

Innovative sparkling wines, traditional grape varieties and autochthonous yeasts: emerging trends for regional products diversification

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Abstract. Italy, like all the major vine-growing and wine-producing countries, has experienced a decline in wine export volumes in recent years. The only wine-derived products that continue to perform well in the global market are sparkling wines. To meet consumer preferences, we have developed new sparkling wines utilizing typical Italian grape wine varieties combined with selected autochthonous yeasts. The selection of yeasts from regional vineyards aims to enhance the wine's perceived "identity," which is associated with the concepts of heritage and terroir. These sparkling wines allow producers to take advantage of the traditional grape varieties already grown in their vineyards, preserving the unique character of the growing area while also meeting consumer demand. The wines have been analyzed using conventional methods to evaluate key parameters such as pH, titratable acidity, volatile acidity, alcohol content, and reducing sugar. Additionally, an aromatic profile analysis was conducted using GC-MS techniques. Sensory analysis has also been performed to assess wine acceptance. The positive reception of these novel sparkling wines with pleasant floral and white fruit notes was linked to their chemical composition, providing valuable insights for the future production of similar innovative products.

1. Introduction

The Apulia region in Southern Italy is well-known for its wine production. Thanks to its favourable Mediterranean climate, a wide variety of grape varieties are cultivated in this area. Currently, the region is most famous for its red, full-bodied wines made using traditional winemaking methods [1]. Nonetheless, changing consumer attitudes have impacted the popularity of traditional Apulian wines. A steady or increasing sales of other alcoholic products, such as beer and fruit-flavoured spirits, indicates a shift in consumers' choice of alcoholic beverages [2]. The only wine-derived product that continues to have strong global sales is sparkling wines [3]. To adapt to changing consumer preferences, we have developed new sparkling wines using typical Italian grape wine varieties and selected indigenous yeasts. By choosing yeasts from regional vineyards, we aim to enhance the wine's perceived "identity," which is closely related to the concepts of heritage and terroir [4]. These sparkling wines enable producers to leverage traditional grape wine varieties already cultivated in their vineyards, preserving the unique character of the growing area while

satisfying consumer demand. We analyzed the wines using conventional methods to evaluate key parameters such as pH, titratable acidity, volatile acidity, alcohol content, and reducing sugars. A sensory analysis was performed to determine wines' favorable traits and preference. Moreover, the aromatic profile was assessed with GC-MS. The favorable acceptance of these novel sparkling wines, which were found to be characterized by pleasant floral and white fruit notes, provides valuable insights for the future production of similar products.

2. Materials and Methods

2.1. Grape composition, basic and sparkling wines production

The grapes were harvested on 30 August 2022 from an experimental vineyard grown under conventional farming conditions belonging to CREA Research Centre for Viticulture and Enology (CREA-VE) located in Southern Italy (Apulia region). Two traditional Italian white grape wine varieties of Southern Italy were harvested, namely

Antinello and Bombino bianco. The titratable acidity (TA) by titration with NaOH and pH were measured on grape with a Crison Basic 20 pH. The total soluble solids (TSS) were determined in Brix degrees with a digital refractometer, Atago PR1 (Atago Co., Tokyo, Japan). The sparkling wines were produced using the Champenoise method. This choice was made based on recent findings that highlighted how consumer expectations can significantly influence product preferences. Sparkling wines labeled as produced using the traditional Champenoise method were usually preferred over others [5]. Base wines were produced in the experimental winery of the CREA-VE of Turi on a pilot scale. For each variety, 100 kg of grapes were inoculated with a commercial *Saccharomyces cerevisiae* strain (VB1, Oenobrand) to produce the base wines. Base wine's chemical/physical characteristics were determined on wines at racking with a multiparametric enzymatic analyzer (Hyperlab Smart, Steroglass, Italy). The base wines were supplemented with the selected yeasts (0.2 g/L) for the secondary fermentation and added with a sugar solution (24 g/L sugarcane) until reaching a stable pressure (5–6 bar) at a low temperature (10–15 °C). The yeast inoculated were either a commercial *S. cerevisiae* strain (18-2007 IOC) or a native *S. cerevisiae* strain (S21). The native S21 yeast strain was previously collected from grape growing in North Apulia vineyards and was selected as a starter culture for its aptitude to carry on in-bottle secondary fermentation to produce white and rosé sparkling wines [6]. Basic parameters were measured on sparkling wines after the *dégorgement* (removal of yeast sediment from bottles) performed prior to the sensory analysis. These parameters were measured following the same procedures used for basic wines. A total of thirteen bottles for each variety were bottled.

