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Characterization of Nero Antico di Pretalucente Wine and Grape Fungal Microbiota: An Expression of Abruzzo Region Cultivar Heritage

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Abstract: The aim of this study was to characterize the ampelographic and genetic profiles of *Vitis vinifera* L. cv. Nero Antico di Pretalucente and to describe the grape-borne fungal communities. The oenological characteristics and the aroma profile of wine obtained by spontaneous fermentation were also investigated. Microsatellite profiles and ampelographic traits indicated that this cultivar presented a unique profile, and therefore it can be considered a cultivar in its own right and autochthonous of Gessopalena village. Next-generation sequencing analysis revealed that *Aureobasidium* spp. was the main genus detected on grapes. At the species level, *Aureobasidium pullulans* was the main species, followed by *Alternaria alternata*. Wines were characterized by a final ethanol content of 12.75% (*v*/*v*), a pH of 3.4, a volatile acidity lower than 0.6 g/L, a content of glycerol of 8.56 g/L, and a concentration of polyphenols and anthocyanins of 977 GAE/L and 266 mg/L, respectively. The intensity and tonality of the wine as well as the active odor compounds found were described. The results obtained could improve the knowledge concerning the agronomic traits and the wine obtained from this ancient and autochthonous grapevine variety cultivated in a foothill area, in order to offer consumers a wine with unique traits.

Keywords: Nero Antico di Pretalucente wine; grape-borne fungal communities; microsatellite profile; ampelographic traits

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1. Introduction

The domestication of the grapevine (*Vitis vinifera* L.) happened in the sixth millennium B.C.E. in the region comprising Eastern Anatolia, the South Caucasus, and Western Asia and was likely concurrent with the development of winemaking techniques based on wild grapes and other luscious fruits [1]. In recent years, developments in the wine market have resulted in a decline in wine consumption in European nations, despite a significant desire for wines that meet health standards and satisfy the senses. Market trends show that consumers are attracted to grapes and wines that are strictly related to the production area [2]. Minor *Vitis vinifera* L. grape varieties that are typical of a certain geographical area seem to be an interesting option to increase sales as they link the product to the territory of origin and differentiate the obtained wines from others, because they are not found anywhere else [2]. Furthermore, the exploitation of minor local varieties may be ecologically interesting and useful in increasing environmental biodiversity. Due to the changing marketing techniques of wine firms and the shifts in wine consumption patterns, some grape types that had been neglected by the wine industry for decades have reemerged in recent years, enabling their economic and social rehabilitation [2]. Local varieties are well

adapted to the environment and allow for more sustainable viticulture. In addition, these varieties could have a selective advantage because, for example, they are able to cope with the climatic changes in their environment due to global warming [3]. The preservation of grapevine biodiversity and genetic variety is unquestionably one of the primary objectives of wine research [4].

The Italian National Catalogue of Grapevine Varieties lists approximately 610 *Vitis vinifera* L. cultivars used for winemaking in a country devoted to viticulture [5]. Occasionally, the prevalence of synonyms (multiple names for the same genotype) and homonyms (identical or very similar cultivar names but different genotypes) is one of the primary problems in contemporary viticulture. Simple sequence repeat (SSR) markers can be used to identify synonyms and homonyms and to analyze the parentage of locally or widely cultivated grapevine varieties in Italy. The characterization of genotypes, together with the study of morphological features, represents a useful tool to clarify synonyms and homonyms, detect the origin of species, and eventually describe new cultivars [6]. According to the regional vineyards register, the Abruzzo region currently has 33,319 hectares of vineyards, principally located in the province of Chieti (23,355 hectares), and in total represents 6% of the total Italian wine production [7]. The wine-growing area in the Abruzzo region is concentrated in the coastal or inland hilly areas (96%). In the mountainous areas of the Abruzzo Apennines, the vineyard area is around 4% [7] and represents a precious reservoir of biodiversity. The main grape varieties present in the Abruzzo region are Montepulciano, Trebbiano Abruzzese, Passerina, Montonico, and Pecorino, among others.

Vitis vinifera L. cv. Nero Antico di Pretalucente is one of these minor cultivars. It is a black grape variety located in ancient vineyards in the Gessopalena and Torricella Peligna villages (Chieti, Italy). The vineyards are at altitudes between 600 m and 900 m above sea level, and grapes are harvested at the end of October. They are called "uva nera antica" (ancient black grapes) by people inhabiting these areas. The word "Pretalucente" refers to the large chalk boulder on which the medieval core of the village of Gessopalena was built and which is characterized by the presence of large selenite crystals that reflect sunlight. Grapes were used by old farmers not only for winemaking but were also stored in houses, hanging from the ceiling, to be consumed during the Christmas holidays. The oenological potential of this cultivar is unknown, and no data are available concerning the fungal communities associated with grapes or how these could influence the characteristics of the wine obtained. Phenotypic and genotypic characteristics were evaluated through an ampelographic study of the *Vitis vinifera* L. cv. Nero Antico di Pretalucente shoots, mature leaves, bunches, berries, and seeds, while an international set of nine SSR markers was employed for its genetic characterization. Furthermore, the fungal communities of *Vitis vinifera* L. cv. Nero Antico di Pretalucente grapes were described using next-generation sequencing technology. The oenological characteristics and aroma profile of the wine produced through spontaneous fermentation were also investigated.

2. Materials and Methods

2.1. Sampling Site

The accession samples for the ampelographic, molecular, and oenological studies were collected in an 18-year-old vineyard located in Gessopalena, Chieti, Abruzzo Region, Central Italy (42°03′22″ N, 14°16′46″ E; 660 m a.s.l.) on a clayey and sandy soil type. The vines were ungrafted, planted with 3.5 × 1.5 m inter- and intra-row spacing, and trained to a classic or bilateral Guyot system. The vineyard was approximately 0.5 ha in size and hosted only the Nero Antico grape variety. The vineyard was managed as organic in accordance with Reg. EC 834/2007 [8], EC 889/2008, and EC1235/2008 [9]. In particular, pest management was achieved only through copper/sulfur-based products.

2.2. Ampelographic Descriptions

The morphological characterization was made during the 2019, 2020, and 2021 growing seasons. The young shoots, leaves, bunches, and berries were evaluated using 19 descriptors

which were selected for their power of discrimination and objectivity, according to the International Organization of Vine and Wine descriptors [10]. The OIV descriptors evaluated were: OIV codes 001, 002, 004, 006, 007, 008, and 016 for shoots; 051 for young leaves; 065, 067, 068, 069, and 080 for mature leaves; 202, 204, and 208 for bunches; 223 and 225 for berries; and 241 for seeds (Table S1).

2.3. Yield and Grape and Berry Characteristics at Harvest

In all of the years of the trial, grapes from 50 vines were individually picked, and the crop weight and bunch number per vine were recorded. The average bunch weight was calculated, and berry fresh weight and number of berries per bunch were measured. Finally, the percentage of skin by weight was also assessed.

