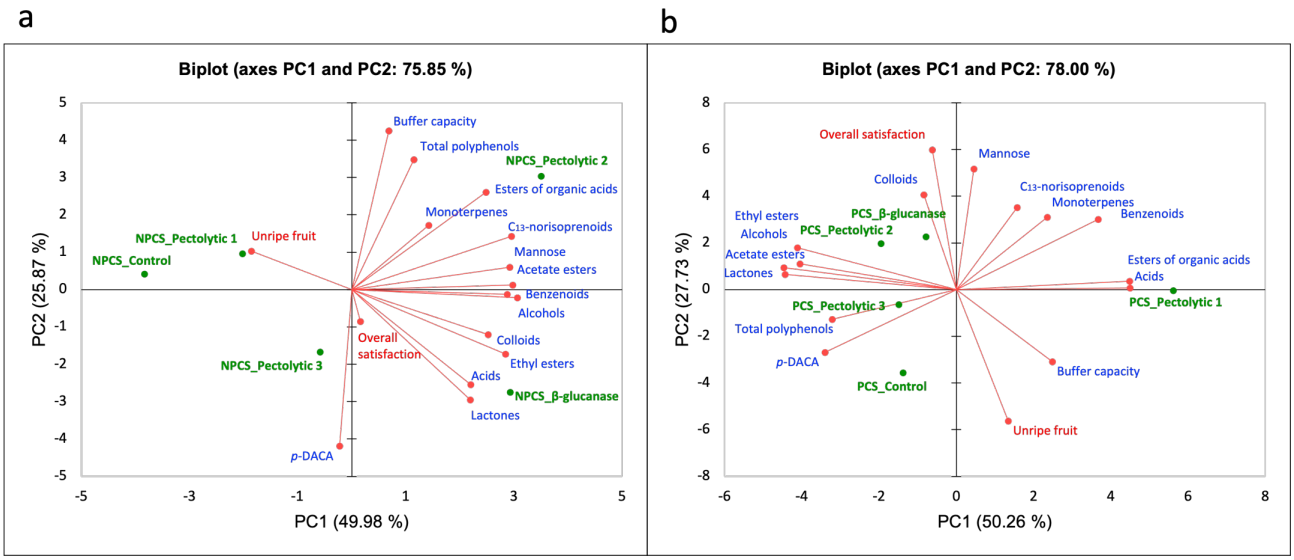

Figure 4: PCA biplots for NPCS wines (a) and PCS wines (b) treated with the addition of different pectolytic and β-glucanase enzymes (green text), based on the two sensory attributes scores (unripe fruit and overall satisfaction) (red text) and compositional parameters (blue text) as variables.

Supplemetary Information

Table S1 Commercial standards used as reference compounds for the identification of volatile organic compounds purchased from Sigma-Aldrich (Milan, Italy).

Standard compound	CAS no.	Purity (%)
Hexanoic acid	142-62-1	99
Octanoic acid	124-07-02	98
Decanoic acid	334-48-5	98
Dodecanoic acid	143-07-7	99
Hexadecanoic acid	57-10-3	99
Isoamyl alcohol	123-51-3	99
Hexanol	111-27-3	99
Benzyl alcohol	100-51-6	99
Ethyl hexanoate	123-66-0	99
Ethyl hexadecanoate	628-97-7	99
Isoamyl acetate	123-92-2	99
Hexyl acetate	142-92-7	99
Phenylethyl acetate	103-45-7	99
Linalool	78-70-6	97
α-Terpineol	98-55-5	95

Table S2 Linear retention indices (LRI) for the volatile organic compounds (VOCs) identified in *Catarratto* wines.

Compound name	Molecular Weight	LRI
Butanoic acid	88.1051	833
Isovaleric acid	102.1317	875
2-Methylbutanoic acid	102.1317	884
Hexanoic acid	116.1583	950
Octanoic acid	144.2114	1175
Decanoic acid	172.2646	1384
Dodecanoic acid	200.3178	1576
Tetradecanoic acid	228.3709	1763
Hexadecanoic acid	256.4241	1962
Octadecanoic acid	284.4772	2179
9-decenoic acid	170.2487	2303
Isoamyl alcohol	88.1482	732
Hexanol	102.1748	833
(*E*)-3-Hexenol	100.1589	840
(*Z*)-3-Hexenol	100.1589	855
Methionol	106.187	980
3-Ethoxypropanol	104.1476	1364
Tryptophol	161.2004	1750
Benzyl alcohol	108.1378	1026
2-Phenylethanol	122.1644	1121
Acetovanillone	166.1740	1212
4-vinylguaicol	150.1745	1356
Ethyl hexanoate	144.2114	1000
Ethyl octanoate	172.2646	1228
Ethyl decanoate	200.3178	1405
Ethyl dodecanoate	228.3709	1643
Diethyl succinate	174.1944	1694
Ethyl tetradecanoate	256.4241	1778
Ethyl hexadecanoate	284.4772	1978
Diethyl malate	190.1938	2041
Ethyl octadecanoate	312.5304	2171
Diethyl 2-hydroxyglutarate	204.2203	2195
Ethyl 9-decenoate	198.3019	2209
Isoamyl acetate	130.1849	866
4-vinylphenol	120.1485	881
Hexyl acetate	144.2114	1010
Phenylethyl acetate	164.2011	1224

Tyrosol	138.1638	2999
3-hydroxy-β-damascone	208.2967	1627
Blumenol C	210.3126	1713
Vomifoliol	224.2961	1796
Linalool	154.2493	1098
α-Terpineol	154.2493	1189
3-Oxo-α-ionol	208.2967	2640
Diendiol I	170.2487	1816
Endiol	172.2646	1821

Table S3 volatile acids composition at the end of alcoholic fermentation

Factor	2-methylbutanoic plus Isovaleric acid	Butanoic acid	Hexanoic acid	Octanoic acid	Decanoic acid	Dodecanoic acid	Tetradecanoic acid	Hexadecanoic acid	Octadecanoic acid	9-decenoic acid
Prefermentative (n = 5)										
PCS	134 ± 9	65 ± 6	2.653 ± 143	5.354 ± 257	2.177 ± 105	153 ± 21	10 ± 2	49 ± 14	4 ± 1	169 ± 19
NPCS	118 ± 22	57 ± 13	1.725 ± 183	3.139 ± 364	989 ± 181	33 ± 10	3 ± 0	11 ± 1	3 ± 1	265 ± 45
Sign.	ns	ns	*	**	**	**	*	*	ns	ns
Enzyme (n= 2)										
Control	101 ± 22	60 ± 14	2.223 ± 943	4.509 ± 1.849	1.676 ± 868	40 ± 31	1 ± 1	11 ± 3	1 ± 1	198 ± 25
Pectolytic 1	106 ± 41	46 ± 17	2.092 ± 204	3.978 ± 1.053	1.506 ± 619	108 ± 66	9 ± 6	56 ± 41	6 ± 2	205 ± 100
Pectolytic 2	160 ± 12	69 ± 13	2.454 ± 208	4.933 ± 349	1.934 ± 254	123 ± 56	8 ± 3	25 ± 11	4 ± 2	294 ± 128
β-Glucanase	107 ± 4	43 ± 8	2.239 ± 363	3.871 ± 1.099	1.259 ± 632	102 ± 80	7 ± 5	31 ± 20	3 ± 2	185 ± 16
Pectolytic 3	155 ± 7	89 ± 5	1.936 ± 601	3.942 ± 1.185	1.540 ± 595	92 ± 66	7 ± 4	28 ± 20	3.1 ± 0.4	203 ± 20
Sign.	ns	ns	Ns	ns	ns	Ns	ns	ns	ns	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, . = 90% of significance, ** = 99% of significance, *** = 99 % of significance. Different letters mean different averages for the Tukey's *post hoc* test with α value of 0.05. < LOQ =less than the limit of quantification. The unit of measurement is µg L^{-1} as 1-heptanol. PCS= prefermentative cold soaked wine NPCS = non-prefermentative cold soaked wine.

Table S4 Ethyl esters of fatty acids at the end of alcoholic fermentation

Factor	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate	Ethyl 9-decenoate	Ethyl dodecanoate	Ethyl tetradecanoate	Ethyl hexadecanoate	Ethyl octadecanoate
Prefermentative (n = 5)								
PCS	638 ± 36	1.080 ± 152	1.300 ± 290	70 ± 9	524 ± 130	12 ± 3	72 ± 15	10 ± 1
NPCS	321 ± 67	463 ± 124	139 ± 35	65 ± 13	21 ± 7	6 ± 3	52 ± 10	16 ± 6
Sign.	**	**	*	ns	*	ns	ns	ns
Enzyme (n= 2)								
Control	340 ± 160	307 ± 175 ab	108 ± 51	53 ± 14	19 ± 9	9 ± 9	22 ± 8	7 ± 1
Pectolytic 1	537 ± 99	884 ± 352 b	964 ± 802	65 ± 4	410 ± 364	11 ± 7	90 ± 3	25 ± 14
Pectolytic 2	611 ± 93	1.072 ± 247 a	936 ± 679	93 ± 12	322 ± 297	8 ± 4	63 ± 19	11 ± 1
β-Glucanase	475 ± 206	877 ± 297 ab	816 ± 683	72 ± 11	341 ± 327	9 ± 5	79 ± 15	14.1 ± 0.3
Pectolytic 3	434 ± 236	718 ± 471 ab	775 ± 689	54 ± 29	271 ± 259	8 ± 6	56 ± 18	9.2 ± 0.4
Sign.	ns	*	Ns	ns	ns	ns	.	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, . = 90% of significance, ** = 99% of significance, *** = 99 % of significance. Different letters mean different averages for the Tukey's *post hoc* test with α value of 0.05. < LOQ =less than the limit of quantification. The unit of measurement is μg L^{-1} as 1-heptanol. PCS= prefermentative cold soaked wine NPCS = non-prefermentative cold soaked wine.

Table S5 Alcohols at the end of alcoholic fermentation

Factor	Isoamyl alcohol	Hexanol	(E)-3-hexenol	3-ethoxypropanol	(Z)-3-hexenol	Methionol	Tryptophol
Prefermentative (n = 5)							
PCS	12.505 ± 1.474	1.078 ± 160	142 ± 74	521 ± 24	135 ± 86	52 ± 8	870 ± 222
NPCS	9.678 ± 1.356	555 ± 231	26 ± 7	263 ± 58	24 ± 6	18 ± 2	585 ± 232
Sign.	*	ns	ns	*	Ns	**	ns
Enzyme (n= 2)							
Control	6.966 ± 358 ab	731 ± 497	44 ± 19	412 ± 191	23 ± 9	40 ± 24	437 ± 12
Pectolytic 1	14.774 ± 1.685 b	393 ± 48	229 ± 207	318 ± 160	246 ± 232	18 ± 4	1.022 ± 671
Pectolytic 2	12.395 ± 491 a	1.344 ± 129	51 ± 1	331 ± 183	51 ± 3	42 ± 19	646 ± 323
β-Glucanase	11.871 ± 1.537 ab	839 ± 427	44 ± 31	399 ± 70	52 ± 28	34 ± 19	1.069 ± 439
Pectolytic 3	9.454 ± 2.995 ab	775 ± 467	52 ± 33	500 ± 42	25 ± 5	42 ± 19	463 ± 147
Sign.	*	ns	ns	ns	ns	ns	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, . = 90% of significance, ** = 99% of significance, *** = 99 % of significance. Different letters mean different averages for the Tukey's *post hoc* test with α value of 0.05. < LOQ =less than the limit of quantification. The unit of measurement is µg L^{-1} as 1-heptanol. PCS= prefermentative cold soaked wine NPCS = non-prefermentative cold soaked wine.

Table S6 Acetate esters at the end of the alcoholic fermentation

Factor	4-vinylphenol	Tyrosol	Isoamyl acetate	Hexyl acetate	Phenylethyl acetate
Prefermentative (n = 5)					
PCS	273 ± 63	884 ± 52	3.791 ± 661	1.019 ± 62	1.846 ± 87
NPCS	397 ± 89	762 ± 113	2.346 ± 410	222 ± 73	849 ± 126
Sign.	ns	ns	.	***	**
Enzyme (n= 2)					
Control	192 ± 28	685 ± 84	1.562 ± 280	591 ± 479	1.193 ± 560
Pectolytic 1	385 ± 53	759 ± 311	3.614 ± 414	443 ± 331	1.281 ± 691
Pectolytic 2	576 ± 163	960 ± 149	4.295 ± 819	775 ± 285	1.399 ± 165
β-Glucanase	268 ± 92	832 ± 29	3.096 ± 1.472	693 ± 426	1.559 ± 503
Pectolytic 3	251 ± 80	880 ± 30	2.776 ± 1.186	602 ± 471	1.307 ± 573
Sign.	ns	ns	Ns	ns	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, . = 90% of significance, ** = 99% of significance, *** = 99 % of significance. Different letters mean different averages for the Tukey's *post hoc* test with α value of 0.05. < LOQ =less than the limit of quantification. The unit of measurement is μg L^{-1} as 1-heptanol. PCS= prefermentative cold soaked wine NPCS = non-prefermentative cold soaked wine.

Table S7 Benzenoids at the end of the alcoholic fermentation

Factor	Benzyl alcohol	2-phenylethanol	Acetovanillone	4-vinylguaiacol
Prefermentative (n = 5)				
PCS	67 ± 4	15.372 ± 552	139 ± 114	1.101 ± 51
NPCS	14 ± 3	11.763 ± 1.903	14 ± 5	993 ± 119
Sign.	***	ns	ns	ns
Enzyme (n= 2)				
Control	31 ± 23	10.661 ± 2.981	15 ± 9	949 ± 112
Pectolytic 1	44 ± 34	11.993 ± 5.004	302 ± 293	1.025 ± 265
Pectolytic 2	46 ± 21	16.400 ± 439	29 ± 2	966 ± 59
β-Glucanase	40 ± 26	14.488 ± 687	16 ± 1	1.275 ± 160
Pectolytic 3	41 ± 30	14.296 ± 791	19 ± 10	1.020 ± 7
Sign.	ns	ns	ns	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, . = 90% of significance, ** = 99% of significance, *** = 99 % of significance. Different letters mean different averages for the Tukey's *post hoc* test with α value of 0.05. < LOQ =less than the limit of quantification. The unit of measurement is μg L^{-1} as 1-heptanol. PCS= prefermentative cold soaked wine NPCS = non-prefermentative cold soaked wine.

Table S8 C_{13}-norisoprenoids and monoterpenes at the end of the alcoholic fermentation

Factor	3-hydroxy-β-damascone	9-Hydroxy-megastigm-7-en-3-one	3-oxo-α-ionol	Blumenol C	Vomifoliol	Linalool	α-terpineol	Nerol	Diendiol I	Endiol
Prefermentative (n = 5)										
PCS	43 ± 25	98 ± 90	124 ± 6	83 ± 11	163 ± 18	8 ± 2	2 ± 2	12 ± 3	28 ± 13	2 ± 2
NPCS	10 ± 4	6 ± 4	154 ± 22	24 ± 8	84 ± 18	63 ± 51	16 ± 12	7 ± 3	60 ± 38	3 ± 1
Sign.	Ns	Ns	ns	**	*	ns	ns	ns	ns	ns
Enzyme (n= 2)										
Control	6 ± 6	13 ± 13	117 ± 3	59 ± 32	73 ± 28	11 ± 1	0 ± 0	9 ± 5	25 ± 2	2 ± 2
Pectolytic 1	74 ± 68	7 ± 1	132 ± 10	71 ± 46	130 ± 74	15 ± 12	9 ± 1	3 ± 3	54 ± 18	<LOQ
Pectolytic 2	17 ± 0	11 ± 11	164 ± 45	34 ± 19	161 ± 14	13 ± 12	3 ± 3	16 ± 2	10 ± 8	3 ± 3
β-Glucanase	21 ± 1	228 ± 228	170 ± 35	35 ± 35	117 ± 37	8 ± 2	4 ± 4	10 ± 6	25 ± 5	5 ± 2
Pectolytic 3	13 ± 6	<LOQ	114 ± 1	68 ± 17	137 ± 44	7 ± 3	0 ± 0	9 ± 6	10 ± 1	4 ± 4
Sign.	Ns	Ns	ns	ns	Ns	ns	ns	ns	ns	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA, ns = not significant, . = 90% of significance, ** = 99% of significance, *** = 99 % of significance. Different letters mean different averages for the Tukey's *post hoc* test with α value of 0.05. < LOQ =less than the limit of quantification. The unit of measurement is μg L^{-1} as 1-heptanol. PCS= prefermentative cold soaked wine NPCS = non-prefermentative cold soaked wine.

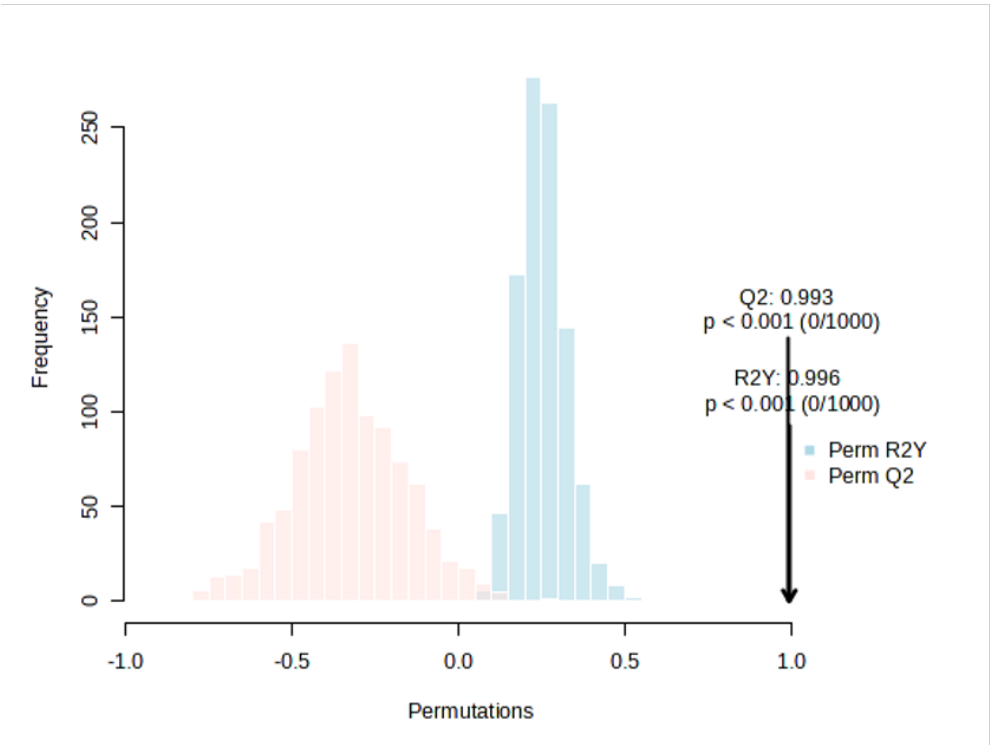

Figure S1 Results of the permutation tests (n = 1000) for supervised O-PLS-DA analysis: (a) is the result of *Catarratto* wines at the end of the alcoholic fermentation with pre-fermentative cold soaking treatment (PCS) and non-prefermentative cold soaking (NPCS).

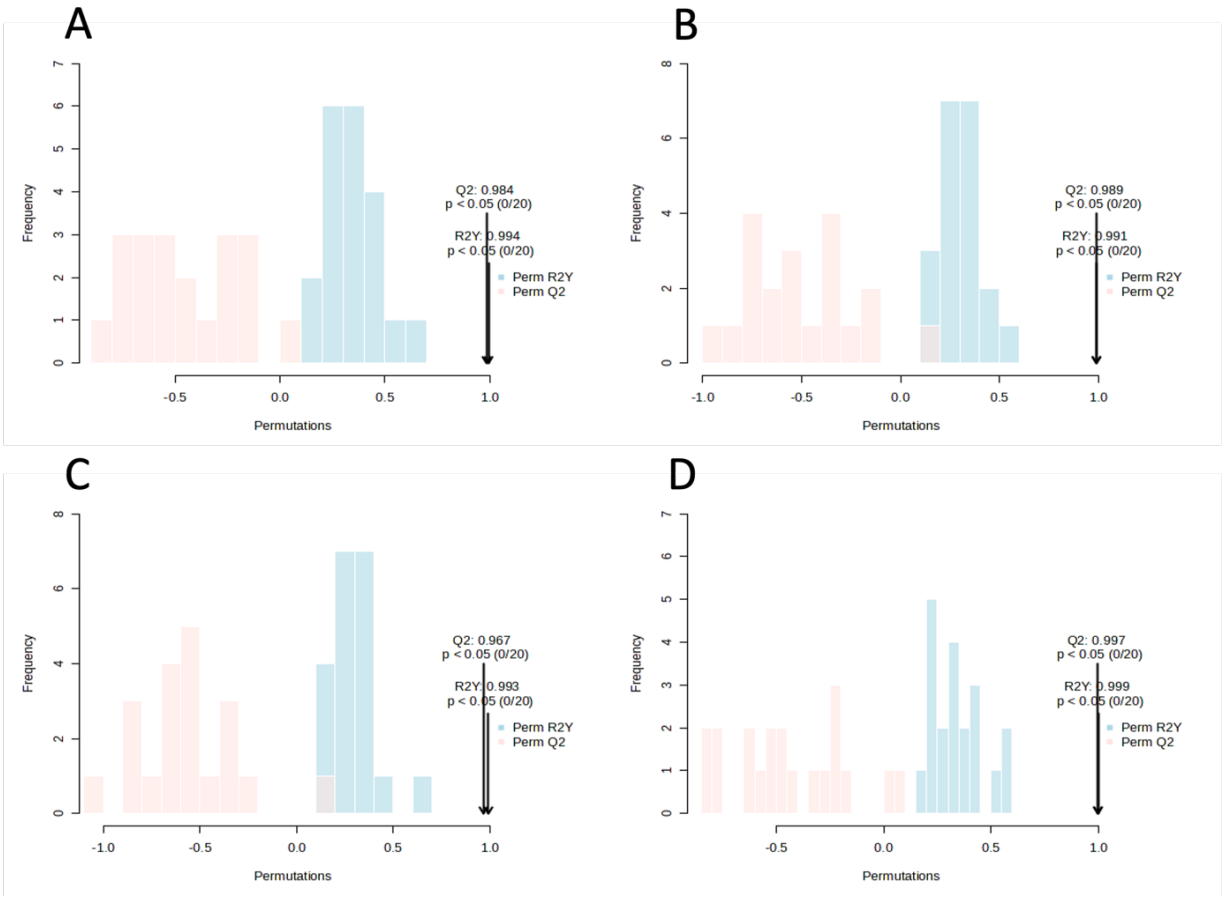

Figure S2 Results of the permutation tests (n = 1000) for supervised O-PLS-DA analysis. A, B, C, D is the results of *Catarratto* wines at the end of the alcoholic fermentation with the enzyme Pectolytic 1, Pectolytic 2, β-glucanase and Pectolytic 3, respectively.

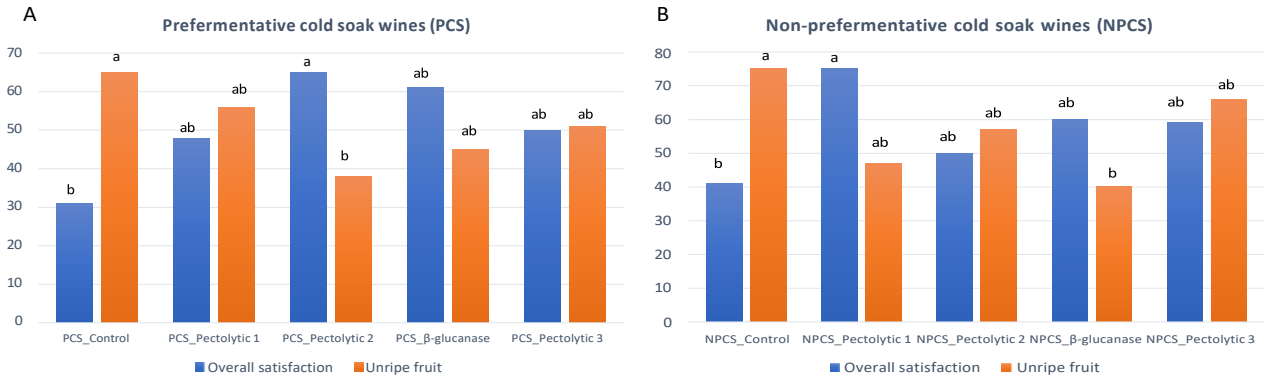

Figure S3 Sorting test of Prefermentative cold soaked (A) and Non-prefermentative cold soaked (B) *Catarratto* wines, with the addition of different pectolytic and β-glucanase enzymes. Data are reported as median of rank, the judges ordered wines from the lowest sensation to the highest. Sign. = Kramer test significant, Different letters mean different averages with *p* value of 0.05.

