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New Sparkling Wines from Traditional Grape Varieties and Native Yeasts: Focusing on Wine Identity to Address the Industry's Crisis

Teodora Basile *, Giambattista Debiase, Francesco Mazzone, Leonardo Scarano, Antonio Domenico Marsico and Maria Francesca Cardone

CREA Research Centre for Viticulture and Enology, Via Casamassima 148, 70010 Turi, Italy; giambattista.debiase@crea.gov.it (G.D.); francesco.mazzone@crea.gov.it (F.M.); leonarscara@gail.com (L.S.); adomenico.marsico@crea.gov.it (A.D.M.); mariafrancesca.cardone@crea.gov.it (M.F.C.)
* Correspondence: teodora.basile@crea.gov.it; Tel.: +39-080-8915711

Abstract: The objective of this research was the production of new sparkling wines using either commercial or native yeast strains along with traditional Italian grape varieties. This approach preserves the unique character of the region, represented by the typical grape varieties, and incorporates the concept of "microbial *terroir*", which is linked to the native yeasts. The wines produced have been analyzed for conventional parameters such as pH, titratable acidity, volatile acidity, alcohol content, and residual sugar, as well as for their aromatic profile through GC-MS analysis. To evaluate the acceptance of the wines, a sensory analysis was conducted, and NIR spectra were collected to identify the factors influencing their preferences. Two varieties, Fiano and Falanghina, produced sparkling wines well appreciated with pleasant floral and white fruit notes. The positive reception of these innovative sparkling wines was connected to their chemical composition, offering valuable insights into the future production of similar products.

Keywords: *terroir*; NIR; GC-MS; winemaking; *Vitis vinifera*

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1. Introduction

Like all major wine-producing nations, Italy has seen a decline in wine export volumes in recent years [1]. The only wine-derived product that still maintains a good selling point worldwide is sparkling wines [2].

Consumer expectations can significantly influence their preference. Indeed, consumers preferred sparkling wines labeled as *Champenoise* over others when information about the production procedure was provided [3]. The traditional *Champenoise* method, used to produce high-quality sparkling wines like Champagne in France and Cava in Spain, was therefore chosen to create these novel sparkling wines [4].

A distinguishing characteristic of wine, setting it apart from other alcoholic beverages, is its perceived "identity", which is linked to the concepts of heritage and *terroir* [2]. The use of native vineyard locally selected yeasts, able to significantly influence the volatile profiles of fermented grape juice has been introduced as an innovative approach to obtaining wines reflecting *terroir* [5,6]. Studies involving native strains associated with the grape/wine growing area have highlighted the close connection existing among microbial consortium, geographical location, grape variety, climatic conditions, and agronomical practices, establishing a new concept of *terroir*, the so-called "microbial *terroir*" [7]. Although the role of

native grape microbiota in wine identity is still being studied, the use of native yeast could be a way to link wines to the culture and history of their production area [8].

Today, several select *Saccharomyces* and non-*Saccharomyces* strains appropriate for the preparation of commercial starter cultures dedicated to the primary, and particularly the secondary fermentation of sparkling wines have been studied, to evaluate the possible uses of selected native strains to improve the unique traits of sparkling wines from distinguished productive areas [9–12].

Native microbial strains could also enhance the functional components of sparkling wines by improving the extraction of polyphenols from grapes while also producing other beneficial compounds (e.g., hydroxytyrosol). Effectively managing winemaking microbiota can create unique wines that not only please the palate but could also promote well-being [13]. The *L. thermotolerans*, a yeast commonly found as part of grape/wine microbiota, was used for base wine fermentation [14]. Studies have examined its application and effectiveness in oenology, leading to the availability of several *L. thermotolerans* strains for mixed-starter fermentations [15–17].

For each wine, the conventional parameters including pH, titratable acidity, volatile acidity, alcohol content, and residual sugars were evaluated. Additionally, the volatile pattern was assessed through GC-MS analysis. Understanding the reasons for the favorable acceptance of these new sparkling wines is important for planning the production of similar new products. Therefore, to evaluate the consumer's appreciation for these novel wines a sensory analysis was conducted in conjunction with NIR spectroscopy evaluation to search for spectral regions that could predict factors influencing testers' preferences. The aim of this study was to produce novel sparkling wines with the *Champenois* method using typical Apulian white grape varieties (Greco bianco, Falanghina, Montonico pinto, and Fiano), which are conventionally used to produce white wine, in combination with native yeasts. The decision to select four Apulian white wine varieties, which are largely present in the region but are seeing a decline in sales as still wines, aims to explore opportunities for creating products that are more appealing to consumers. This approach could provide vine growers with a chance to diversify their offerings.

2. Materials and Methods

2.1. Chemicals and Reference Compounds

Solid anhydrous sodium sulfate (analytical grade), 2-octanol standard (purity over 97%), and NaOH solution (0.1 M) were purchased from Merck (Darmstadt, Germany). Kits for the multiparametric analyzer were purchased from Steroglass (San Martino in Campo, Italy). MS grade dichloromethane was supplied by Carlo Erba (Milan, Italy). Water was obtained from a Milli-Q purification system by Millipore (Langenselbold, Germany).

2.2. Grape Composition, Harvest and Climatic Data

The research was conducted in 2022–2024 on grapes collected in 2022 from the "Lamarossa" experimental vineyard of CREA Research Centre for Viticulture and Enology (CREA-VE), which consists of 11 hectares located in the Apulia region, Southern Italy (S.P. 65 snc—70018 Rutigliano, Italy). Four traditional Italian grape varieties of Southern Italy were harvested on 30 August 2022: Falanghina, Fiano, Greco bianco, and Montonico pinto. Vines were grown under conventional farming conditions with a Guyot pruning system.

Climatic data for the geographical coordinates of the vineyard, 40°57′23″ N 17°00′25″ E, and the altitude of 44 m were obtained from https://open-meteo.com/ [18]. Since focusing on one production year, the weather was monitored until August 2022 (the month of harvest), focusing on the start of the annual growth cycle in the spring-summer, between April 2022 and August 2022. Moreover, some bioclimatic indices were computed:

1. Huglin Index (IH), which calculates the sum of temperatures above a threshold of 10 °C from the beginning of April to the end of August [19];
2. Night Coolness Index (IF), which is determined using the means of the minimum temperatures recorded in the 30 days before the harvest date in August [20];
3. Hydrothermal Index of Branas, Bernon, and Levandoux (BBLI), which is calculated by summing the products of the monthly mean temperature and the monthly accumulated precipitation for the period from April to August [21]. Basic parameters on grapes at harvest were measured. For pH and total acidity (TA) a Crison Basic 20 pH meter was used (TA by titration with NaOH), while total soluble solids (TSS in Brix) were determined with a digital refractometer Atago PR1 (Atago Co., Tokyo, Japan).

2.3. Physiochemical Composition of Base and Sparkling Wines

The production of base wines was performed on a pilot scale in the experimental winery of the CREA-VE of Turi. For each treatment, 100 kg of grapes were employed. For each grape variety, two different types of base wines were produced, one using only a commercial *Saccharomyces cerevisiae* strain (VB1, Oenobrands) while the other with a sequential inoculation using a *Lachancea thermotolerans* (Lt) yeast strain collected from an experimental vineyard in Southern Italy [22] followed by the same *S. cerevisiae* strain (VB1, Oenobrands) to complete the fermentation. As is typical for non-*Saccharomyces* yeasts, pure cultures of *L. thermotolerans* cannot fully ferment all grape sugars to complete the wine fermentation process. Therefore, they require the simultaneous or sequential addition of another co-starter, usually a strain of *S. cerevisiae* [23]. Some strains of *L. thermotolerans* can create mixed-culture dry wines with similar or lower pH and ethanol levels than those fermented solely with *S. cerevisiae*. They partially convert sugars into lactic acid and produce less acetic acid, positively affecting the wines' pH [14,23]. Unfortunately, due to complications during the fermentation process, it was not possible to obtain a base wine using *L. thermotolerans* for the Falanghina variety. Two other strains of *S. cerevisiae* were then used for the secondary fermentation: a commercial *S. cerevisiaes* train (18-2007 IOC) and a native *S. cerevisiae* strain directly collected from grape berries growing in North Apulia vineyards (S21) which was previously selected as starter culture due to its performances in base wine fermentation to produce white and rosé Apulian sparkling wines [24]. Selected base wine's chemical/physical characteristics were determined according to methods recommended by the International Organization of Vine and Wine (OIV) [25]. Total acidity (by titration with NaOH) g/L expressed as tartaric acid and pH were determined with a Crison Basic 20 pH meter, while volatile acidity g/L expressed as acetic acid, malic acid g/L, lactic acid g/L, total SO_2 mg/L, residual sugar g/L, and polyphenolic content mg/L were all determined on wines at racking with a multiparametric enzymatic analyzer (Hyperlab Smart, Steroglass, Italy). The selected base wines were re-fermented by adding a sugar solution (24 g/L sugarcane) and supplemented with selected yeasts (0.2 g/L) until reaching a stable pressure value (5–6 bar) at low temperature (10–15 °C). For the second fermentation, two different *S. cerevisiae* strains were used: a native yeast isolated from a grape, which has previously been tested on white and rosé sparkling wines for its aptitude to carry on in-bottle secondary fermentation, and a commercial one. Basic parameters (pH, TA, volatile acidity, malic acid, lactic acid, alcohol, and residual sugars) on sparkling wines prior to the sensory analysis were also measured following the same procedures used for basic wines. Thirteen bottles for each combination of yeasts were produced for each sparkling wine. A depiction of the experimental design is provided in Figure 1.