2.4. Plant Material Sampling

Plant material was collected in May 2021. Young leaves were collected from different rows using sterile scissors and immediately placed on dry ice for transport to the laboratory, where they were quickly immersed in liquid nitrogen and stored at −80 °C until analysis. Grape samples of *Vitis vinifera* L. cv. Nero Antico di Pretalucente were collected aseptically in September 2021. Three sampling points were identified at distal spatial points of different rows, where samples of grape berries were collected and processed independently (representing three biological replicates). Healthy and undamaged grapes (about 1 kg) were collected using sterile scissors and placed in sterile plastic bags, transported to the laboratory in refrigerated boxes, and processed within 12 h. To prevent cross-contamination, sampling tools were sterilized with 75% ethanol before sampling.

2.5. DNA Extraction and Microsatellite Analysis

Genomic DNA from 300 mg of frozen leaf tissue was extracted using the DNeasy Plant Pro kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The yield and quality of nucleic acid extraction were determined using a NanoDrop™ 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA 02451). A DNA sample was analyzed at the nine international reference SSR loci for *Vitis vinifera* L. accessions: VVS2 [11], VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32 [12,13], VrZAG62, and VrZAG79 [14]. PCR amplification was performed in a Biometra Thermocycler T-Gradient ThermoBlock (Analytik Jena GmbH, Jena, Germany) in a 25 µL final volume using the PCR Core kit (Roche Diagnostics GmbH by Sigma-Aldrich, Mannheim, Germany) and adapting the method previously described by Panara et al. [15]. Briefly, the reaction mixture contained an adequate volume of PCR grade water, 100 µg of bovine serum albumin (Promega, Madison, WI, USA), a 2.5 mM final concentration of $MgCl_2$, 2.5 µL of 10x PCR-reaction buffer without $MgCl_2$, 200 µM of each dNTP final concentration, 0.625 U of Taq DNA Polymerase, 0.2 µM of each primer pair, and 150 ng of extracted DNA. All the forward primers were fluorescein-5′-labeled. PCR products were verified at 2% (*w*/*v*) agarose gel electrophoresis. The length of amplified SSR loci was assessed via capillary electrophoresis at BMR Genomics srl (Padua, Italy). The resulting genetic profile was compared with the SSR profile accessions already available in the following databases: European Vitis Database (www.eu-vitis.de, accessed on 21 November 2022), Italian Vitis Database (www.vitisdb.it, accessed on 21 November 2022), Greek Vitis Database (www.biology.uoc.gr/gvd, accessed on 21 November 2022), Swiss Vitis Microsatellite Database (www1.unine.ch/svmd/, accessed on 21 November 2022), and Vitis International Variety Catalogue (www.vivc.de, accessed on 21 November 2022).

2.6. Next-Generation Sequencing Analysis

Grape berries were processed using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Total genomic DNA was measured, and its purity at $A_{260\,nm}/A_{280\,nm}$ was evaluated. Using the ITS3 (5′-GCATCGATGAAGAACGCAGC) and ITS4 (5′-TCCTCCGCTTATTGATAGATAC-3′)

primers, the fungal community was amplified. In a final volume of 25 µL, PCR mixtures contained 1x reaction buffer (5x Go Taq DNA Polymerase, Milan, Promega), 1 µL of each primer (10 µM), and 2 µL of DNA template. Initial denaturation at 95 °C for 3 min was followed by 40 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min, with a final elongation step at 72 °C for 5 min. At BMR Genomics srl, DNA sequencing and taxonomy analysis were performed.

2.7. Laboratory Scale Wine Fermentations

The grapes were crushed, and the grape mash (must and skins) was placed in sterilized 500 mL glass bottles with sterile Müller valves. Each of the 400 mL glass bottles included must and sterilized paraffin oil in order to allow the carbon dioxide evolved during the fermentation process to escape from the fermenting juice, to ensure semi-anaerobic conditions and to prevent external contamination, according to the procedure reported by Englezos et al. [16]. The spontaneous fermentations, without SO_2 addition, were conducted at 25 °C under static conditions. The solids (cap) were not handled due to the small volume employed. The fermentation kinetic was evaluated daily by monitoring the loss of weight due to CO_2 emission. Once a steady weight was achieved, the fermentation was deemed complete. Samples were aseptically collected with sterile serological pipettes at different stages of the alcoholic fermentation (T0—after crushing; T7—after 7 days of spontaneous fermentation; and at the end of alcoholic fermentation) for yeast cell count and physical and chemical analysis. Three biological and three technical replicates were conducted.

2.8. Viable Yeasts Count

Fifty milliliters of must were suspended in 450 milliliters of physiological solutions (NaCl 0.85% *w*/*v*) and diluted serially. To detect total yeasts and non-*Saccharomyces* yeasts, cell suspensions were plated on YPD agar (Yeast Extract 10 g/L, Peptone 20 g/L, Dextrose 20 g/L, Agar 18 g/L) and Lysine medium (LM, Oxoid, Milan, Italy). YPD is a medium supporting the growth of all yeasts, while LM allowed the enumeration of non-*Saccharomyces*, since *S. cerevisiae* is not able to use lysine for its growth. Plate counts were performed in triplicate at different times (T0—after crushing, T7—after 7 days of spontaneous fermentation, and at the end of alcoholic fermentation). Plates were incubated for 48 h at 30 °C.

2.9. Main Physical–Chemical Parameters

FOSS WineScan™ FT120 rapid scanning Fourier Transform Infrared Spectroscopy with FOSS WineScan software version 2.2.1 was used to analyze the primary physicochemical parameters. Previously, the equipment was calibrated using wine samples tested according to established OIV protocols. The pH was determined using a pH meter. The total polyphenol content was evaluated using the Folin–Ciocalteu colorimetric technique, calibrated against gallic acid standards, and expressed as mg gallic acid equivalents per liter (GAE/L). Briefly, the samples were diluted, and then Folin–Ciocalteu reagent and sodium carbonate were added. The solution was reacted at 40 °C for 30 min. A spectrophotometer (Jenway 6305 UV/vis, Jenway, Essex, UK) was used to determine the absorbance of the solution at 760 nm. Each analysis was conducted in duplicate.

2.10. Volatile Profile

Volatile organic compounds (VOCs) were measured by solid-phase microextraction coupled with gas-chromatography (GC-MS-SPME) utilizing a Clarus SQ8S chromatography/mass (GC-MS) spectrometer (Perkin Elmer, Boston, MA, USA) as described previously [17]. By comparing the retention duration of pure chemicals (Sigma-Aldrich, Milan, Italy) analyzed under identical conditions, volatile substances were found. The internal standard was 2-methylhexanol. A provisional identification was determined by comparing MS fragmentation patterns to those in the National Institute of Standards and Technology database (NIST version 2005). All analyses were conducted in duplicate.

2.11. Odor Activity Values (OAVs)

The odor activity value (OAV) was determined to assess the contribution of a chemical ingredient to the wine's scent. It was determined by dividing the concentration of an individual compound by the perceptual threshold (OTV) stated in the literature.

2.12. Statistical Analysis

The program Prism 7.0 (GraphPad Software Inc., La Jolla, CA, USA) was used to analyze data and create graphs. The results were reported as the mean value ± standard deviation.

3. Results and Discussion

3.1. Ampelographic Descriptions

Over the decades, a program of rediscovery and recovery of minor grapevine varieties has been launched in Abruzzo Region (central Italy) in order to meet consumers' demand for products strictly linked to the territory of origin and with unique sensorial traits. *Vitis vinifera* L. cv. Nero Antico di Pretalucente can be considered a minor grapevine variety located in ancient vineyards in the Gessopalena and Torricella Peligna villages. The ampelographic characteristics assessed in *Vitis vinifera* L. cv. Nero Antico di Pretalucente, reported in Table S1, constitute a unique profile as emerges from the morphological characteristics of the shoot, leaf, cluster and berry shape. The bunches are medium-large and loose, and the berries are medium in size, with thick skin.