CHAPTER 4: Oenological characterization and grape heterogeneity assessment of ancient minor white and red cultivars (*Vitis vinifera* L.) from Sicily

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Abstract

Minor grape varieties often possess distinctive traits that contribute to wines with specific territorial identity. Sicily hosts a wide range of autochthonous cultivars, including *Lucignola* (rose-berry), *Vitrarolo* and *Catanese Nera* (red-berry), officially registered in the National Catalogue in 2018, as well as other relic cultivars such as *Dunnuni* and *Rucignola* (white-berry), *Anonima* and *Bracau* (red-berry), which remain potential candidates for registration. Among the cultivars investigated, *Vitrarolo* was also studied across different terroirs. These varieties represent a reservoir of biodiversity closely tied to the island's ancient viticultural heritage yet remain scarcely studied. In this work, the metabolite profiles of the above cultivars (*Vitis vinifera* L.) were investigated over two vintages focusing on berry heterogeneity, physicochemical traits, phenolic composition, and volatile organic compounds. Analysis of the glycosylated volatile compounds highlighted 2-phenylethanol abundance in *Rucignola* (354 ± 16 μg kg^{-1})and *Dunnuni* (505 ± 6 μg kg^{-1}) whereas *Lucignola* displayed relatively high terpene levels (262 ± 3 μg kg^{-1}). *Vitrarolo* exhibited the high flavonol and anthocyanin profile. *Catanese Nera* was characterized by higher levels of acylated anthocyanins (37.42 ± 0.10 %), while *Anonima* and *Bracau* displayed lower overall phenolic concentrations. In *Vitrarolo*, cultivation across different terroirs revealed clear environmental influences on its phenolic and volatile composition. Hydroxycinnamic acids were also identified and quantified. Multivariate analysis confirmed clear discrimination among red cultivars, with quercetin-3-glucoside, caftaric acid, terpenes, kaempferol, and anthocyanins as the most discriminant markers. These findings provide the first comprehensive metabolic characterization of minor Sicilian grapevines. The data obtained represent a useful tool for

varietal differentiation and for the development of appropriate vinification procedures to enhance wine quality, thereby contributing to the conservation and valorization of these cultivars.

Keywords: minor Sicilian grape cultivars, phenolic compounds, volatile organic compounds, wine quality, biodiversity

Introduction

Sicily, having long served as a crossroads of peoples and civilizations, has historically facilitated intense exchanges of plant material with countries throughout the Mediterranean region. Sicily, in addition to having one of the largest vineyard areas in Italy, is also one of the oldest centers for the domestication of the vine [1, 2]. Thanks to its variety of climates and soils, ranging from arid to rainy areas, with volcanic, sandy, clayey, calcareous, and even humid soils, Sicily has allowed the many grapevine varieties (*V. vinifera* L.) introduced over the millennia to adapt to the most suitable areas. However, many of these varieties are at risk of extinction due to the lack of proper conservation programs. Despite this, there is growing interest in minor native varieties, as they often exhibit longer vegetative cycles and delayed ripening, which make critical phenological phases more manageable and possess key traits to produce wines with a strong territorial identity [3]. In hot-arid regions, during the berry ripening phase, prolonged periods of high temperatures combined with drought conditions, (phenomena that are becoming increasingly frequent as a consequence of climate change), impair the vine's photosynthetic capacity, preventing the berries from completing ripening [4]. Consequently, at harvest, clusters may simultaneously contain unripe, ripe, and overripe berries. As a result, the physiological heterogeneity in ripening, which becomes evident at veraison and would normally diminish as ripening progresses, persists, thereby affecting the qualitative characteristics of the final wine [5, 6]. In addition, these ancient genotypes of Sicily are capable of tolerating extreme climatic conditions, therefore they could be essential for improving Mediterranean viticulture in future genetic enhancement programs [7]. Furthermore, many ancient Sicilian grape varieties were abandoned due to their naturally low sugar content, which did not align with the production standards of the 18th and 19th centuries, when viticulturists were primarily focused on quantity and high sugar content. Nowadays, with changing climate conditions and increasing temperatures, there is a high accumulation of sugars level in the grapes and low titratable acidity, leading to wines that are too

alcoholic and lack freshness. Meanwhile, consumer preferences are shifting towards lighter wines with lower alcohol content driven by a growing interest in healthy living [8, 9]. Internationally, winemakers are implementing various strategies to reduce grape sugar levels, utilizing both vineyard management techniques [10] and advanced technologies (i.e. spinning cone column distillation) in the winery [11]. One approach to produce lower-alcohol wines involves early grape harvesting [12]. However, grapes harvested at this stage, or heterogeneous grapes ripening at harvest, often have high acidity and exhibit "unripe", "green" and vegetative aromas and flavors, which can impact the overall sensory profile of the final wine [6, 13, 14]. Sicilian viticulture is characterized by the complexity of its indigenous cultivars, which can be classified based on their regional distribution. Among the main varieties, widely cultivated across the different wine-growing areas of the region, there are *Carricante*, *Catarratto*, *Grillo*, *Grecanico* and *Inzolia* as white berry cultivars. *Nero d'Avola*, *Nerello Cappuccio*, *Nerello Mascalese*, *Perricone*, and *Frappato* as red berry cultivars. In addition to these, there are numerous minor accessions where at a high phenotypic variability between biotypes corresponds to an equally significant genetic diversity. This variability has no parallels in any other European vine and reflects an ancient practice of propagation through seeds and the cultivation of multiple varieties within the same vineyard [15]. Among the minor grape varieties, in 2018, three red cultivars from Sicily have been registered in the National Catalogue of Italian grapevines for wine production, namely *Vitrarolo*, *Catanese Nera*, and *Lucignola* [16]. Scientific reports on the metabolic profile of ancient Sicilian red and white grape varieties are quite limited or missing in literature. This is especially disappointing because these are small-scale productions grown in only a few vineyards, and they are valuable resources that should be preserved. To understand and potentially promote the unique characteristics of ancient grape varieties (*V. vinifera* L.), it is essential to have a thorough understanding of their chemical composition. To this aim, this study reports data on the physicochemical characteristics of seven ancient Sicilian grape cultivars over a period of two year. Among the studied genotypes, there are four red-berry cultivars (*Anonima*, *Bracau*, *Catanese Nera* e *Vitrarolo*), one is rose-pale berry (*Lucignola*) and two are white berry cultivars (*Rucignola* and

Dunnuni). Our chemical characterization with a particular focus on compounds of enological interest is critical to develop specific vinification processes of the above mentioned ancient grape varieties. Additionally, the understanding of the molecular basis of the adaptation of these varieties to harsh soil and often unfavorable climatic conditions may suggest possible genetic strategies to ameliorate other grape varieties adaption to climate changes. Moreover, these grape varieties were innovatively characterized by evaluating ripening heterogeneity at harvest time, to account more carefully in vineyard zoning and varietal selection strategies.

Material and methods

Vineyards

Samples from seven cultivars of *Vitis vinifera* L. were collected during the 2023 and 2024 vintages in an experimental vineyard situated in Mazara del Vallo (Trapani), Italy. These included four red berry cultivars (*Anonima*, *Bracau*, *Catanese Nera*, and *Vitrarolo*), one rose-pale berry cultivar (*Lucignola*), and two white berry cultivars (*Rucignola and Dunnuni*) (Table 1). Given the growing viticultural and enological interest in the *Vitrarolo* cultivar, and the establishment of new vineyards over the past decade by several winegrowing enterprises, this variety was additionally monitored in two distinct environments, Contessa Entellina (western Sicily, Palermo) and Sclafani Bagni (north-central Sicily, Palermo), to capture potential environmental effects on its ripening dynamics and compositional traits. The experimental vineyard in Mazara del Vallo is situated at 37°47'17"N, 12°38'13"E, at 117 m a.s.l. The commercial vineyard in Contessa Entellina is located at 37°43'32"N, 13°02'19"E, at 250 m a.s.l., while the vineyard in Sclafani Bagni is located at 37°43'08"N, 13°49'45"E, at 590 m a.s.l.

Grape berries sampling and density sorting by means of densimetric flotation

Grape berries of ancient Sicilian grape cultivars (*Vitis vinifera* L.) were collected between August the 1st and the 2nd decade of September in 2023 and between the 2nd and the 3rd decade of September in

2024. The harvest date for each variety was determined by monitoring the evolution of primary metabolites, including sugar accumulation and total acidity, together with the sanitary condition of the grapes. In some cases, grapes were collected before reaching full technological maturity to prevent deterioration and ensure representative sampling, in order to avoid parasitic attacks resulting from the exceptional weather conditions of the season. Approximately 1000 grape berries were randomly collected from each vineyard, including their pedicels. The berries were sampled from both the middle and lower portions of the clusters, encompassing those exposed and unexposed to sunlight, to obtain a more representative sample and to account for possible variability in ripening. Subsequently, the berries were sorted based on their apparent density using flotation in solutions with varying salt concentrations [17]. Six NaCl solutions were prepared with incremental concentration differences of 20 g L^{-1}, ranging from -90 g L^{-1} to 170 g L^{-1} NaCl. Berries were first placed in the densest solution. Those that sank had the same or slightly higher density as the solution, while floating berries had lower density. The sunken berries were separated, counted, and the floating berries were transferred to a less dense solution. This process was repeated until all berries had sunk. After sorting, the berries were washed with water, visually inspected, and any with damaged skins were discarded. The berries from each density class were then weighed and counted. Two subsamples of 20 berries from each density class per vineyard were used to prepare skin and seed extracts for phenolic composition analysis. The remaining berries were divided into two replicates, crushed, and the juice was centrifuged at 4000 rpm using a PK 131 centrifuge with a T516 swing-out rotor (ALC International, MI, Italy). The supernatant was collected to determine the key chemical-physical parameters of the grapes in each density class. These parameters included reducing sugar content (g L^{-1}), pH, titratable acidity (g L^{-1}), tartaric acid (g L^{-1}), malic acid (g L^{-1}), K content (%), and readily assimilable nitrogen (APA). The analyses were conducted using a Winescan™ instrument (FOSS, Hilleroed, Denmark), calibrated according to the EEC 2676 standard procedure [18].

Grape skin and seeds extraction

Skins and seeds extracts were obtained following the procedure described by Squadrito [19], with some modifications. Skins and seeds were carefully separated by hand from the pulp of 20 berries and placed into individual plastic containers. Each was immersed in 20 mL of a buffer solution at pH 3.2, prepared by dissolving 5 g of tartaric acid, 2 g of $Na_2S_2O_5$, and 125 mL of 95% ethanol in deionized water, adjusting the pH with 22.2 mL of 1 M NaOH, and bringing the total volume to 1 L. Samples were kept at room temperature for 4 hours before being homogenized at 5,000 rpm for 1 minute using an Ultraturrax T25 high-speed homogenizer (IKA Labortechnik, Staufen, Germany). After homogenization, they were sonicated for 3 minutes and centrifuged at 4,000 rpm using a PK 131 centrifuge with a T516 swing-out rotor (ALC International, Milan, Italy). The resulting supernatant was transferred to a 50 mL volumetric flask. After each centrifugation, the pellet was resuspended in 5 mL of pH 3.2 buffer solution. The residual pellet was washed three times by resuspending it in 5 mL of the same buffer solution and centrifuging after each resuspension. All collected supernatants were pooled and diluted with buffer to a final volume of 50 mL. The use of $Na_2S_2O_5$ in the extraction buffer served to inhibit polyphenol oxidase activity, thereby preventing enzymatic browning and oxidation of phenolic compounds. Analyses were performed in triplicate.

Phenolic compounds analysis

Analysis of total flavonoids and total anthocyanins

Total flavonoid content in both skins and seeds, as well as total anthocyanin content in skins, was quantified using UV–Vis spectrophotometry. Measurements were performed with a UV-1800 spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Prior to analysis, extracts were diluted in hydrochloric ethanol (ethanol:water:hydrochloric acid 37%, in a 70:30:1 v/v/v

ratio) following the protocol described by Corona et al. [20]. The dilution factors applied were 50 for seed extracts and 25 for skin extracts. Results were expressed as mg kg^{-1}. The analyses were performed in triplicates.

Analysis of monomer anthocyanins, hydroxycinnamoyl tartaric acids (HTCAs) and flavonols

To analyze the profiles of monomeric anthocyanins, hydroxycinnamic tartaric acid esters (HCTAs), and flavonols, skin extracts were first acidified by adding 0.5 mL of 1 M phosphoric acid (H_3PO_4) to 4.5 mL of extract. The acidified solutions were then filtered through 0.45 µm nylon membrane filters and transferred into 1.5 mL vials for analysis. Chromatographic separation and identification were performed using a high-performance liquid chromatography (HPLC) system (Agilent Series 1200, Milan, Italy) equipped with a diode array detector (Hewlett-Packard 1100 D.A.D.). The separation was achieved on a C18 reverse-phase column (Econosphere™ C18, 5 µm, 250 × 4.6 mm i.d., Lokeren, Belgium, part no. 70066). An injection volume of 20 µL was used, in accordance with the method described by Squadrito et al. [19].

Monomeric Anthocyanins Analysis

Monomeric anthocyanins were analyzed using a reverse-phase HPLC method with a binary solvent system. The mobile phase consisted of solvent A (formic acid:water, 10:90 v/v) and solvent B (formic acid:methanol:water, 10:50:40 v/v/v). The elution was carried out with the following gradient program: 45% B for 15 minutes; a linear increase to 70% B over 20 minutes; followed by a gradient to 90% B over 10 minutes, then to 99% B in 5 minutes. The column was held at 99% B for 4 minutes, then returned to 45% B in 2 minutes, and re-equilibrated at 45% B for an additional 5 minutes. The mobile phase flow rate was set to 0.48 mL min^{-1}, and the column oven was maintained at 45 °C. Detection was performed at 520 nm using a diode array detector. Anthocyanins were identified based on retention times and UV–Vis absorption spectra, as previously reported by López et al. [21].

Quantification was carried out by peak integration, and the results were expressed as the relative percentage of each monomeric anthocyanin with respect to the total anthocyanin content.

HCTAs and Flavonols Analysis

Hydroxycinnamic tartaric acid esters (HCTAs) and flavonols were analyzed using a gradient elution method with a binary solvent system. Solvent A consisted of 10^{-3} M phosphoric acid (H_3PO_4) in water, while solvent B was methanol. The gradient elution program was as follows: 5% B for 5 minutes; a linear increase to 10% B over 5 minutes; from 10% to 30% B in 10 minutes; then from 30% to 60% B over the following 10 minutes; and finally, from 60% to 100% B in 10 minutes. The gradient was then returned from 100% to 5% B in 5 minutes to re-equilibrate the column. The mobile phase flow rate was maintained at 0.48 mL min^{-1}, and the column temperature was set to 40 °C. HCTAs and flavonols were identified based on retention times and UV–Vis spectra by comparison with literature data [22] and authentic standards previously isolated in our laboratory. Quantification was performed by external calibration using commercially available standards: chlorogenic acid (for HCTAs) and quercetin (for flavonols) purchased from Sigma-Aldrich (St. Louis, MO, USA) with a purity of 95%. Results were expressed as mg per kg of fresh berry weight (mg kg^{-1}). The analyses were carried out in triple.

Volatile organic compounds (VOCs)

VOCs were determined following the method reported by Corona et al. [23]. Briefly, 25 mL of wine were spiked with 0.25 mL of a hydroalcoholic solution of 1-heptanol (35 mg L^{-1}), used as internal standard, and diluted to a final volume of 100 mL with distilled water. The solution was then passed through a 1 g C18 SPE cartridge (Isolute, Uppsala, Sweden, part no. 221-0100-C), previously conditioned with 3 mL of methanol followed by 4 mL of distilled water. After washing with 30 mL of distilled water, the free VOCs were eluted using 12 mL of dichloromethane. The glycosidically

bound VOCs retained on the cartridge were subsequently eluted with 30 mL of methanol. The methanolic eluate was evaporated under reduced pressure, and the residue was reconstituted with 5 mL of citrate–phosphate buffer (pH 5.0). A pectolytic enzyme with high glycosidase activity (0.2 mL) was added, and the solution was incubated at 40 °C for 24 hours. After enzymatic hydrolysis, 0.5 mL of internal standard (same as used for the free fraction) was added, and the sample was centrifuged and passed through a new 1 g C18 SPE cartridge (previously activated with 5 mL of methanol and 10 mL of distilled water). The cartridge was washed with 10 mL of water, and the hydrolyzed VOCs were eluted with 10 mL of dichloromethane. The resulting solution was dehydrated and concentrated to 0.5 mL before GC-MS injection. Analyses were performed using an Agilent 6890 Series GC system coupled with an Agilent 5973 Network Mass Selective Detector (Agilent Technologies, Milan, Italy), equipped with a DB-WAX column (30 m × 0.25 mm i.d., 0.25 µm film thickness, part no. 122-7032). The oven temperature was initially held at 40 °C for 2 min (splitless injection), then ramped to 60 °C at 4 °C/min and held for 2 min, followed by an increase to 190 °C at 2 °C/min, then to 230 °C at 5 °C/min, and held at 230 °C for 15 min. The injector and FID detector temperatures were set at 250 °C, and the transfer line was maintained at 230 °C. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. Ionization energy was 70 eV (electron impact mode). Volatile compounds were identified by comparing GC retention times and MS spectra with those of pure commercial standards. When standards were not available, identification was based on the NIST/EPA/NIH Mass Spectral Library (Version 2.0d, 2005). Quantification of volatile compounds was expressed in µg L^{-1}. The analyses were performed in triplicate.

Statistical analysis

A factorial analysis of variance (ANOVA) was applied to assess the vintage effect within each grape variety studied. For the *Vitrarolo* cultivar, the environmental factor and the interaction between vintage and environment were also considered. The analysis was carried out using Rstudio (RStudio Team, Boston, MA, USA) version [4.0.3]. Differences with p values of less than 5 % ($p < 0.05$) were

considered statistically significant. In case of significant differences, a post hoc HSD Tukey's test was conducted. The results were expressed as mean values with corresponding standard error. To point out the differences of the grape varieties, the Partial Least Squares-Discriminant Analysis (PLS-DA) was performed by using MetaboAnalist 6.0 web-based tool suite [24].

Results and discussion

Density classes distribution

The distribution percentages of all grape berries of the relic varieties in the different density classes at harvest were determined (Figure 1). For *Vitrarolo* grape variety, this analysis was also conducted across the three vineyard zones (Figure 2). The distribution of density classes holds a dual significance. Firstly, it reflects the variability in ripeness levels among the berries at the time of harvest. Secondly, as the apparent density of a berry is largely influenced by its reducing sugar content, each density class can be interpreted as representing a distinct stage of berry maturation. The distribution of the berries across the density classes displayed a bell-shaped pattern. This type of distribution can be mathematically characterized by its mode, skewness, and kurtosis. The mode, which represents the most frequently occurring value, corresponds to the density class with the highest proportion of berries. Skewness, a measure of asymmetry in the distribution, provides insights into the maturation rate. A high (positive) skewness indicates a greater proportion of berries in more advanced ripening classes (i.e., higher density), whereas a low (negative) skewness reflects a greater proportion of berries in less advanced ripening classes (i.e., lower density). Lastly, kurtosis, which quantifies the sharpness of the distribution's peak, reflects the uniformity of ripeness. Low kurtosis values are associated with flatter distributions, where several classes exhibit similar relative frequencies, reflecting a greater heterogeneity in berry ripeness. Conversely, high kurtosis values indicate sharper, more peaked distributions, in which a limited number of classes account for a higher

percentage of the total, signifying more uniform ripeness. In the 2023 vintage, in almost all grape varieties, the berries were distributed across three density classes (except for *Anonima)*, with high kurtosis values indicating greater ripeness uniformity. In the 2024 vintage, the berries tended to be distributed across more density classes, with lower kurtosis values, reflecting less ripeness uniformity. Overall, all relic varieties showed approximately bell-shaped density distributions, confirming the presence of ripening heterogeneity at harvest. Between 2023 and 2024, a general shift toward higher mean densities was observed across cultivars, indicating more advanced ripening in 2024. *Rucignola* and *Dunnuni* exhibited the most pronounced increases, while *Lucignola* and *Catanese Nera* displayed more symmetric distributions and moderate kurtosis values, reflecting improved ripening uniformity. In contrast, *Anonima* showed a reduction in distribution sharpness from 2023 to 2024, suggesting greater variability. The overall trend highlights a more advanced and homogeneous ripening in 2024, likely favored by more suitable climatic conditions and the absence of the downy mildew (*Plasmopara viticola*) outbreak that had affected vineyards in 2023, leading to earlier harvests and less mature fruit. Importantly, an earlier harvest date does not significantly affect the assessment of ripening heterogeneity, as the distribution of ripeness levels within clusters tends to remain stable over short time intervals, being mainly governed by intrinsic varietal and environmental factors rather than by short-term ripening progression. Regarding *Vitrarolo* distribution, the density classes with the highest weight showed similar ripeness levels in 2024 compared to 2023, particularly in Contessa Entellina and Sclafani Bagni environments. The heterogeneity in grape ripening affects the chemical composition and quality of wine [25, 26], as many sensory attributes depend on the phenolic composition of the grapes at the time of harvest [27]. The presence of a certain, non-negligible percentage of underripe berries can contribute with bitter and astringent characteristics to wine, which are generally undesirable by tasters [6]. Grapes below 1075 kg m^{-3} density class are associated with green and bitter notes in wines [28]. In 2023, a significantly larger percentage of grapes was present below this density class indicating lower ripeness level. By 2024, this percentage decreased,

suggesting more advanced ripeness across varieties, probably due to the above-average temperatures that characterized that vintage.

Grapes physical-chemical parameters

The main chemical-physical parameters of grape berries, including reducing sugars (g L^{-1}), pH, titratable acidity (g L^{-1}), malic acid (g L^{-1}), tartaric acid (g L^{-1}), K (g L^{-1}), berry weight (g) and APA (assimilable nitrogen), are listed in Table 1. As expected, all the studied grape varieties are characterized by a moderate sugar accumulation, ranging from 161 to 210 g L^{-1} for red berry cultivars, from 180 to 185 g L^{-1} for rose-pale berry cultivar, and from 165 to 195 g L^{-1} for white cultivars. Low-alcohol, fresh, and easy-to-drink wines cater to the demands of emerging markets [9]. However, some of these varieties, despite their low sugar accumulation levels, do not retain acidity, showing low values for total acidity and high values of pH. Wine quality is largely dependent on the wine titratable acidity and pH values, because of their effect in modulating microbial activity, stabilizing color in red wines and influencing phenolic and aromatic oxidation [29]. Across all cultivars, clear interannual differences were observed, with 2024 generally showing higher sugar concentrations and pH values, together with lower total and malic acidity, indicating a more advanced ripening stage compared to 2023. In the white cultivars *Rucignola* and *Dunnuni*, sugar levels increased markedly (+10–17 g L^{-1}), while total acidity and malic acid decreased (−2 to −3 g L^{-1} and −0.7 to −0.6 g L^{-1}), accompanied by higher pH values (+0.3 units). *Lucignola* showed a strong decrease in acidity (from 8.07 to 5.73 g L^{-1}) and malic acid (from 2.97 to 1.27 g L^{-1}), along with a substantial reduction in berry weight (−43%), suggesting greater dehydration or concentration effects. Among red cultivars, *Anonima*, *Bracau*, and *Catanese Nera* exhibited significantly higher sugar contents and lower total and malic acid levels in 2024, consistent with enhanced ripening under warmer and drier conditions. Both *Anonima* and *Catanese Nera* also showed a marked decrease in APA (−30–40%), likely linked to reduced berry size or nitrogen availability. Sugar accumulation in grape berries is a complex

physiological process involving multiple developmental stages. During ripening, the increase in sugar content is primarily driven by active phloem unloading, which transports photoassimilates from the leaves to the berries, contributing both to sugar accumulation and berry enlargement [30]. However, at later stages of ripening, a decrease in berry weight may coincide with a continued rise in sugar concentration [31]. *Vitrarolo* exhibits distinct physicochemical characteristics across different environments over the two years studied. Sclafani Bagni site showed the highest reducing sugar content (230 ± 12 g L^{-1}), together with elevated tartaric acid levels (7 ± 1 g L^{-1}). Contessa Entellina environment displayed reducing sugar levels of 211 ± 8 g L^{-1}, combined with significantly higher total acidity (5.19 ± 0.07 g L^{-1}) and tartaric acid (7.9 ± 0.5 g L^{-1}), both statistically significant ($p < 0.01$). In contrast, the Marsala site showed the lowest sugar content (192 ± 9 g L^{-1}) and total acidity (3.4 ± 0.9 g L^{-1}). The significant year × terroir interaction observed for most parameters ($p < 0.001$) confirms a strong environmental modulation of grape composition. In general, it can be observed that the studied grape varieties tend to lose acidity as the sugar concentration in the berries increases. However, in the 2023 vintage, some varieties, such as *Lucignola*, *Bracau*, and *Anonima*, exhibited relatively low sugar levels and moderate acidity. Such compositional traits are particularly valuable for producing wines that meet current consumer preference, which favor freshness and balanced acidity.