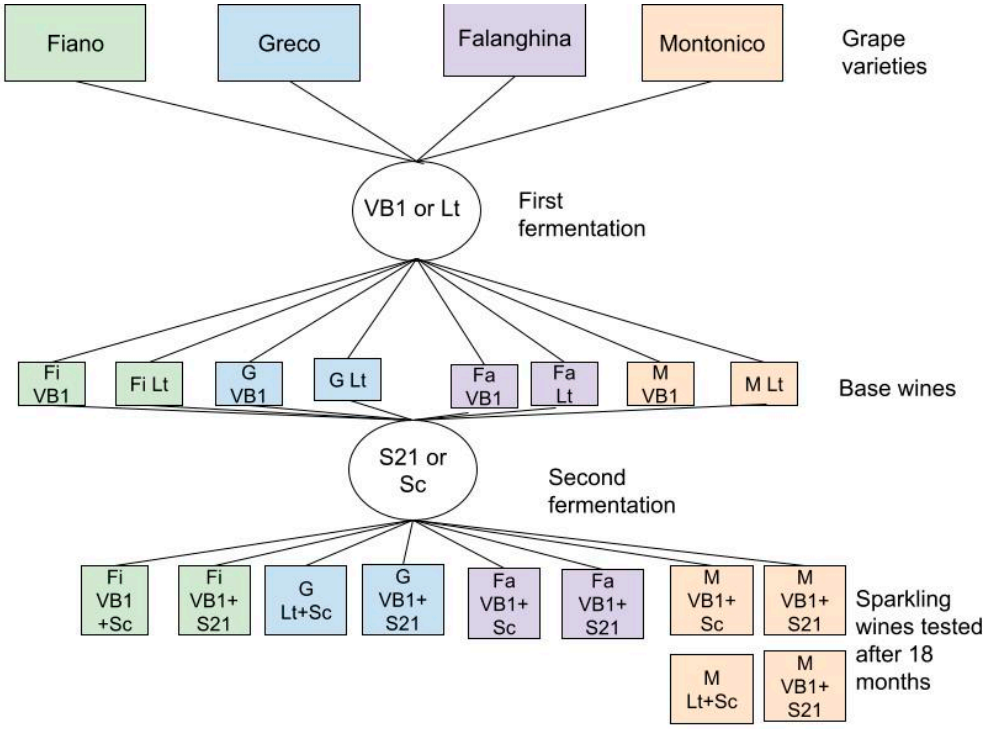


Figure 1. Flow chart of the experimental design. Acronyms used in this table: Fi is Fiano; Fa is Falanghina; G is Greco; M is Montonico; VB1 is commercial *S. cerevisiae* strain VB1, Oenobrands; Lt is *L. thermotolerans;* Sc is commercial *S. cerevisiae* strain 18-2007 IOC; S21 is native *S. cerevisiae* strain S21.

2.4. GC-MS Analysis

The sparkling wines were degassed using an ultrasonic bath; then, NaCl was added at a dose of 50 g/L. A 50 mL sample aliquot was added with 250 µL of a solution of 8.20 mg/L of 2-octanol (CAS 4128-31-8) in dichloromethane used as an internal standard. The sample was extracted 2 times in a separating funnel with 5 mL of dichloromethane for an extraction period of 20 min/extraction. Ten milliliters of the organic phase was dried over anhydrous Na_2SO_4, filtered on a 0.2 µm Nylon filter, and concentrated in a roto-evaporator to 1 mL. One microliter of extract obtained was injected into a GC/MS system—a 6890N gas chromatograph interfaced with a 5973 mass selective detector equipped with a multi-sampler 7683B series injector (Agilent, Palo Alto, CA, USA). The column used was HP-INNOWax (30 m × 0.25 mm i.d. × 0.25 µm film thickness, Agilent) silica capillary column. Splitless injection was used. The carrier gas was helium at a flow rate of 1 mL min^{-1}. The oven temperature program was: 50 °C (for 1 min), then increased to 220 °C, at 2 °C min^{-1}, and held for 10 min. The MS detector was set as follows: transfer line temperature 220 °C; emission source temperature 230° while the MS temperature was set at 150 °C. The mass range was m/z 30–300. All mass spectra were acquired in the electron impact (EI) mode (Ei = 70 eV, source temperature, 180 °C). The identification of the volatile compounds of interest and their quantification was carried out as described by Perestrelo et al. 2006. Identification was achieved by comparisons with mass spectra presented in the NIST MS library Database (2017) or in the literature. The concentration was calculated as µg/L of 2-octanol (internal standard) [26].

To assess the contribution of different detected compounds to the perceived aroma, the odor activity value (OAV), as the ratio of the compound's concentration in the sample to its odor perception threshold (OPT), was calculated for each detected compound. Compounds with OAVs of 1 or higher are considered major contributors, while those below 1 are minor contributors [27]. Although OAVs provide a simple and straightforward assessment of a compound's importance, OPT values are often derived from the literature without consider-

ing the specific sample matrix, leading to potential inaccuracies. Additionally, interactions with other aromatic substances and matrix complexity can mask aroma compounds and elevate their OPTs [28,29].

2.5. Sensory Analysis

To follow the organoleptic evolution of the product during aging on lees tasting at different months: 6, 12, and 18 months were performed. Prior to the first sensory evaluation, five experienced wine judges selected the sensorial descriptors to characterize the wines. The first two tastings performed by experienced testers allowed us to choose the most promising wines to finally taste after one and a half years. The *dégorgement* (removal of yeast sediment from bottles) was performed prior to sensory testing. A total of eight sparkling wines were selected among those produced from the 4 different grape varieties to be tasted during the final sensory evaluation: Falanghina (one wine), Fiano (one wine), Greco (two wines), and Montonico (four wines).

The tasters panel was composed of 14 experienced tasters selected among personnel at CREA-VE, sommeliers, winemakers, wine professionals, and grape growers (11 males and 3 females; mean age of 49.3 $\pm$ 11.4). All of the individuals had at least three years of experience working in the wine industry and were considered experts based on the criteria by Parr et al. [30]. The panel was composed of subjects already familiar with the testing procedure. The testers were requested not to smoke or eat for one hour prior to the sensory sessions. The wines were evaluated after 18 months of bottle aging at 10 °C, while the room temperature was 18 °C. Wines were presented simultaneously and anonymously (through coding) to the assessors, in order to eliminate the effects of presentation order and any potential biases from the initial sample presentation. A mandatory tasting order was maintained (based on a complete block design) with sample randomization order performed using the statistical software program R. Assessors were allowed to taste the product samples multiple times if they desired. Still, they were required to provide a response for each sample (forced choice) [31]. The attributes characterizing the wines for aroma and flavor by mouth were: fruity (as white fruits and ripened fruits), floral, balsamic, herbaceous, phenolic aromas, sourness, astringency (relative to tannins or procyanidins [32], body (mouthcoat and/or ethanol), persistency (how long the first taste sensation produced by the wine persists on the palate), sapidity (sensation of minerality due to dissolved mineral substances), typicity (the degree to which a wine reflects its varietal origins and the grape from which it was produced), and pleasantness. These attributes, together with color (relative to color intensity and tone; moderately pale straw yellow as usual for *Champenois* products), *perlage* (presence and persistence of bubbles), and bubble size (dimension of the bubbles to eyes and mouth) were evaluated on intensity scales from 1 to 10 (where 1 means absent/negative while 10 intense/excellent).

The mean scores of all the attributes were submitted to a Quantitative Descriptive Analysis (QDA). The sensory session was conducted under natural light with 50 mL wine at 10 °C in 125 mL ISO wine glasses, labeled with three-digit random numbers, and covered with plastic film.

2.6. NIR Data

NIR absorption measurements were carried out on a TANGO FT-NIR spectrometer (Bruker, Germany). NIR spectra were collected by data acquisition software OPUS/QUANT software version 2.0 (Bruker Optik GmbH, Ettlingen, Germany) between 12,000 and 4000 cm^{-1} (833–2500 nm), with 8 cm^{-1} resolution and 64 scans. A background spectrum was automatically recorded, before each sample. Both the temperature and relative humidity of the room were kept constant with an air conditioning system.

2.7. Statistical Analysis

The statistical procedures described in detail in the following paragraphs, including analysis of variance, pre-treatments of the original NIR spectra, and Principal Component Analysis (PCA) were performed using R Statistical Software (v4.4.2; R Core Team 2024, R Foundation for Statistical Computing, Vienna, Austria) [33]. The R packages used are listed in alphabetical order: corrplot [34], ggbiplot [35], ggplot2 [36], IMIFA [37], and mdatools [38]. Chemical data were subjected to an analysis of variance at 95% significance level ($p < 0.05$). Prior to PCA data were normalized with Pareto scaling to eliminate differences introduced by the different measurement units [39].

3. Results and Discussion

3.1. Climatic Data

The chemical composition of grapes and thus wine is influenced by climatic conditions. Due to the forecasted change in climate worldwide linked to climate change, the knowledge of actual climatic parameters will allow for undertaking preventative measures (e.g., early harvest) to ensure wine's constant quality [40]. The most important climatic indicators used in viticulture together with meteorological parameters are reported in Table 1.

Table 1. Climatic parameters and bioclimatic indicators for the experimental vineyard.

Parameters and Bioclimatic Indicators	Value
Mean annual temperature (°C)	18.3
Mean temperature in the vegetation period (°C) †	24.1
Rainfall annual (mm)	548.9
Rainfall in the vegetation period (mm)	165.2
Et0 (evapotranspiration) in the vegetation period (mm)	870
HI (Huglin index) in the vegetation period	2689
CI (cool night index) 30 days before harvest in August	23
BBLI (from April to August)	3528

† Vegetation period: April–August 2022.

The annual precipitation in the vineyard amounts to 548.9 mm while the mean annual air temperature is 18.3 °C, which rises to 24.1 °C during the growing season. January is the coldest month, with an average temperature of 9.0 °C, while July is the hottest, with an average temperature of 29.5 °C. In the growing season from April to August, the recorded values of rainfall were very low, below 200 mm this combined with a significant value of evapotranspiration (Et0), which almost exceeded 800 mm, decreased the water available for vines.

The air temperature plays an important role in grape maturation during ripening, influencing the aroma and color compound development. Cool nights are especially important for color and polyphenols development [41]. The HI value (heat accumulation during the growing season) is above 2600 and the CI above 20 °C indicates hot days and nights before harvest, suggesting high heat transferred to and accumulated by vines. The absence of a significant day–night temperature variation led to higher sugar and pigment concentrations in grapes, resulting in an earlier harvest [42]. The hydrothermic index of Branas, Bernon, and Levadoux (BBL) based on rainfall and temperature rates, accounts for the influence of temperature and rainfall on grape production. This index indicates the possibility of the vine being attacked by pathogens such as mildew. While high temperatures and drought helped control fungal diseases and reduced the need for phytosanitary interventions, it influenced production, particularly for non-irrigated vineyards.