3.2. Yield, Vine Phenology, and Grape and Berry Characteristics

Pooling the data across trial seasons 2019, 2020, and 2021 showed that *Vitis vinifera* L. cv. Nero Antico di Pretalucente is a grapevine variety characterized by a late budburst and medium fertility of the buds, with 1.4 bunches produced for each bud maintained in the vine with winter pruning (Table 1). Under the conditions examined, the yield per vine was 6.7 kg attributable above all to the high average bunch weight. The flowering is on average, during the first ten days of June, the veraison is late in that is about half way through September, and the ripening is quite late. In all years, the harvest was, in fact, carried out during the third week of October (Table 2). Interestingly, there was a high percentage of skin, at about 16% of fresh weight. In other black grape varieties, this percentage usually ranges from 10% to 12%, which gives a greater resistance against filamentous fungi and harmful insects.

Table 1. Yield component at harvest (average values 2019–2021).

Characteristics	Number
Bud fertility	1.4 ± 0.2
Bunches per vine (n°)	16 ± 3
Yield/vine (kg)	6.7 ± 0.5
Bunch weight (g)	420 ± 19
Berry weight (g)	3.1 ± 0.4
Berries per bunch (n°)	135 ± 14
Berry skin (%)	16 ± 1.10

Table 2. Phenological stage dates for *Vitis vinifera* L. cv. Nero Antico di Pretalucente vines.

Phenological Stage	Date 2019	Date 2020	Date 2021
Budburst (green shoot tips clearly visible = 50% of total buds)	18 April	20 April	17 April
Flowering (50% of flowers open and caps fallen)	7 June	8 June	5 June
Veraison (50% of berries had changed color)	13 September	16 September	11 September

Table 2. *Cont.*

Phenological Stage	Date 2019	Date 2020	Date 2021
Ripening (100% berries completely black)	19 October	20 October	18 October

3.3. Analysis of SSR Regions

SSR markers have been widely used for the identification of synonyms and homonyms, as well as the analysis of the parentage of grapevine varieties. The Italian Vitis Database (http://www.vitisdb.it, accessed on 21 November 2022) and the Registro Nazionale delle Varietà di Vite collect ampelographic and ampelometric descriptions, as well as biochemical and microsatellite profiles of approximately 800 grapevine varieties cultivated on the Italian Peninsula. This information is used to establish the parentage and relatedness of grape varieties. In this study, nine SSR loci were studied to assess the genetic profile of *Vitis vinifera* L. cv. Nero Antico di Pretalucente (Table 3). These loci were chosen due to their combination of polymorphism, reproducibility, and their codominant nature and have been added to the OIV register. It is assumed that two different plants having the same profile for the 9 SSR loci represent the same grape genotype; two related varieties in the first degree share at least one allele per marker [18]. The allele sizes expressed in base pairs for the nine SSR loci are reported in Table 3. The loci VVS2, VVMD7, VrZAG79, VVMD25, VVMD28, and VVMD32 were heterozygous, while VVMD5, VVMD27, and VrZAG62 were homozygous. The Italian Vitis Database revealed a unique SSR profile, suggesting that *Vitis vinifera* L. cv. Nero Antico di Pretalucente could be considered a new cultivar. Moreover, the frequency of homozygous loci could be due to natural events of self-fertilization linked to its geographic isolation. This event has also been previously described for Tannat, an ancient "cépage" planted in the Madiran region of France [19].

Table 3. Allele sizes in base pair of nine SSR loci of *Vitis vinifera* L. cv. Nero Antico di Pretalucente.

Accession	SSR Locus								
	VVS2	**VVMD5**	**VVMD7**	**VVMD27**	**VrZAG62**	**VrZAG79**	**VVMD25**	**VVMD28**	**VVMD32**
Nero Antico di Pretalucente	133:151	234:234	241:247	183:183	190:190	240:248	254:262	233:257	251:271

3.4. Viable Yeast Count

The plate count was used to monitor the ratio between total yeast and non-*Saccharomyces*. The total yeast count after crushing (T0) was approximately 8 Log CFU/g, while non-*Saccharomyces* yeasts reached 7.3 log CFU/g, indicating that the majority of yeasts present did not belong to the *Saccharomyces* genus (Figure 1). This result is consistent with prior findings that non-*Saccharomyces* yeasts are the most prevalent on grapes [20]. A lower concentration is generally found in grapes. However, Guerzoni and Marchetti [21] and Sabate et al. [22] found a population ranging from 10^2 to 10^7 CFU/g of grape. This issue could be related to the occurrence of some apparently healthy berries that are not completely intact, which could result in an increase in the total number of copiotrophic species. The total yeasts reached 8.7 log CFU/mL after seven days of fermentation, but the quantity of non-*Saccharomyces* decreased by approximately one log (6.9 log CFU/mL). At the end of fermentation, non-*Saccharomyces* yeasts were almost not detected, but the total yeast concentration was 7.7 log CFU/mL.

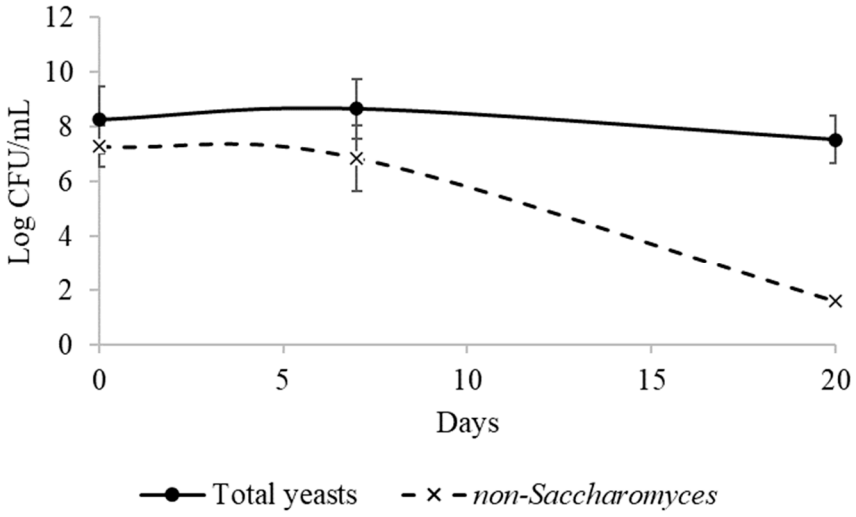

Figure 1. Yeast cell counts detected during the vinification process.

3.5. Fungal Taxonomy Diversity

Grape berries host several microbes which are involved in the must fermentation, shaping a wine's characteristics [23]. Therefore, it is important to identify the grape-borne microorganisms, especially in poorly studied cultivars, such as *Vitis vinifera* L. cv. Nero Antico di Pretalucente, in order to improve our knowledge concerning the "microbial terroir". The fungal genera detected are reported in Figure 2A. *Aureobasidium* spp. (31.2%) was the main genus detected, followed by *Metschnikowia* spp. (19.1%), *Hanseniaspora* spp. (14.8%), *Botrytis* spp. (12.4%), *Cladosporium* spp. (8.7%), *Pichia* spp. (3.5%), *Alternaria* spp. (2.9%), *Epicoccum* spp. (2.9%), *Vishniacozyma* spp. (3.9%), and *Candida* spp. (0.6%).