Phenolic compounds

Total flavonoids, total anthocyanins and non-anthocyanic flavonoids

The total amount of non-anthocyanic flavonoids (NAF) ranged from 2939 to 2435 mg kg^{-1} in *Lucignola* (pale-rose berry cultivar), from 3078 to 4335 mg kg^{-1} for red berry cultivars, and from 1480 to 3242 mg kg^{-1} for white grape varieties. Across most grape varieties, significant differences in NAF were observed between 2023 and 2024 (Table 2). In red grape varieties such as *Anonima* and

Bracau, NAF levels increased significantly, while in white grape varieties, namely *Dunnuni* and *Rucignola*, these compounds showed a decline. NAF in the skins ranged from 916 to 1281 mg kg^{-1} for red berry cultivar, from 935 to 1033 mg kg^{-1} for pale-rose berry cultivar (*Lucignola*), and from 314 to 441 mg kg^{-1} for white grape varieties. Seed total flavonoids ranged from 1903 to 2397 mg kg^{-1} for red berry cultivars, from 1402 to 2003 mg kg^{-1} for rose-pale berry cultivar, and from 1039 to 2067 mg kg^{-1} for white grape cultivars. NAF in the skins increased across all grape varieties, whereas seeds total flavonoids decreased, except in *Anonima* and *Bracau*, where they remained stable or increased. The skin/seed NAF ratio ranges from 0.39 to 0.80 for red grape cultivars, from 0.47 to 0.74 for pale-rose berry cultivar (*Lucignola*), and from 0.11 to 0.42 for white grape cultivars. The skin/seed ratio of non-anthocyanic flavonoids (NAFs) is a useful indicator of the relative contribution of skins and seeds to the phenolic pool of grapes. Flavonoids from skins are mainly associated with color stabilization and softer tannic structure, whereas seed-derived flavonoids are richer in catechins and proanthocyanidins, often contributing to astringency and bitterness [32]. Thus, the skin/seed NAF ratio provides valuable information on grape phenolic maturity and has practical implications for winemaking decisions [33]. For this reason, during the vinification of *Anonima* and *Catanese Nera*, as well as for *Vitrarolo* (2023 vintage) and *Lucignola* (2024 vintage), extended maceration could be applied to enhance wine structure and longevity. Conversely, for *Lucignola* and *Vitrarolo* in the other vintages and in *Bracau*, shorter maceration times may be preferable to limit the extraction of seed tannins, or alternatively, harvesting at a more advanced stage of ripening could be adopted to reduce their extractability. In white grape cultivars, the skin/seed ratio of non-anthocyanic flavonoids is generally lower than in red cultivars, reflecting the predominance of seed-derived flavan-3-ols over skin flavonols. However, in the 2024 vintage *Dunnuni* showed a comparatively high ratio, which could be attributed to the greater extractability of flavan-3-ols from berry skin cells in riper grapes, caused by histochemical modifications occurring in berry tissues [34]. In such cases, it is advisable to avoid or limit the extraction of these compounds from the skins, as they are not only contributing to bitterness and astringency, but are also responsible for chemical oxidation of wine [35]. Although,

white grape varieties can be subjected to pre-fermentative cold maceration, to minimize the extraction of phenolic compounds while promoting the release of aromatic constituents present in the skins [36]. Polyphenols also have significance from a taxonomic perspective. It is well established that the distribution of certain flavonoid classes, such as anthocyanins, is tightly regulated by genetic factors, and this distribution can differ greatly across various grape cultivars [37, 38]. Total anthocyanins amount ranged from 154 to 39 mg kg^{-1} for pale-rose berry cultivar (*Lucignola*), from 172 to 700 mg kg^{-1} for red berry cultivars. The accumulation of total anthocyanin was significantly year-dependent. A marked increase in total anthocyanins was observed in 2024 for most red grape varieties, except for *Lucignola*, which showed a decline. The accumulation of anthocyanins in grape berries is strongly influenced by climatic conditions and vintage variability, with temperature, solar radiation, and water availability playing key roles in modulating their biosynthesis [39]. Among the vineyard environments, Sclafani Bagni demonstrated the highest anthocyanin levels and significantly higher skin NAF compared to other locations. In contrast, Marsala exhibited the lowest anthocyanin content and a lower skin/seed flavonoid ratio, highlighting environmental influences on phenolic composition and grape quality.

Monomer anthocyanins profiles

In grape skins from all the cultivars, tri-oxygenated anthocyanins (malvidin-3-glucoside, petunidin-3- glucoside, and delphinidin-3-glucoside) prevailed over di-oxygenated forms (peonidin-3-glucoside and cyanidin-3-glucoside) (Table 4). Among the tri-hydroxylated forms, malvidin-3-glucoside was the dominant compound, consistent with its recognized role in wine color stability [40]. The anthocyanin profiles of relic red berry cultivars highlight not only varietal biosynthetic tendencies but also important properties that can be exploited to optimize pre-fermentative operations, fermentation maceration techniques, and wine aging strategies. Distinct varietal patterns were observed: *Anonima*, *Bracau*, and *Vitrarolo* exhibited high percentages of peonidin-3-glucoside, elevated levels of p-

coumaroylated derivatives, and an acetylated/p-coumaroylated derivative ratio markedly below 1. Conversely, *Catanese Nera* was characterized by a predominance of acetylated derivatives, with the same ratio also below 1. The anthocyanin profiles of grapes and their resulting wines can differ significantly due to two key factors: the oxidation of anthocyanins mediated by grape polyphenol oxidases (PPOs), especially during the pre-fermentative stage, and the different diffusion rates of individual anthocyanins and their acylated derivatives from skins into must [41]. Anthocyanins with faster diffusion, such as peonidin-3-glucoside and particularly cyanidin-3-glucoside, are largely oxidized, whereas malvidin-3-glucoside, though rapidly diffusing, is less prone to oxidation and contributes to polymeric pigments. Delfinidin-3-glucoside and petunidin-3-glucoside, more oxidizable than malvidin-3-glucoside, are partially preserved as their diffusion occurs mainly during fermentation, when PPOs activity is reduced due to limited oxygen [29]. p-Coumaroylated derivatives diffuse more slowly due to the hydrophobicity of p-coumaric acid esterified to the glucose moiety, and their low presence in wines is attributed to poor extractability rather than oxidation [42]. Acetylated derivatives (mainly of malvidin-3-glucoside) follow the same trend as malvidin. Overall, traditional vinification results in lower extraction of p-coumaroylated anthocyanins (mainly derivatives of malvidin-3-glucoside) compared to malvidin-3-glucoside and its acetylated form, whose extraction should instead be enhanced, as these molecules play a key role in wine color stability. Enhanced extraction of these compounds can be achieved by vinifying grapes at optimal cellular maturity and using fermentative maceration techniques that promote the hydrolysis of cell wall polysaccharides and membrane proteo-phospholipids. For varieties rich in dihydroxylated anthocyanins, such as *Lucignola* grape, the rapid diffusion of these compounds from skins into must increases susceptibility to PPO-induced oxidation [43]. Thus, minimizing PPO activity in pre-fermentation phase, through grape cooling and reduced oxygen exposure is essential (e.g., avoiding aerative pump-overs during the prefermentative stage or in the early phase of fermentative maceration).

Hydroxycinnamoyl tartaric acids (HCTAs) and Flavonols

All the studied varieties were characterized by the prevalence of quercetin-3-glucoside over the other flavonols and by higher caffeoyl-tartaric acid compared with *p*-coumaroyl-tartaric acid (Table 5). Caffeoyl-tartaric acid, showed particularly high levels in red cultivars such as *Vitrarolo* and *Catanese Nera* in 2023 (81 mg kg^{-1} and 48 mg kg^{-1}, respectively), with a significant decrease in 2024, highlighting a strong vintage effect. In contrast, quercetin-3-glucoside, the predominant flavonol, increased markedly in 2024 across almost all cultivars. For instance, values in *Catanese Nera* rose from 3.6 mg kg^{-1} in 2023 to 8 mg kg^{-1} in 2024, while in *Vitrarolo* they more than doubled from 6 mg kg^{-1} to 15 mg kg^{-1}. The flavonol pattern is mostly controlled by genotype [44] as well as by environmental conditions, particularly solar radiation and water stress [45]. The influence of these two classes of polyphenols and their profiles on the management of the winemaking process should not be underestimated. Flavonols are powerful copigments able to slow down anthocyanin degradation in wine, while hydroxycinnamic acids, especially those from the pulp that are present in the must at crushing, serve as PPOs substrates. In must PPOs quickly oxidize phenolic acids into highly reactive quinones, responsible for browing reactions [45]. Moreover, these quinones are prone to nucleophilic attacks by sulfur dioxide and sulfur containing aromatic compounds, resulting in musts and future wines less rich in varietal aroma and less protected by sulfur dioxide [46]. Data obtained in this study suggest that, especially in *Rucignola* and *Dunnuni* grapes, during the first phases of winemaking, it is important to limit the negative activity of PPOs by a proper management of temperature and sulfur dioxide. In addition, from a human health-related point of view, these compounds are of some interest on account of their antioxidant and anti-inflammatory properties [47].

Volatile organic compounds

The analysis of free volatile organic compounds showed that, for the three classes of aromatic compounds, terpenes, benzenoids, and norisoprenoids, as reported in Tables S1, S2, and S3, respectively, many of the identified compounds were either absent or present at very low concentrations. These varieties are generally classified as non-floral (neutral) types, characterized by low concentrations of free aroma compounds. Therefore, the analysis of flavour precursors was employed as a strategy to assess the actual aromatic potential of these ancient varieties, providing useful insights for future technological improvements related to skin or pomace contact time and the possible use of enzymatic treatments during winemaking to enhance the release of bound aroma compounds [48]. The analysis of VOCs released by enzymatic hydrolysis of glycosidic precursors enabled the identification and quantification of 27 compounds, classified into three chemical families: terpenes, benzenoids and C_{13}-norisoprenoids, as reported in Tables 6, 7, and 8, respectively. The same aroma compounds as in aromatic grapes were found, namely terpenes, benzenoids and norisoprenoids, but the amounts were rather small. Geraniol, linalool and α-terpineol, the dominant terpenes in Muscat varieties (aromatic grapes) [36], did not show relevant amounts in all the cases. Cultivars are usually classified into two groups: aromatic varieties, rich in terpenic compounds (e.g., Gewurztraminer and Muscat), and non-aromatic varieties, generally poor in terpenes. However, among the autochthonous and relic cultivars analyzed, some such as *Rucignola* (white cultivar) and *Lucignola* (pale-rose berry cultivar), although not strictly aromatic, displayed relatively high terpene levels (29 ± 1 and 262 ± 3 μg kg^{-1}, respectively), while in the red cultivars, these compounds were not detected. In aromatic cultivars, the predominance of free linalool suggests limited yeast impact on the varietal terpenic profile, whereas cultivars characterized by free geraniol (Table S1) are more affected by yeast metabolism [49]. For the latter, winemaking practices that promote the release of glycosylated geraniol from skins are important to offset yeast consumption of free geraniol and to increase its conversion into citronellol, a compound contributing to the characteristic aroma of these wines. Di-oxygenated compounds derived from linalool and geraniol are well represented (Table 6), the most abundant being the furanic and pyranic oxides of linalool and the two isomers of 8-

hydroxylinalool. The p-ment-1-ene-7,8-diol, a very important aroma compound that derives from α-terpineol, was found in higher amounts in *Rucignola* and *Bracau* over the other cultivars. The group of benzenoids (Table 7) includes methyl salicylate, benzyl alcohol, 2-phenylethanol, vanillin, acetovanillone, zingerone and dihydroconiferyl alcohol. Volatile benzenoids (except for 2-phenylethanol) are formed during the phenylpropanoid synthesis by the phenylalanine ammonia-lyase (PAL) enzyme [50]. This enzyme catalyzes the conversion of phenylalanine into trans-cinnamic acid, which in turn is converted into benzyl alcohol and other derived compounds [51]. In many neutral grape varieties these compounds, particularly benzyl alcohol and 2-phenylethanol, are the major constituents of the glycosidic aroma fraction. However, their contribution to wine aroma is generally negligible, as both compounds have relatively high odor thresholds, and 2-phenylethanol is mainly produced as a secondary metabolite of yeast during fermentation, with grape-derived glycosides representing only a minor fraction. Consequently, indirect measures of the aromatic potential of neutral grapes [52] may not reflect the true varietal aromatic potential but rather the general secondary metabolic activity of the grape. Among the benzenoids, 2-phenylethanol, characterized by a pleasant rose-like aroma [53], was detected at the highest concentration in *Rucignola*, followed by *Dunnuni* and *Lucignola*, and at lower levels in all the other cultivars. Norisoprenoids are products of enzymatic and non-enzymatic degradation reactions of carotenoids. These breakdown products are carbonyl compounds with 9, 10, 11, and 13 C-atoms, some of which have powerful aroma properties [54]. The qualitative and quantitative profiles of carotenoids and norisoprenoids in grapes are affected by several factors including plant variety, climatic conditions, stage of maturity, soil characteristics and viticultural practices. C_{13}-Norisoprenoids are considered as those that contribute mostly to the aroma of wines made with non-aromatic grapes. Norisoprenoids were identified in all the varieties studied. These includes 3,4-dihydro-3-oxoactinidol (I, II, III), 3-oxo-α-ionol, 4-oxo-β-ionol, 3-hydroxy-7,8-dihydro-β-ionol and vomifoliol, while blumenol C was found under limit of detection in *Lucignola* and *Vitrarolo* cultivars (Table 8). The results indicate differences in the synthesis of varietal glycosylated aroma compounds among the minor Sicilian red

and white grape varieties studied. Differences seem to be higher in white and the rosé berry varieties. These differences concern both the amounts of terpenic, norisoprenoid, and benzenoid compounds and the ratios between isomeric forms. Such ratios, commonly used to classify grape varieties according to their flavour potential [55], were evaluated (Table 8). Overall, all the cultivars investigated can be considered neutral, as the levels of compounds released remain far below those typically observed in aromatic varieties, where monoterpene concentrations may reach up to 14 mg L^{-1}. The linalool/α-terpineol ratio was greater than 1 in both *Rucignola* and *Lucignola*. However, only *Lucignola* exhibited a linalool/geraniol ratio higher than 1. An environmental influence on the varieties studied can be observed, although investigations over multiple years are required to reach definitive conclusions. The qualitative profiles and the ratios between isomeric compounds remained largely consistent across different viticultural environments (Table 9), suggesting potential applicability for varietal discrimination. By contrast, quantitative data revealed marked differences (Tables 6, 7, and 8), which are consistent with previous findings [36,56].

Multivariate statistical analyses

Multivariate statistical analyses were applied exclusively to red-berry cultivars, as the inclusion of both red and white grapes (the latter represented by only two grape varieties) would have resulted in a trivial separation driven primarily by differences in color-related variables rather than by subtler varietal traits. A PLS-DA (Partial Least Squares Discriminant Analysis) approach was employed to investigate patterns of variability and to assess the discriminatory power of the measured variables. The analysis was first conducted separately for the 2023 dataset and subsequently for the 2024 dataset, in order to capture year-specific trends and potential vintage effects (Figure 3 and 4). Consequently, a combined PLS-DA including both vintages (2023 and 2024) was performed, allowing the evaluation of varietal separation across vintages and providing a more robust assessment of the consistency of discriminant markers (Figure 5). In Figures 3, 4, and 5, panels A show the 3D

score plots, which clearly illustrate the separation of the grape varieties, while panels B report the Variable Importance in Projection (VIP) scores. The VIP scores rank the contribution of each variable to the separation among groups, and the accompanying heatmaps (colored squares) indicate the relative abundance of each variable in the different cultivars, with the color scale ranging from low (blue) to high (red) values. The significativity associated to this analysis, obtained by permutation test (n = 1000), is shown in Figure S1, S2 and S3. In the PLS-DA conducted separately for the 2023 and 2024 vintage, the variables responsible for varietal separation were quercetin-3-glucoside, caftaric acid, terpenes, kaempferol, and anthocyanins. A slightly different separation pattern was observed between vintages, indicating a vintage effect on the discriminatory power of these compounds [57]. When the datasets from 2023 and 2024 vintage were analyzed together through PLS-DA, a clear separation among the red-berry cultivars was observed (Figure 5A), confirming the robustness of varietal discrimination across vintages. The PLS-DA of the combined model clearly discriminated the red-berry cultivars according to their varietal origin, with a cumulative explained variance of 68.8% across the first three components (Component 1 = 27.4%, Component 2 = 33.6%, Component 3 = 7.8%). Distinct clustering patterns were observed, indicating well-defined chemical profiles among the cultivars. *Lucignola* samples (light blue) formed a separate and compact group, clearly differentiated from the others along Component 2, mainly due to their higher contents of glycosylated terpenes and flavonols (e.g., quercetin-3-glucoside and kaempferol-3-glucoside). *Anonima* (red) and *Catanese* Nera (blue) were positioned in proximity but remained distinct, driven by differences in anthocyanin composition, particularly cyanidin-3-glucoside and malvidin-3-glucoside. *Bracau* (green) clustered apart along Component 1, characterized by higher levels of hydroxycinnamic acids such as trans-fertaric and caffeoyl derivatives. The Variable Importance in Projection (VIP) scores (Figure 5B), confirmed that glycosylated terpenes, quercetin-3-glucoside, and caffeoyl-tartaric acid were among the main discriminant metabolites, followed by petunidin-3-glucoside and total acetylated anthocyanins. These compounds together explain most of the varietal separation observed in the model, highlighting the combined influence of phenolic composition and

bound aroma precursors in differentiating the ancient red grape cultivars. The heatmaps further revealed cultivar-specific patterns, with *Lucignola* showing higher terpene levels, *Vitrarolo* (Marsala and Sclafani Bagni) enriched in flavonols, and *Anonima* and *Bracau* displaying comparatively lower levels of these compounds. These findings suggest that despite inter-annual variability, consistent varietal markers can be identified, providing reliable indicators for distinguishing minor Sicilian cultivars [58].

Conclusion

Seven ancient and autochthonous Sicilian grape varieties, including white (*Rucignola*, *Dunnuni*), rose-berry (*Lucignola*), and red cultivars (*Anonima*, *Bracau*, *Catanese Nera*, and *Vitrarolo*), were thoroughly analyzed and characterized in terms of their physicochemical, phenolic, and volatile composition. *Vitrarolo* cultivar was additionally investigated across different terroirs, allowing the evaluation of environmental influences on its metabolic profile. For the first time, comprehensive insights into the metabolic composition of these ancient grape varieties have been provided. More specifically, *Lucignola* and *Rucignola* stood out for their relatively high glycosylated terpene content. *Vitrarolo* exhibited the most abundant and diverse flavonol and anthocyanin profiles. *Catanese Nera* was distinguished by its higher proportion of acylated anthocyanins, whereas *Anonima* and *Bracau* were characterized by comparatively lower levels of total phenolics. In the case of *Vitrarolo* grapes, the comparison across different terroirs confirmed that environmental conditions significantly shape its phenolic and volatile composition, underlining the importance of site-specific factors in defining varietal expression. In conclusion, our study provided a useful characterization of these cultivars, especially regarding phenolic and volatile compounds, which are key tools for variety differentiation. Furthermore, the presented data are of practical relevance to winemakers, as phenolic and volatile markers can guide the development of specific viticultural and enological practices to address climate change-related challenges. Indeed, the metabolic profiles, and in particular those of specialized

metabolites, may underpin the resilience of these vines to the harsh pedoclimatic conditions of the Sicilian coast. Consequently, winemakers may trace back viticultural factors influencing the accumulation of specific phytochemicals in grapes, with the aim of modulating their transfer into wines through appropriate vinification strategies. Ultimately, this approach is expected to enhance wine quality, reinforce territorial identity, and increase the reputation of both wineries and the broader region. In the future, the contribution of tannins should be investigated in greater detail, given their quantitative and qualitative importance in wine evolution processes. This includes the determination of mean degree of polymerization, the epigallocatechin/epicatechin ratio, and their evolution during berry growth and ripening. Such knowledge would be essential for the development of varietal winemaking techniques specifically tailored to the content and profile of the different classes of grape polyphenols.

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Acknowlegments

This study is part of Clara Vitaggio's PhD thesis supervised by Prof. Onofrio Corona. The authors gratefully acknowledge the winemaker of Assuli Winery (Marsala) and the winemakers of Donnafugata Winery (Contessa Entellina) and Tasca d'Almerita Winery (Sclafani Bagni) for kindly providing the grape samples used in this study.

Supplementary materials

Table 1 Berry color classification of the analyzed ancient Sicilian grape cultivar

Cultivar	berry color
Rucignola	white berry cultivar
Dunnuni	white berry cultivar
Lucignola	rose-pale berry cultivar
Anonima	red berry cultivar
Bracau	red berry cultivar
Catanese Nera	red berry cultivar
Vitrarolo	red berry cultivar

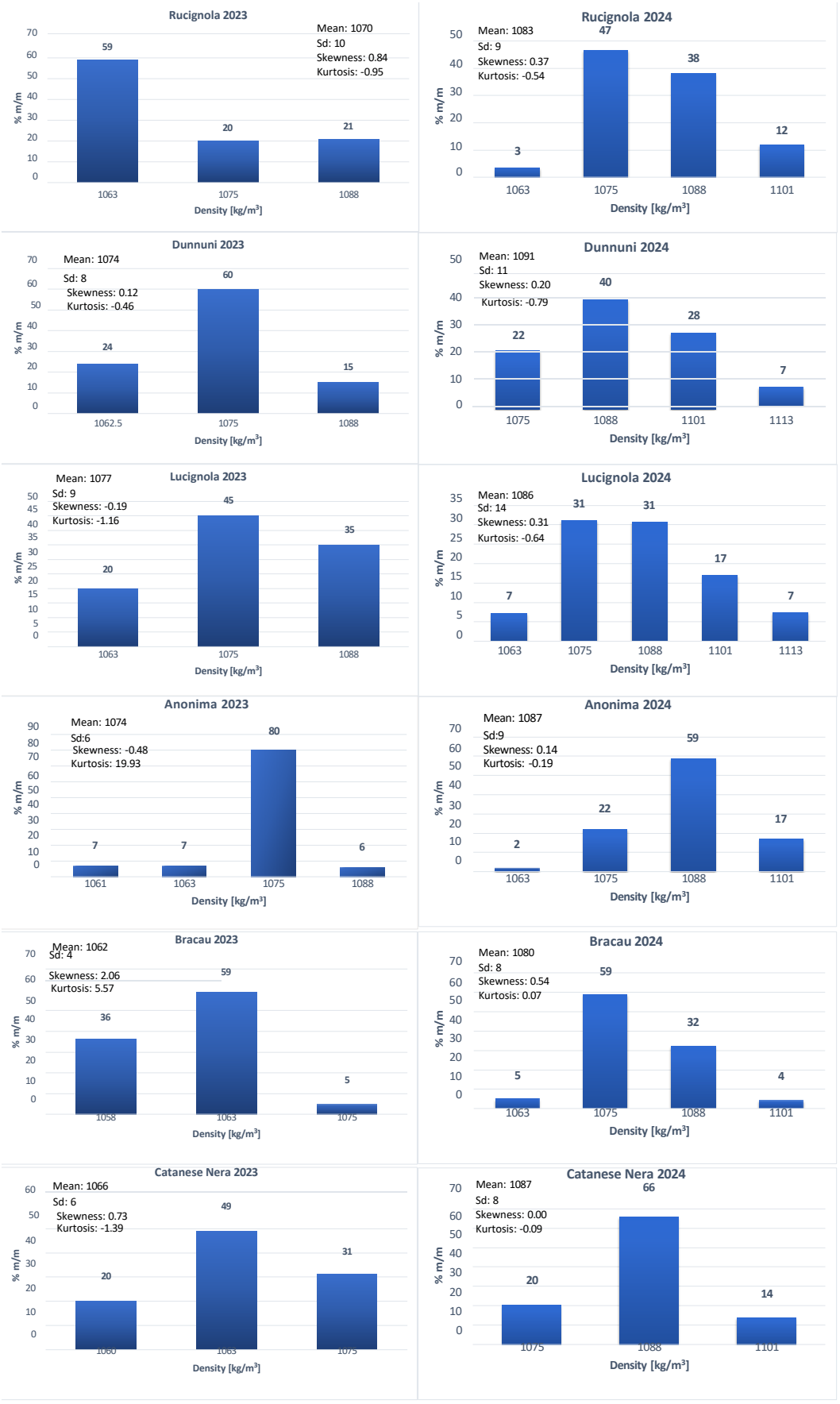


Figure 1 Density class distributions of ancient grape berries (red, pale-rose and white berry cultivar) grown in Marsala in 2023 and 2024 vintages.