2.2. GC-MS analysis

The CO₂ was removed from the sparkling wines by immersion of the samples in an ultrasonic bath. Then 50 mL of the degassed samples were added first with NaCl (50 g/L) and after with 250 µL of the 2-octanol (CAS 4128-31-8) internal standard solution (8.20 mg/L in dichloromethane). After two extractions in a separating funnel (5 mL of CH₂Cl₂, extraction period of 20 min each), 10 mL of the organic phase was dried over anhydrous Na₂SO₄ and filtered on a 0.2 µm Nylon filter. The sample was concentrated in a roto-evaporator to 1 mL and then injected into 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. The GC/MS analyses were performed following a previously reported procedure [7]. The identification of the detected compounds was achieved by comparisons with mass spectra presented in the NIST MS library Database (2017) or from the literature. The 2-octanol was used as an internal standard, with concentrations of the detected compounds expressed in µg/L.

For each detected compound, the odor activity value (OAV) was calculated as the ratio of each compound's concentration in the sample to its odor perception threshold (OPT) [8]. OAV is conventionally used to assess the contribution of different detected compounds to the perceived aroma in a simple and straightforward way without taking into consideration interactions with other aromatic substances and matrix complexity, factors that can mask or increase aroma compounds's OPT. Among the available OPTs to calculate the OAV, we used those recorded in matrices as close as possible to wine. Anyway, since such an OPT is not available in the literature for all the detected compounds, we decided to take into consideration all the compounds, even those with OAV below 1 [9,10].

2.3. Sensory analysis

Five experienced wine judges selected the sensorial descriptors to characterize the wines prior to the sensory evaluation, which was performed after 18 months of aging on wines lees at 10 °C.

The attributes characterizing the wines for aroma and flavor by mouth included:

- Fruity : as white fruits and ripened fruits;
- Floral: Notes that resemble flowers.
- Balsamic: Aromas reminiscent of balsamic vinegar.
- Herbaceous: Herbal scents.
- Phenolic aromas: These are compounds that might impart unique scents and flavors.
- Sourness: A flavor descriptor indicating acidity.
- Astringency: This relates to the presence of tannins or procyanidins that create a drying sensation.
- Body: This refers to the mouthfeel, which can be influenced by the wine's texture and alcohol content.
- Persistency: The duration that the initial taste sensation lasts on the palate.
- Sapidty: A sensation of minerality due to dissolved mineral substances.
- Typicity: The extent to which a wine reflects its varietal origins and the specific grape variety used.
- Pleasantness: Overall enjoyment of the wine.
- Color: This is relative to intensity and tone, referring to a moderately pale straw yellow as usual for Champenois products.
- Perlage: The presence and persistence of bubbles.
- Bubble size: The dimension of the bubbles as perceived visually and orally.

All of these attributes were rated on an intensity scale from 1 to 10, where 1 indicates absent or negative while 10 signifies intense or excellent.