At the species level, *A. pullulans* was the main species (34.2%), followed by *A. alternata* (25.9%), *H. uvarum* (16.8%), *C. cladosporoides* (14.9%), *V. victoriae* (4.8%), and *E. nigrum* (3.4%) (Figure 2B). For the other genera detected, it was not possible to assign the corresponding species. NGS analyses are principally targeted on highly conserved regions. It is not easy to obtain correct taxonomic assignments at lower taxonomic levels [24]. In fact, this method is more reliable for genus-level identification, or even higher taxonomic levels [25]. Moreover, the correspondence of OTUs with species can be unreliable because some species have genes that are 97% similar, which will result in merged OTUs containing multiple species [26]. DNA may not be recovered from all genotypes; in fact, less abundant ones could not be detected as in the case of *S. cerevisiae* on grapes [26]. In fact, it is found in low concentration on grapes [27].

Aureobasidium pullulans is a yeast-like fungus that can live as an endophyte or an epiphyte. It has been isolated from plant surfaces, soil, and grape berries [28,29]. According to prior studies, it is one of the most prevalent yeast species isolated from grape berries at all maturity stages and other vine tissues from both diseased and healthy vines [27]. The high relative abundance identified in this investigation is consistent with these findings. Its presence on *Vitis vinifera* L. cv. Nero Antico di Pretalucente may be of interest due to its biocontrol potential against significant grape diseases such as gray mold and bunch rot caused by the *Aspergillus* species [28]. In addition, it can breakdown and eliminate ochratoxin A, eliminating wine contamination [30]. *Alternaria* species are the predominant component of wine grape mycobiota in a variety of wine-producing countries across the world [31], mostly due to their unique lifestyle, which produces highly melanized hyphae capable of resisting and growing under extreme UV radiation. It is capable of producing chemicals that are harmful to grapes [31].

Cladosporium cladosporioides is known as the causal agent of Cladosporium rot in vineyards and can develop as a symbiont with many plants [32].

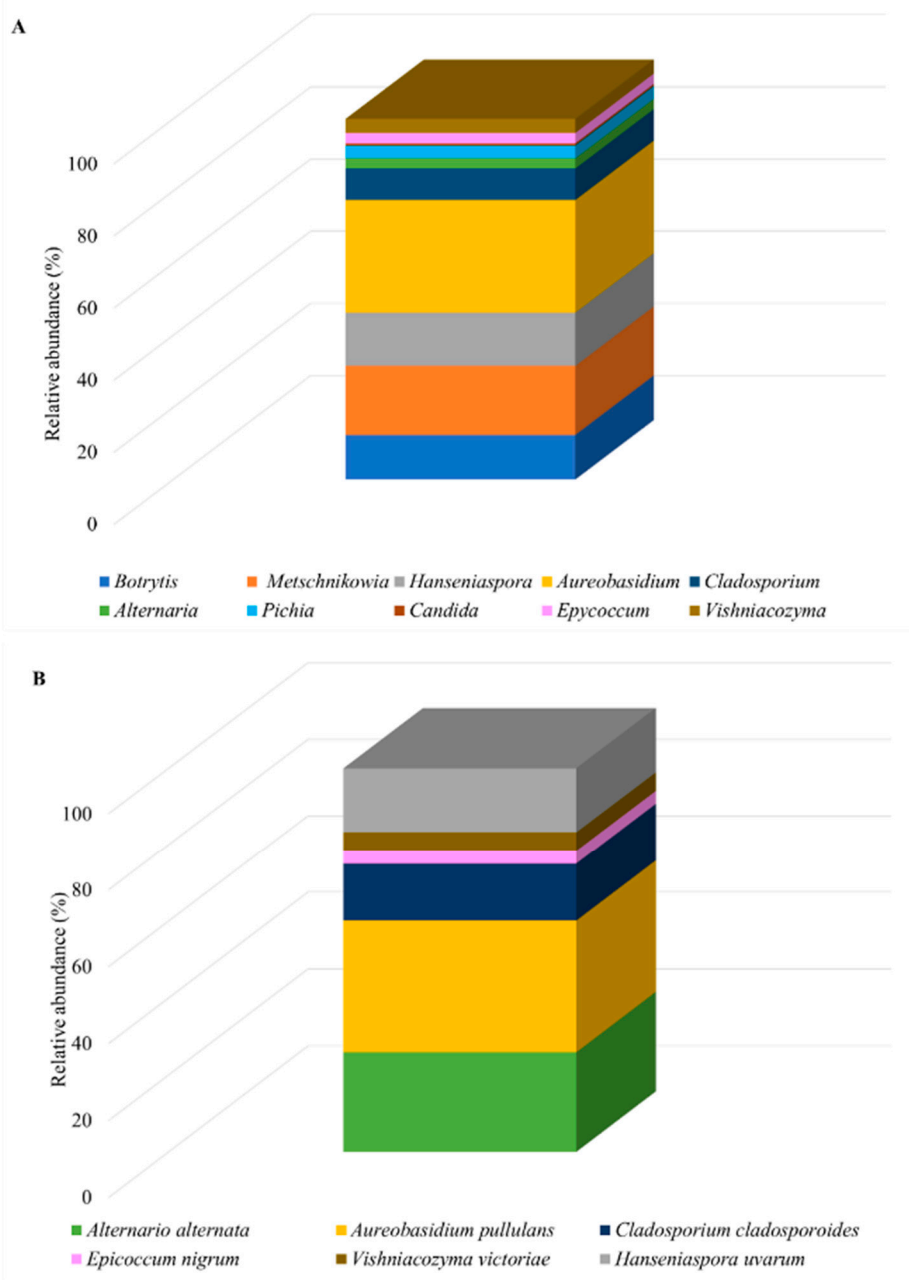

Figure 2. Relative abundance of *Vitis vinifera* L. cv. Nero Antico di Pretalucente grape fungal community detected by NGS at harvest time. (**A**) Main genera detected; (**B**) main species detected.

The presence of *H. uvarum*, *E. nigrum*, and *V. victoriae* is notable because of their biocontrol properties. According to Barata et al. [27], *H. uvarum* is regarded a copiotrophic group with higher nutritional requirements, necessitating a high availability of nutrients, as is the case with harvest-ready grape berries. It is a slightly fermentative yeast with anti-*Alternaria* spp. action [33]. In addition, Liu et al. [33] demonstrated the organism's biological control activity against *B. cinerea* on Kyoho wine grapes.

Recently, *V. victoriae* was identified in biodynamic Montepulciano grapes [29]. It demonstrates biological control activity against the bunch rot pathogen *B. cinerea*, and *Penicillium expansum*, the causative agent of table grape postharvest rot [34].

Epicoccum nigrum is connected with various crops, such as grapevine [35]. It exhibits biological control activity against grape fungal diseases, including *B. cinerea* and *Plasmopara viticola*, respectively, the causative agents of grape grey mold and downy mildew [35]. The co-existence of *E. nigrum* and *A. pullulans* in the same grapevine tissues has been

reported [35], suggesting that they coexist in the same host. Their co-occurrence might positively influence the grape microbial community and wine characteristics. For example, they can inhibit or retard the establishment of pathogens, e.g., *B. cinerea*, and at the same time reduce ochratoxin accumulation, inhibiting *Aspergillus* spp.