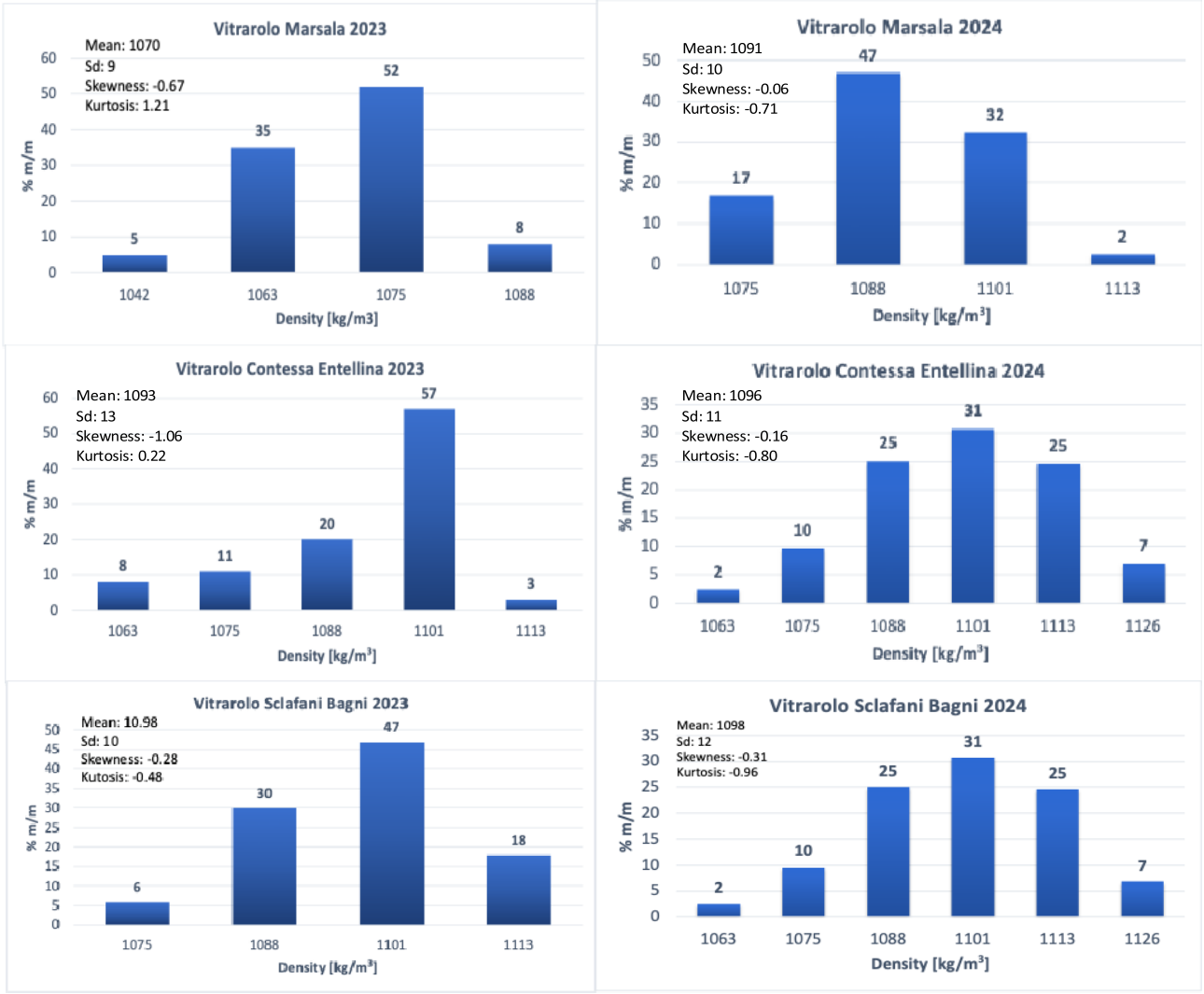


Figure 2 Density class distributions of *Vitrarolo* berries (red berry cv) grown in different environments in 2023 and 2024 vintages.

Table 2 Grapes chemical physical parameters of white, rose, and red berry grape varieties across the 2023 and 2024 vintages.

	Reducing sugars g L^{-1}	pH	Titratable acidity g L^{-1}	Malic acid g L^{-1}	Tartaric acid g L^{-1}	K g L^{-1}	Berry weight g	APA
Rucignola (white berry cultivar)								
Year (n = 4)								
2023	165.3 ± 0.2	3.39 ± 0.01	5.83 ± 0.01	1.55 ± 0.02	7.45 ± 0.01	1.80 ± 0.02	1.73 ± 0.01	116 ± 3
2024	182.4 ± 0.4	3.58 ± 0.01	3.87 ± 0.02	0.80 ± 0.03	6.33 ± 0.01	1.97 ± 0.00	2.13 ± 0.01	105 ± 6
Sign.	***	***	***	***	***	*	***	
Dunnuni (white berry cultivar)								
Year (n = 4)								
2023	184.5 ± 0.3	3.28 ± 0.01	6.13 ± 0.01	1.22 ± 0.01	7.91 ± 0.01	1.63 ± 0.02	1.99 ± 0.01	114 ± 5
2024	194.6 ± 0.2	3.63 ± 0.01	4.27 ± 0.01	0.65 ± 0.02	7.33 ± 0.02	2.13 ± 0.01	2.42 ± 0.03	98 ± 4
Sign.	***	***	***	***	***	***	**	
Lucignola (pale-rose berry cultivar)								
Year (n = 4)								
2023	185.3 ± 0.1	3.29 ± 0.01	8.07 ± 0.01	2.97 ± 0.01	7.94 ± 0.02	1.96 ± 0.01	2.05 ± 0.01	131 ± 3
2024	180.2 ± 0.1	3.38 ± 0.01	5.73 ± 0.00	1.27 ± 0.01	7.58 ± 0.01	2.05 ± 0.01	2.01 ± 0.01	75 ± 3
Sign.	***	***	***	***	**	**	**	**
Anonima (red berry cultivars)								
Year (n = 4)								
2023	186.1 ± 0.1	3.40 ± 0.01	5.99 ± 0.02	0.92 ± 0.02	8.14 ± 0.04	2.00 ± 0.04	2.02 ± 0.06	134 ± 4
2024	197.8 ± 0.1	3.44 ± 0.01	4.14 ± 0.01	0.38 ± 0.03	6.55 ± 0.02	1.74 ± 0.01	2.00 ± 0.01	92 ± 4
Sign.	***	*	***	**	***	*		**
Bracau (red berry cultivars)								
Year (n = 4)								
2023	161.36 ± 0.01	3.33 ± 0.01	5.87 ± 0.03	1.53 ± 0.02	7.00 ± 0.01	1.47 ± 0.01	2.47 ± 0.01	121 ± 1
2024	180.9 ± 0.3	3.41 ± 0.01	4.50 ± 0.01	0.42 ± 0.02	6.78 ± 0.00	1.79 ± 0.01	2.22 ± 0.06	101 ± 3
Sign.	***	**	***	***	***	***	*	*
Catanese Nera (red berry cultivars)								

Year (n = 4)								
2023	169.1 ± 0.3	3.29 ± 0.01	5.36 ± 0.01	1.30 ± 0.01	7.22 ± 0.01	1.78 ± 0.01	2.00 ± 0.01	137 ± 1
2024	192.2 ± 0.3	3.47 ± 0.01	4.44 ± 0.01	0.69 ± 0.03	6.77 ± 0.01	1.90 ± 0.01	2.28 ± 0.03	121 ± 3
Sign.	***	***	***	**	***	**	**	*
Vitrarolo (red berry cultivars)								
Year (n = 6)								
2023	212 ± 24	3.46 ± 0.13	4.7 ± 0.4	0.7 ± 0.2	7 ± 1	2.1 ± 0.3	1.8 ± 0.3	109 ± 34
2024	209 ± 12	3.49 ± 0.15	4 ± 1	0.1 ± 0.1	7 ± 2	1.80 ± 0.07	1.4 ± 0.2	57 ± 17
Sign.			*	**		*	**	***
Terroir (n = 4)								
Contessa Entellina	211 ± 8 b	3.37 ± 0.08 b	5.19 ± 0.07 a	0.4 ± 0.5	7.9 ± 0.5 a	1.91 ± 0.03	1.7 ± 0.4	59 ± 19
Marsala	192 ± 9 b	3.47 ± 0.16 ab	3.4 ± 0.9 b	0.5 ± 0.4	6 ± 1 b	1.9 ± 0.1	1.81 ± 0.08	75 ± 30
Sclafani Bagni	230 ± 12 a	3.59 ± 0.02 a	4.4 ± 0.3 ab	0.3 ± 0.2	7 ± 1 a	2.1 ± 0.5	1.3 ± 0.1	114 ± 42
Sign.	**	.	**		**			
Interaction Year:Terroir (n = 2)								
2023:Contessa Entellina	217 ± 1	3.43 ± 0.04 c	5.1 ± 0.1 b	0.8 ± 0.1 a	7.5 ± 0.2 b	1.9 ± 0.1 b	2.0 ± 0.1 a	75 ± 4 c
2024:Contessa Entellina	203.8 ± 0.4	3.30 ± 0.04 e	5.3 ± 0.1 a	0.0 ± 0.2 b	8.4 ± 0.2 a	1.9 ± 0.2 c	1.3 ± 0.1 e	43 ± 1 d
2023:Marsala	184 ± 22	3.33 ± 0.01 d	4.2 ± 0.3 d	0.8 ± 0.1 a	6.4 ± 0.5 d	1.9 ± 0.1 b	1.9 ± 0.1 b	100.9 ± 0.3 b
2024:Marsala	199 ± 3	3.61 ± 0.01 a	2.6 ± 0.0 e	0.2 ± 0.1 b	5.0 ± 0.2 e	1.8 ± 0.2 d	1.7 ± 0.2 c	49 ± 4 d
2023:Sclafani Bagni	234 ± 19	3.61 ± 0.01 a	4.7 ± 0.0 c	0.4 ± 0.3 ab	8.4 ± 0.1 a	2.5 ± 0.1 a	1.4 ± 0.1 d	150 ± 1 a
2024:Sclafani Bagni	225 ± 1	3.58 ± 0.01 b	4.1 ± 0.2 d	0.2 ± 0.1 b	6.9 ± 0.2 c	1.7 ± 0.1 d	1.3 ± 0.2 f	78 ± 1 c
Sign.	.	***	***	*	***	***	***	***

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$).

Table 3 Non-anthocyanic flavonoids (NAF), total flavonoids, and total anthocyanins (red cultivars only) in white, rose, and red berry grape varieties across the 2023 and 2024 vintages.

	NAF (mg kg^{-1})	Seeds total flavonoids (mg kg^{-1})	skin/seeds FNA	Total anthocyanins (mg kg^{-1})
Rucignola (white berry cultivar)				
Year (n = 4)				
2023	3242 ± 99	2927 ± 93	0.11 ± 0.01	/
2024	2994 ± 17	2607 ± 7	0.15 ± 0.01	/
Sign.	ns	*	**	
Dunnuni (white berry cultivar)				
Year (n = 4)				
2023	1829 ± 74	1505 ± 72	0.21 ± 0.001	/
2024	1480 ± 32	1039 ± 59	0.42 ± 0.05	/
Sign.	*	*	*	
Lucignola (pale-rose berry cultivar)				
Year (n = 4)				
2023	2939 ± 27	2003 ± 72	0.47 ± 0.04	154 ± 8
2024	2435 ± 74	1402 ± 4	0.74 ± 0.05	39.4 ± 0.2
Sign.	*	**	*	**
Anonima (red berry cultivar)				
Year (n = 4)				
2023	3078 ± 104	1903 ± 85	0.62 ± 0.02	334 ± 23
2024	3720 ± 172	2067 ± 62	0.80 ± 0.03	700 ± 86
Sign.	*		*	*
Bracau (red berry cultivar)				
Year (n = 4)				
2023	3314 ± 145	2397 ± 151	0.39 ± 0.03	172 ± 14

2024	4335 ± 105	2954 ± 98	0.47 ± 0.02	401 ± 33
Sign.	*	*	ns	*
		Catanese Nera (red berry cultivar)		
Year (n = 4)				
2023	3360 ± 9	2078 ± 11	0.62 ± 0.01	286 ± 12
2024	3262 ± 134	1991 ± 88	0.64 ± 0.01	515 ± 20
Sign.	ns	Ns	ns	**
		Vitrarolo (red berry cultivar)		
Year (n = 6)				
2023	3393 ± 298	2172 ± 137	0.7 ± 0.3	540 ± 250
2024	3248 ± 625	2074 ± 439	0.5 ± 0.1	651 ± 173
Sign.	ns	Ns	**	*
Terroir (n = 4)				
Contessa Entellina	3629 ± 313 a	2306 ± 301	1 ± 0.1	614 ± 133 b
Marsala	2812 ± 362 a	1914 ± 376	0.8 ± 0.1	357 ± 88 c
Sclafani Bagni	3520 ± 261 b	2149 ± 163	0.9 ± 0.2	815 ± 33 a
Sign.	*	Ns	.	***
Interaction Year:Terroir (n = 2)				
2023:Contessa Entellina	3366 ± 124 ab	2048 ± 9 bc	0.4 ± 0.1 c	509 ± 88 b
2024:Contessa Entellina	3892 ± 35 a	2564 ± 85 a	1 ± 0.2 a	721 ± 19 a
2023:Marsala	3109 ± 75 b	2225 ± 74 ab	0.4 ± 0.1 c	281 ± 22 c
2024:Marsala	2516 ± 192 c	1604 ± 188 c	0.6 ± 0.1 b	433 ± 3 bc
2023:Sclafani Bagni	3704 ± 262 a	2245 ± 206 ab	0.7 ± 0.1 c	831 ± 42 a
2024:Sclafani Bagni	3338 ± 41 ab	2054 ± 25 c	0.3 ± 25 c	800 ± 26 a
Sign.	**	**	***	*

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$).

Table 4 Monomer anthocyanins profile (%) of rose, and red berry grape varieties across the 2023 and 2024 vintages.

Factor	Delphinidin 3-glucoside	Cyanidin 3-glucoside	Petunidin 3-glucoside	Peonidin 3-glucoside	Malvidin 3-glucoside	Acetylated anthocyanins	p-coumaroylated anthocyanins	acetylated/p-coumaroylated	Anthocyanins tri-substituted	Anthocyanins di-substituted	tri/di-substitured
Lucignola (pale-rose berry cultivar)											
Year (n = 4)											
2023	24.84 ± 1.08	14.41 ± 1.11	14.99 ± 0.16	8.32 ± 0.09	31.56 ± 2.19	2.31 ± 0.10	3.60 ± 0.02	0.64 ± 0.03	71.38 ± 1.27	22.72 ± 1.20	3.15 ± 0.22
2024	18.80 ± 0.78	20.69 ± 0.20	8.03 ± 0.71	13.25 ± 0.56	23.77 ± 1.85	6.83 ± 0.01	5.74 ± 0.18	1.10 ± 0.06	50.59 ± 3.34	33.95 ± 0.36	1.49 ± 0.08
Sign.	*	*	*	*	ns	***	**	*	*	*	*
Anonima (red berry cultivar)											
Year (n = 4)											
2023	2.84 ± 0.11	2.31 ± 0.05	3.96 ± 0.07	23.13 ± 0.10	45.09 ± 0.22	7.19 ± 0.07	15.48 ± 0.05	0.31 ± 0.16	51.89 ± 0.04	25.44 ± 0.16	2.04 ± 0.01
2024	2.48 ± 0.06	1.36 ± 0.01	1.71 ± 0.05	5.98 ± 0.70	38.67 ± 1.04	7.79 ± 0.08	42.01 ± 0.37	0.19 ± 0.01	42.86 ± 1.15	7.34 ± 0.71	5.91 ± 0.73
Sign.	ns	**	**	**	*	*	***	ns	*	**	*
Bracau (red berry cultivar)											
Year (n = 4)											
2023	2.72 ± 0.10	1.71 ± 0.25	4.24 ± 0.06	16.55 ± 1.86	51.12 ± 2.21	8.82 ± 0.34	14.84 ± 0.61	0.51 ± 0.04	58.09 ± 2.37	18.26 ± 2.11	3.24 ± 0.50
2024	1.93 ± 0.16	1.47 ± 0.15	1.56 ± 0.03	6.54 ± 0.02	31.80 ± 1.29	8.41 ± 0.26	46.06 ± 0.57	0.18 ± 0.01	35.29 ± 1.48	8.00 ± 0.18	4.41 ± 0.09
Sign.	.	ns	***	*	*	ns	***	*	*	*	ns
Catanese Nera (red berry cultivar)											
Year (n = 4)											
2023	1.85 ± 0.08	1.46 ± 0.03	2.14 ± 0.22	12.28 ± 0.27	50.25 ± 0.43	28.47 ± 0.11	8.56 ± 0.06	0.64 ± 0.01	61.24 ± 0.13	16.74 ± 0.30	3.66 ± 0.07
2024	1.66 ± 0.04	0.62 ± 0.15	1.74 ± 0.26	10.81 ± 1.67	36.19 ± 1.26	37.42 ± 0.10	11.75 ± 0.18	0.18 ± 0.01	39.58 ± 1.57	11.44 ± 1.81	3.57 ± 0.70
Sign.	ns	*	*	ns	**	*	***	***	**	ns	ns
Vitrarolo (red berry cultivar)											
Year (n= 6)											
2023	2.38 ± 0.45	1.93 ± 0.23	4.91 ± 0.59	19.46 ± 2.36	54.36 ± 1.08	3.79 ± 0.54	12.57 ± 1.53	0.37 ± 0.10	61.64 ± 1.43	21.39 ± 2.58	3.11 ± 0.38
2024	2.03 ± 0.10	1.09 ± 0.05	4.74 ± 1.15	9.73 ± 2.07	49.27 ± 1.73	5.84 ± 0.48	21.40 ± 2.15	0.26 ± 0.01	56.04 ± 2.78	10.82 ± 2.08	6.31 ± 1.22
Sign.	ns	**	ns	*	*	*	**	ns	ns	**	*
Terroir (n = 4)											

Contessa Entellina	2.02 ± 0.45	1.39 ± 0.16	6.61 ± 1.05 a	10.87 ± 2.90	54.98 ± 1.51	4.83 ± 1.05	18.36 ± 2.64	0.26 ± 0.02	63.60 ± 0.33 a	12.26 ± 3.06	6.38 ± 1.59
Marsala	1.76 ± 0.04	1.86 ± 0.45	2.66 ± 0.54 b	21.52 ± 3.09	48.55 ± 1.98	6.03 ± 0.33	17.15 ± 5.33	0.47 ± 0.13	52.98 ± 2.48 b	23.38 ± 3.53	2.38 ± 0.25
Sclafani Bagni	2.84 ± 0.38	1.29 ± 0.15	5.21 ± 0.34 ab	11.38 ± 2.52	51.91 ± 1.94	3.59 ± 0.42	15.46 ± 0.34	0.22 ± 0.02	59.95 ± 2.58 ab	12.68 ± 2.67	5.37 ± 1.08
Sign.	ns	ns	*	ns	ns	.	ns	ns	*	.	.
Interaction Year:Terroir (n = 2)											
2023:Contessa Entellina	2 ± 2	1.7 ± 0.1 b	5 ± 2	15.9 ± 0.2	56 ± 4	3 ± 1 d	14 ± 1 c	0.22 ± 0.03 cd	63.6 ± 0.2 a	17.6 ± 0.1	3.62 ± 0.01 b
2024:Contessa Entellina	2.1 ± 0.3	1.12 ± 0.02 b	8 ± 1	5.9 ± 0.4	54 ± 3	6.62 ± 0.03 a	23 ± 1 b	0.30 ± 0.02 b	64 ± 1 a	7.0 ± 0.3	9.1 ± 0.3 a
2023:Marsala	1.7 ± 0.1	2.6 ± 0.2 b	4 ± 3	26.9 ± 0.2	51.9 ± 0.1	5.47 ± 0.04 b	7.9 ± 0.1 d	0.69 ± 0.01 a	57.2 ± 0.1 b	29.5 ± 0.1	1.9 ± 0.1 b
2024:Marsala	1.8 ± 0.1	1.1 ± 0.1 b	2 ± 2	16 ± 1	45 ± 2	6.60 ± 0.02 a	26.4 ± 0.5 a	0.25 ± 0.01 bc	49 ± 1 c	17.3 ± 0.4	2.82 ± 0.01 b
2023:Sclafani Bagni	3.5 ± 0.1	1.5 ± 0.2 a	6 ± 1	16 ± 2	55 ± 1	2.9 ± 0.1 d	15.9 ± 0.2 c	0.19 ± 0.00 d	64 ± 1 a	17 ± 2	3.8 ± 0.5 b
2024:Sclafani Bagni	2.2 ± 0.1	1.1 ± 0.3 b	5 ± 1	7 ± 1	49 ± 3	4.3 ± 0.1 c	15 ± 1 c	0.25 ± 0.00 bcd	56 ± 3 b	8 ± 2	7 ± 2 a
Sign.	ns	**	.	ns	ns	**	***	***	*	ns	*

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). BDL= below detection limit.

Table 5 Hydroxycinnamoyl tartaric acids (HCTAs) and flavonol contents (g kg^{-1} of berries) in white, rose, and red berry grape varieties across the 2023 and 2024 vintages.