The data calculated for the vineyard location aligns with the trend observed in 2022 in Italy. Indeed in 2022, Italy faced high temperatures and persistent drought, severely impacting water resources. In Southern Italy, temperatures were about 1.06 °C above the previous decade's average. This increase was primarily observed during the summer months. Moreover, the Apulia region also experienced below-normal annual precipitation [43].

3.2. Grape, Basic, and Sparkling Wines Chemical Composition

Grapes were harvested prior to reaching technological maturity, as usual for grapes to be used in the production of sparkling wines. The conventional basic parameters of grapes, recorded in duplicate at harvest, are reported in Table 2.

Table 2. Grape basic parameters.

Parameters	Fiano	Greco Bianco	Falanghina	Montonico Pinto
TSS (Brix)	21.7 ± 1.0 b	16.6 ± 1.2 a	14.8 ± 1.0 a	21.9 ± 2.0 b
TA (g/L)	10.5 ± 0.4 a	17.1 ± 0.3	21.6 ± 0.3	10.4 ± 0.4 a
pH	3.18 ± 0.10 b	2.93 ± 0.08 a	2.83 ± 0.08 a	3.13 ± 0.10 b

Mean ± standard deviation. Different letters in the same row indicate significant differences ($p \leq 0.05$).

The base wines (Table 3) showed values in line with those usually obtained for each variety, as found in the literature: Fiano bianco (alcohol 12–13.5% vol, pH 3.1–3.5, TA 6.5–9.5 g/L), Greco bianco (alcohol 11.5–13%vol, pH 3.0–3.4, TA 6.5–9.5 g/L), Falanghina (alcohol 11–12.5% vol, pH 3.1–3.4, TA 6.5–10 g/L), and Montonico (alcohol 12–13.5%vol, pH 3.1–3.3, TA 7–10 g/L) [44].

The Lt and VB1 base wines of each variety did not differ for pH, TA, ethanol, and malic acid content. A difference in the lactic acid content was observed, with Fiano Lt wine showing the highest content, probably linked to Lt yeast's lactic acid production ability. For Montonico wines the lactic acid was higher in VB1 compared to Lt, while in the Greco wines, the lactic acid was always absent. Moreover, Greco wines showed the lowest alcohol content among the base wines produced; notably, the alcohol % is in the lowest range of the values usually expected for this variety. Moreover, residual sugars were absent only in Greco VB1, which indicates that fermentation was fully completed for this wine. The high sugar levels in the Lt base wines suggest that the fermentation was not efficient for both Fiano and Greco when using the Lt yeast. The highest volatile acidity was found in Greco Lt wine. The Lt strain used probably was not able to deliver a low production of acetic acid for the Fiano and Greco varieties. Previous studies have highlighted that the composition of wines is influenced by both the yeast strain used and the inoculation method [23]. In our case, the *L. thermotolerans* yeast employed did not produce base wines with all the desirable characteristics obtained with some other strains. This may be related to the ability of the specific isolates of *L. thermotolerans* used to ferment the selected grape varieties.

The results of the secondary fermentation step can be better understood by examining the chemical composition of the base wines. In addition to the lack of nutrients (like sugar), which are added, these wines pose difficulties for yeast survival due to harsh conditions. These conditions include low pH levels, high ethanol content, elevated polyphenolic content, and the presence of SO_2 [24,45].

Even if no difference in alcohol content was found between base wines from the same varieties, the Montonico and Fiano ones show the highest amount of alcohol compared to all the other base wines. This represents a negative feature in a base wine since the fermentation capacity of yeasts decreases with the increase in the ethanol content.

The SO_2 level was always higher in VB1 wines, with Montonico showing the highest value. Moreover, for Fiano and Montonico total polyphenols were higher in VB1 wines, while

for Greco it was the Lt wine which showed the highest value among of all the base wines. Concerning volatile acidity, expressed as acetic acid, it was quite low in all wines ranging from 0.24 to 0.44 g/L. These data indicate how Montonico base wines showed the least favorable conditions to ensure tolerance of yeasts to the medium.

The main parameters measured on the selected sparkling wines are reported in Table 4. A few wines (two Fiano and two Falanghina wines obtained using Lt in the first fermentation, and two Greco wines) were discarded by our oenologist due to perceived defects.

Despite small differences for sparkling wines from the same variety, the two Greco wines (10.5% vol) showed the lowest alcohol content, while in Montonico wines it was quite high for sparkling wine since sparkling wines generally range from 10.5% to 12.5% alcohol volume. Residual sugars were found in small amounts in Greco wines, and the highest in Montonico Lt + 18-2007 (19 g/L) strictly followed by Montonico Lt + S21 (18 g/L) and Vb1 + S21 (15 g/L). The high levels of residual sugars in Montonico wines suggest suboptimal secondary fermentation, which can be linked to the harsh conditions for yeast survival caused by the base wine composition. Overall, the Montonico variety seemed the least appropriate to produce sparkling wines with the yeasts used in this work. Among the other sparkling wines Greco wines, even with low alcohol content and effective secondary fermentation (almost no residual sugar), were not the optimal product since were characterized by the highest acidity. Based only on the chemical base parameters, Fiano and Falanghina wines seem the most promising varieties for sparkling wine production.

3.3. Sensory Analysis

Among all the sparkling wines, ten were subjected to a sensory evaluation by a panel of expert wine testers. A Pearson correlation was performed to understand the relationship among the scores of the various sensory parameters. The main parameters highly positively correlated to the perceived pleasantness of the wines are linked to the visual aspect, like color, *perlage*, and bubble size. This is in agreement with the known relevance of foam characteristics (foamability, persistence, in-mouth aggressiveness, and bubble size) and color for consumers' appreciation of sparkling wines [46]. Also, aroma plays an important role in consumer's liking, indeed another parameter positively correlated with pleasantness is white flowers aroma. Instead, ripened and balsamic aromas negatively influenced the perceived pleasantness of the wines (Figure 2).

The mean aroma-intensity scores for the wines are reported in a radar plot (Figure 3). In the sensory analysis, Fiano and Falanghina wines obtained with both yeast combinations were equally appreciated. Instead, for both Greco and Montonico varieties all the wines obtained using Lt in the first fermentation step were appreciated the least or not at all. Color was also a very important discriminator factor for wine appreciation. The two least appreciated wines showed the lowest rating for this parameter. Indeed, the color of these two wines was way darker than all the others which was probably negatively linked to the supposed appearance of sparkling wines in the eyes of the tasters. The sugar type added during the second fermentation has a great influence on sparkling wines in terms of consumer's liking. In the *liqueur de tirage* added for secondary fermentation, the concentration of cane sugar was specifically chosen to enable good foam quality in all wines. The difference observed among wines in the foam characteristics could be attributed to a poor secondary fermentation performed by yeasts in Montonico base wines probably linked to the high alcohol representing harsh conditions for yeast survival.

Table 3. Base wine's chemical composition.

Parameters	Fiano Lt †	Fiano VB1	Greco Lt	Greco VB1	Falanghina VB1	Montonico Lt	Montonico Vb1
pH	3.13 ± 0.10 c	3.11 ± 0.09 c	2.74 ± 0.10 a	2.82 ± 0.09 ab	2.86 ± 0.07 ab	3.08 ± 0.08 c	3.12 ± 0.08 c
TA g/L	6.2 ± 0.3 a	6.8 ± 0.3 a	10.1 ± 0.3 d	9.9 ± 0.2 d	8.9 ± 0.3 c	7.5 ± 0.3 b	7.8 ± 0.3 b
Volatile acidity g/L	0.38 ± 0.03 c	0.24 ± 0.02 a	0.45 ± 0.02 d	0.29 ± 0.03 ab	0.39 ± 0.02 c	0.28 ± 0.03 ab	0.34 ± 0.03 bc
Malic acid g/L	1.36 ± 0.11 a	1.55 ± 0.12 a	3.42 ± 0.27 c	3.30 ± 0.26 c	2.93 ± 0.23 c	2.34 ± 0.18 b	2.28 ± 0.15 b
Lactic acid g/L	0.17 ± 0.01 d	0.00	0.00	0.00	0.01 ± 0.01 a	0.04 ± 0.01 b	0.10 ± 0.01 c
Alcohol %vol	12.80 ± 0.30 bc	12.90 ± 0.50 bc	10.00 ± 0.40 ab	9.80 ± 0.30 a	12.1 ± 0.40 b	13.60 ± 0.50 c	13.50 ± 0.40 c
Total SO_2 mg/L	41.0 ± 0.3 a	48.0 ± 0.2 b	59.0 ± 0.2 c	57.0 ± 0.3 b	57.0 ± 0.2 b	57.0 ± 0.2 b	73.0 ± 0.3 d
Residual sugars g/L	0.89 ± 0.08 c	0.50 ± 0.02 b	2.37 ± 0.20 d	0.00	0.24 ± 0.02 a	3.03 ± 0.03 e	4.47 ± 0.02 f
Total polyphenols mg/L	150 ± 6 a	163 ± 4 b	530 ± 12 g	238 ± 5 c	285 ± 6 d	365 ± 3 e	431 ± 11 f

Mean ± standard deviation. Different letters in the same row indicate significant differences ($p \leq 0.05$). † Lt is *L. thermotolerans*; VB1 is commercial *S. cerevisiae* strain VB1, Oenobrands.

Table 4. Sparklingwine's chemical composition.