3.6. Main Physico-Chemical Parameters of Wines

Vitis vinifera L. cv. Nero Antico di Pretalucente fermentation was spontaneously carried out in the laboratory, and the kinetics are reported in Figure 3. The fermentation started slowly, but in general, a typical fermentation kinetic, without stops in fermentation or too-long fermentation periods, was observed. Generally, spontaneous fermentation presents a longer initial phase and lasts longer than inoculated fermentations, which could positively impact the production of aroma compounds, especially in neutral grape varieties such as Trebbiano Abruzzese [22,36,37]. Despite these differences in the fermentation rate and lag phase, spontaneous fermentations, often, evolve correctly. For instance, Rodríguez et al. [37] revealed that the kinetic of inoculated fermentation was comparable to those presented by spontaneous fermentations carried out at laboratory scale. Uzkuç et al. [38] obtained that spontaneous fermentations took 4 and 7 days longer in Karalahna and Cabernet Sauvignon wines compared to inoculated fermentations. However, the fermentation process completed after 20 days without sluggish or stuck fermentations. Similar results have also been obtained by other authors [37–39].

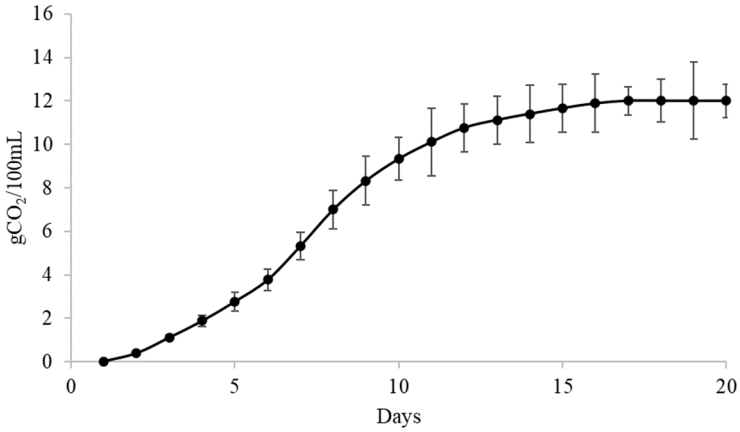

Figure 3. Fermentation kinetic of *Vitis vinifera* L. cv. Nero Antico musts. Data are means ± standard deviations of two independent experiments, each carried out in triplicate.

The oenological parameters are reported in Table 4. The obtained wines had a final ethanol content of 12.75% (*v*/*v*), and the residual sugars were 0.71 g/L. The majority of sugars were consumed after 7 days (194.66 g/L), suggesting the development of fermentative strains. Autochthonous non-*Saccharomyces* yeasts trigger the alcoholic fermentation. Uzuk et al. [38] highlighted that spontaneously fermented Karalahna and Cabernet Sauvignon wines showed a higher ethanol content that wines resulting from inoculated fermentations. The great biodiversity of non-*Saccharomyces* yeasts occurring during the early stages of fermentation is related to several factors influencing the grape microbiota, e.g., vineyard localization, climatic conditions, cultivar, agronomic practices, stage of ripening, health of the grapes, harvesting procedures and the specific weather conditions in each vintage year [40]. An indigenous *S. cerevisiae* population is able to dominate the fermentation process, and the increasing ethanol content due to its development inhibits most of the non-*Saccharomyces* species due to their lower ethanol resistance [40]. The wines showed a pH of 3.4, and a volatile acidity lower than 0.6 g/L. These values are similar to those obtained in inoculated fermentations, suggesting the correct outcome of the fermentation process. A higher concentration of this last parameter is considered undesirable because it increasingly gives an acetic taste to the wine. The content of glycerol reached 8.56 g/L at the

end of fermentation, suggesting the presence of glycerol-producing strains. However, the levels of glycerol depend upon many factors. In this study, the content of glycerol could be related to the presence of non-*Saccharomyces* and to the fermenting temperature (25 °C) [41]. In fact, glycerol is generally more abundant at temperatures of about 20–25 °C. Ciani and Comitini [42] demonstrated that increasing the fermentation temperature from 16 to 20 °C favored the accumulation of glycerol. This compound positively influences wine quality. In fact, when it is present in concentrations above the threshold taste level of 5.2 g/L in wine, it contributes to sweetness. In addition, it has been linked to mouth-feel sensations by imparting "fullness" (also known as "viscosity" or "weight") to wine. Glycerol is also believed to improve the overall balance between alcoholic strength, acidity, astringency, and sweetness, so imparting a degree of roundness and smoothness to the tongue [43]. The obtained data revealed that spontaneous malolactic fermentation occurred. In fact, the malic acid was almost completely converted into lactic acid (Table 4). Wines were characterized by the content of polyphenols and anthocyanins similar to those obtained for other red wines [44]. The intensity and tonality reveal a light red color with purple nuances. The low intensity and tonality values obtained can be attributed to the fact that the fermentation trials were performed in a low volume (500 mL) and, therefore, the cap was not managed.

Table 4. Main oenological parameters determined at different times (T0, T7, and at the end of fermentation—T20).

Oenological Parameters	T0	T7	T20
Alcohol (% *v*/*v*)	-	11.44±1.34	12.75±2.07
Residual sugars (g/L)	213.63 ± 12.78	18.97 ± 2.08	0.71 ± 0.08
pH	3.41 ± 0.56	3.37 ± 1.14	3.48 ± 0.24
Total acidity (g/L) *	5.10 ± 0.09	5.11 ± 0.43	5.28 ± 0.14
Volatile acidity (g/L) **	0.07 ± 0.01	0.38 ± 0.03	0.54 ± 0.03
Malic acid (g/L)	2.52 ± 0.12	2 ± 0.05	0.17 ± 0.02
Lactic acid (g/L)	-	0.3 ± 0.01	1.31 ± 0.05
Glycerol (g/L)	-	8.09 ± 1.99	8.56 ± 1.98
Anthocyanins (mg/L)	32 ± 5.78	85 ± 12.92	266 ± 6.64
Polyphenols (GAE/L)	368 ± 8.93	728 ± 7.88	977 ± 12.72
Intensity			3.8 ± 0.65
Tonality			0.76 ± 0.17

* expressed as tartaric acid. ** expressed as acid acetic.

3.7. Characterization of Aroma Compounds in Vitis vinifera L. cv. Nero Antico di Pretalucente Wine

The unique traits of a wine mainly rely on its volatile footprint. Therefore, the VOCs of spontaneously fermented Nero Antico di Pretalucente wine were determined (Table 5). A total of 45 compounds were detected, including: 13 higher alcohols, 18 esters, 6 organic acids, 3 ketones, 2 terpens, and 3 aldehydes. The most abundant compounds were esters (68 mg/L), and ethyl acetate (fruity, sweet, weedy, green), isoamyl acetate (sweet, banana, fruity), and diethyl succinate (fruity, cooked apple, ylang) were the main ones.

Table 5. Volatile compounds detected in Nero Antico di Pretalucente wines.