A total of four sparkling wine types were tested: Bombino S21, Bombino IOC, Antinello S21, and Antinello IOC. The panel was composed of 14 experienced tasters already familiar with the testing procedure, with at least three years of experience working in the wine industry: sommeliers, wine professionals, winemakers, grape growers and personnel at CREA-VE (3

females and 11 males with a mean age of 49.3 ± 11.4). All of the tasters were considered experts based on the criteria by Parr et al. [11]. The tasting was conducted at 18 °C under natural light pouring 50 mL of each wine at 10 °C in 125 mL ISO wine glasses, labeled with three-digit random numbers, and covered with plastic disk. Wines were presented to the assessors simultaneously and anonymously using a coding system. This approach aimed to eliminate the effects of the order in which the samples were presented and reduce any potential biases that might arise from initial impressions. Assessors were encouraged to taste the wine samples multiple times if they wished; however, they were required to provide a response for each sample (forced choice) [12]. The mean scores for all attributes were then analyzed using Quantitative Descriptive Analysis (QDA).

2.4. Statistical Analysis

The statistical procedures, including analysis of variance, post hoc analysis, and calculation of Pearson correlation coefficient (R packages corrplot [13]), were performed using R Statistical Software (v4.4.2; R Core Team 2024, R Foundation for Statistical Computing, Vienna, Austria) [14].

3. Results and Discussion

3.1. Grape and wine chemical composition

Grapes were harvested before reaching technological maturity, as usual for grapes to be used in the production of sparkling wines. The standard basic parameters of the grapes, recorded in duplicate, are reported in Table 1.

Table 1. Grape basic parameters.

Variety	TSS (Brix)	TA (g/L)	pH
Antinello bianco	16.2 ± 1.0	9.9 ± 0.3	3.13 ± 0.10
Bombino bianco	16.7 ± 1.0	10.2 ± 0.4	3.01 ± 0.10

The base wines chemical composition was in line with that of conventional wines: Antinello bianco (alcohol 11.0–12.5 % vol, pH 3.10 – 3.35, TA 6 – 8 g/L), and Bombino bianco (11. – 12.5 % vol, pH 3.15 – 3.40, TA 5.5 – 7.0 g/L) [15]. No differences were found among the four sparkling wines in terms of pH, total or volatile acidity, and tartaric acid content (Table 2). Antinello wines showed a significantly higher malic acid content and, consequently, a smaller lactic acid content. Bombino wines were characterized by a higher alcoholic content compared to the Antinello wines. Anyway, the alcohol content of all the wines was in line with the amount generally found in sparkling wines, which ranges from 10.5% to 12.5% alcohol volume. Residual sugars were found in small amounts in all the wines except for Bobino S21 and Antinello IOC. The high levels of residual sugars in Bobino S21 wine could be attributed to a not ineffective secondary fermentation with S21 yeasts. Instead, it performed an effective secondary fermentation in

Antinello S21. Based only on the chemical base parameters, both varieties seem promising for sparkling wine production.

Table 2. Sparkling wine's chemical composition.

Parameter	Bombino S21	Bombino IOC	Antinello S21	Antinello IOC
pH	3.05 ± 0.08	3.03 ± 0.10	3.00 ± 0.07	3.03 ± 0.1
TA g/L	6.5 ± 0.3	6.1 ± 0.3	6.0 ± 0.3	6.2 ± 0.3
Volatile acidity g/L	0.39 ± 0.04	0.40 ± 0.05	0.40 ± 0.04	0.35 ± 0.03
Malic acid g/L	0.89 ± 0.12 a	0.88 ± 0.11 a	2.20 ± 0.11 b	2.40 ± 0.11 b
Lactic acid g/L	0.08 ± 0.01 b	0.07 ± 0.01 b	0.06 ± 0.01 ab	0.04 ± 0.01 a
Tartaric acid g/L	2.96 ± 0.2 c	2.87 ± 0.1 a	2.93 ± 0.1 b c	2.91 ± 0.1 b
Alcohol %vol	11.6 ± 0.4 a b	12.0 ± 0.5 b	10.4 ± 0.6 a	11.0 ± 0.5 ab
Residual sugars g/L	10.5 ± 0.1 d	6.5 ± 0.1 b	2.0 ± 0.1 a	9.6 ± 0.1 c

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

Concerning the sensory analysis, all four wine types were scored between 6 and 7 for overall pleasantness, showing a good appreciation of these novel products (Figure 1).