Parameters	FiVB1 + Sc †	FiVB1 + S21	GLt + Sc	G VB1 + S21	FaVb1 + Sc	Fa Vb1 + S21	M Lt + S21	MVB1 + Sc	MLt + Sc	MVB1 + S21
pH	3.09 ± 0.06 bc	3.08 ± 0.08 bc	2.79 ± 0.05 a	2.77 ± 0.06 a	2.93 ± 0.10 ab	3.08 ± 0.05 bc	3.15 ± 0.05 c	3.10 ± 0.05 c	3.11 ± 0.07 c	3.12 ± 0.05 c
TAg/L	6.4 ± 0.3 a	6.7 ± 0.3 a	9.5 ± 0.3 d	9.5 ± 0.3 d	8.7 ± 0.3 c	6.7 ± 0.3 a	6.5 ± 0.3 a	7.0 ± 0.3 a	8.1 ± 0.3 b	6.8 ± 0.3 a
Volatile acidity g/L	0.28 ± 0.04 ab	0.27 ± 0.03 a	0.28 ± 0.03 ab	0.24 ± 0.03 a	0.44 ± 0.03 d	0.38 ± 0.03 cd	0.39 ± 0.04 cd	0.30 ± 0.03 a	0.41 ± 0.05 d	0.34 ± 0.03 bc
Malic acid g/L	1.41 ± 0.11 a	1.32 ± 0.12 a	2.80 ± 0.12 d	2.70 ± 0.10 cd	2.54 ± 0.12 c	2.53 ± 0.15 cd	1.80 ± 0.11 b	2.10 ± 0.11 b	2.04 ± 0.12 b	1.98 ± 0.11 b
Lactic acid g/L	0.00	0.00	0.00	0.00	0.00	0.01 ± 0.01 a	0.18 ± 0.01 c	0.00	0.10 ± 0.01 b	0.12 ± 0.01 b
Tartaric acid g/L	3.2 ± 0.1 c	3.2 ± 0.1 c	4.1 ± 0.1 d	4.2 ± 0.1 d	2.9 ± 0.1 b	3.0 ± 0.0 b	2.6 ± 0.1 a	2.6 ± 0.1 a	2.6 ± 0.0 a	2.6 ± 0.0 a
Alcohol %vol	12.80 ± 0.60 bc	12.70 ± 0.40 bc	10.50 ± 0.50 a	10.60 ± 0.30 a	12.00 ± 0.40 b	12.70 ± 0.50 bc	13.00 ± 0.50 c	13.20 ± 0.50 c	13.00 ± 0.40 c	12.90 ± 0.50 bc
Residualsugars g/L	10.1 ± 0.1 c	13.4 ± 0.1 e	1.2 ± 0.0 a	1.2 ± 0.0 a	9.0 ± 0.1 c	7.2 ± 0.1 b	18.1 ± 0.2 g	13.0 ± 0.1 d	19.1 ± 0.1 h	15.4 ± 0.1 f

Values are reported as mean ± standard deviation. Different letters in the same row indicate significant differences ($p \leq 0.05$). † Acronyms used in this table: Fi is Fiano; Fa is Falanghina; G is Greco; M is Montonico; VB1 is commercial *S. cerevisiae* strain VB1, Oenobrands; Lt is *L. thermotolerans*. Sc is commercial *S. cerevisiae* strain 18-2007 IOC; S21 is native *S. cerevisiae* strain S21.

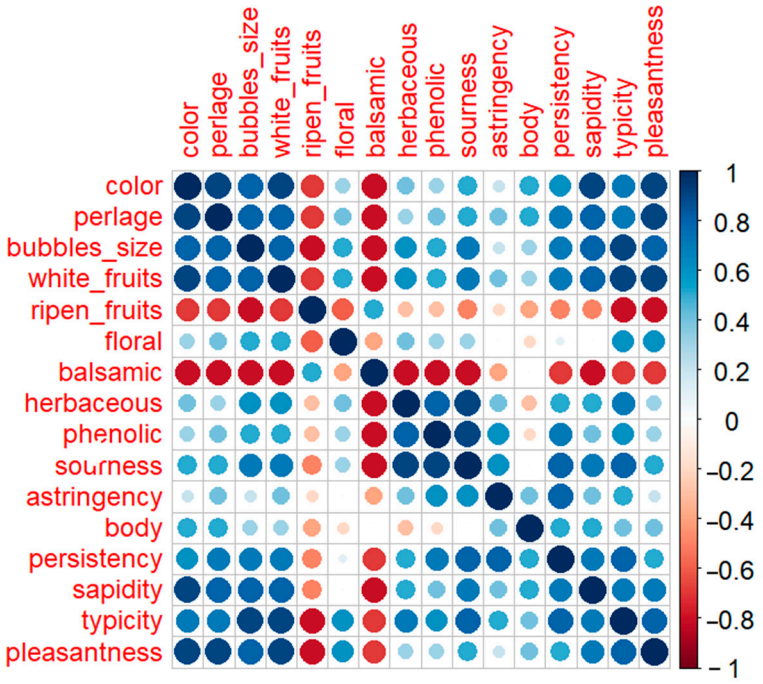


Figure 2. Correlation plot of sensory analysis outcome.

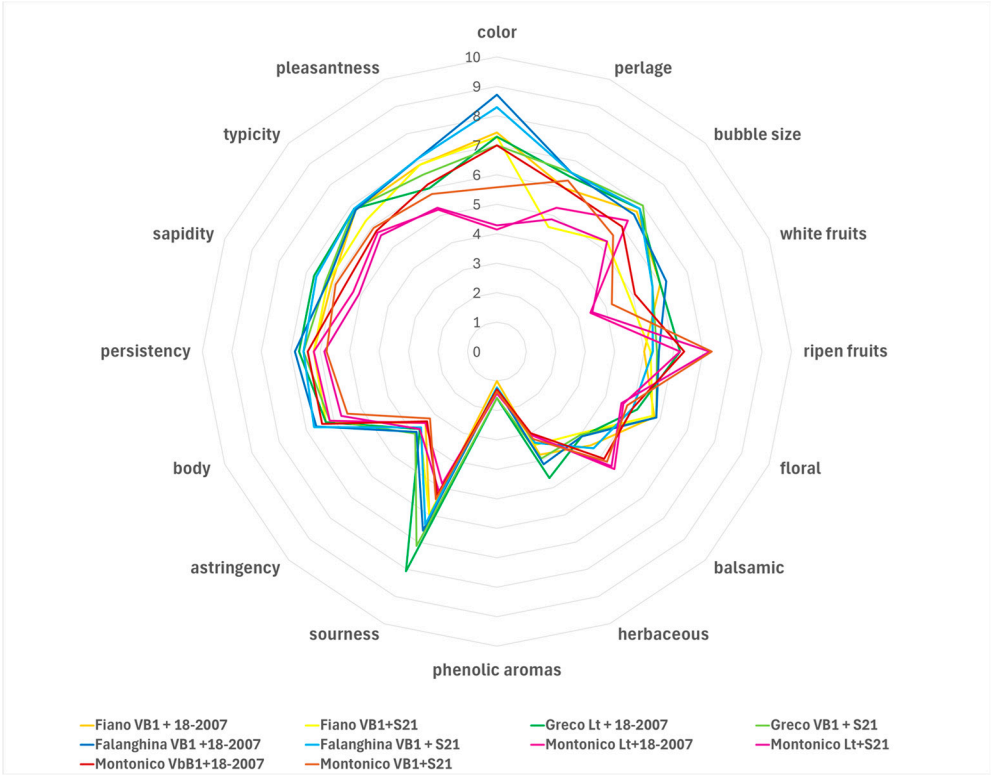


Figure 3. Quantitative descriptive analysis.

3.4. Volatilomic Profile

The volatile composition of sparkling wines is shown in Table 5. A total of 62 volatile compounds were detected and quantified in each wine. Based on their chemical structure, they were identified as: 20 alcohols (including polyols and polyphenols), 14 carboxylic acids, 15 esters, 3 terpenoids, 3 lactones, 3 methoxyphenols, and 4 others (aldehydes, ketones, and amides).

Table 5. Concentration of major volatile compounds in sparkling wines in μg/L.