Higher Alcohols	mg/L
(S)-3,4-Dimethylpentanol	0.24 ± 017
1-Butanol, 2-methyl	4.15 ± 0.12
1-Butanol, 3-methyl	27.12 ± 1.99
1-Hexanol	0.24 ± 0.03
1-Pentanol	2.13 ± 0.32
2,3-Butanediol	0.21 ± 0.023
2-Heptanol	0.77 ± 0.11
Phenylethyl alcohol	20.37 ± 4.78

Table 5. *Cont.*

Higher Alcohols	mg/L
1-butanol	3.23 ± 0.98
1-octanol	0.65 ± 0.06
1-nonanol	0.77 ± 0.05
1-dodecanol	3.08 ± 0.84
1-propanol	2.88 ± 0.56
TOT	**65.84**
Esters	
Isoamyl acetate	9.05 ± 1.02
Phenethyl acetate	0.03 ± 0.01
Ethyl isovalerate	0.23 ± 0.18
Pentanoic acid, 2-methyl-, butyl ester	4.67 ± 0.94
Pentanoic acid, 2,2-dimethyl-, methyl ester	2.66 ± 1,03
Pentanoic acid, 3-methyl-, ethyl ester	2.88 ± 0.06
Pentanoic acid, 4-methyl-, ethyl ester	1.98 ± 0.05
Undecanoic acid, 11-bromo-ethylester	0.77 ± 0.12
2-pheylethyl ethanoate	3.55 ± 0.05
Hexyl-ethanoate	2.93 ± 0.66
Ethyl phenyl acetate	3.54 ± 1.12
Ethyl decanoate	6.63 ± 2.14
Ethyl 9-decenoate	0.03 ± 0.01
Ethyl acetate	14.77 ± 1,32
Ethyl hexanoate	1.12 ± 0.25
Ethyl octanoate	2.34 ± 2.76
Diethyl succinate	8.18 ± 1.93
Ethyl lactate	2.77 ± 0.07
TOT	**68.11**
Organic acids	
n-Decanoic acid	0.06 ± 0.01
Hexanoic acid	0.12 ± 0.05
Butanoic acid	0.55 ± 0.04
Propanoic acid	0.24 ± 0.03
Acetic acid	0.06 ± 0.02
Octanoic acid	1.12 ± 0.09
TOT	**2.15**
Ketones	
β-ionone	0.02 ± 0.01
2,3-butanedione	0.06 ± 0.02
3-hexanone	0.03 ± 0.01
TOT	**0.11**
Terpenes	
Nerol	0.41 ± 0.02
Terpinen-4-ol	0.03 ± 0.01
TOT	**0.44**
Aldehydes	
Benzaldehyde	0.34 ± 0.06
Decanal	0.44 ± 0.03
Nonanal	1.12 ± 0.05
TOT	**1.90**

Several studies revealed that spontaneous fermentations are associated with greater wine body, unusual or odd aromas and flavors, and greater complexity [45]. The develop-

ment of indigenous yeasts is associated with a higher amounts of esters in spontaneously fermented wines than inoculated wines [46,47]. The synthesis of esters may be a result of the high expression of acyltransferase and alcohol acetyltransferase during spontaneous fermentation, which is greater when the fermentation rate is slower [38]. For example, ethyl acetate, which is one of the most important volatile compounds in wines, is produced in greater quantities when the rate of spontaneous fermentation is lower and is primarily dependent on the metabolic activity of non-*Saccharomyces* yeasts, which produce more ethyl acetate than *Saccharomyces* strains [48]. The occurrence of the *Pichia*, *Candida*, and *Metschnikowia* genera detected in this study could be associated with ethyl acetate production. In fact, they have been described as producers of esters, especially ethyl acetate [49].

A final concentration of 65 mg/L of higher alcohols was reported. Below 300 mg/L, they contribute positively to wine complexity; over 400 mg/L, they have a negative effect on wine quality. These chemicals are secondary products of yeast metabolism and can be generated through either the anabolic pathway from glucose or the catabolic pathway from their respective amino acids [50]. Since the content of amino acids is dependent on the grape variety, all of these volatile chemicals are dependent on the grape variety [50]. 1-butanol-3-methyl (fusel, alcoholic, whiskey, fruity, banana) and phenylethyl alcohol (sweet, flowery fresh, bready, rose, honey) were the most abundant. These two compounds are often associated with the development of some non-*Saccharomyces* such as *Hanseniaspora* spp. or *Pichia* spp. Their production seems to be strain-dependent and related to the presence of *S. cerevisiae* [49]. However, it is important to underline that the amount of phenylethyl alcohol in wines is dependent on grape variety, ripeness, and winemaking processes [51], which suggests that *Vitis vinifera* L. cv. Nero Antico di Pretalucente grapes may contain its precursors. In addition, spontaneous fermentation could positively influence its buildup. Garde-Cerdan and Ancin-Azcilicueta [48] obtained a considerable yield of 2-phenyl ethanol from the spontaneously fermented musts of Parellada.

The effect of the fermentation process (spontaneous vs. inoculated) on higher alcohols is still a matter of debate. Varela et al. [45] stated that spontaneously fermented Chardonnay wines had higher concentrations of higher alcohols than inoculated wines. In a recent study, it is suggested that inoculated Cabernet Sauvignon wines had a greater concentration of higher alcohols [52]. In another study, no significant differences were found in the concentration of total higher alcohols between spontaneous and inoculated wines [48].

Terpenes add floral notes to wine and belong to the secondary plant constituents. Several studies reported that they could be used for grape characterization [49] since, even if microorganisms are able to synthetize terpenes, their concentration does not show significant changes during the fermentation [49]. Nerol showed OAV of >1 suggesting that it can be considered a terpen associated with the Nero Antico di Petralucente cultivar.

Organic acids were also detected, with octanoic acid being the main one. However, they were present in low amounts, and none of them reached the OAV. Yeast and bacteria produce this class of VOCs during fatty acid metabolism. They can contribute to the complexity of the wine bouquet even at sub-sensory threshold levels, but have a negative effect on the wine scent when present in excess of their thresholds [53].

Due to their low sensory threshold values, aldehydes play a crucial role in wine scent. They are the primary products of lipid oxidation and the degradation or autooxidation of unsaturated fatty acids via hydroperoxides, and they may be transformed to alcohols or acids during fermentation [53]. The content of aldehydes might be due to the occurrence of *Hanseniaspora*. In fact, the genus *Hanseniaspora* has been characterized as a high producer of aldehydes such as acetaldehyde, benzaldehyde, 4-ethylbenzaldehyde, and benzene acetaldehyde [49]. It is intriguing to emphasize nonanal's presence; in fact, it is considered a potentially active odorant of wine even at a low concentration [54].

Three ketones have been identified: β-ionone, 2,3-butanedione, and 3-hexanone. Ketones are primarily produced through lipid oxidation, as well as citrate and glucose metabolism. Of relevance is the presence of β-ionone, which is characterized by a distinct

aroma of violets. Like the monoterpenes, the norisoprenoids are varietal aroma compounds and occur predominantly as glycosidically bound precursors [55]. Consequently, their presence in Nero Antico di Pretalucente wines is directly tied to the aroma traits of this variety.