Rucignola (white berry cultivar)

Factor	caffeoyl tartaric acid	p-coumaroyl tartaric acid	feruloyl tartaric acid	myricetin 3-glucuronide	myricetin 3-glucoside	quercetin 3-glucuronide	quercetin 3-glucoside	kaempferol 3-glucuronide	kaempferol 3-glucoside
Year (n = 4)									
2023	28 ± 1	5.7 ± 0.3	0.8 ± 0.1	BDL	0.17 ± 0.02	BDL	0.8 ± 0.1	BDL	BDL
2024	31 ± 4	8 ± 1	0.7 ± 0.1	BDL	0.02 ± 0.01	8 ± 3	5 ± 2	0.8 ± 0.1	1 ± 1
Sign.	ns	ns	ns	ns	**	.	.	ns	ns
Dunnuni (white berry cultivar)									
Year (n = 4)									
2023	30 ± 1	8.0 ± 0.3	2.1 ± 0.2	BDL	0.14 ± 0.01	0.21 ± 0.02	8.0 ± 0.4	BDL	BDL
2024	24 ± 3	10 ± 1	1.6 ± 0.2	BDL	-	6 ± 1	6 ± 1	0.18 ± 0.02	0.72 ± 0.05
Sign.	.	.	.	ns	**	*	*	ns	ns
Lucignola (pale-rose berry cultivar)									
Year (n = 4)									
2023	38.5 ± 0.2	26.2 ± 0.3	1.8 ± 0.1	BDL	0.8 ± 0.1	BDL	8 ± 1	13 ± 4	0.26 ± 0.05
2024	18 ± 1	24 ± 1	1.26 ± 0.04	0.6 ± 0.1	2.7 ± 0.2	5.2 ± 0.5	7 ± 1	0.11 ± 0.04	0.5 ± 0.1
Sign.	**	ns	*	**	**	**	ns	*	*
Anonima (red berry cultivar)									
Year (n = 4)									
2023	41 ± 3	20 ± 2	1.1 ± 0.1	BDL	0.6 ± 0.1	0.2 ± 0.4	3.2 ± 0.3	3.4 ± 0.3	1.1 ± 0.2
2024	15 ± 1	12 ± 1	0.4 ± 0.1	1.3 ± 0.2	6 ± 1	8 ± 2	4.5 ± 0.3	BDL	2.4 ± 0.2
Sign.	**	*	*	*	*	*	*	**	*
Bracau (red berry cultivar)									
Year (n = 4)									
2023	44.0 ± 0.2	24.9 ± 0.1	0.72 ± 0.03	BDL	0.6 ± 0.1	0.2 ± 0.1	2.5 ± 0.3	4 ± 1	0.7 ± 0.4
2024	37 ± 2	30 ± 1	0.8 ± 0.1	1.9 ± 0.1	9 ± 1	13.1 ± 0.2	9 ± 1	BDL	3.9 ± 0.4
Sign.	*	*	ns	**	**	***	**	**	**
Catanese Nera (red berry cultivar)									
Year (n = 4)									
2023	48 ± 1	28.38 ± 0.02	1.03 ± 0.04	BDL	0.93 ± 0.02	0.23 ± 0.01	3.60 ± 0.04	5.3 ± 0.2	1.09 ± 0.02
2024	25 ± 1	22.6 ± 0.3	0.65 ± 0.02	1.9 ± 0.4	9 ± 2	12 ± 1	8 ± 1	BDL	4.1 ± 0.4

Sign.	**	**	**	*	*	**	*	***	**
					Vitrarolo (red berry cultivar)				
Year (n = 6)									
2023	81 ± 9	31 ± 2	2 ± 1	0.03 ± 0.02	0.5 ± 0.4	0.1 ± 0.1	6 ± 6	12 ± 2	2 ± 1
2024	70 ± 13	28 ± 5	1.6 ± 0.2	1.7 ± 0.5	13 ± 2	28 ± 5	15 ± 1	BDL	6 ± 1
Sign.	*	*	ns	***	***	***	**	***	***
Terroir (n = 4)									
Contessa Entellina	83 ± 2 a	32 ± 1 a	1.9 ± 0.2 a	1 ± 1	7 ± 7	15 ± 17 a	7 ± 8 c	5 ± 6	3 ± 2 b
Marsala	63 ± 10 b	26 ± 4 b	1.3 ± 0.3 b	1 ± 1	6 ± 6	11 ± 13 b	10 ± 6 b	6 ± 7	4 ± 3 a
Sclafani Bagni	80 ± 11 a	30 ± 3 a	1.8 ± 0.3 a	1 ± 1	8 ± 8	16 ± 19 a	14 ± 1 a	6 ± 7	5 ± 1 a
Sign.	**	**	*	ns	ns	***	***	ns	***
Interaction Year:Terroir (n = 2)									
2023:Contessa Entellina	84 ± 3	32 ± 1 a	2.03 ± 0.04 ab	0.1 ± 0.1	0.17 ± 0.01 c	BDL	BDL	10.6 ± 0.2	1.4 ± 0.3 d
2024:Contessa Entellina	82.0 ± 0.1	33.3 ± 0.1 a	1.8 ± 0.2 ab	2 ± 1	13 ± 1 ab	29.6 ± 1.6 a	14.2 ± 0.4 a	BDL	4.7 ± 0.1 bc
2023:Marsala	71 ± 5	29 ± 3 ab	1.1 ± 0.2 c	BDL	0.3 ± 0.1 c	0.14 ± 0.01 c	5 ± 2 b	12 ± 4	1.4 ± 0.3 d
2024:Marsala	56 ± 6	23 ± 1 b	1.5 ± 0.1 bc	1.3 ± 0.1	11 ± 1 b	22 ± 1 b	15 ± 2 a	BDL	7.1 ± 0.3 a
2023:Sclafani Bagni	89 ± 3	33 ± 2 a	2.1 ± 0.2 a	0.1 ± 0.1	1.0 ± 0.2 c	0.18 ± 0.01 c	14 ± 2 a	12 ± 1	4.0 ± 0.5 c
2024:Sclafani Bagni	72 ± 10	28 ± 1 ab	1.56 ± 0.05 abc	1.8 ± 0.3	14.8 ± 0.2 a	33 ± 1 a	14.7 ± 0.3 a	BDL	5.7 ± 0.2 b
Sign.	ns	*	**	ns	*	***	***	ns	***

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). BDL= below detection limit. Caffeoyl tartaric acid, p-coumaroyl tartaric acid and feruloyl tartaric acid represent the sum of the cis- and trans-isomers.

Table 6 Glycosylated terpenes content (μg kg^{-1}) of white, rose, and red berry grape varieties across the 2023 and 2024 vintages.

Factor	trans-furan linalool oxide	cis-furan linalool oxide	Linalool	α-terpineol	trans-pyran linalool oxide	cis-pyran linalool oxide	Nerol	Geraniol	trans-8-hydroxylinalool	cis-8-hydroxylinalool	Geranic acid	p-menthen-7,8-diol
Rucignola (white berry cultivar)												
Year (n = 4)												
2023	22 ± 1	6.0 ± 0.2	29 ± 1	24.0 ± 0.4	16.5 ± 0.5	2.0 ± 0.3	26 ± 1	102 ± 1	48 ± 1	287 ± 1	165.0 ± 0.1	40.0 ± 0.1
2024	21.54 ± 0.02	5.2 ± 0.1	28 ± 1	23.4 ± 0.3	15.83 ± 0.08	2.05 ± 0.01	27 ± 1	107 ± 1	51.1 ± 1.1	327 ± 5	149 ± 1	38.9 ± 0.3
Sign.	ns	*	ns	ns	ns	ns	ns	*	.	*	ns	.
Dunnuni (white berry cultivar)												
Year (n = 4)												
2023	1.00 ± 0.03	2.0 ± 0.2	BDL	BDL	BDL	1.00 ± 0.01	15 ± 1	139 ± 3	11 ± 1	4 ± 1	129 ± 1	BDL
2024	1.22 ± 0.02	2.1 ± 0.1	BDL	BDL	0.97 ± 0.06	0.81 ± 0.05	13 ± 0	116 ± 3	10.3 ± 0.4	7.0 ± 2.0	92.0 ± 3.8	BDL
Sign.	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	*	ns
Lucignola (pale-rose berry cultivar)												
Year (n = 4)												
2023	24 ± 1	11.0 ± 0.2	262 ± 3	87 ± 1	42.00 ± 0.01	6.00 ± 0.03	44 ± 1	175 ± 1	49 ± 1	55.0 ± 0.1	128.0 ± 0.2	5.0 ± 0.1
2024	23.27 ± 0.02	9.16 ± 0.02	224 ± 1	70 ± 1	31.08 ± 0.25	4.64 ± 0.12	36 ± 1	144 ± 2	39.5 ± 0.2	48 ± 1	91 ± 2	3.5 ± 0.1
Sign.	ns	ns	ns	ns	ns	ns	*	ns	ns	*	ns	ns
Anonima (red berry cultivar)												
Year (n = 4)												
2023	4.00 ± 0.01	15.00 ± 0.01	BDL	BDL	7.00 ± 0.02	3.00 ± 0.03	10.0 ± 0.1	70 ± 2	9.0 ± 0.3	2.0 ± 0.2	45.0 ± 0.2	B. L
2024	3.85 ± 0.05	13.80 ± 0.05	BDL	BDL	6.23 ± 0.07	3.10 ± 0.04	10.8 ± 0.2	74 ± 1	10.8 ± 0.2	2.2 ± 0.2	43.5 ± 0.3	BDL
Sign.	ns	**	*	*	**	ns	*	.	*	ns	*	ns
Bracau (red berry cultivar)												
Year (n = 4)												
2023	6.00 ± 0.04	21.00 ± 0.03	BDL	BDL	10.00 ± 0.04	2.00 ± 0.05	15.00 ± 0.20	96 ± 2	34.0 ± 0.2	5.0 ± 0.1	75 ± 2	30 ± 0.01
2024	5.67 ± 0.05	19.67 ± 0.12	BDL	BDL	9.71 ± 0.11	1.98 ± 0.02	15.59 ± 0.32	102 ± 1	36.0 ± 1.0	6.9 ± 0.3	68.4 ± 0.1	20 ± 0.08

Sign.	*	**	ns	ns	ns	ns	ns	ns	ns	*	.	ns
					Catanese Nera (red berry cultivar)							
Year (n = 4)												
2023	3.00 ± 0.03	11.5 ± 0.5	BDL	BDL	5.5 ± 0.5	2.00 ± 0.01	11 ± 1	72 ± 1	9 ± 1	3 ± 1	44 ± 3	0.5 ± 0.5
2024	2.91 ± 0.04	10.56 ± 0.04	BDL	BDL	5.1 ± 0.3	2.2 ± 0.2	10.5 ± 0.2	74 ± 5	10 ± 1	2.3 ± 0.4	40 ± 3	0.41 ± 0.01
Sign.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
					Vitrarolo (red berry cultivar)							
Year (n = 6)												
2023	5.0 ± 0.4	8 ± 1	BDL	BDL	3.8 ± 0.3	2 ± 1	7.0 ± 0.4	31 ± 3	2.3 ± 0.2	5 ± 1	41 ± 4	1.2 ± 0.3
2024	4.6 ± 0.4	7 ± 1	BDL	BDL	4.2 ± 0.3	3.1 ± 0.2	7.1 ± 0.3	31 ± 3	3.1 ± 0.2	6 ± 1	45 ± 5	1.2 ± 0.3
Sign.	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
Terroir (n = 4)												
Contessa Entellina	6.0 ± 0.1	8.3 ± 0.2	BDL	BDL	4.8 ± 0.3	3.1 ± 0.1	6.1 ± 0.1	24.6 ± 0.4	3.4 ± 0.2	2.6 ± 0.4	41 ± 2	1.0 ± 0.1
Marsala	3.8 ± 0.1	5.7 ± 0.2	BDL	BDL	4.0 ± 0.1	3 ± 1	7.8 ± 0.2	40 ± 1	2.3 ± 0.2	5.1 ± 0.1	33 ± 1	0.5 ± 0.2
Sclafani Bagni	4.6 ± 0.2	7.8 ± 0.1	BDL	BDL	3.3 ± 0.1	2.3 ± 0.2	7.3 ± 0.2	26.5 ± 0.2	2.4 ± 0.3	9 ± 1	55 ± 2	1.98 ± 0.02
Sign.	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Interaction Year:Terroir (n = 2)												
2023:Contessa Entellina	6.0 ± 0.1 a	8.5 ± 0.7	BDL	BDL	4.5 ± 0.7 ab	3.0 ± 0.1	6.0 ± 0.1 c	24.0 ± 0.1	3.0 ± 0.1 b	2.0 ± 0.1	37 ± 3 c	1.0 ± 0.1
2024:Contessa Entellina	5.9 ± 0.2 b	8.2 ± 0.1	BDL	BDL	5.0 ± 0.1 ab	3.3 ± 0.2	6.3 ± 0.2 c	25.3 ± 0.6	3.8 ± 0.2 a	3.3 ± 0.2	45 ± 1 b	1.1 ± 0.2
2023:Marsala	4.0 ± 0.1 e	6.0 ± 0.1	BDL	BDL	4.0 ± 0.1 abc	4.0 ± 0.2	8.0 ± 0.2 a	41 ± 1	2.0 ± 0.0 d	5.0 ± 0.2	34 ± 1 c	0.5 ± 0.7
2024:Marsala	3.6 ± 0.3 f	5.5 ± 0.1	BDL	BDL	4.0 ± 0.4 abc	3.5 ± 0.3	7.6 ± 0.3 a	40 ± 2	2.5 ± 0.1 c	5.2 ± 0.2	32 ± 2 c	0.4 ± 0.2
2023:Sclafani Bagni	5.0 ± 0.1 c	8.0 ± 0.2	BDL	BDL	3.0 ± 0.2 c	2.0 ± 0.1	7.0 ± 0.2 b	27 ± 1	2.0 ± 0.1 d	7.0 ± 0.2	53 ± 1 a	2.0 ± 0.1
2024:Sclafani Bagni	4.3 ± 0.1 d	7.6 ± 0.3	BDL	BDL	3.5 ± 0.1 bc	2.5 ± 0.1	7.6 ± 0.2 a	26.5 ± 0.3	2.9 ± 0.1 bc	10 ± 1	58 ± 2 a	2.0 ± 0.1
Sign.	***	ns	ns	ns	***	ns	**	ns	**	ns	*	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). BDL= below detection limit. Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). The unit of measurement is µg kg $^{-1}$ as 1-heptanol.

Table 7 Glycosylated benzenoids content (µg kg^{-1}) of white, rose, and red berry grape varieties across the 2023 and 2024 vintages.

Factor	Methyl salicylate	Benzyl alcohol	2 phenylethanol	Vanillin	Acetovanillone	Zingerone	Dihydroconiferyl alcohol
Rucignola (white berry cultivar)							
Year (n = 4)							
2023	3.00 ± 0.02	106 ± 3	354 ± 16	15.0 ± 0.1	7 ± 1	6.0 ± 0.0	163 ± 4
2024	2.986 ± 0.002	105 ± 1	344 ± 2	12.5 ± 0.7	10.0 ± 0.4	5.6 ± 0.0	154 ± 2
Sign.	*	ns	Ns	ns	ns	ns	ns
Dunnuni (white berry cultivar)							
Year (n = 4)							
2023	15.0 ± 0.0	505 ± 6	218 ± 7	18 ± 1	17.0 ± 2.0	5.0 ± 0.1	109 ± 3
2024	21.02 ± 0.56	431 ± 13	164 ± 5	10.5 ± 0.4	23.9 ± 1.7	2.3 ± 0.2	95 ± 5
Sign.	ns	*	*	ns	ns	**	ns
Lucignola (pale-rose berry cultivar)							
Year (n = 4)							
2023	1.00 ± 0.01	97 ± 1	204 ± 1	19 ± 1	15.0 ± 1.0	4.0 ± 0.2	98 ± 1
2024	1.14 ± 0.03	79 ± 2	157 ± 4	10.6 ± 0.1	14.8 ± 1.1	1.6 ± 0.1	85.8 ± 0.2
Sign.	*	*	Ns	ns	ns	ns	ns
Anonima (red berry cultivar)							
Year (n = 4)							
2023	3.00 ± 0.01	90 ± 2	77 ± 5	7.00 ± 0.01	4.0 ± 0.2	4 ± 1	15 ± 1
2024	3.23 ± 0.01	90 ± 1	69 ± 1	5.94 ± 0.28	6.9 ± 0.1	3.6 ± 0.1	21.0 ± 0.5
Sign.	**	ns	Ns	.	**	ns	*
Bracau (red berry cultivar)							
Year (n = 4)							
2023	11.50 ± 0.50	209 ± 1	150 ± 1	14 ± 1	9.5 ± 0.5	10 ± 1	104 ± 6
2024	13.72 ± 0.01	207 ± 2	144 ± 1	11 ± 0	13.1 ± 1.0	8.6 ± 0.0	116.9 ± 3.3
Sign.	*	.	**	.	.	ns	ns
Catanese Nera (red berry cultivar)							
Year (n = 4)							
2023	5.0 ± 0.2	123 ± 2	82 ± 4	8 ± 1	6.0 ± 0.5	5.0 ± 0.4	22 ± 3

2024	5.81 ± 0.18	128 ± 3	75 ± 4	7 ± 1	8.0 ± 0.4	4.9 ± 0.3	22 ± 4
Sign.	*	ns	Ns	ns	*	ns	ns
Vitrarolo (red berry cultivar)							
Year (n = 6)							
2023	4 ± 1	174 ± 39	140 ± 10	8 ± 1	4 ± 1	1.0 ± 0.2	55 ± 13
2024	3 ± 1	174 ± 38	129 ± 11	6.7 ± 0.3	5 ± 0	1.0 ± 0.1	59 ± 11
Sign.	ns	ns	Ns	.	ns	ns	ns
Terroir (n = 4)							
Environment							
Contessa Entellina	3.9 ± 0.1	171 ± 2	167 ± 3	8.2 ± 0.5	5.0 ± 0.3	1.1 ± 0.1	44 ± 2
Marsala	5.6 ± 0.2	281 ± 4	120 ± 4	7 ± 1	5.2 ± 0.5	0.8 ± 0.1	32 ± 3
Sclafani Bagni	1.02 ± 0.01	69.3 ± 0.2	117 ± 4	6.3 ± 0.2	3.2 ± 0.5	1.1 ± 0.1	95 ± 2
Sign.	ns	ns	Ns	ns	ns	ns	ns
Interaction Year:Terroir (n =2)							
2023:Contessa Entellina	4.0 ± 0.2 c	169.0 ± 4.2	171.0 ± 4.2	9.0 ± 0.1	5.5 ± 0.7 a	1.0 ± 0.1 ab	40.5 ± 4.9
2024:Contessa Entellina	3.7 ± 0.1 d	172.1 ± 2.8	162.0 ± 3.1	7.3 ± 0.1	4.5 ± 0.2 ab	1.3 ± 0.2 a	47.2 ± 0.8
2023:Marsala	6.0 ± 0.2 a	283.0 ± 14.1	126.0 ± 5.7	8.0 ± 1.4	4.5 ± 0.7 ab	1.0 ± 0.2 ab	27.5 ± 0.7
2024:Marsala	5.2 ± 0.1 b	279.2 ± 0.1	113.9 ± 3.0	6.7 ± 0.7	5.9 ± 0.3 a	0.7 ± 0.1 b	36.2 ± 6.5
2023:Sclafani Bagni	1.0 ± 0.1 e	69.0 ± 0.3	123.0 ± 7.1	6.5 ± 0.7	2.5 ± 0.7 b	1.0 ± 0.1 ab	96.5 ± 4.9
2024:Sclafani Bagni	1.0 ± 0.2 e	69.6 ± 0.7	110.3 ± 1.3	6.1 ± 0.1	3.9 ± 0.1 ab	1.2 ± 0.2 a	94.3 ± 2.3
Sign.	***	ns	Ns	ns	*	*	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). BDL= below detection limit. Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). The unit of measurement is µg kg $^{-1}$ as 1-heptanol.

Table 8 Glycosylated norisoprenoids content (μg kg^{-1}) of white, rose, and red berry grape varieties across the 2023 and 2024 vintages.

Factor	3,4-dihydro-3-oxoactinidol I	3,4-dihydro-3-oxoactinidol II	3,4-dihydro-3-oxoactinidol III	3-oxo-α-ionol	4-oxo-β-ionol	Blumenol C	3-hydroxy-7,8-dihydro-β-ionol	Vomifoliol
	Rucignola (white berry cultivar)							
Year (n = 4)								
2023	31 ± 1	78 ± 1	57.0 ± 0.4	438 ± 3	30.0 ± 0.3	4 ± 1	11 ± 1	133 ± 1
2024	30 ± 1	74.16 ± 0.05	53.5 ± 0.2	386 ± 1	24.6 ± 0.3	1.9 ± 0.5	10.6 ± 0.4	115 ± 4
Sign.	ns	*	ns	ns	ns	ns	ns	.
	Dunnuni (white berry cultivar)							
Year (n = 4)								
2023	17 ± 1	54 ± 1	51 ± 1	462 ± 7	26 ± 1	4.0 ± 0.2	10.00 ± 0.20	136 ± 5
2024	13 ± 0	41 ± 1	39 ± 1	331 ± 13	18.7 ± 0.6	1.0 ± 0.4	8.3 ± 0.3	99 ± 3
Sign.	*	*	*	*	ns	*	*	*
	Lucignola (pale-rose berry cultivar)							
Year (n = 4)								
2023	20 ± 1	73 ± 1	54 ± 1	745 ± 3	44 ± 1	BDL	3 ± 1	162 ± 5
2024	15 ± 1	56.35 ± 0.05	42 ± 1	553 ± 3	29.4 ± 0.3	BDL	2.6 ± 0.1	138 ± 7
Sign.	*	ns	ns	ns	ns	ns	ns	ns
	Anonima (red berry cultivar)							
Year (n = 4)								
2023	11 ± 1	30 ± 1	20.00 ± 0.01	245 ± 8	8 ± 1	1 ± 1	1.00 ± 0.02	52 ± 1
2024	10.6 ± 0.1	29 ± 1	20.40 ± 0.01	235 ± 2	8 ± 1	1.25 ± 0.02	1.83 ± 0.02	51 ± 1
Sign.	ns	ns	**	ns	ns	ns	***	ns
	Bracau (red berry cultivar)							
Year (n = 4)								
2023	32 ± 1	87 ± 2	52.50 ± 0.50	686 ± 31	28 ± 2	1.1 ± 0.1	10.00 ± 1.00	101 ± 1
2024	32.3 ± 0.9	85 ± 0	53.09 ± 0.18	666 ± 6	25 ± 1	1.78 ± 0.19	9.62 ± 0.29	95 ± 8
Sign.	ns	ns	ns	ns	ns	.	ns	ns
	Catanese Nera (red berry cultivar)							
Year (n = 4)								

2023	11 ± 1	27 ± 1	19 ± 1	205 ± 6	9 ± 1	1.1 ± 0.1	1.0 ± 0.2	60 ± 1
2024	10 ± 1	27 ± 2	19 ± 1	194 ± 12	8.1 ± 0.5	1.13 ± 0.01	1.4 ± 0.3	56 ± 3
Sign.	ns	ns	ns	ns	ns	ns	ns	ns
				Vitrarolo (red berry cultivar)				
Year (n = 6)								
2023	13 ± 1	24 ± 2	16 ± 1	582 ± 46	11 ± 1	BDL	4.0 ± 0.4	90 ± 7
2024	13 ± 1	23 ± 2	16 ± 2	547 ± 43	10.5 ± 0.4	BDL	5 ± 1	85 ± 8
Sign.	ns	ns	ns	ns	ns	ns	ns	ns
Terroir (n = 4)								
Contessa Entellina	15.0 ± 0.1	26.8 ± 0.5	16.9 ± 0.2	673 ± 18	12.1 ± 0.3	BDL	5.2 ± 0.2	88 ± 2
Marsala	10.1 ± 0.3	16.8 ± 0.5	11.9 ± 0.4	435 ± 13	10.4 ± 0.4	BDL	2.8 ± 0.1	67 ± 2
Sclafani Bagni	13.9 ± 0.3	27.4 ± 0.2	19.6 ± 0.2	584 ± 5	9.7 ± 0.2	BDL	4.8 ± 0.5	106 ± 2
Sign.	ns	ns	ns	ns	ns	ns	ns	ns
Interaction Year:Terroir (n = 2)								
2023:Contessa Entellina	15.01 ± 0.01	27.5 ± 0.7	17.0 ± 0.1	703.0 ± 1.4	12.5 ± 0.7	BDL	5.0 ± 0.1 ab	91.0 ± 5.7
2024:Contessa Entellina	15.1 ± 0.3	26.2 ± 0.4	16.7 ± 0.5	643.5 ± 13.9	11.7 ± 0.5	BDL	5.5 ± 0.4 a	85.2 ± 2.8
2023:Marsala	10.5 ± 0.7	17.5 ± 0.7	12.5 ± 0.7	454.5 ± 21.9	11.0 ± 0.1	BDL	3.0 ± 0.1 cd	71.0 ± 4.2
2024:Marsala	9.8 ± 0.2	16.1 ± 0.5	11.3 ± 0.4	416.3 ± 11.2	9.8 ± 0.1	BDL	2.7 ± 0.4 d	63.9 ± 0.2
2023:Sclafani Bagni	13.5 ± 0.7	27.5 ± 0.7	19.5 ± 0.7	587.0 ± 15.6	9.5 ± 0.7	BDL	4.01 ± 0.01 bc	107.0 ± 7.1
2024:Sclafani Bagni	14.2 ± 0.1	27.3 ± 0.3	19.7 ± 0.2	580.2 ± 7.2	10.0 ± 0.2	BDL	5.6 ± 0.2 a	105.2 ± 0.2
Sign.	ns	ns	ns	.	.	ns	**	ns

Note: Data are reported as mean plus and minus standard error of the mean. Sign. =ANOVA Statistical significance is indicated for each factor and their interaction (ns = not significant, . = 90% of significance, * = 95% of significance, ** = 99% of significance, *** = 99.9% of significance). BDL= below detection limit. Different letters indicate statistically significant differences according to post-hoc tests ($p < 0.05$). The unit of measurement is µg kg $^{-1}$ as 1-heptanol.