CAS	Molecules	Fi Vb1 + Sc †	Fi Vb1 + S21	G Lt + Sc	G Vb1 + S21	Fa Vb1 + Sc	Fa Vb1 + S21	M Lt + Sc	M Lt +S21	M Vb1 + Sc	M Vb1 + S21
Acids											
64-19-7	Acetic Acid	3128.8 ± 472.1 bcd	n.d.	3317.0 ± 500.5 bc	1971.0 ± 297.4 ab	3417.2 ± 515.6 cd	3390.7 ± 511.6 cd	2853.8 ± 430.6 abc	1503.2 ± 226.8 a	4397.9 ± 663.6 d	4354.6 ± 657.1 d
79-31-2	Isobutyric Acid	188.1 ±21.7 d	132.4 ± 15.3 bcd	165.1 ± 19.1 d	77.6 ± 9.0 ab	134.5 ± 15.5 bcd	91.5 ± 10.6 abc	148.9 ± 17.2 cd	57.6 ± 6.6 a	323.3 ± 37.3 f	252.5 ± 29.1 e
107-92-6	Butyric Acid	399.6 ± 37.8 cd	502.9 ± 47.5 e	473.8 ± 44.8 de	351.9 ± 33.3 cb	340.9 ± 32.2 cb	346.1 ± 32.7 cb	260.5 ± 24.6 b	151.8 ± 14.4 a	358.6 ± 33.9 cb	402.2 ± 38.0 cde
503-74-2	Isovaleric Acid	307.8 ± 20.5 cd	312.8 ±20.8 cd	352.7 ± 23.5 de	238.3 ± 15.9 bc	245.0 ± 16.3 bc	228.1 ± 15.2 bc	181.8 ± 12.1 b	103.4 ± 6.9 a	374.0 ± 24.9 de	401.4 ± 26.7 e
142-62-1	Hexanoic Acid	3170.4 ±270.8 ef	3220.7 ± 275.1 f	3469.9 ± 296.4 f	2707.9 ± 231.3 de	2454.6 ± 209.7 d	2674.5 ± 228.4 d	1634.1 ± 139.6 b	1239.0 ± 105.8 a	1622.4 ± 138.6 b	1932.5 ± 165.1 c
124-07-2	Octanoic Acid	3493.2 ±366.5 e	2946.3 ± 309.2 e	3393.9 ± 356.1 e	2501.9 ± 262.5 d	1981.0 ± 207.9 c	2498.2 ± 262.1 d	1343.1 ± 140.9 a	1375.4 ± 144.3 ab	1554.5 ± 163.1 b	2104.9 ± 220.9 cd
112-05-0	Nonanoic Acid	13.4 ±.5 b	13.9 ± 2.6 bc	15.8 ± 3.0 c	12.5 ± 2.4 bc	12.4 ± 2.4 bc	13.3 ± 2.5 bc	10.3 ± 2.0 b	6.5 ± 1.2 a	11.2 ± 2.1 bc	12.2 ± 2.3 bc
334-48-5	N-Decanoic Acid	770.6 ± 95.9 e	585.0 ± 72.8 d	573.5 ± 71.4 d	495.9 ± 61.8 c	368.7 ± 45.9 bc	326.5 ± 40.7 bc	130.5 ± 16.2 a	130.6 ± 16.3 a	294.2 ± 36.6 b	413.2 ± 51.4 c
14436-32-9	9-Decenoic Acid	19.6 ±2.0 bc	14.1 ± 1.4 a	20.3 ± 2.1 c	17.9 ± 1.8 b	154.2 ± 15.7 ef	175.8 ± 17.9 fg	213.5 ± 21.8 gh	229.2 ± 23.4 h	89.3 ± 9.1 d	125.5 ± 12.8 e
65-85-0	Benzenemethanoic Acid	20.0 ± 3.2 b	21.3 ± 3.4 b	21.9 ± 3.5 bc	14.2 ± 2.3 a	32.0 ± 5.1 d	54.8 ± 8.8 e	54.9 ± 8.8 e	30.3 ± 4.9 d	30.0 ± 4.8 cd	32.1 ± 5.2 d
143-07-7	Lauric Acid	10.6 ± 1.3 b	10.9 ± 1.4 b	n.d.	5.7 ± 0.7 a	n.d.	5.5 ± 0.7 a	n.d.	n.d.	4.7 ± 0.6 a	n.d.
103-82-2	Benzenacetic Acid	77.5 ±15.0 cd	82.7 ± 16.0 cd	67.7 ± 13.1 b c	49.1 ± 9.5 b	46.8 ± 9.0 b	67.6 ± 13.1 bc	37.0 ± 5.0 b	24.7 ± 4.8 a	77.9 ± 15.1 cd	88.2 ± 15.0 d
544-63-8	Myristic Acid	13.8 ± 1.9 c	11.9 ± 1.6 c	11.8 ± 1.6 c	12.3 ± 1.7 c	13.7 ± 1.9 c	13.2 ± 1.8 c	7.2 ± 1.0 b	5.1 ± 0.7 a	10.5 ± 1.4 c	12.4 ± 1.7 c
57-10-3	Palmitic Acid	166.4 ± 26.6 c	151.0 ± 23.2 c	132.3 ± 29.1 bc	154.3 ± 24.0 c	141.9 ± 21.0 c	146.4 ± 22.2 c	92.8 ± 20.4 ab	68.9 ± 15.2 a	146.3 ± 22.2 c	165.7 ± 30.1 c
Alcohols											
78-83-1	Isobutanol	2179.6 ± 197.1 e	1866.1 ± 168.8 cd	1839.0 ± 166.3 cd	1212.9 ± 109.7 a	1333.1 ± 120.6 a	1597.7 ± 144.5 bc	2016.4 ± 182.4 de	1488.8 ± 134.7 b	1946.8 ± 156.1 d	2873.2 ± 159.9 f
763-32-6	3-Methyl-3-Buten-1-ol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.7 ± 0.2	n.d.	n.d.	n.d.
626-89-1	1-Pentanol- 4-Methyl	49.5 ± 7.0 cd	55.4 ± 7.8 d	43.7 ± 6.1 cd	39.3 ± 5.5 c	25.7 ± 3.6 ab	25.6 ± 3.6 ab	28.5 ± 4.0 b	21.6 ± 3.0 ab	21.2 ± 3.0 a	26.3 ± 3.7 ab
589-35-5	3-Methyl-1-Pentanol	181.8 ± 15.8 d	182.9 ± 15.9 d	119.5 ± 10.4 c	n.f.	83.0 ± 7.2 b	78.3 ± 6.8 b	61.4 ± 5.3 a	n.f.	85.2 ± 7.4 b	n.f.
928-97-2	(E)-3-Hexen-1-ol	57.0 ± 7.5 e	59.3 ± 7.8 e	46.3 ± 6.1 de	38.1 ± 5.0 d	10.7 ± 1.4 a	12.2 ± 1.6 ab	20.0 ± 2.6 c	14.9 ± 2.0 ab	33.1 ± 4.4 d	45.5 ± 6.0 de
928-96-1	(Z)-3-Hexen-1-ol	23.7 ± 1.5 b	27.4 ± 1.7 c	48.9 ± 3.1 e	39.8 ± 2.5 e	31.0 ± 2.0 cd	30.9 ± 2.0 cd	20.9 ± 1.3 b	14.7 ± 0.9 a	33.1 ± 2.1 d	30.5 ± 1.9 cd
71-36-3	1-Butanol	131.6 ± 12.1 f	156.0 ± 13.0 f	81.1 ± 7.7 e	68.7 ± 6.5 de	56.7 ± 5.4 c	67.3 ± 6.4 cde	36.3 ± 3.4 b	23.6 ± 2.2 a	64.9 ± 6.1 cd	85.0 ± 8.0 e
1569-50-2	3-Penten-2-ol	3.5 ± 0.9 b	3.1 ± 0.8 b	2.3 ± 0.6 ab	n.d.	n.d.	n.d.	3.1 ± 0.8 b	1.7 ± 0.4 a	2.3 ± 0.6 ab	3.4 ± 0.8 b
123-51-3	3-Methylbutan-1-ol	7687.8 ± 397.3 a	10,802.7 ± 558.2 b	20,426.7 ± 1055.5 f	15,401.2 ± 795.9 e	12,328.3 ± 637.1 c	14,769.6 ± 763.2 de	13,682.5 ± 707.0 cd	10,606.0 ± 548.1 b	15,257.0 ± 788.4 e	21,620.4 ± 1117.2 f
565-67-3	2-Methyl-3-Pentanol	43.8 ± 2.0 d	100.0 ± 4.6 f	45.1 ± 2.1 d	70.5 ± 3.2 e	29.0 ± 1.3 c	102.1 ± 4.7 f	11.6 ± 0.5 a	15.9 ± 0.7 b	44.2 ± 2.0 d	112.6 ± 5.2 f
513-85-9	2,3-Butanediol	6256.1 ± 973.4 e	7063.0 ± 1098.9 e	2265.5 ± 352.5 ab	1783.4 ± 277.5 a	2685.7 ± 417.9 b	3286.0 ± 511.3 bc	2868.0 ± 446.2 b	1679.7 ± 261.3 a	4308.7 ± 670.4 cd	5107.7 ± 794.7 de
111-27-3	1-Hexanol	960.2 ± 81.9 e	958.8 ± 81.7 e	980.1 ± 83.6 e	775.3 ± 66.1 d	445.1 ± 37.9 b	449.1 ± 38.3 b	265.8 ± 22.7 a	205.9 ± 17.6 a	496.8 ± 42.4 bc	568.8 ± 48.5 c
111-35-3	3-Ethoxy-1-Propanol	136.0 ± 11.7 g	172.5 ± 14.8 h	72.0 ± 6.2 f	65.6 ± 5.6 ef	49.8 ± 4.3 cd	47.4 ± 4.1 c	13.8 ± 1.2 b	3.5 ± 0.3 a	56.3 ± 4.8 de	47.2 ± 4.1 c
513-85-9	2, 3-Butanediol (R,R,R)	1404.1 ± 243.1 e	1678.7 ± 250.6 e	446.0 ± 77.2 bc	316.2 ± 54.7 ab	575.2 ± 99.6 c	609.3 ± 105.5 c	501.0 ± 86.7 c	247.8 ± 42.9 a	943.3 ± 163.3 d	931.1 ± 161.2 d
505-10-2	3-(Methylthio)-1-propanol	371.8 ± 1.8 d	463.8 ± 39.6 e	355.3 ± 30.3 d	262.8 ± 22.4 c	257.0 ± 22.0 c	278.5 ± 23.8 c	169.6 ± 14.5 b	96.5 ± 8.2 a	387.9 ± 33.1 d	414.5 ± 35.4 de
100-51-6	Benzylalchol	19.9 ± 1.0 g	23.4 ± 1.1 h	9.7 ± 0.5 d	9.2 ± 0.4 cd	7.1 ± 0.3 b	7.9 ± 0.4 bc	8.5 ± 0.4 c	5.7 ± 0.3 a	11.7 ± 0.6 e	14.4 ± 0.7 f
60-12-8	2-Phenylethanol	32,237.3 ± 1896.1 c	29,835.0 ± 1847.3 c	35,421.1 ± 1993.2 c	30,068.6 ± 1861.8 c	23,662.9 ± 1465.2 b	29,991.7 ± 1857.0 c	16,383.9 ± 1014.5 a	15,652.7 ± 969.2 a	23,874.4 ± 1478.3 b	28,908.4 ± 1790.0 c
112-53-8	Lauric alcohol	274.2 ± 22.8 g	222.0 ± 18.5 ef	194.5 ± 16.2 de	242.8 ± 20.2 fg	174.3 ± 14.5 cd	158.3 ± 13.2 bc	140.1 ± 11.7 b	97.0 ± 8.1 a	217.7 ± 18.1 ef	241.0 ± 20.1 fg
96-76-4	2,4-Di-t-Butylphenol	384.3 ± 54.0 d	370.6 ± 52.0 d	329.4 ± 46.2 bcd	357.5 ± 50.2 cd	309.0 ± 43.4 bcd	286.6 ± 40.2 bc	256.4 ± 36.0 b	172.0 ± 24.1 a	347.0 ± 48.7 bcd	357.6 ± 50.2 cd
501-94-0	2-(4-Hydroxyphenyl) Ethanol	4447.5 ± 578.9 def	5731.8 ± 746.1 ef	4620.9 ± 601.5 ef	2877.4 ± 374.5 b	2843.2 ± 370.1 b	3325.5 ± 432.9 bc	3489.9 ± 454.3 bcd	2066.6 ± 269.0 a	3368.8 ± 438.5 bc	3770.3 ± 490.8 cde