OAVs were computed in order to assess the contribution of observed volatile compounds to the olfactory perception of wines. Only compounds with OAVs of >1 contribute to wine fragrance. The detected VOCs have distinct odors that were described using a database (www.thegoodscentscompany.com, accessed on 21 November 2022). Fruity, sweet, and green were the most common scents, occurring in the vast majority of compounds with a descriptor. A total of 15 aroma compounds showed an OAV of >1 (Table 6). The highest values were for decanal, β-ionone, ethyl octanoate, and ethyl isovalerate, suggesting their contribution to the overall aroma of Nero Antico di Pretalucente wines. In particular, nerol and β-ionone could be considered important aromas of this variety.

The concentration and the type of volatile compounds are affected by indigenous microorganisms. Indigenous yeasts are better adapted to the micro-conditions of fermentation and the composition of the grape must, which is beneficial to producing wines with unique characteristics [56]. Previous studies highlighted that spontaneous fermentation improved the aroma complexity of some wines, e.g., Cabernet Sauvignon, Chardonnay and Vidal, proving the contribution of non-*Saccharomyces* to the formation of wine flavors [57].

Table 6. Volatile compounds with OAV >1.

Higher Alcohols	OTV (mg/L)	Odour Description	OAV
2-Heptanol	0.2 **	Fresh lemon, grass, herbal, sweet, floral, fruity	3.85
Phenylethyl alcohol	14 **	Sweet, floral, fresh, bready, rose, honey	1.46
1-octanol	0.12 ***	Waxy, green, aldehydic and floral with a sweet, fatty, coconut nuance	5.42
1-dodecanol	1 **	Earthy, soapy, waxy, fatty, honey, coconut	3.08
Esters			
Isoamyl acetate	0.16 **	Sweet, banana, fruity, ripe, estery	56.56
Ethyl isovalerate	0.001 **	Fruity, sweet, apple, pineapple	230
Ethyl decanoate	0.2 *	Sweet, waxy, fruity, apple, grape, oily, brandy	33.15
Ethyl acetate	12.26 **	Ethereal, fruity, sweet, weedy, green	1.20
Ethyl hexanoate	0.014 *	Sweet, fruity, pineapple, waxy, green, banana	78.57
Ethyl octanoate	0.005 *	Fruity, wine, sweet, apricot, banana, brandy, pear	468
Diethyl succinate	1.2 **	Mild, fruity, cooked, apple, ylang	6.82
Ketons			
β-ionone	0.00009 *	Floral, woody, sweet, fruity, berry, tropical, violet	222
Terpenes			
Nerol	0.40	Neroli, citrus, magnolia	1.03
Aldehydes			
Decanal	0.001 **	Sweet, aldehydic, waxy, orange, peel, citrus, floral	440
Nonanal	0.015 **	Waxy, aldehydic, rose, fresh, orange, peel	74.67

* Gomez-Mıguez et al. [58]. ** Welke et al. [59]. *** Fracassetti et al. [60].

4. Conclusions

The results obtained in this study contributed to the description and valorization of a local grape variety grown in a mountains area, where the interest for this cultivation is strongly improved, thanks also to several young producers, who are planting new vineyards of *Vitis vinifera* L. cv. Nero Antico di Pretalucente. This is a strength of the Italian viticultural–oenological system, which can offer many unique and unrepeatable products linked to a specific region on the global market. On the basis of SSR and ampelographic profiles, *Vitis vinifera* L. cv. Nero Antico di Pretalucente can be considered a new cultivar and can represent a reservoir of microbial biodiversity. Further studies are necessary to

prepare a culture collection of wine microbes associated with this cultivar in order to preserve the microbial biodiversity and select strains useful to protect the characteristics of wines obtained from ancient cultivars, maintaining the link with the land of origin. This study represents a first step for the valorization of this variety which could also be used with success for the production of white and rosé wines, as well as sparkling wines, thanks to the high level of organic acids in the must at harvest. *Vitis vinifera* L. cv. Nero Antico di Pretalucente could help to showcase the regional terroir, and could expand the wine list of the Abruzzo Region together with other native vines already cultivated with success. Moreover, it could improve the development of these difficult mountain areas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9020150/s1, Table S1: Descriptors proposed by the International Organisation of Vine and Wine (OIV, 2007) for the description of young shoots, shoots, young leaves, adult leaves, bunches and berries in one black-berried grapevine accession from the Abruzzo Region.

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References

1. Batiuk, D. The fruits of migration: Understanding the 'longue dureé' and the socio-economic relations of the Early Transcaucasian Culture. *J. Anthropol. Archaeol.* **2013**, *32*, 449–477. [CrossRef]
2. García-Muñoz, S.; Asproudi, A.; Cabello, F.; Borsa, D. Aromatic characterization and enological potential of 21 minor varieties (*Vitis vinifera* L.). *Eur. Food Res. Technol.* **2011**, *233*, 473. [CrossRef]
3. Frioni, T.; Bertoloni, G.; Squeri, C.; Garavani, A.; Ronney, L.; Poni, S.; Gatti, M. Biodiversity of local *Vitis vinifera* L. germplasm: A powerful tool toward adaptation to global warming and desired grape composition. *Front. Plant Sci.* **2020**, *11*, 608. [CrossRef] [PubMed]
4. Zombardo, A.; Storchi, P.; Valentini, P.; Ciofini, A.; Migliaro, D.; Crespan, M. Recovery, Molecular Characterization, and Ampelographic Assessment of Marginal Grapevine Germplasm from Southern Umbria (Central Italy). *Plants* **2021**, *10*, 1539. [CrossRef]
5. Crespan, M.; Migliaro, D.; Larger, S.; Pindo, M.; Palmisano, M.; Manni, A. Grapevine (*Vitis vinifera* L.) varietal assortment and evolution in the Marche region (central Italy). *OENO ONE* **2021**, *3*, 17–37. [CrossRef]
6. D'Onofrio, C.; Tumino, G.; Gardiman, M.; Crespan, M.; Bignami, C.; de Palma, L.; Barbagallo, M.G.; Muganu, M.; Morcia, C.; Novello, V.; et al. Parentage atlas of Italian grapevine varieties as inferred from SNPgenotyping. *Front. Plant Sci.* **2021**, *11*, 605934. [CrossRef]
7. Assovini. Available online: https://www.assovini.it/italia/abruzzo (accessed on 21 November 2022).
8. Regulation, Council. No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. *Off. J. Eur. Union* **2007**, *189*, 139–161.
9. European Union Regulation (EC). Commission Regulation (EC) No. 889/2008 laying down detailed rules for the implementation of Council Regulation (EC) No. 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. *Off. J. Eur. Union* **2008**, *8*, 173–256.
10. OIV-International Organization of Vine and Wine. Second Edition of the OIV Descriptor List for Grape Varieties and Vitis Species. 2019. Available online: www.oiv.it/public/medias/2274/code-2e-edition-finale.pdf (accessed on 21 November 2022).