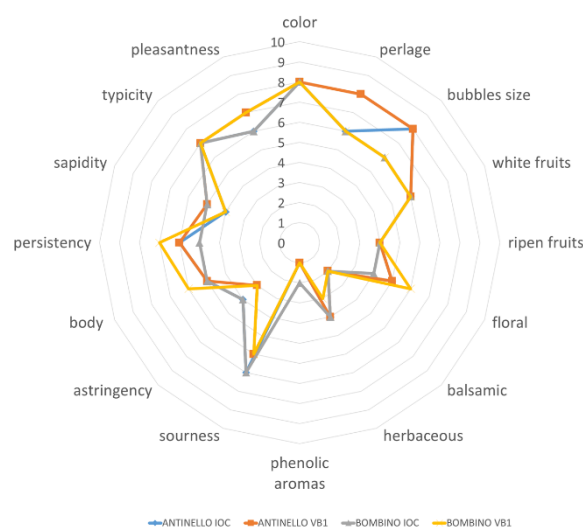


Figure 1. Quantitative Descriptive Analysis.

For both varieties, the wines produced with native yeasts scored a higher pleasantness. Concerning the single descriptors, all wines were characterized by high scores for floral and white fruits, and conversely low scores for ripen fruits, phenolic, balsamic, and herbaceous. The main difference was not in the aroma notes, since all wines showed more or less intense floral and white fruit notes. To better understand the relationship among the parameters we calculated the Pearsons Correlation coefficients (Figure 2).

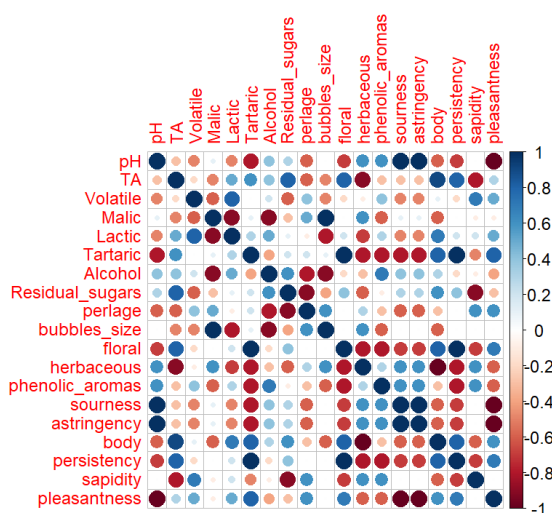


Figure 2. Correlation plot

The pleasantness is highly negatively correlated with high sourness and astringency, and in a lesser extent with herbaceous and phenolic aromas, as well as high alcoholic content and pH. A positive correlation was found instead for tartaric and lactic acid as well as for perlage, floral notes, body and persistency. Perhaps the main reason for the preference towards S21 wines was the lack of floral notes and presence of phenolic ones, as well as the sourness, lack of persistency, and less visible perlage of the IOC wines. Among the two most appreciated S21 wines, the sensory analysis showed a slight preference for the sparkling wine produced with Antinello grape in combination with the native S21 *S. cerevisiae*, which obtained the highest scoring for all the favorable parameters, including also the visual ones (perlage and bubble size).

3.2. GC/M profile

The volatile composition of the most appreciated sparkling wine, Antinello S21, is shown in Table 3 alongside each compound's CAS number, OAV, OPT, and odor descriptor. Among the 55 volatile metabolites detected and quantified, they were identified based on their chemical structure as: 18 alcohols, 13 carboxylic acids, 13 esters, 2 terpenoids, 3 lactones, 3 methoxyphenols, and 3 others (aldehydes, ketones, and amides).