Table 5. *Cont.*

CAS	Molecules	Fi Vb1 + Sc †	Fi Vb1 + S21	G Lt + Sc	G Vb1 + S21	Fa Vb1 + Sc	Fa Vb1 + S21	M Lt + Sc	M Lt +S21	M Vb1 + Sc	M Vb1 + S21
Esters											
105-54-4	Butanoicacid, Ethyl Ester R	227.6 ± 41.3 e	176.5 ± 32.0 de	197.3 ± 35.8 de	174.4 ± 31.6 de	22.7 ± 4.1 a	61.1 ± 11.1 c	42.1 ± 7.6 b	38.4 ± 7.0 b	144.0 ± 26.1 d	183.8 ± 33.3 de
106-32-1	Octanoic Acid Ethylester	845.1 ± 157.4 e	569.5 ± 106.1 de	537.8 ± 100.1 d	393.3 ± 73.2 cd	174.5 ± 32.5 b	116.6 ± 21.7 a	108.1 ± 20.1 a	137.9 ± 25.7 ab	281.9 ± 52.5 c	413.9 ± 77.1 d
123-92-2	Isoamyl Acetate	100.4 ± 18.2 e	62.9 ± 11.4 bcd	108.2 ± 19.7 e	56.9 ± 10.3 bc	25.4 ± 4.6 a	19.2 ± 3.5 a	43.7 ± 7.9 b	20.6 ± 3.7 a	79.6 ± 14.4 cde	82.8 ± 15.0 de
123-66-0	Ethylexanoate	575.3 ± 66.5 f	441.6 ± 51.1 e	504.1 ± 58.3 ef	350.2 ± 40.5 d	152.7 ± 17.7 b	128.2 ± 14.8 b	92.4 ± 10.7 a	101.0 ± 11.7 a	227.7 ± 26.3 c	296.7 ± 34.3 cd
617-35-6	Ethylpyruvate	735.7 ± 7.4 d	823.7 ± 8.2 e	901.3 ± 9.0 f	683.8 ± 6.8 c	741.6 ± 7.4 d	1090.1 ± 10.9 g	284.8 ± 2.8 b	210.3 ± 2.1 a	1135.5 ± 11.4 h	2026.5 ± 20.31 i
97-64-3	Ethyllactate	7551.6 ± 109.5 f	7602.0 ± 110.2 f	5191.2 ± 75.3 e	3937.0 ± 57.1 b	4449.8 ± 64.5 c	5143.5 ± 74.6 d	3663.4 ± 53.1 b	2376.8 ± 34.5 a	10,776.4 ± 156.2 g	15,012.7 ± 217.6 h
5405-41-4	Ethyl-3-hydroxybutanoate	n.d.	54.7 ± 5.6 d	32.5 ± 3.3 b	26.2 ± 2.7 a	32.1 ± 3.3 ab	34.9 ± 3.6 b	n.d.	n.d.	47.9 ± 4.9 c	54.3 ± 5.6 d
110-38-3	Ethyldecanoate	194.2 ±36.1 g	131.0 ± 24.3 f	86.1 ± 16.0 e	37.0 ± 6.9 c	23.9 ± 4.4 b	13.1 ± 2.4 a	12.7 ± 2.4 a	16.5 ± 3.1 b	54.4 ± 10.1 d	93.2 ± 17.3 ef
123-25-1	Diethylsuccinate	14,348.0 ± 3346.8	11,410.7 ± 2661.6 bc	12,718.3 ± 2966.7 c	9127.0 ± 2129.0 b	9787.8 ± 2283.1 b	11,714.0 ± 2732.4 c	4225.3 ± 985.6 a	4208.2 ± 981.6 a	12,465.4 ± 2907.7 c	16,969.8 ± 1823.4
67233-91-4	Ethyl-9-decenoate	n.d.	n.d.	n.d.	n.d.	8.2 ± 2.0 a	7.5 ± 1.8 a	11.8 ± 2.9 ab	14.6 ± 3.6 b	7.5 ± 1.8 a	17.0 ± 4.2 b
103-45-7	Phenylethylacetate	77.7 ± 12.0 e	63.4 ± 11.1 de	48.6 ± 8.5 cd	36.0 ± 6.3 bc	62.4 ± 11.0 de	52.1 ± 9.2 d	24.4 ± 4.3 a	26.6 ± 4.7 ab	50.0 ± 8.8 cd	54.4 ± 9.6 d
626-11-9	Diethyl-DL-Malate	8082.0 ± 1205.2 cd	7451.9 ± 1111.3 b	17,044.7 ± 2541.8 f	12,292.8 ± 1833.2 e	11,033.1 ± 1645.3 de	13,616.2 ± 2030.5 ef	5549.4 ± 827.6 a	4503.6 ± 671.6 a	7979.0 ± 1189.9 bc	10,753.4 ± 1603.6 d
87-91-2	(+)-Diethyl-L-Tartrate	1555.3 ± 351.6 c	2020.3 ± 456.7 cd	3964.2 ± 896.0 e	3064.2 ± 692.6 e	1842.6 ± 416.5 c	2290.8 ± 517.8 d	840.5 ± 190.0 b	511.4 ± 115.6 a	1472.1 ± 332.8 c	1592.0 ± 359.8 c
1070-34-4	Ethylhydrogensuccinate	28,759.4 ± 5682.0 cd	17,437.1 ± 3445.1 b	27,043.5 ± 5343.0 cd	17,528.8 ± 3463.2 b	17,693.8 ± 3495.8 b	21,445.7 ± 4237.0 c	16,577.6 ± 3275.2 b	11,962.3 ± 2363.4 a	27,545.3 ± 5442.1 cd	32,455.5 ± 5412.2 d
3943-74-6	Methylvanillate	n.d.	3.0 ± 0.3 a	5.2 ± 0.3 b	n.d.	6.6 ± 0.4 c	7.4 ± 0.4 c	n.d.	n.d.	n.d.	n.d.
Terpenes											
98-55-5	A-Terpineol	4.9 ± 0.4 b	5.4 ± 0.5 b	n.d.	n.d.	3.8 ± 0.3 a	3.5 ± 0.3 a	14.5 ± 1.3 c	12.8 ± 1.1 c	37.9 ± 3.4 d	53.2 ± 4.7 e
14049-11-7	EpoxylinalolR	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.3 ± 0.1 b	2.5 ± 0.1 a	7.7 ± 0.3 c	10.8 ± 0.4 d
14049-11-7	EpoxylinalolS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11.4 ± 0.4 b	8.1 ± 0.3 a	23.4 ± 0.8 c	32.7 ± 1.1 d
Lactones											
96-48-0	Butyrolactone	1407.2 ± 18.0 g	1675.9 ± 21.4 h	1359.8 ± 17.4 f	1235.5 ± 15.8 d	1287.1 ± 16.4 e	1443.3 ± 18.4 g	754.1 ± 9.6 b	478.7 ± 6.1 a	1143.5 ± 14.6 c	1260.4 ± 16.1 de
599-04-2	Pantolactone	68.0 ± 11.9 bcd	92.3 ± 16.1 d	74.2 ± 13.0 bcd	57.8 ± 10.1 ab	58.4 ± 10.2 ab	61.8 ± 10.8 bc	79.8 ± 13.9 cd	45.3 ± 7.9 a	65.6 ± 11.5 bcd	70.2 ± 12.3 bcd
1126-51-8	γ-Carboethoxy-γ-Butyrolactone	1952.8 ± 127.7 e	2235.6 ± 146.2 e	1708.8 ± 111.7 d	1441.1 ± 94.2 c	1497.7 ± 97.9 c	1605.7 ± 105.0 d	927.8 ± 60.7 b	668.3 ± 43.7 a	1687.2 ± 110.3 d	1951.4 ± 127.6 e
Metoxyphenols											
2785-89-9	*p*-Ethylguaiacol	n.d.	3.7 ± 1.4 b	4.3 ± 1.6 b	3.5 ± 1.3 b	4.1 ± 1.6 b	3.8 ± 1.5 b	2.1 ± 0.8 ab	1.4 ± 0.5 a	491.3 ± 187.5 d	36.1 ± 13.8 c
7786-61-0	*p*-Vinylguaiacol	84.1 ± 4.4 b	147.7 ± 7.7 f	118.7 ± 6.2 d	106.4 ± 5.5 c	126.9 ± 6.6 e	131.8 ± 6.9 e	85.2 ± 4.4 b	45.3 ± 2.4 a	187.4 ± 9.8 g	218.4 ± 11.4 h
498-02-2	Acetylguaiacol	30.6 ± 1.6 c	34.0 ± 1.8 c	31.9 ± 1.7 c	25.4 ± 1.3 b	13.7 ± 0.7 a	14.3 ± 0.7 a	31.0 ± 1.6 c	23.3 ± 1.2 b	56.8 ± 3.0 d	58.6 ± 3.1 d
Other											
513-86-0	Acetoin	386.8 ± 24.3 d	1185.6 ± 74.6 e	248.2 ± 15.6 bc	242.5 ± 15.3 bc	274.3 ± 17.3 c	1587.4 ± 99.9 f	170.6 ± 10.7 a	381.2 ± 24.0 d	237.2 ± 14.9 b	1262.7 ± 79.5 e
877-95-2	N-(2-Phenylethyl)Acetamide	136.2 ± 6.2 g	150.6 ± 6.8 h	99.0 ± 4.5 f	82.7 ± 3.7 e	51.2 ± 2.3 d	50.8 ± 2.3 d	19.0 ± 0.9 c	13.6 ± 0.6 a	16.9 ± 0.8 b	16.6 ± 0.8 b
121-33-5	Vanillin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.6 ± 0.2a	3.6 ± 0.3b	n.d.	n.d.

Different letters in the same row indicate significant differences. ANOVA ($p \leq 0.05$) followed by Tukey post hoc test. n.d. Compound not detected. † Acronyms used in this table: Fi is Fiano; Fa is Falanghina; G is Greco; M is Montonico; VB1 is commercial *S. cerevisiae* strain VB1, Oenobrands; Lt is *L. thermotolerans;* Sc is commercial *S. cerevisiae* strain 18-2007 IOC; S21 is native *S. cerevisiae* strain in S21.