Table 9 Ratios between aroma compounds of white, rose and red berries grapes varieties across the 2023 and 2024 vintages

	Benz alc/2 phenyletan	3OHβ dam/3oxo αion	OxA/OxB	Lin/αterp	OxC/OxD	Lin/ger	T8OHlin/C8OHlin
Rucignola (white berry cultivar)							
Year (n = 4)							
2023	< 1	< 1	> 1	> 1	> 1	< 1	< 1
2024	< 1	< 1	> 1	> 1	> 1	< 1	< 1
Dunnuni (white berry cultivar)							
Year (n = 4)							
2023	< 1	< 1	< 1		< 1	< 1	> 1
2024	< 1	< 1	> 1		> 1	< 1	> 1
Lucignola (pale-rose berry cultivar)							
Year (n = 4)							
2023	< 1	< 1	> 1	> 1	> 1	> 1	> 1
2024	> 1	< 1	> 1	> 1	> 1	> 1	> 1
Anonima (red berry cultivar)							
Year (n = 4)							
2023	> 1	< 1	< 1		> 1	< 1	> 1
2024	> 1	< 1	< 1		> 1	< 1	> 1
Bracau (red berry cultivar)							
Year (n = 4)							
2023	> 1	< 1	< 1		> 1	< 1	> 1
2024	> 1	< 1	< 1		> 1	< 1	> 1
Sign.	ns	Ns	ns		ns	ns	ns
Catanese Nera (red berry cultivar)							
Year (n = 4)							
2023	> 1	< 1	< 1		> 1	< 1	> 1
2024	> 1	< 1	< 1		> 1	< 1	> 1
Vitrarolo (red berry cultivar)							
Year (n = 6)							
2023	> 1	< 1	> 1		> 1	< 1	> 1

2024	> 1	< 1	> 1	> 1	< 1	> 1
Terroir (n = 4)						
Contessa Entellina	> 1	< 1	> 1	> 1	< 1	> 1
Marsala	> 1	< 1	> 1	> 1	< 1	< 1
Sclafani Bagni	> 1	< 1	> 1	> 1	< 1	< 1
Interaction Year:Terroir (n = 2)						
2023:Contessa Entellina	> 1	< 1	> 1	> 1	< 1	> 1
2024:Contessa Entellina	> 1	< 1	> 1	> 1	< 1	> 1
2023:Marsala	> 1	< 1	> 1	> 1	< 1	< 1
2024:Marsala	> 1	< 1	> 1	> 1	< 1	< 1
2023:Sclafani Bagni	> 1	< 1	> 1	> 1	< 1	< 1
2024:Sclafani Bagni	> 1	< 1	> 1	> 1	< 1	< 1

Note: OxA = trans isomer of the furanic oxide of linalool; OxB = cis isomer of the furanic oxide of linalool; OxC = trans isomer of the pyranic oxide of linalool; OxD = cis isomer of the pyranic oxide of linalool; ger = geraniol; T8OHlin = Z isomer of 2,6-dimethyl-2,7-octadiene-1,6-diol; C8OHlin = E isomer of 2,6-dimethyl-2,7-octadiene-1,6-diol; 3OHβ dam = 3-hydroxy-β-damascone; 3oxo αion = 3-oxo-α-ionol; Benzyl alcohol = Benz alc; 2-phenylethanol = 2 phenyletan; Lin = linalol; αterp = α-terpineol (one of the compounds was not detected).

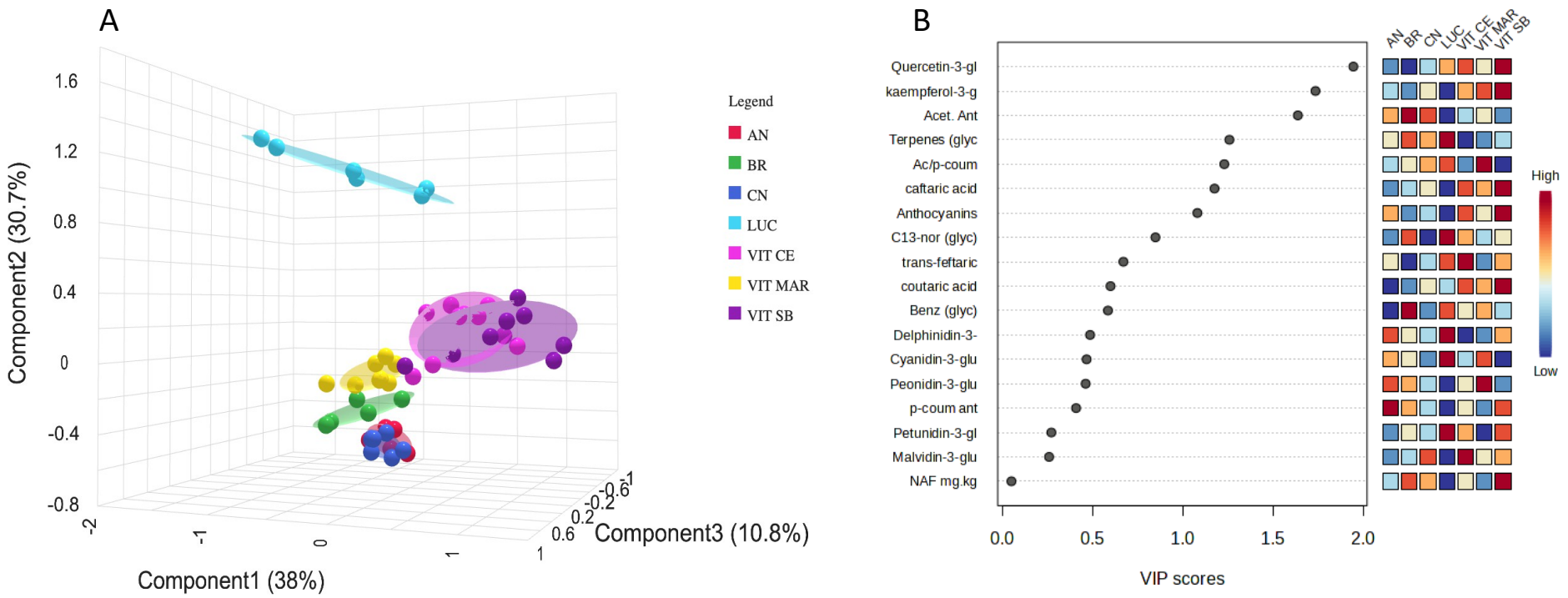


Figure 3 PLS-DA analysis of Sicilian minor red grape varieties (vintage 2023). (A) 3D score plot showing the distribution of the cultivars based on all studied variables: AN = Anonima; BR = Bracau; CN = Catanese Nera; LUC = Lucignola; VIT CE = Vitrarolo (Contessa Entellina); VIT MAR = Vitrarolo (Marsala); VIT SB = Vitrarolo (Sclafani Bagni). (B) Variable Importance in Projection (VIP) scores highlighting the most discriminant variables, with corresponding heatmaps of their relative levels across cultivars.

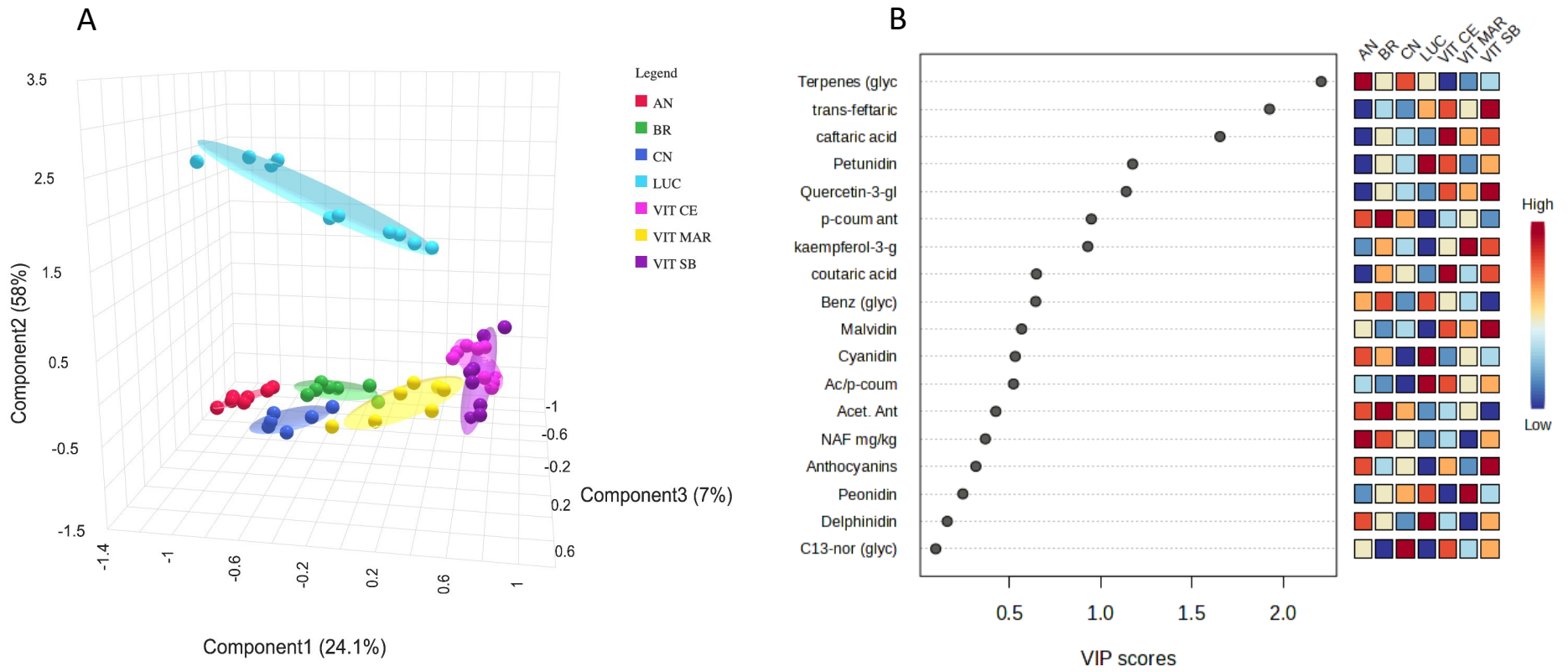


Figure 4 PLS-DA analysis of Sicilian minor red grape varieties (vintage 2024). (A) 3D score plot showing the distribution of the cultivars based on all studied variables: AN = Anonima; BR = Bracau; CN = Catanese Nera; LUC = Lucignola; VIT CE = Vitrarolo (Contessa Entellina); VIT MAR = Vitrarolo (Marsala); VIT SB = Vitrarolo (Sclafani Bagni). (B) Variable Importance in Projection (VIP) scores highlighting the most discriminant variables, with corresponding heatmaps of their relative levels across cultivars.

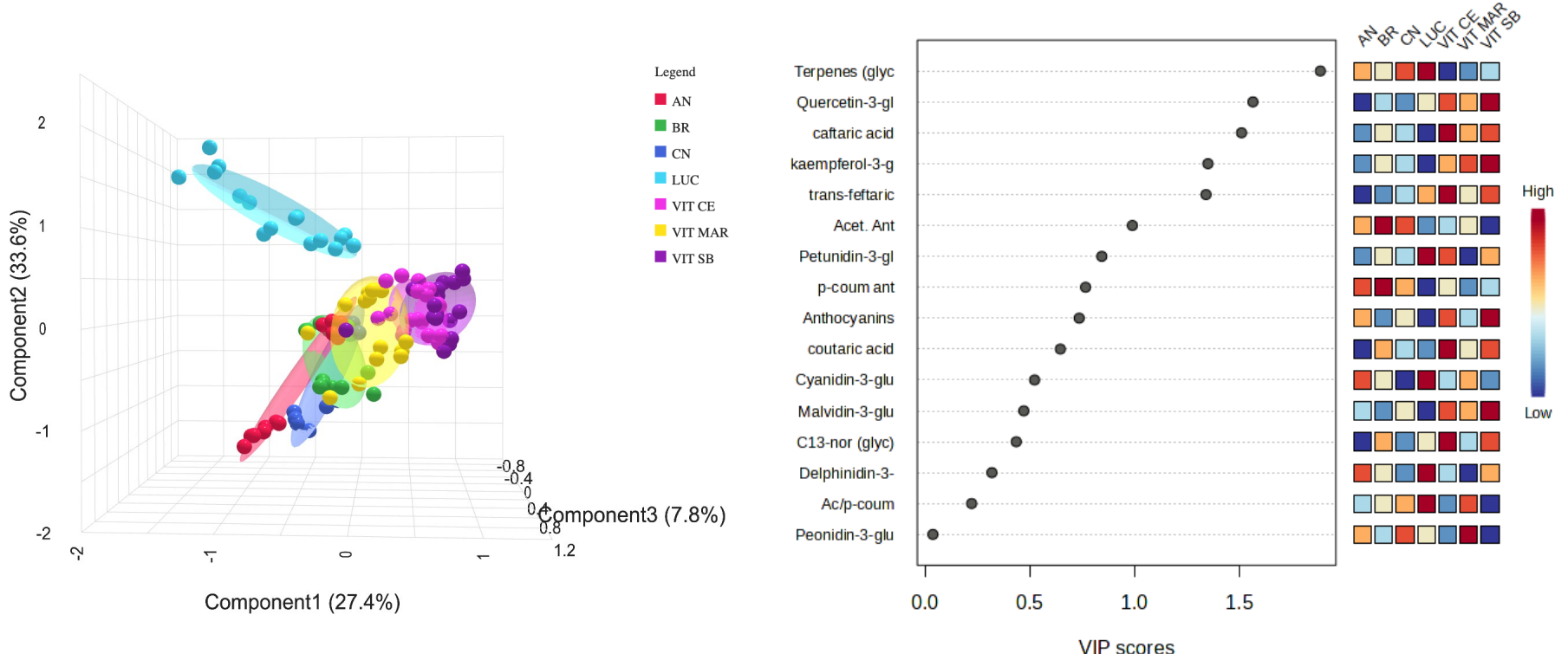


Figure 5 PLS-DA analysis of Sicilian minor grape varieties combining datasets from vintages 2023 and 2024. (A) 3D score plot showing the separation of cultivars based on all studied variables: AN = Anonima; BR = Bracau; CN = Catanese Nera; LUC = Lucignola; VIT CE = Vitrarolo (Contessa Entellina); VIT MAR = Vitrarolo (Marsala); VIT SB = Vitrarolo (Sclafani Bagni). (B) Variable Importance in Projection (VIP) scores identifying the most discriminant variables, with heatmaps illustrating their relative abundance across cultivars.

Table S1 Free terpenes content (μg kg^{-1}) of white, rose, and red berry grape varieties across the 2023 and 2024 vintages.

Factor	trans-furan linalool oxide	cis-furan linalool oxide	Linalool	Hotrienol	α-terpineol	trans-pyran linalool oxide	cis-pyran linalool oxide	Nerol	Geraniol	Diendiol I	Endiol	Diendiol II	trans-8-hydroxylinalool	cis-8-hydroxylinalool	Geranic acid	p-menthen-7,8-diol
Rucignola (white berry cultivar)																
Year (n = 4)																
2023	BDL	BDL	2.5 ± 2.1	BDL	BDL	0.5 ± 0.7	BDL	BDL	BDL	BDL	BDL	BDL	BDL	3.2 ± 2.8	65.3 ± 2.1	1.4 ± 1.4
2024	BDL	BDL	0.9 ± 0.1	BDL	BDL	0.5 ± 0.1	BDL	1.5 ± 0.1	7.5 ± 1.1	BDL	BDL	BDL	6.4 ± 0.2	4.2 ± 0.1	49.4 ± 3.8	0.6 ± 0.1
Dunnuni (white berry cultivar)																
Year (n = 4)																
2023	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	9.6 ± 1.4	BDL
2024	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	4.9 ± 0.2	BDL	BDL	BDL	12.8 ± 0.1	1.7 ± 0.1	6.7 ± 0.7	BDL
Lucignola (pale-rose berry cultivar)																
Year (n = 4)																
2023	1.7 ± 2.1	BDL	2.1 ± 2.1	BDL	BDL	5.6 ± 2.1	0.4 ± 0.5	BDL	BDL	BDL	BDL	BDL	BDL	BDL	9.5 ± 1.4	BDL
2024	0.3 ± 0.1	BDL	3.5 ± 0.1	BDL	BDL	3.5 ± 0.1	0.6 ± 0.1	BDL	8.9 ± 2.3	2.4 ± 0.1	BDL	BDL	12.1 ± 0.2	3.2 ± 0.5	9.4 ± 0.5	BDL
Anonima (red berry cultivar)																
Year (n = 4)																
2023	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	19.2 ± 0.1	BDL	BDL	BDL	BDL	BDL	3.8 ± 0.1	BDL
2024	BDL	BDL	BDL	BDL	BDL	0.4 ± 0.1	BDL	BDL	6.8 ± 0.2	BDL	BDL	0.7 ± 0.1	15.3 ± 0.6	0.4 ± 0.5	4.9 ± 1.5	BDL
Bracau (red berry cultivar)																
Year (n = 4)																
2023	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	4.2 ± 0.1	BDL
2024	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	5.1 ± 0.1	BDL	BDL	BDL	17.3 ± 0.1	1.2 ± 0.2	4.4 ± 0.1	BDL
Catanese Nera (red berry cultivar)																
Year (n = 4)																
2023	BDL	BDL	BDL	BDL	BDL	1.9 ± 1.4	1.8 ± 2.1	BDL	BDL	BDL	BDL	BDL	BDL	BDL	2.4 ± 2.8	BDL
2024	BDL	BDL	BDL	BDL	BDL	0.6 ± 0.1	BDL24/11/25 18:58:00	BDL	5.7 ± 0.7	BDL	BDL	BDL	12.5 ± 0.2	BDL	4.7 ± 0.9	BDL
Vitrarolo (red berry cultivar)																
Year (n = 6)																

2023	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	5.3 ± 4.7	BDL	BDL	BDL	6.1 ± 5.7	4.8 ± 5.2	7.7 ± 3.6	1 ± 2
2024	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	4.3 ± 6.7	BDL	BDL	BDL	8.2 ± 6.9	6.1 ± 3.4	14.3 ± 10.5	BDL
Terroir (n = 4)																
Contessa Entellina	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	4.7 ± 5.7	BDL	BDL	BDL	4.4 ± 1.3	5.5 ± 2.6	14.5 ± 5.2	0.5 ± 1.0
Marsala	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	9.6 ± 4.3	BDL	BDL	BDL	15.0 ± 3.1	9.8 ± 2.3	15.8 ± 9.1	1 ± 2
Sclafani Bagni	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	2.2 ± 1.5	1.0 ± 1.1	2.8 ± 1.7	BDL
Interaction Year:Terroir (n = 2)																
2023:Contessa Entellina	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	9.5 ± 2.8	BDL	BDL	BDL	4.0 ± 2.1	3.5 ± 2.1	10.7 ± 2.8	1 ± 1
2024:Contessa Entellina	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	4.7 ± 0.6	7.5 ± 0.4	18.2 ± 4.2	BDL
2023:Marsala	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	6.5 ± 2.8	BDL	BDL	BDL	12.9 ± 2.8	10.8 ± 2.8	8.6 ± 1.4	2 ± 2
2024:Marsala	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	12.8 ± 2.7	BDL	BDL	BDL	17.0 ± 1.9	8.8 ± 1.9	23.0 ± 6.3	BDL
2023:Sclafani Bagni	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1.5 ± 2.1	BDL	3.8 ± 2.1	BDL
2024:Sclafani Bagni	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	2.9 ± 0.3	1.9 ± 0.1	1.8 ± 0.1	BDL

Note: Data are reported as mean plus and minus standard error of the mean. BDL= below detection limit. The unit of measurement is μg kg $^{-1}$ as 1-heptanol.

Table S2 Free benzenoids content (μg kg^{-1}) of white, rose, and red berry grape varieties across the 2023 and 2024 vintages.

Factor	Methyl salicylate	Benzyl alcohol	2-phenylethanol	Vanillin	Ethyl vanillate	Acetovanillone	Zingerone	Dihydroconiferyl alcohol
Rucignola (white berry cultivar)								
Year (n = 4)								
2023	BDL	51.8 ± 2.8	122.5 ± 2.1	53.9 ± 2.1	BDL	6.6 ± 1.4	BDL	13.9 ± 2.1
2024	BDL	47.3 ± 2.3	112.8 ± 3.2	42.4 ± 3.8	BDL	6.4 ± 0.4	BDL	6.1 ± 0.6
Dunnuni (white berry cultivar)								
Year (n = 4)								
2023	BDL	206.1 ± 2.1	127.0 ± 1.4	120.5 ± 1.4	BDL	37.4 ± 2.1	BDL	12.6 ± 2.1
2024	BDL	169.1 ± 0.8	83.9 ± 0.1	76.5 ± 3.9	BDL	26.8 ± 0.4	BDL	7.0 ± 0.2
Lucignola (pale-rose berry cultivar)								
Year (n = 4)								
2023	BDL	23.2 ± 2.8	77.2 ± 2.8	116.2 ± 2.1	BDL	22.8 ± 2.1	BDL	19.0 ± 2.8
2024	BDL	17.3 ± 0.2	65.4 ± 0.1	94.4 ± 0.7	BDL	18.9 ± 0.1	BDL	8.0 ± 0.2
Anonima (red berry cultivar)								
Year (n = 4)								
2023	BDL	22.4 ± 0.3	64.6 ± 0.1	70.9 ± 0.3	BDL	5.1 ± 0.1	BDL	10.9 ± 0.1
2024	BDL 1	18.3 ± 0.4	50.0 ± 0.2	56.5 ± 1.3	BDL	7.9 ± 0.7	BDL	4.6 ± 0.2
Bracau (red berry cultivar)								
Year (n = 4)								
2023	BDL	30.2 ± 0.2	42.3 ± 0.2	52.0 ± 0.1	1.2 ± 0.1	8.3 ± 0.1	BDL	12.8 ± 0.1
2024	BDL	25.2 ± 0.7	45.1 ± 8.4	48.9 ± 0.1	2.0 ± 0.4	11.6 ± 0.1	BDL	9.1 ± 0.1
Catanese Nera (red berry cultivar)								
Year (n = 4)								
2023	BDL	23.9 ± 2.1	51.7 ± 2.8	42.7 ± 2.8	BDL	10.1 ± 1.4	BDL	5.3 ± 2.1
2024	BDL	21.2 ± 0.4	38.4 ± 0.8	31.7 ± 0.3	BDL	7.5 ± 0.5	BDL	3.0 ± 0.3
Vitrarolo (red berry cultivar)								
Year (n = 6)								
2023	1.6 ± 2.2	33.2 ± 18.8	127.6 ± 48.5	33.2 ± 20.2	BDL	4.6 ± 3.0	BDL	8.2 ± 3.5

2024	0.2 ± 0.2	34.5 ± 16.3	123.9 ± 35.7	43.8 ± 32.7	BDL	7.7 ± 3.2	BDL	7.4 ± 1.0
Terroir (n = 4)								
Contessa Entellina	BDL	25.3 ± 2.6	37.3 ± 0.9	24.0 ± 3.9	BDL	6.0 ± 1.7	BDL	8.8 ± 1.0
Marsala	1.4 ± 2.0	20.2 ± 5.1	30.8 ± 5.6	72.5 ± 15.4	BDL	8.9 ± 3.8	BDL	5.7 ± 2.2
Sclafani Bagni	1.2 ± 2.0	56.0 ± 1.4	309.1 ± 11.6	19.1 ± 1.1	BDL	3.6 ± 2.5	BDL	8.9 ± 3.0
Interaction Year:Terroir (n = 2)								
2023:Contessa Entellina	BDL	27.0 ± 2.8	37.4 ± 1.4	20.7 ± 1.4	BDL	5.8 ± 2.8	BDL	9.1 ± 1.4
2024:Contessa Entellina	BDL	23.5 ± 0.2	37.2 ± 0.4	27.2 ± 0.7	BDL	6.1 ± 0.5	BDL	8.5 ± 0.7
2023:Marsala	2.5 ± 2.8	16.1 ± 2.8	26.1 ± 2.8	59.2 ± 2.8	BDL	6.0 ± 2.8	BDL	4.2 ± 2.1
2024:Marsala	0.4 ± 0.1	24.4 ± 0.1	35.4 ± 0.7	85.7 ± 0.7	BDL	11.9 ± 0.2	BDL	7.3 ± 0.4
2023:Sclafani Bagni	2.3 ± 2.8	56.5 ± 2.1	319.1 ± 2.1	19.7 ± 1.4	BDL	1.9 ± 2.7	BDL	11.4 ± 1.4
2024:Sclafani Bagni	0.2 ± 0.1	55.4 ± 0.2	299.2 ± 0.1	18.5 ± 0.2	BDL	5.2 ± 0.2	BDL	6.5 ± 0.5

Note: Data are reported as mean plus and minus standard error of the mean. BDL= below detection limit. The unit of measurement is µg kg^{-1} as 1-heptanol.