Table 3. Concentration of major volatile compounds of Antinello S21 with OAV, OPT, and odor descriptor

Compound (CAS)	Concentration (µg/L)	OAV	Odor descriptor	OPT ‡ (mg/L)
Carboxylic acids				
Acetic acid (64-19-7)	3124.4 ±500.3	<1	Vinegar [16]	200mg/L [16] [a]
Isobutyric acid (79-31-2)	280.3 ±17.2	<1	Rancid, butter, cheese, pungent [17]	30 mg/L [17] [b]

Butyric acid (107-92-6)	315.4 ±32.5	<1	Rancid, cheese [16]	10mg/L [16] [a]
Isovaleric acid (503-74-2)	425.4 ±25.6	<1	Rancid, acidic [16]	3mg/L [16] [a]
Hexanoic acid (142-62-1)	2152.3 ±22.3	1	Cheese, fatty [18]	3 mg/L [18] [b]
Octanoic acid (124-07-2)	2229.2 ±255.1	<1	Fatty, rancid [18]	10 mg/L [18] [b]
Nonanoic acid (112-05-0)	12.3 ±2.4	<1	Fatty [19]	3 mg/L [19] [a]
n-decanoic acid (334-48-5)	345.2 ±40.7	<1	Fatty, rancid [18]	6 mg/L [18] [b]
9-decenoic acid (14436-32-9)	150.1 ±15.8	4	Waxy, fatty, soapy [19]	0.040 mg/L [19] [a]
Benzoic acid (65-85-0)	18.9 ±3.2		n.f.	n.f.
Benzenacetic acid (103-82-2)	67.3 ±13.1	<1	Honey, pollen, roses [19]	1mg/l [19] [a]
Myristic acid (544-63-8)	10.2 ±1.2	<1	Waxy, fatty, soapy [19]	10mg/L [19] [c]
Palmitic acid (57-10-3)	133.7 ±23.1	<1	Waxy, fatty [19]	10mg/L [19] [c]
Alcohols				
n-Butanol (71-36-3)	35.5 ±12.1	<1	Medicinal [18]	150mg/L [18] [b]
Isobutanol (78-83-1)	2294.6 ±196.1	<1	Alcohol, nail polish [18]	75 mg/L [18] [b]
3-methyl-1-butanol (123-51-3)	17005.8 ±755.4	<1	Solvent [18]	60mg/L [18] [b]
4-methyl-1-pentanol (626-89-1)	16.4 ±1.3	<1	Almond, toasted [19]	50 mg/L [19] [a]
2-methyl-3-pentanol (565-67-3)	16.6 ±1.1		Ethereal [20]	n.f.
1-Hexanol (111-27-3)	666.0 ±50.3	<1	Herbaceous, grass, woody [18]	1.1 mg/L [18] [b]
(E)-3-hexen-1-ol (928-97-2)	9.6 ±1.0		Green, grassy [20]	n.f.

(Z)-3-hexen-1-ol (928-96-1)	162.2 ±4.3	<1	Green, bitter, fatty [18]	1 mg/L [18][b]
3-penten-2-ol (1569-50-2)	2.4 ±0.3		n.f.	n.f.
2,3-butanediol (R,S) (513-85-9)	2678.3 ±351.3	<1	Fruity [17]	150 mg/L [17][b]
3-ethoxy-1-propanol (111-35-3)	41.1 ±4.0	<1	Fruity [19]	0.100 mg/L [19][a]
2, 3-butanediol [R-(R*,R*)] (513-85-9)	541.9 ±39.9	<1	Fruity [17]	150 mg/L [17][b]
3-(Methylthio)-1-propanol (505-10-2)	485.7 ±39.7	1	Cooked vegetable [19]	0.500 mg/L [19][a]
Benzyl alcohol (100-51-6)	12.0 ±0.6	<1	Sweet, fruity [19]	200 mg/L [19][a]
2-phenyl ethanol (60-12-8)	26167.3 ±1323.2	<1	Rose, honey [18]	200 mg/L [18] [b]
Lauric alcohol (112-53-8)	241.4 ±20.3	<1	Unpleasant (high concentration), flowery (low concentration) [17]	1 mg/L [17] [b]
2,4-di-t-Butylphenol (96-76-4)	363.7 ±52.0	1	n.f.	0.5 mg/L [22] Solvent unknown
2-(4-Hydroxyphenyl)ethanol (501-94-0)	2552.8 ±368.2		n.f.	n.f.
Esters				
Ethyl butyrate (105-54-4)	100.0 ±20.1	<1	Strawberry, apple, banana [18]	0.4 mg/L [18] [b]
Ethyl octanoate (106-32-1)	155.4 ±31.7	<1	Sweet, floral, fruity, banana, pear, brandy [18]	0.58 mg/L [18] [b]
Isoamyl acetate (123-92-2)	52.4 ±10.3	<1	Banana, fruity, sweet[18]	0.16 mg/L [18] [b]