The composition of base wine and the choice of yeast strain are the most important factors that influence the sensory quality of sparkling wines, and the qualitative–quantitative nature of volatile organic compounds (VOCs) released by *S. cerevisiae* yeasts is strain-dependent [12,47]. The native *S. cerevisiae* S21 strain was selected for its ability to produce a variety of appreciated VOCs during in-bottle secondary fermentation together with a low production of acetic acid [24]. Indeed, wines made with the S21 strain showed a lower acetic acid content, with the Fiano Vb + S21 wine displaying no detectable acetic acid. The mean value of acetic acid in our sparkling wines was comparable to the levels reported in the literature (0.6–0.9 g/L level) and below the sensitive threshold (about 0.8 g/L of acetic acid) [24]. Acetic acid and ethyl acetate are the main contributors to perceived volatile acidity, and the presence of the latter increases the acidity sensory perception of wines. In all our wines, ethyl acetate was not detected.

All the higher alcohols, esters, and volatile acids that characterized sparkling wines previously obtained with the S21 yeast strain were detected in the samples [24]. The main alcohols previously detected were 3-methyl butanol (or isoamyl alcohol) and phenylethanol, which are the main alcohols also detected in all our sparkling wines. Among ethyl esters of fatty acids, the ethyl lactate and monoethyl succinate followed by diethyl succinate and diethyl malate were the prevalent ones, both in the literature and in our wines. Also, hexanoic and octanoic acids were found as the most abundant carboxylic acids in accordance with results in previous articles.

Concerning differences in the concentration of the main VOCs between S21 wines and those obtained with commercial yeasts from the same variety, S21 wines did not produce peculiar flavor. The differences observed in the volatile composition of wines obtained from the different *S. cerevisiae* yeast strains (commercial or native) appear to be quantitative rather than qualitative, which agrees with previous studies [48,49].

Another reason for the difference with previous works could be attributable to the different grape varieties tested in the previous study (Nero di Troia and Bombino bianco) with the S21 yeasts. Indeed, both https://www.ajevonline.org/content/37/1/84 (accessed on 24 January 2025) the composition of the base wine and the choice of yeast strain could influence the final product. As a recent review underlines, in some cases, the native yeast strains can have a significant impact on the aroma and metabolome of sparkling wines when compared to commercial strains [50]. However, in other cases, the sensory characteristics of the sparkling wines appear to be more influenced by the properties of the base wine than by the yeast strain used [46].

Among the detected VOCs, three metoxy phenols were found. *In our case*, after grape pressing, the musts were clarified using commercial pectinase enzymes (0.01 g/L Rapidase® Clear by DSM, Kaiseraugst, Switzerland). These pectinases possess enzymatic activities that could have transformed cinnamic acids (such as *p*-cumaric and ferulic) into metoxy phenols derivatives [51].

3.5. Odor Activity Value and Principal Component Analysis

All detected VOCs, regardless of their OAV, were included along with the main wine parameters as variables for a Principal Component Analysis (PCA) [52–54]. Supplementary Table S1 shows the CAS number, OAV, OPT, and odor descriptor for all the metabolites identified in this work. Since the data were recorded in different units and exhibited large deviations, a Pareto scaling was applied to give equal weight to each variable before the PCA. Unlike conventional autoscaling, Pareto scaling employs the square root of the standard deviation rather than the standard deviation itself. This approach reduces the impact of large fold changes more than that of smaller fold changes, thereby lessening the dominance of large fold changes in the cleaned data [39]. Additionally, the method used

for handling missing values involved replacing them with the mean of the non-missing values for each variable (metabolite or parameter) [55].

A total of 65.8% variance was explained by the first two PCs, as shown in Figure 4. The plot shows a clear distinction among wines. The loading values were used to understand the chemical differences responsible for the wine's placement in the PCA plot (Figure 5). Loadings range from −1 to 1, with a high absolute value indicating that a variable has a strong influence on that PCA component. The closer to 0 is the variable, the smaller its contribution on that PC. The least appreciated wines (MLtS21 et MLt18) are placed in the second quadrant, which is characterized by high volatile acidity, residual sugar, alcohol content, and pH. Moreover, these wines were rich in VOCs mainly linked to unpleasant aromas (rancid, acidic, medicinal, alcohol) such as acetic acid, isobutyric acid, isovaleric acid, isobutanol, and all three guaiacols detected by GC-MS. Some sweet/floral aromas (vanillin, benzenacetic acid ethyl-9-decenoate, alfa terpineol, epoxylinalol S), a few fruity ones (2,3-butandiol, ethyl lactate, ethyl 3-hydroxybutanoate) and two earthy ones (ethyl pyruvate, epoxylinalol R) also contributed to the positive PC1 and negative PC2. Indeed, in the sensory analysis, these least appreciated wines were lacking in herbaceous, floral, and white fruit notes. Instead, they exhibited notes of ripened fruits and balsamic flavors, which are negatively correlated with pleasantness (Figure 1). The two wines in the third quadrant (MVb18 et MVb21), which were slightly more appreciated than the other two Montonico wines, also showed high pH, alcohol, and residual sugar content. Anyway, these wines had a lower volatile acidity and were characterized by a larger number of VOCs, which included only a few unpleasant ones, like methoxy phenols, among several other pleasant compounds. Despite the differences in VOCs, the other wines that were perceived as more pleasant showed lower alcohol %, residual sugar, lactic acid, methoxy phenols content, and pH. Therefore, higher pH, along with high alcohol % and residual sugar appear to be significant factors contributing to the lack of appreciation for Montonico wines. Among the three varieties that produced the most appreciated wines (Fiano, Falanghina, and Greco), the two Falanghina are the only ones located in the first quadrant (positive PC1). Those wines show a higher volatile acidity and the presence of 9-decenoic acid (waxy-type odor and a waxy-type flavor) in a concentration with higher OAV compared to the Greco and Fiano ones (Supplementary Table S1). Fiano and Greco negative PC1 coordinates indicate the contribution of a large number of VOCs. Among those with higher loading on PC1 and higher OAV, there are the diethylsuccinate, ethylhexanoate, isoamyl acetate, ethyl butyrate, and 3-ethoxy-1-propanol with fruity aromas and the 1-hexanol with herbaceous notes. Indeed, the Greco and Fiano wines obtained a high rating for floral parameters and a low rating for ripened fruit parameters by tasters. Since the least liked Montonico wines received a high rating for ripened fruit parameter, it confirms how this type of aroma is not an appreciated note in sparkling wines. For all wines with positive PC2, testers perceived intense notes of white fruits (both the Falanghina and the Greco wines, as well as Fiano VB1 + 18). However, the positive PC2 is not characterized by molecules that have a distinct floral character; instead, there are cis-3-hexenol (green, bitter, fatty) and 4-methyl 1-pentanol (almond, toasted). Wine aroma results from a combination of various molecules, some volatile and some non-volatile, which can influence each other in a complex way. Therefore, it is challenging to associate "floral" and "fruity" aromas with just a few specific compounds [56]. The strong contribution to the positive PC2 axis of tartaric and malic acids along with their correspondent diethyl esters (diethyl malate and diethyl tartrate), explains the sour taste detected in Greco wines. Indeed, the higher the concentration of tartaric acid and malic acid, the stronger the puckering sensation in the mouth, which leads to increased salivation. Interestingly, a compound that contributes to the negative PC1 axis is tyrosol. This phenolic compound commonly present in wine and formed by yeasts during fermentation possesses a remarkable value due to antioxidant properties and beneficial effects at the cardiovascular level [57].

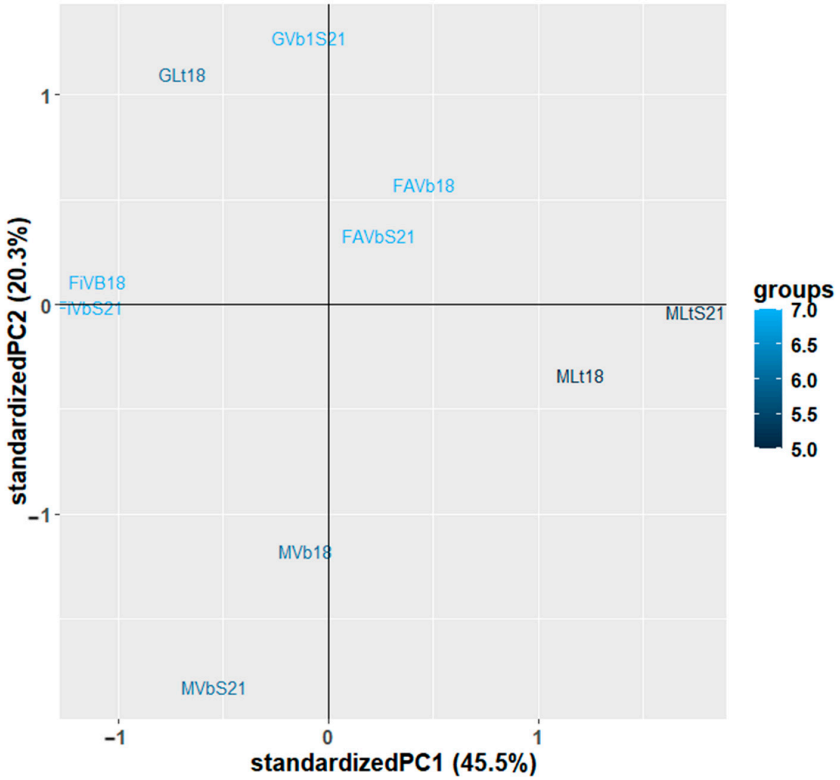


Figure 4. PCA plot of VOC and chemical data in the sparkling wines tested in the sensory analysis colored by perceived pleasantness.