Table S3 Free norisoprenoids content (μg kg^{-1}) of white, rose, and red berry grape varieties across the 2023 and 2024 vintages.

Factor	3,4-dihydro-3-oxoactinidol I	3,4-dihydro-3-oxoactinidol II	3,4-dihydro-3-oxoactinidol III	3,4-dihydro-3-oxoactinidol IV	3-hydroxy-β-damascone	3-oxo-α-ionol	4-oxo-β-ionol	Blumenol C	3-hydroxy-7,8-dihydro-β-ionol	Vomifoliol
Rucignola (white berry cultivar)										
Year (n = 4)										
2023	BDL	BDL	BDL	4.7 ± 1.4	BDL	11.3 ± 2.1	BDL	BDL	BDL	BDL
2024	BDL	BDL	BDL	3.5 ± 0.3	BDL	1.7 ± 0.1	BDL	BDL	BDL	BDL
Dunnuni (white berry cultivar)										
Year (n = 4)										
2023	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	10.2 ± 2.1
2024	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	7.2 ± 0.2
Lucignola (pale-rose berry cultivar)										
Year (n = 4)										
2023	BDL	BDL	BDL	BDL	BDL	14.8 ± 1.4	BDL	BDL	BDL	BDL
2024	BDL	BDL	BDL	BDL	BDL	3.6 ± 0.1	BDL	BDL	BDL	BDL
Anonima (red berry cultivar)										
Year (n = 4)										
2023	BDL	BDL	BDL	BDL	BDL	8.3 ± 0.1	BDL	BDL	BDL	BDL
2024	BDL	BDL	BDL	BDL	BDL	1.9 ± 0.1	BDL	BDL	BDL	BDL
Bracau (red berry cultivar)										
Year (n = 4)										
2023	BDL	BDL	BDL	BDL	BDL	11.7 ± 0.1	BDL	BDL	BDL	BDL
2024	BDL	BDL	BDL	BDL	BDL	1.1 ± 0.3	BDL	BDL	BDL	BDL
Catanese Nera (red berry cultivar)										
Year (n = 4)										
2023	BDL	BDL	BDL	BDL	BDL	12.8 ± 2.1	BDL	BDL	BDL	BDL
2024	BDL	BDL	BDL	BDL	BDL	0.7 ± 1.0	BDL	BDL	BDL	BDL
Vitrarolo (red berry cultivar)										
Year (n = 6)										
2023	BDL	BDL	BDL	BDL	BDL	19.3 ± 11.6	BDL	BDL	BDL	16.9 ± 5.0
2024	BDL	BDL	BDL	BDL	BDL	9.1 ± 4.9	BDL	BDL	BDL	20.0 ± 3.8

Terroir (n = 4)										
Contessa Entellina	BDL	BDL	BDL	BDL	BDL	9.9 ± 1.8	BDL	BDL	BDL	20.8 ± 4.6
Marsala	BDL	BDL	BDL	BDL	BDL	8.9 ± 6.5	BDL	BDL	BDL	13.8 ± 2.8
Sclafani Bagni	BDL	BDL	BDL	BDL	BDL	23.7 ± 11.7	BDL	BDL	BDL	20.8 ± 1.7
Interaction Year:Terroir (n = 2)										
2023:Contessa Entellina	BDL	BDL	BDL	BDL	BDL	9.6 ± 2.8	BDL	BDL	BDL	17.1 ± 2.8
2024:Contessa Entellina	BDL	BDL	BDL	BDL	BDL	10.2 ± 1.1	BDL	BDL	BDL	24.5 ± 0.1
2023:Marsala	BDL	BDL	BDL	BDL	BDL	14.4 ± 2.1	BDL	BDL	BDL	11.7 ± 2.1
2024:Marsala	BDL	BDL	BDL	BDL	BDL	3.4 ± 0.3	BDL	BDL	BDL	16.0 ± 0.3
2023:Sclafani Bagni	BDL	BDL	BDL	BDL	BDL	33.8 ± 1.4	BDL	BDL	BDL	22.1 ± 1.4
2024:Sclafani Bagni	BDL	BDL	BDL 1	BDL	BDL	13.7 ± 2.9	BDL	BDL	BDL	19.6 ± 0.1

Note: Data are reported as mean plus and minus standard error of the mean. BDL= below detection limit. The unit of measurement is µg kg^{-1} as 1-heptanol.

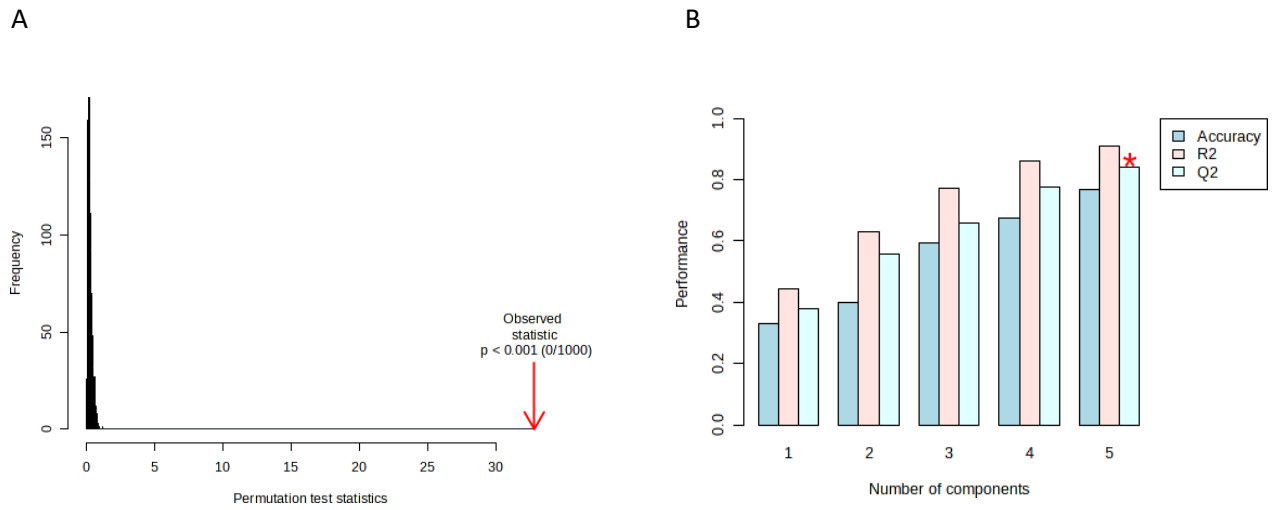


Figure S1 Permutation test (A) and cross validation (B) (n = 1000) for the PLS-DA analysis carried out on red minor Sicilian grapes composition in vintage 2023.

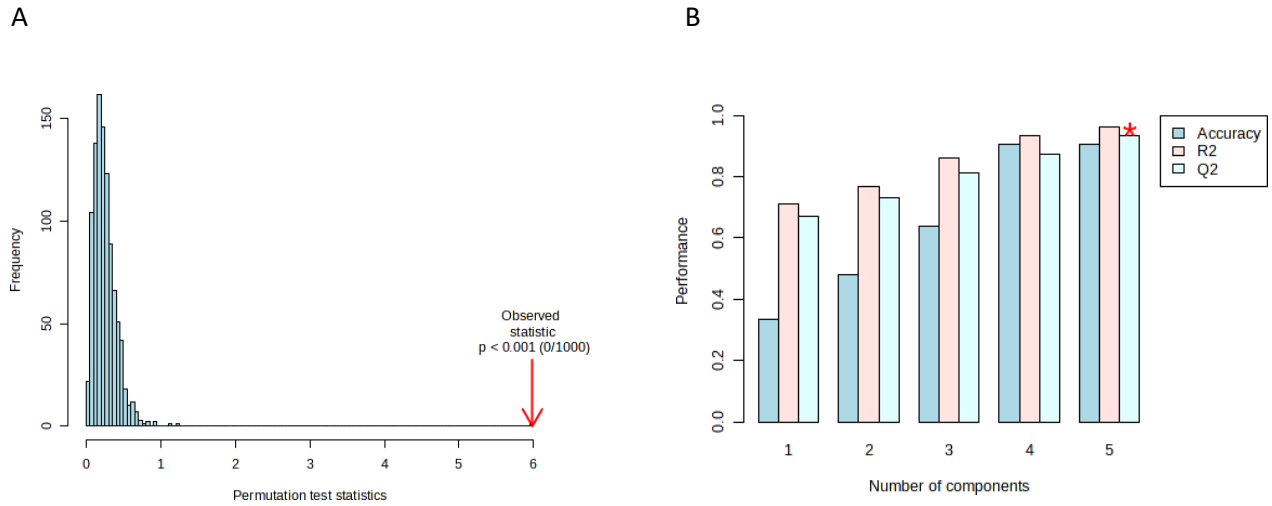


Figure S2 Permutation test (A) and cross validation (B) (n = 1000) for the PLS-DA analysis carried out on red minor Sicilian grapes composition in vintage 2024.

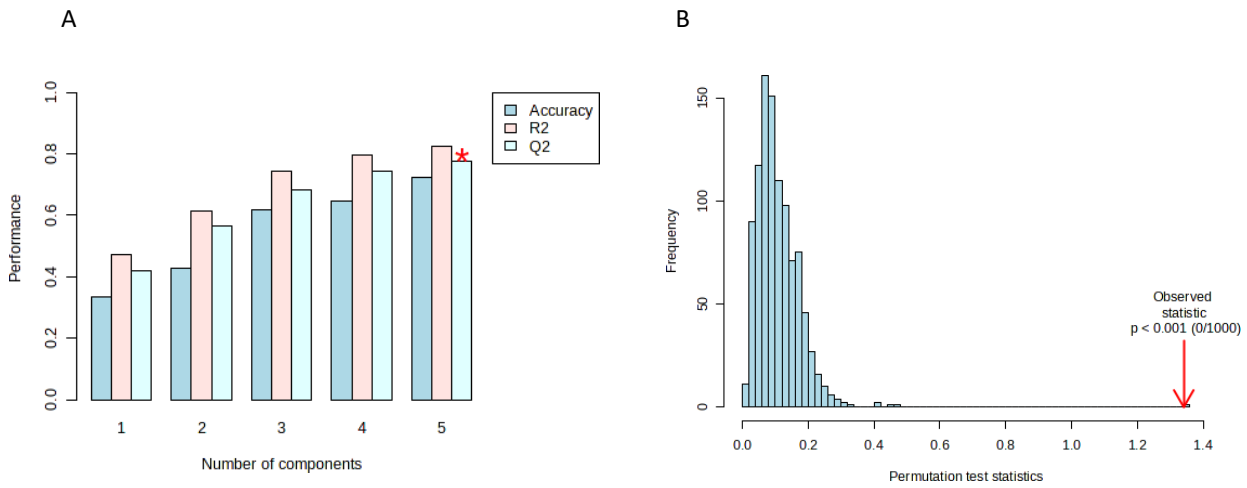


Figure S3 Cross validation (A) and permutation test (B) (n = 1000) for the PLS-DA analysis carried out on red minor Sicilian grapes composition in 2023 and 2024 vintage.

CHAPTER 5: Sensory characterization of wines from Sicilian ancient grape varieties

Introduction

Climate change accelerates grape ripening, raising sugar levels and resulting in higher alcohol wines, which threaten balance, freshness, and consumer preference (Palliotti et al., 2014). Many heritage cultivars, particularly those from hot or arid regions such as Sicily in Italy, naturally produce lower-sugar musts or maintain higher acidity, making them suited to counteract alcohol production under global warming conditions. Lower-alcohol wines made from ancient varieties offer a compelling alternative to conventional high-alcohol wines, aligning with evolving consumer preferences for healthier lifestyles and addressing the challenges posed by climate change on traditional viticulture (Sekhon and Sun, 2024). This approach also allows for the preservation of distinctive regional characteristics, offering a wider spectrum of flavors and aromas that can be diluted or lost in wines subjected to excessive ripeness to achieve higher alcohol levels (Gomis-Bellmunt et al., 2022). Furthermore, the production of low-alcohol wines from ancient varieties presents a sustainable viticultural strategy, mitigating the environmental impact associated with high-alcohol wine production while simultaneously expanding the stylistic diversity of wine offerings to the global market (Sekhon & Sun, 2024). Lower-alcohol wines made from ancient varieties can maintain aromatic freshness, phenolic complexity, and terroir expression, which are often lost in high-alcohol wines produced under climate stress (Kumar et al., 2024). This is particularly relevant given the increasing global temperatures, which often lead to grapes with higher sugar concentrations and, consequently, elevated ethanol levels in the final wine (Caldeira et al., 2025). This often necessitates premature harvesting to control alcohol content, which can compromise the full development of phenolic maturity and aromatic complexity essential for typicity and quality (Cuenca-Martínez et al., 2025). Conversely, ancient grape varieties, often characterized by naturally lower sugar accumulation

and higher acidity, present a promising avenue for producing wines with balanced alcohol levels while preserving their intrinsic sensory attributes and regional identity (Martínez-Moreno et al., 2023) (Puig-Pujol et al., 2023). This research aims to investigate the sensory attributes of wines produced from ancient Sicilian grape varieties, thereby contributing to a deeper understanding of their organoleptic profiles and commercial potentiality. The study focused on sensory characteristics of 10 unique wines obtained from ancient grapes not registered in the official Denominazione di Origine Controllata (DOC) framework in two consecutive vintages. These non-commercial wines reflect native grape biodiversity that may not conform to formal regulatory classifications yet hold significant cultural and enological value. This work employed a methodology of sensory analysis to characterize these wines by consumers called Check-All-That-Apply (CATA) for evaluating consumer perceptions and liking drivers, providing an overall understanding of these unique Sicilian wines. The study of wine sensory characteristics coupled with adapted viticultural practices, contribute to wines that not only align with consumer demand for moderation but also offer a unique expression of their specific origin.

Methodology

Sensorial analysis

Ten wines obtained from Nave (R1), Rucignola (R2), Dunnuni (R3), Lucignola (R4), Anonima (R5), Catanese nera (R6), Bracao (R7), Vitrarolo [sb (R8), mar (R9), ce (R10)] grape varieties in two consecutive vintages (2023 and 2024) were used for this study. The sensory analysis was carried with sixty not-trained assessors, which regularly drink wine, recruited by an on-line form by Better Sensing company (Portugal). Two separate sessions were organized for 2023 and 2024 vintages, including the tasting of 10 wines by 26 and 34 consumers, respectively. Twenty mL of wine was presented in ISO-glass identified with a three-digit-number. The samples were randomized across sessions according to a Williams' Latin square design, balanced for order and first-order carry-over effects.

Consumers were encouraged to drink still mineral water between each tasting and eat breadsticks, if necessary. Firstly, consumers were asked to fill out a socio-demographic questionnaire and answer some questions aimed at determining their attitude towards the lower alcohol wines. Then, they evaluated the sensory characteristics using the CATA questionnaire with different attributes for wines (Fruity intensity, Red fruit, Dried fruit, Vegetal/Herbaceous, Spicy, Off-Flavor, Acid, Salty, Complex, Taste persistence, Bitter, Astringent, Sweet, Floral, Aromatic herbs, Persistent flavor, Citrus aroma). The liking of color, smell, and flavor, and overall liking were evaluated on a 9-point hedonic scale (1 = 'dislike extremely', 9 = 'like extremely'). The protocol used for data collection complied with national ethical requirements and was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. In particular, all subjects provided their informed consent to participate in the study, and all data were collected anonymously.

Results

Demographic data and consumer perception

Table 1 and Table 2 represent the demographic data and the perception of lower alcohol wines of 60 consumers participating in the survey on ancient Sicilian wines from 2023 and 2024 vintages.

Table 1: socio-demographic data of consumers.

Question	Categories	%
Which category below includes your age?	18-20	5
	21-29	18
	30-39	22
	40-49	37
	50-59	8
	60 o +	10
What is your gender?	female	55
	male	45

What is your marital status?	Divorced	5
	Married	43
	Never married	47
	Widowed	5
What is the highest level of school you have completed or the highest degree you have received?	Associate degree	3
	Bachelor degree	20
	Graduate degree	33
	High school degree or equivalent	13
	PhD	22
	Some college but no degree	8
What is your nationality?	Portuguese	45
	Brazilian	23
	Italian	10
	American	3
	English	3
	French	3
	Polish	3
	South Africa	2
	Greece	2
	Nepali	2
	UK	2
	Swiss	2
What is your current employment status?	Full-time employment	53
	Home-maker	2
	Part-time employment	5
	Retired	10
	Self-employed	12
	Student	12
	Unemployed	7
How often do you drink wine?	2–3 times a month	7

	2–3 times a week	35
	4-5 times a week	15
	Daily	7
	Once a week	22
	Rarely	15
What type(s) of wine do you usually consume?	Red	73
	White	73
	Rosé	27
	Sparkling	32
	Sweet or Fortified	18

Table 2: socio-demographic data and wine attitude.

Question	Categories	%
Which category below includes your age?	18-20	5
	21-29	18
	30-39	22
	40-49	37
	50-59	8
	60 o +	10
What is your gender?	Female	55
	Male	45
What is your marital status?	Divorced	5
	Married	43
	Never married	47
	Widowed	5
What is the highest level of school you have completed or the highest degree you have received?	Associate degree	3
	Bachelor degree	20
	Graduate degree	33
	High school degree or equivalent	13
	PhD	22

	Some college but no degree	8
What is your nationality?	Portuguese	45
	Brazilian	23
	Italian	10
	American	3
	English	3
	French	3
	Polish	3
	South African	2
	Grecian	2
	Asian	2
	British	2
	Swiss	2
What is your current employment status?	Full-time employment	53
	Home-maker	2
	Part-time employment	5
	Retired	10
	Self-employed	12
	Student	12
	Unemployed	7
How often do you drink wine?	2–3 times a month	7
	2–3 times a week	35
	4-5 times a week	15
	Daily	7
	Once a week	22
	Rarely	15
What type(s) of wine do you usually consume?	Red	73
	White	73
	Rosé	27
	Sparkling	32
	Sweet or Fortified	18

Respondents were almost equally represented by gender (female by 55%), with an age comprised between 40 and 49 years old (37%), never married (47%), with a good level of education (Graduate degree 33%), mainly of Portuguese (45%), Brazilian (23%), and Italian (10%) nationality, full-time employed, which drink wine 2-3 times a week (35%). The red and white wines were equally preferred (73%) by this panel, with a peculiar interest in sparkling wines (32%), while rosé (27%) and sweet or fortified wines (18%) were less preferred. In Figure 1, the price represented the most important attribute for choosing wine (60%).

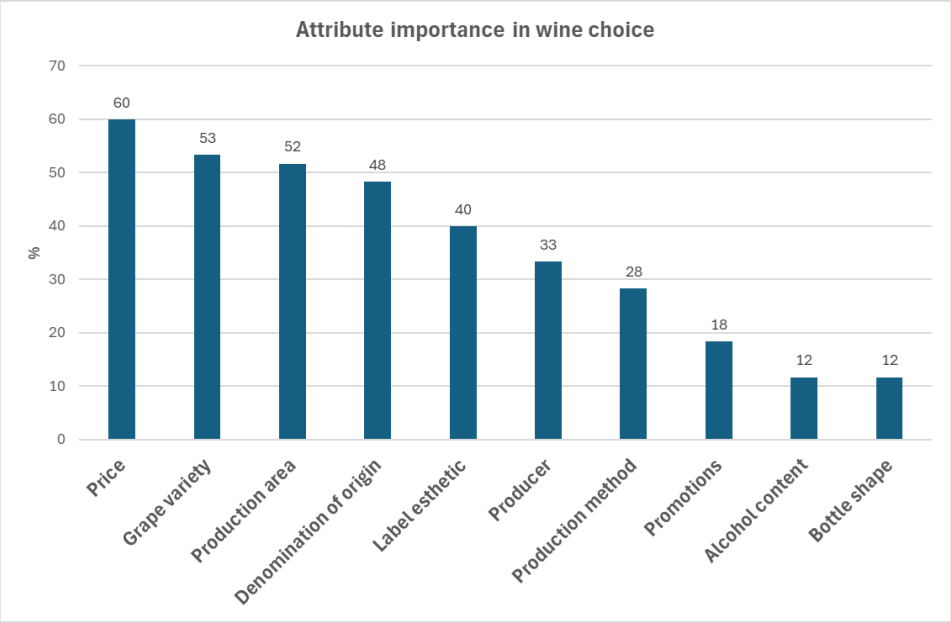


Figure 2: attributes for wine choice by consumers.

However, consumer choices were also influenced by grape variety (53%), production area (52%), and denomination of origin (48%). The alcohol content represented only 12% of the choice for purchasing wine. Among others, consumers preferences for wine attributes such as harvest type, organic certification, geographical indications, and price significantly influence their purchasing decisions (Dominici et al., 2019).

Table 3: perception of lower alcohol wines.

Question	Categories	Frequencies
What is your preferred alcohol content in wine?	11–13%	25
	13–15%	17
	9–11%	3
	Below 9%	1
	No preference	14
Have you ever tried a lower alcohol wine (below 11% ABV)?	No	7
	Not sure	12
	Yes	41
If yes, how would you describe your experience?	Neutral	17
	Somewhat negative	3
	Somewhat positive	13
	Very positive	14

The perception and preference on alcohol level are shown in Table 3. The favorite percentage of alcohol is between 11 and 13% (v/V) by 25% of respondents, however 14% has no specific preferences. The 41% of panel had previously tried lower alcohol wine being the experience neutral (17%), but in general positive (27% being the sum of somewhat and very positive answers). This indicates a significant segment of consumers open to exploring wines with reduced alcohol content, aligning with evolving market trends towards lighter and healthier beverage options (Ghvanidze et al., 2019). However, in this study the health reasons represented only 20% of choices (Figure 2).

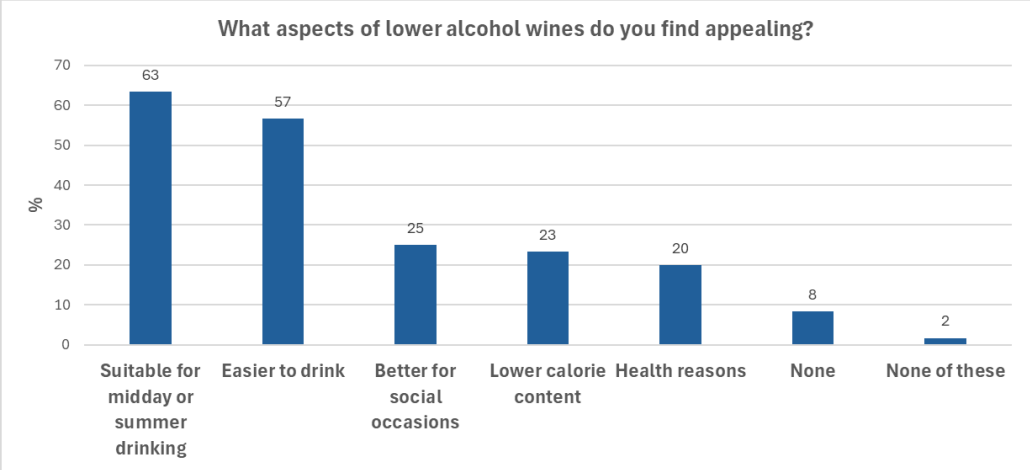


Figure 3: appealing features for drinking lower alcohol wines.