Ethyl hexanoate (123-66-0)	145.1 ±13.7	2	Fruity, green apple,banana, brandy, wine-like [18]	0.08 mg/L [18] [b]
Ethyl pyruvate (617-35-6)	568.7 ±5.9		Herbaceous, oil painting, forage [21]	n.f.
Ethyl Lactate (97-64-3)	10759.4 ±154.3	<1	Fruity, buttery [18]	150 mg/L [18] [b]
Ethyl 3-hydroxybutanoate (5405-41-4)	37.5 ±153.2	<1	Fruity [25]	14 mg/L [25]
Ethyl decanoate (110-38-3)	24.6 ±4.2	<1	Brandy, fruity, grape [18]	0.5 mg/L [18] [b]
Diethyl succinate (123-25-1)	9384.3 ±2021.0	8	Fruity, melon [18]	1.2 mg/L [18] [b]
Ethyl 9-decenoate (67233-91-4)	7.6 ±1.7	<1	Rose [19]	0.100 mg/L [19][a]
Diethyl-DL-malate (626-11-9)	6335.6 ±827.9		n.f.	n.f.
(+)-Diethyl-L-tartrate (87-91-2)	1022.9 ±321.5		n.f.	n.f.
Ethyl hydrogensuccinate (1070-34-4)	24234.9 ±5323.1		n.f.	n.f.
Terpenes				
alfa terpineol (98-55-5)	5.8 ±0.6	<1	Liliac, flora, sweet [18]	1mg/L [18] [b]
Epoxylinolol (14049-11-7)	2.6 ±0.1		Earthy [24]	n.f.
Lactones				
Butyrolactone (96-48-0)	1301.8 ±18.1	<1	Caramel, sweet [18]	20mg/L [18] [b]
D-(-)-pantolactone (599-04-2)	53.5 ±10.0		n.f.	n.f.
5-Oxotetrahydrofuran-2-carboxylic acid, ethylester (1126-51-8)	1127.9 ±109.7		n.f.	n.f.
Methoxyphenols				

p-ethyl-guaiacol (2785-89-9)	56.4 ±1.6	2	Medicine, wood, clove, smoke [19]	0.033 mg/L [19] [a]
p-vinyl-guaiacol (7786-61-0)	54.2 ±2.3	1	Spices, curry [19]	0.040 mg/L [19] [a]
Acetyl-guaiacol (498-02-2)	45.5 ±2.0		n.f.	n.f.
Miscellanea				
n-(2-phenylethyl)acetamide (877-95-2)	74.9 ±3.2		n.f.	n.f.
Acetoin (513-86-0)	38.1 ±5.6	<1	Buttery, cream [18]	150 mg/L [18] [b]
Vanillin (121-33-5)	9.7 ±0.5	<1	Vanilla	0.2 mg/L [23] [d]

‡ Olfactory perception threshold (OPT) determined in [a] in 10–12% (v/v) ethanol, [b] 10% (v/v) ethanol solution adjusted to pH 3.5 with tartaric acid, [c] water, [d] synthetic wine. n.f. value not found in the literature.