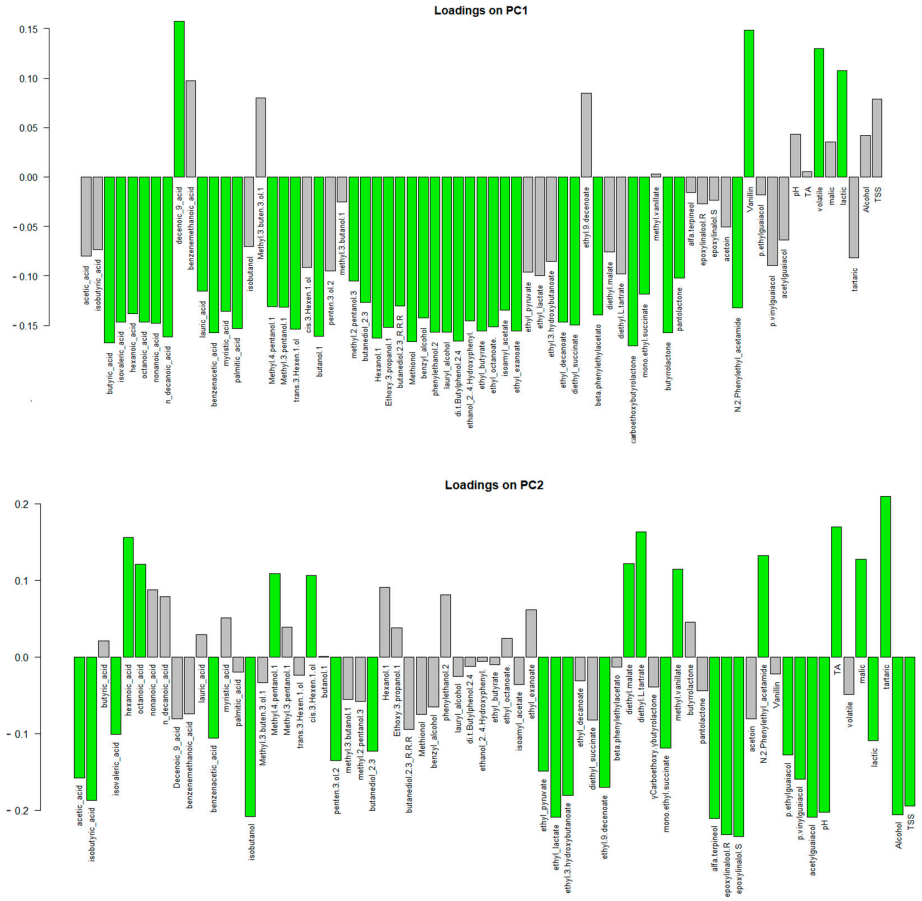


Figure 5. Loadings of the PCA on PC1 (**top**) and PC2 (**bottom**). Green bars represent parameters with a loading > 0.1; instead, gray bars represent parameters with loading below 0.1.

3.6. NIR

A NIR analysis was performed to search for specific regions able to provide insights into the outcomes of the sensory analysis. Unfortunately, it was not possible to analyze Fiano Vb1 + S21 and Falanghina VB1 + 18-2007 wines. However, Fiano VB1 + 18-2007IOC and FalanghinaVB1 + S21 were included as the other two wines of the same variety exhibited similar levels of appreciation in the sensory testing. A spectroscopic data scatter correction, the Standard Normal Variate (SNV) was applied to the NIR data [58]. APCA plot with NIR spectra as variables allowed the selection of the wave numbers that contributed more to the differentiation of the wines, as illustrated in Figure 6. In the plot the PC1 axis is the most important for the differentiation, accounting for 90.3% of the variance. The wines are not grouped by the perceived pleasantness nor by the grape variety. Since CO_2 absorbs in the NIR range, the dissolved gas was removed through sonication in cold water. Ice was added to the water bath to keep the temperature low and prevent heating of the samples. It is possible to hypothesize that the absence of clustering observed in the PCA could be attributed to the removal of CO_2. Indeed, the presence of CO_2 plays an important role in the perceived pleasantness of the wines analyzed.



Figure 6. PCA plot of wines based on NIR spectral data.

In order to better understand the placement of wines in the plot, the wavenumbers with the highest weight on PC1 and PC2 (72 negative and 32 positive values) were selected and were used to build a correlation plot (Pearson correlation) with the sensory outcome (Figure 7). Through the correlation values, it is clearly visible the contribution of specific wavenumbers to selected sensory parameters. The 7324–5292 cm^{-1} range was positively correlated with astringency, a parameter associated with the presence of tannins or procyanidins. These molecules show a broad variety of complex structures [32]. The wavelengths around 7000 cm^{-1} and 5300 cm^{-1} correspond to the first overtones of COH

groups, the first overtones of CH_2 and CH_3 groups, all functional groups compatible with tannins structure [54].

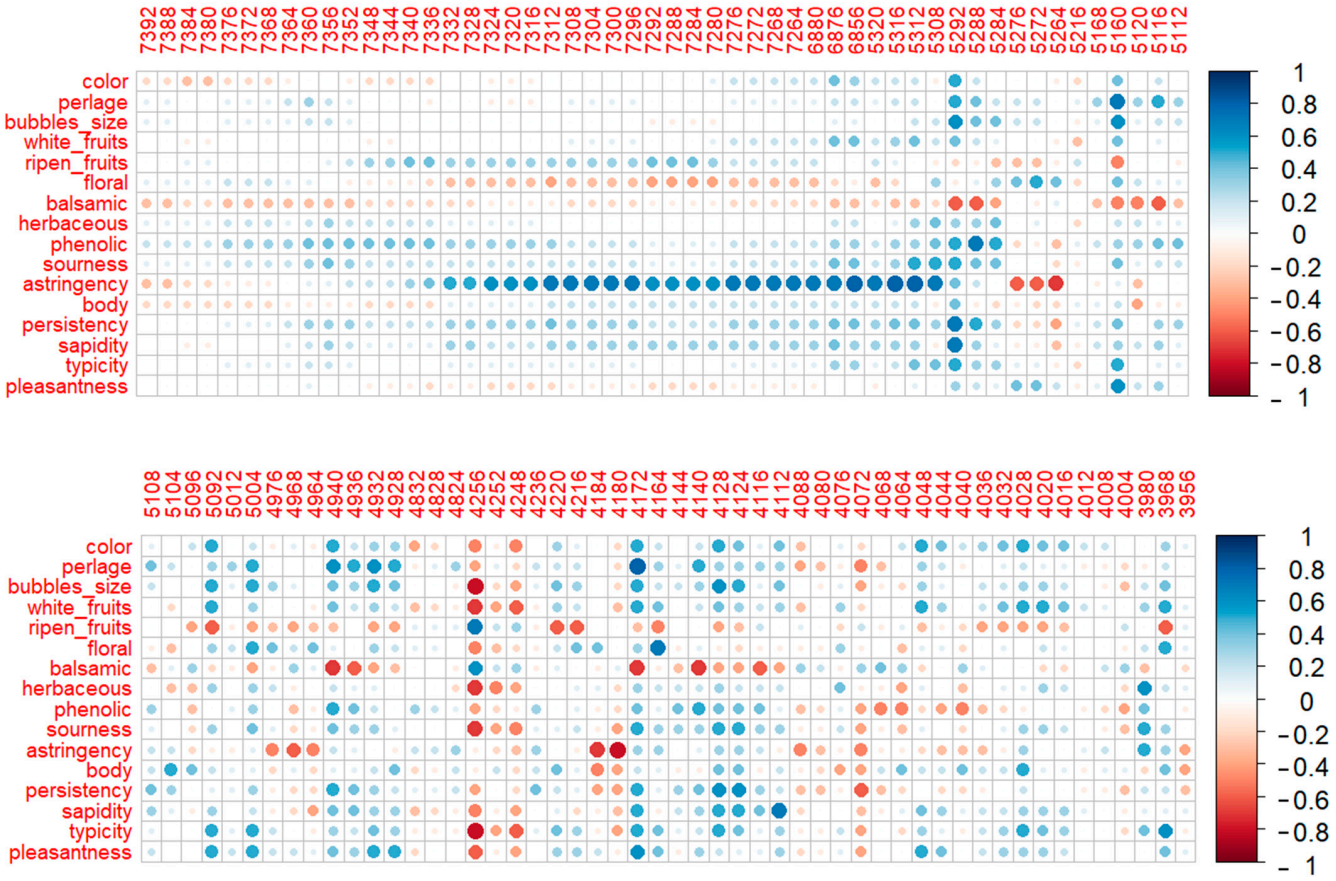


Figure 7. Correlation plot among sensory and NIR data. The plot has been split in half to ease its readability: correlation with the first 52 wave numbers (**top**) and correlation with the other 52 wave numbers (**bottom**).

The 5292, 5288, 5116, 4940, 4936, 4172, 4144, 4140, and 4116 cm^{-1} wave numbers that are associated with the first overtones of CH_2 and CH_3 groups, and COH combination (stretching and bending) vibrations [54], were highly negatively correlated with balsamic. A few wavelengths were highly correlated with several parameters: 5292, 5160, 4256, 4172, and less 4940, 4128, and 4124 cm^{-1}, which, again, can be attributed to the vibration of several chemical groups. Attributing specific NIR peaks to individual molecules is not straightforward, and this attribution can sometimes be misleading. The peaks that differentiate the samples may be associated with the vibrations of molecular bonds in compounds that are not directly linked to the measured parameters. Instead, these peaks could result from interactions with surrounding compounds. Unfortunately, the limited number of samples available makes it impossible to conduct a reliable prediction or classification analysis.

4. Conclusions

In this work, novel sparkling wines using native grape varieties typically used for white wine production, in combination with native yeasts were produced and analyzed. Novel product acceptance was tested showing how two varieties, Fiano and Falanghina, produced sparkling wines well appreciated with pleasant floral and white fruit notes. In contrast, wines primarily characterized by flavors of ripe fruits and balsamic notes did not receive as much appreciation. Visual characteristics, such as color, size, and the persistence of CO_2 bubbles, also contributed to the positive evaluation of the wines.

Unfortunately, the native yeast strains tested did not show significant differences compared to commercial yeasts in terms of the variety of volatile compounds produced; instead, they mainly affected the relative amounts of specific VOCs. This work shows how the use of classic grape varieties in combination with native yeasts allows for the creation of various alcoholic products that retain a recognizable identity while being more in tune with evolving purchasing trends.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/beverages11010025/s1, Table S1: Aroma compounds identified in the sparkling wines with their odor activity value (OAV), odor perception threshold (OPT), and odor descriptor [59–67].

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