The appealing aspects of lower alcohol wines were more related to easiness of drinking (57%) and suitability in summertime (63%). The overall positive reception of lower alcohol wines suggests a market opportunity, as consumers increasingly seek diverse options that balance enjoyment with mindful consumption (García-Martín et al., 2011). While the alcohol content was not a primary determinant for purchasing decisions, the notable acceptance of lower alcohol wines among a significant portion of respondents highlights an emerging consumer segment that values attributes such as ease of consumption and seasonal appropriateness over strict adherence to traditional alcohol levels (Afonso et al., 2025).

Sensory characteristics

Table 4 shows the total citation frequency (%) of the sensory attributes characterizing the Sicilian ancient wines of 2023 and 2024 vintages by consumers using the CATA questionnaire.

Table 4: total citation frequency (%) of the sensory attributes of Sicilian ancient wines of 2023 and 2024 vintages by consumers using the CATA questionnaire.

Attributes	2023	2024
Acid	**59%**	**54%**
Aromatic herbs	38%	26%
Astringent	**54%**	42%
Bitter	32%	41%
Citrus aroma	25%	22%
Complex	43%	36%
Dried fruit	42%	31%
Floral	36%	37%
Fruity intensity	**62%**	**50%**
Off-Flavour	23%	18%
Persistent flavour	**54%**	**54%**
Red fruit	**55%**	45%
Salty	24%	28%
Spicy	36%	30%
Sweet	20%	19%
Taste persistence	**57%**	**63%**
Vegetal/Herbaceous	43%	34%

The most cited attributes in 2023 and 2024 wines were fruity intensity (63% and 50%, respectively), persistent flavor (54%), and acid (59% and 54%, respectively). The 2023 vintage exhibited a higher citation frequency across several attributes, including aromatic herbs, astringency, complex, dried fruit, fruity intensity, red fruit, and vegetal/herbaceous notes. Conversely, the 2024 vintage showed an increased citation for bitter together with a more pronounced taste persistence, but less complexity.

Table 5: the significance (p-value) of attributes characterizing the 2023 and 2024 wines.

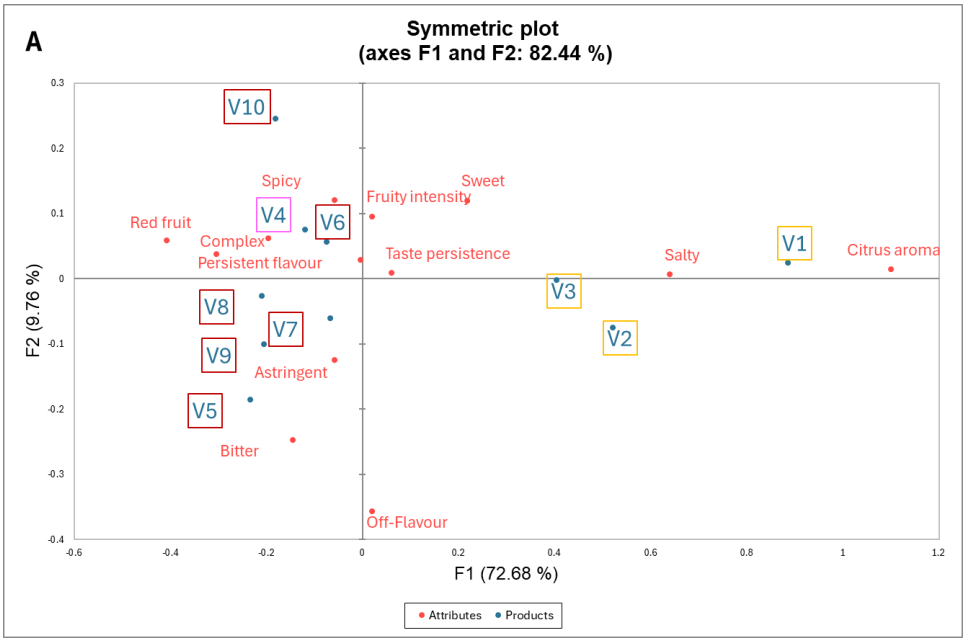
Attributes	2023	2024
	p-values	
Acid	0.186	0.000
Aromatic herbs	0.452	0.054
Astringent	0.001	0.001
Bitter	0.001	0.406
Citrus aroma	<0.0001	<0.0001
Complex	<0.0001	0.009
Dried fruit	0.188	0.218
Floral	0.546	0.020
Fruity intensity	<0.0001	0.313
Off-Flavour	0.041	<0.0001
persistent flavour	0.010	0.027
Red fruit	<0.0001	<0.0001
Salty	0.042	0.040
Spicy	0.028	0.001
Sweet	0.092	0.050
Taste persistence	0.053	0.005
Vegetal/Herbaceous	0.459	0.237

In table 5, the most significant attributes ($p<0.05$) that differentiate between 2023 and 2024 wines were citrus aroma and red fruity. Meanwhile, the descriptors such as taste persistence, floral odor, and acidity were significant only in 2024 wines, indicating these were more characteristic of this vintage. The cultivars considered in this study and their respective growing environments are shown in Table 6.

Table 6 Code names, cultivars, and growing environments of the grapevine samples analyzed

Code name	Cultivar	Terroir
V1	Nave	Marsala
V2	Rucignola	Marsala
V3	Dunnuni	Marsala
V4	Lucignola	Marsala
V5	Anonima	Marsala
V6	Catanese nera	Marsala
V7	Bracao	Marsala
V8	Vitrarolo SB	Sclafani Bagni
V9	Vitrarolo MAR	Marsala
V10	Vitrarolo CE	Contessa Entellina

The sensory characteristics of ancient Sicilian wines were compared according to 2023 and 2024 vintage by Correspondence Analysis in Figure 3A and 4B, respectively.



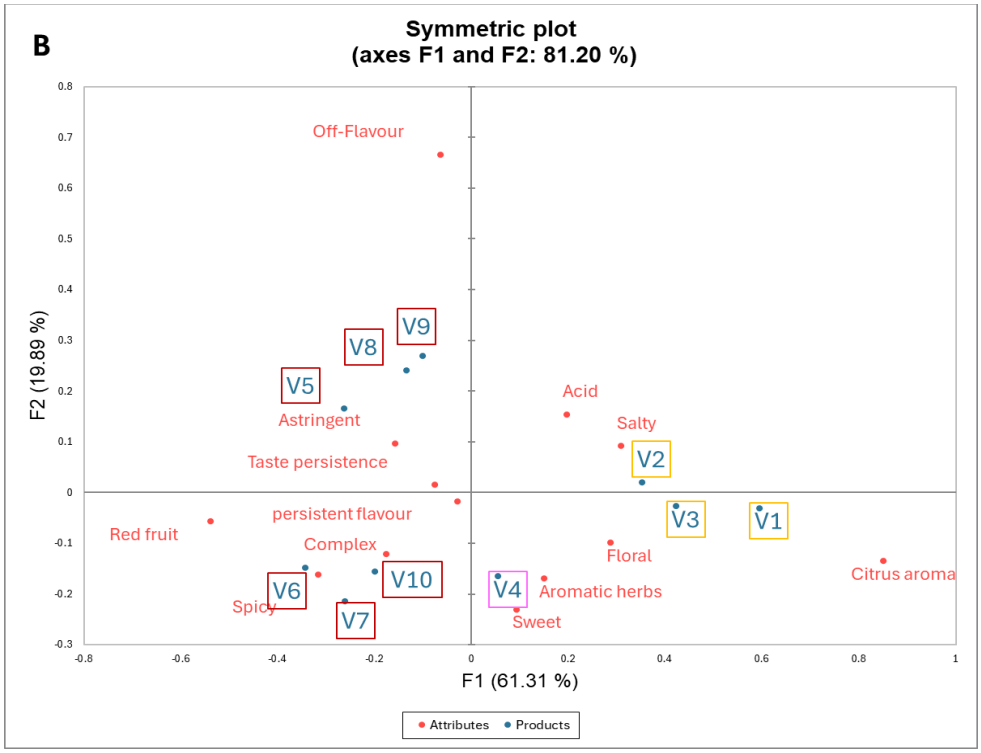


Figure 4: Correspondence Analysis of 2023 (A) and 2024 (B) wines. V1 = Nave, V2 = Rucignola, V3 = Dunnuni, V4 = Lucignola, V5 = Anonima, V6 = Catanese Nera, V7 = Bracau, V8 = Vitrarolo SB (Sclafani Bagni), V9 = Vitrarolo MAR (Marsala), V10 = Vitrarolo CE (Contessa Entellina)

In figure 3A, 2023 wines clearly separated on the symmetric plots according to 82.44% of total inertia in the two dimensions. The main separation was between the white wines (V1, V2, V3), characterized by citrus aroma and saltiness, from the others. The V4 (Lucignola), V6 (Catanese Nera), and V10 (Vitrarolo CE) were more complex and persistent in flavor, with red fruity and spicy nuances. The V5, V7 and V9 were the more bitter and astringent. In figure 4B, 2024 separated on the symmetric plots according to the 81.20% of total inertia in the two dimensions. Also in this case, the white wines (V1, V2, V3) characterized by citrus aroma were separated from the red ones (V6, V7, V10), which in turn were associated with red fruity, spicy and complex sensations. Another cluster was represented by V5, V8, and V9 red wines which were particularly astringent. The 2024 rosé wine (V4), differently than 2023 vintage, differentiated from red wines, being more floral, citric and sweet.

Consumers Liking

The liking of wines for the color, smell and flavor/taste is shown in Figure 4 for 2023 (A) and 2024 (B) vintages. The 2024 wines generally received higher scores for color than 2023 wines across all wines. For example, wines V6 and V10 in 2024 scored around 6.5, noticeably higher than in 2023. V9 was rated low in both years, but especially in 2024. The 2023 wines had slightly more consistent smell than 2024 ones. The 2023 wines had relatively high and consistent flavor/taste ratings, especially for V8 and V10, both around 6. In 2024, taste ratings were lower overall, particularly in V4, V5, and V9, which scored below 4.

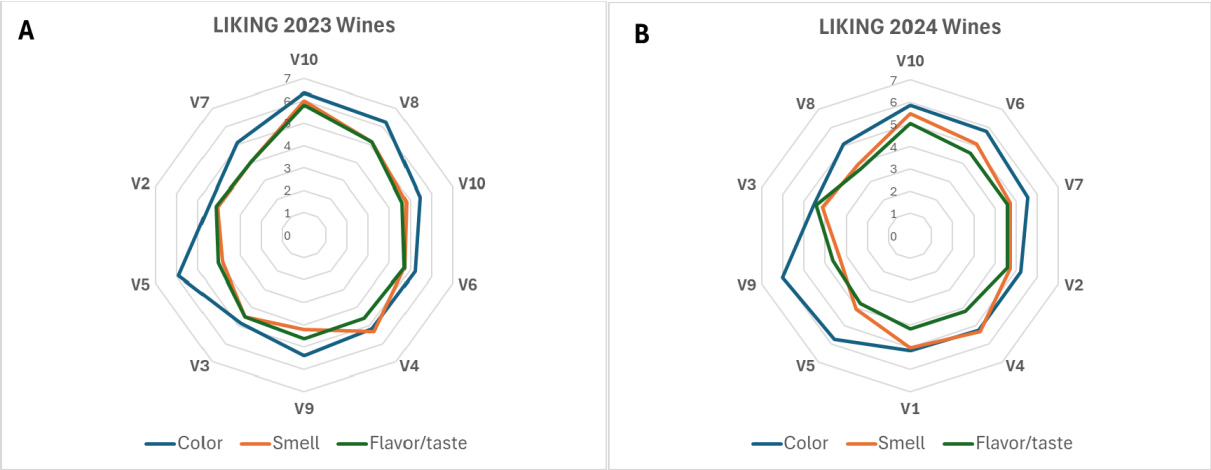


Figure 4: The consumer liking for color, smell and flavor/taste of 2023 (A) and 2024 (B) ancient Sicilian wines. V1 = Nave, V2 = Rucignola, V3 = Dunnuni, V4 = Lucignola, V5 = Anonima, V6 = Catanese Nera, V7 = Bracau, V8 = Vitrarolo SB (Sclafani Bagni), V9 = Vitrarolo MAR (Marsala), V10 = Vitrarolo CE (Contessa Entellina).

The overall liking was evaluated by consumers using hedonic rating was shown in Figure 5.

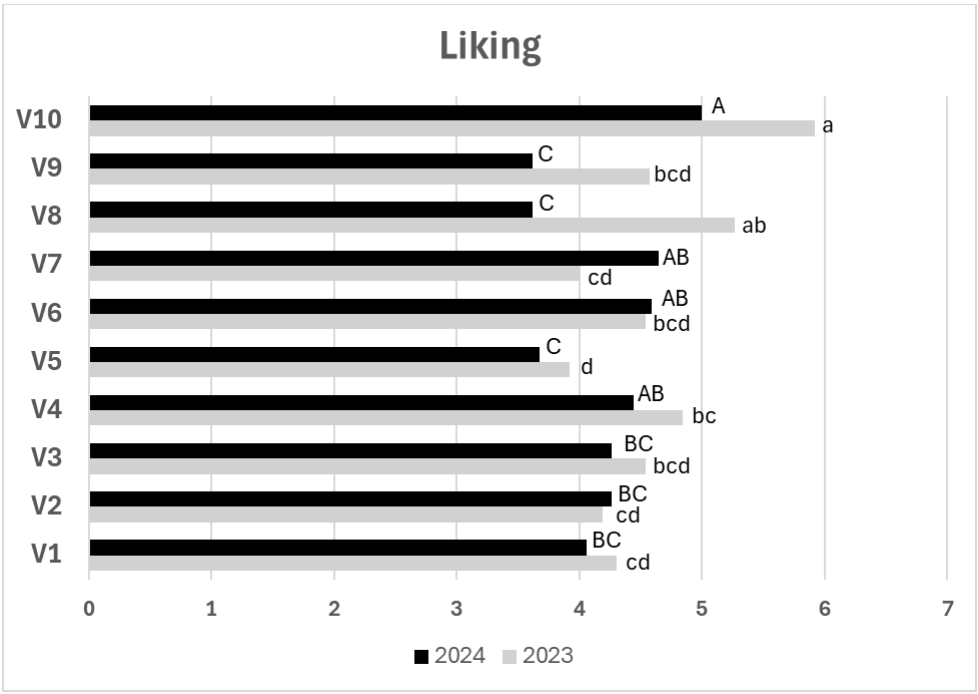


Figure 5: the consumer liking for 2023 and 2024 wines. Different letters mean statistically significant differences across wines of the same vintages (Fisher LSD test with $p<0.05$) V1 = Nave, V2 = Rucignola, V3 = Dunnuni, V4 = Lucignola, V5 = Anonima, V6 = Catanese Nera, V7 = Bracau, V8 = Vitrarolo SB (Sclafani Bagni), V9 = Vitrarolo MAR (Marsala), V10 = Vitrarolo CE (Contessa Entellina)

The most preferred wine among the two consecutive vintages was the V10 (Vitrarolo ce), while the white wines were generally less preferred. The attributes mainly associated with the consumers preferences were fruity intensity, complexity, red fruits, persistent flavor, sweetness, and taste persistence for 2023 wines, while complexity, floral, aromatic herbs, persistent flavor, taste persistence, red fruits and spicy for 2024 wines, as shown in Figure 6A and 6B.

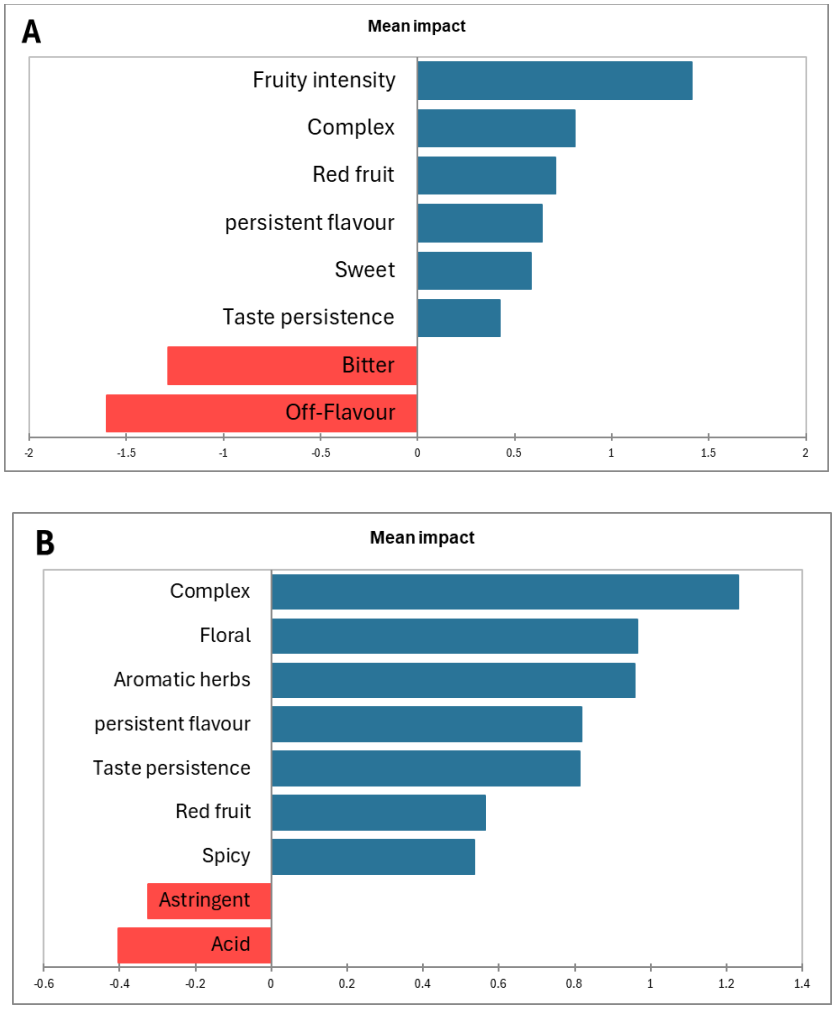


Figure 6: the mean impact of the attributes of 2023 (A) and 2024 (B) wines associated with consumer liking.

The common drivers of liking were complexity, persistent flavor and taste persistence, indicating that the mouthfeel had a great impact on consumer preferences more than aroma and odor characteristics in the wines of two consecutive vintages. The negative drivers of liking were bitter and off-flavors for 2023 wines, and astringent and acid for 2024 ones.

According to these results, there are great potentialities for ancient Sicilian wines obtained by Vitrarolo grape variety (SB and CE) to be introduced in the market.

Conclusion

The contemporary wine industry is navigating a dual challenge: meeting the growing consumer demand for low-alcohol options and preserving the rich biodiversity and heritage of ancient grape varieties. Instead of solely relying on dealcoholization of conventional wines, which can strip away desirable characteristics (Vitaggio et al., 2025), selecting and cultivating ancient varieties with naturally lower sugar accumulation or unique phenolic and aromatic compounds could lead to inherently balanced low-alcohol wines. This study demonstrates that the wines obtained from Sicilian ancient grape varieties had appealing sensory characteristics in terms of aroma, odor, taste and complexity, making some of them suitable for the new market demand. Among these, *Vitrarolo*, *Lucignola* and *Nave* wines proved to possess great potential for market introduction and valorization. In addition, this wine offers a dual benefit under climate change: naturally lowering alcohol content while enhancing resilience, biodiversity, and market sustainability. By understanding the specific sensory attributes that appeal to consumers, producers can effectively enhance the quality and market appeal of these distinctive wines. The emphasis on mouthfeel in consumer preferences suggests that efforts to refine texture and persistence will be key to maximizing consumer liking.

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CHAPTER 6: Conclusion and future perspectives

This doctoral research provided an integrated evaluation of the oenological potential of traditional and ancient Sicilian grape cultivars by combining viticultural, technological, and biodiversity-oriented approaches. The studies demonstrated how the interaction between soil properties, cultivar genotype, and oenological practices determines the overall quality, typicity, and resilience of wines under Mediterranean climatic conditions. The first investigation highlighted the strong influence of calcareous soil texture on grape ripening behavior and composition. Marlstone soils promoted greater homogeneity of ripening, as reflected by lower Technological and Phenolic Variability Indices (TVI and PVI), while autochthonous cultivars such as *Nero d'Avola* and *Grillo* showed enhanced adaptability and stability compared to international varieties. These findings underscore the importance of soil–cultivar matching to optimize grape quality and ensure resilience against climatic variability. The second study, focused on *Catarratto*, revealed that targeted oenological interventions—such as pre-fermentative cold soaking and enzymatic treatments—can effectively modulate the sensory and chemical profile of wines produced from early-harvested grapes. In particular, β-glucanase application enhanced yeast polysaccharide release, improving mouthfeel and reducing bitterness associated with unripe sensory notes. This provides winemakers with practical strategies to manage premature harvesting conditions increasingly common under climate change scenarios. The third study broadened the understanding of Sicily's ampelographic heritage by characterizing several minor and ancient cultivars. These varieties exhibited unique metabolic profiles, high phenolic diversity, and notable adaptability to water stress and high temperatures, confirming their potential as both cultural assets and adaptive genetic resources for sustainable viticulture. Overall, the results of this thesis demonstrate that the integration of quantitative tools for ripening assessment, innovative winemaking practices, and the valorization of local germplasm can collectively enhance wine quality while promoting environmental sustainability. The adoption of such

interdisciplinary approaches strengthens the role of native Sicilian cultivars as key components of climate-resilient viticulture and as a model for other Mediterranean regions. Future research should aim to extend these findings by testing a broader range of indigenous varieties, soils, and climatic conditions, and by linking grape compositional traits to the sensory and volatile profiles of the resulting wines. This holistic understanding will be crucial to preserve viticultural biodiversity while driving innovation toward sustainable, terroir-expressive, and climate-adaptive wine production.

The sensory analysis provided crucial insights into the consumer acceptance and market viability of wines derived from these ancient Sicilian varieties. Wines produced from Sicilian ancient grape varieties demonstrated appealing sensory characteristics concerning aroma, odor, taste, and complexity. *Vitrarolo*, *Lucignola* and *Nave* wines specifically proved to possess great potential for market introduction and valorization. The study confirmed that this utilization offers a dual benefit under climate change: naturally lowering alcohol content while enhancing resilience, biodiversity, and market sustainability. The common drivers of consumer liking across two consecutive vintages (2023 and 2024) were complexity, persistent flavor, and taste persistence. This indicated that mouthfeel attributes had a greater impact on consumer preferences than aroma and odor characteristics. Conversely, negative drivers of liking included bitter and off-flavors for 2023 wines, and astringent and acid notes for 2024 wines. The emphasis on mouthfeel suggests that producers should focus efforts on refining the texture and persistence of these distinctive wines to maximize consumer liking and market appeal. Future research should extend the application of the developed TVI and PVI indices to a wider array of cultivars and regions to further assess the complex role of soil type in ripening variability. Furthermore, the potential of PCS and enzyme techniques should be investigated in other *Vitis vinifera* L. varieties, including red grapes, to fully evaluate their effects on phenolic compounds and unripe sensory perceptions during fermentative maceration. Lastly, detailed investigations into the quantitative and qualitative characteristics of tannins in ancient cultivars—such as the determination of their mean degree of polymerization—are recommended to inform

specialized varietal vinification protocols.

Other publications

In the following section are listed publications related to collaborations to other research projects occurred during the PhD course:

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*Oral presentation

Acknowledgments

A conclusione di questo lavoro di tesi desidero esprimere i miei più sentiti ringraziamenti ai miei tutors, prof. Onofrio Corona e dott. Matteo Pollon, per avermi sempre incoraggiata, spronata e per la fiducia che hanno riposto in me lungo tutto il percorso.

La loro guida e i loro preziosi insegnamenti mi hanno permesso di crescere non solo dal punto di vista professionale, ma anche personale.

Un sentito ringraziamento va inoltre al prof. Luciano Cinquanta, che, al pari dei tutors, è sempre stato presente e disponibile durante l'intero percorso di dottorato, offrendo supporto, competenza e costante attenzione.

Grazie a Valentina e Manuel per il prezioso sostegno, la collaborazione e il contributo dato al lavoro di questi anni.

Un ringraziamento speciale va alla mia famiglia, per la costante presenza, l'incoraggiamento e la fiducia che mi hanno accompagnata in ogni tappa di questo cammino.