The aroma profile is a combination of varietal aromas from the grape variety, together with fermentative (created during the fermentation processes) and post-fermentative aromas (during the aging period) which are produced depending on the yeast strain employed [26]. In particular, the native *S. cerevisiae* S21 strain was selected for its low production of acetic acid [6]. Indeed, in Antinello S21, acetic acid was below the reported aroma threshold in wine [7]. Not only was acetic acid, the main contributor to volatile acidity, low, but also ethyl acetate, a compound that increases the perceived volatile acidity, was not even detected.

It was previously reported that the *S. cerevisiae* S21 strain is capable of producing several favourable aroma compounds in sparkling wines during the in-bottle secondary fermentation [6]. Among the compounds already detected in sparkling wines produced with the S21 strain, several alcohols such as 3-methyl butanol (or isoamyl alcohol), hexanol, 3-hexenol, phenylethanol, and methionol we detected in Antinello S21. All these compounds contribute to the aroma with herbaceous notes, except for the sweet and flowery notes of phenylethanol. Among the esters of fatty acids, the prevalent class of compounds found was ethyl esters. Compounds like ethyl octanoate, isoamyl acetate, ethyl lactate, ethyl decanoate, ethyl 9-decenoate, and two with AOV>1, namely ethyl hexanoate and diethyl succinate, were the prevalent ones, both in the literature and our wines. All these molecules contributed to perceived sweet/fruity/floral aromas. Linear carboxylic acids with 7 to 9 C atoms were found, in accordance with results in previous articles, together with the 9-decenoic acid, all contributing to a fatty-waxy flavour. An explanation for the presence of

methoxyphenols in these sparkling wines is the procedure followed for the must clarification after grape pressing. Indeed, the commercial pectinase enzymes (0.01g/L Rapidase® Clear by DSM) used possess enzymatic activities. These enzymes could have modified cinnamic acids (such as p-cumaric and ferulic) in methoxy phenols derivatives as previously reported. Despite the attribution of odor descriptors to specific compounds, it could be misleading to simply associate "floral" or "fruity" aromas with just a few volatile molecules since the perceived aroma results from a complex interaction of several compounds present in the wine [27]. For sparkling wines, not only may some non-volatile molecules mask aroma compounds of interest, but also the dissolved CO₂ could modify other molecules OPTs [28].

4. Conclusion

The aim of our investigation was to combine classic Apulian grape wine varieties with autochthonous yeasts selected from the same vineyards to create sparkling wines with distinctive flavors that reflect their regional origins, linking the product to its territory. Indeed, since the qualitative and quantitative nature of aroma compounds released by *S. cerevisiae* yeasts in wine is strain-dependent, in our study, we chose to use indigenous yeast strains for wine production rather than commercial ones. The results of this first year of investigation show how both the traditional Italian white grape wine varieties used were suitable for producing novel sparkling wines. Although there was only a slight preference for the wines made with native *S. cerevisiae* yeast, this suggests that further investigation could be valuable. We may consider using other native yeast strains to enhance different aroma profiles and strengthen the connection to the terroir, or use other grape wine varieties that are experiencing a decline in consumer favorability. Due to the fast pace of innovative products released on the market, proposing novel sparkling wines with an identity could be a strategy to cope with the sector crisis.

5. Funding

This research was funded by REVINE Project REVINE “Regenerative agricultural approaches to improve ecosystem services in Mediterranean vineyards” founded within PRIMA - Partnership for research and innovation in the Mediterranean area (Italian MUR DM no. 1966/2021, Project ID 20114-2, PRIMA/0011/2020) and Spumantizzazione E Frizzantatura dei vini Pugliesi, SPUMAPULIA-P.S.R. Puglia 2014/2020, Misura 16-Cooperazione, Sottomisura 16.2. D.A.G. n°194 12 September 2018, published in BURP n°121 20 September 2018.

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