11. Thomas, M.R.; Scott, N.S. Microsatellite repeats in grapevine reveal DNA polymorphism when analysed as sequence-tagged sites (STSs). *Theor. Appl. Genet.* **1993**, *86*, 985–990. [CrossRef]
12. Bowers, J.E.; Dangl, G.S.; Vignani, R.; Meredith, C.P. Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome* **1996**, *39*, 628–633. [CrossRef]
13. Bowers, J.E.; Dangl, G.S.; Meredith, C.P. Development and characterization of additional microsatellite DNA markers for grape. *Am. J. Enol. Vitic.* **1999**, *50*, 243–246. [CrossRef]
14. Sefc, K.M.; Regner, F.; Turetsch, E.; Glossel, J.; Steinkellner, H. Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. *Genome* **1999**, *42*, 367–373. [CrossRef]
15. Panara, F.; Petoumenou, D.; Calderini, O.; Dini, F.; D'Onofrio, C.; Bedini, L.; Palliotti, A. Ampelographic and genetic characterisation of ancestral grapevine (*Vitis vinifera* L.) accessions present in the Umbria Region (Central Italy). *J. Hortic. Sci. Biotechnol.* **2013**, *88*, 525–530. [CrossRef]
16. Englezos, V.; Rantsiou, K.; Cravero, F.; Torchio, F.; Ortiz-Julien, A.; Gerbi, V.; Rolle, L.; Cocolin, L. *Starmerella bacillaris* and Saccharomyces cerevisiae mixed fermentations to reduce ethanol content in wine. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 5515–5526. [CrossRef] [PubMed]
17. Perpetuini, G.; Tittarelli, F.; Battistelli, N.; Suzzi, G.; Tofalo, R. Contribution of *Pichia manshurica* strains to aroma profile of organic wines. *Eur. Food Res. Technol.* **2020**, *246*, 1405–1417. [CrossRef]
18. Panara, F.; Bergamini, C.; Palliotti, A.; Calderini, O. Use of molecular markers (SSRS) and public databases in *Vitis Vinifera* L. as the main case of efficient crop cultivar identification. *JOJ Hortic. Arboric.* **2018**, *2*, 1. [CrossRef]
19. Durquety, P.M.; Houbart, J.P. Two Tannat sports: "meunier" and "bulle". *Progr. Agric. Vitic.* **1982**, *99*, 83–87.
20. Jolly, N.P.; Varela, C.; Pretorius, I.S. Not your ordinary yeast: Non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Res.* **2014**, *14*, 215–237. [CrossRef]
21. Guerzoni, E.; Marchetti, R. Analysis of yeast flora associated with grape sour rot and of the chemical disease markers. *Appl. Environ. Microbiol.* **1987**, *53*, 571–576. [CrossRef]
22. Sabate, J.; Cano, J.; Esteve-Zarzoso, B.; Guillamón, J.M. Isolation and identification of yeasts associated with vineyard and winery by RFLP analysis of ribosomal genes and mitochondrial DNA. *Microbiol. Res.* **2002**, *157*, 267–274. [CrossRef]
23. Setati, M.E.; Jacobson, D.; Bauer, F.F. Sequence-based analysis of the *Vitis vinifera* L. cv Cabernet Sauvignon grape must mycobiome in three South African vineyards employing distinct agronomic systems. *Front. Microbiol.* **2015**, *6*, 1358. [CrossRef] [PubMed]
24. Tedersoo, L.; Anslan, S.; Bahram, M.; Põlme, S.; Riit, T.; Liiv, I.; Kõljalg, U.; Kisand, V.; Nilsson, R.H.; Hildebrand, F.; et al. Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. *MycoKeys* **2015**, *10*, 1–43. [CrossRef]
25. Purahong, W.; Wubet, T.; Krüger, D.; Buscot, F. Molecular evidence strongly supports deadwood-inhabiting fungi exhibiting unexpected tree species preferences in temperate forests. *ISME J.* **2017**, *12*, 289–295. [CrossRef] [PubMed]
26. Jayawardena, R.S.; Purahong, W.; Zhang, W.; Wubet, T.; Li, X.H.; Liu, M.; Zhao, W.; Hyde, K.D.; Liu, J.H.; Yan, J. Biodiversity of fungi on *Vitis vinifera* L. revealed by traditional and high-resolution culture-independent approaches. *Fungal Divers.* **2018**, *90*, 1–84. [CrossRef]
27. Barata, A.; Malfeito-Ferreira, M.; Loureiro, V. The Microbial Ecology of Wine Grape Berries. *Int. J. Food Microbiol.* **2012**, *153*, 243–259. [CrossRef]
28. Bozoud, D.; Tsaltas, D. The multiple and versatile roles of *Aureobasidium pullulans* in the vitivinicultural sector. *Fermentation* **2018**, *4*, 85. [CrossRef]
29. Perpetuini, G.; Rossetti, A.P.; Battistelli, N.; Zulli, C.; Cichelli, A.; Arfelli, G.; Tofalo, R. Impact of vineyard management on grape fungal community and Montepulciano d'Abruzzo wine quality. *Food Res. Int.* **2022**, *158*, 111577. [CrossRef]
30. De Curtis, F.; De Cicco, V.; Lima, G. Efficacy of Biocontrol Yeasts Combined with Calcium Silicate or Sulphur for Controlling Durum Wheat Powdery Mildew and Increasing Grain Yield Components. *Field Crops Res.* **2012**, *134*, 36–46. [CrossRef]
31. Prendes, L.P.; Merín, M.G.; Andreoni, M.A.; Ramirez, M.L.; Morata de Ambrosini, V.I. Mycobiota and toxicogenic *Alternaria* spp. strains in Malbec wine grapes from DOC San Rafael, Mendoza, Argentina. *Food Control* **2015**, *57*, 122–128. [CrossRef]
32. Briceño, E.X.; Latorre, B.A. Characterization of Cladosporium Rot in Grapevines, a Problem of Growing Importance in Chile. *Plant Dis.* **2008**, *92*, 1635–1642. [CrossRef]
33. Liu, H.M.; Guo, J.H.; Cheng, Y.J.; Luo, L.; Liu, P.; Wang, B.Q.; Deng, B.X.; Long, C.A.A. Control of gray mold of grape by *Hanseniaspora uvarum* and its effects on postharvest quality parameters. *Ann. Microbiol.* **2010**, *60*, 31–35. [CrossRef]
34. Dimakopoulou, M.; Tjamos, S.E.; Antoniou, P.P.; Pietri, A.; Battilani, P.; Avramidis, N.; Markakis, E.A.; Tjamos, E.C. Phyllosphere grapevine yeast *Aureobasidium pullulans* reduces Aspergillus carbonarius (sour rot) incidence in wine-producing vineyards in Greece. *Biol. Control* **2008**, *46*, 158–165. [CrossRef]
35. Martini, M.; Musetti, R.; Grisan, S.; Polizzotto, R.; Borselli, S.; Pavan, F.; Osler, R. DNA-dependent detection of the grapevine fungal endophytes *Aureobasidium pullulans* and *Epicoccum nigrum*. *Plant Dis.* **2009**, *93*, 993–998. [CrossRef]
36. Rossetti, A.P.; Perpetuini, G.; Battistelli, N.; Zulli, C.; Arfelli, G.; Suzzi, G.; Cichelli, A.; Tofalo, R. Capturing the fungal community associated with conventional and organic Trebbiano Abruzzese grapes and its influence on wine characteristics. *Food Biosci.* **2023**, *52*, 102382. [CrossRef]
37. Rodríguez, M.E.; Lopes, C.A.; Barbagelata, R.J.; Barda, N.B.; Caballero, A.C. Influence of *Candida pulcherrima* Patagonian strain on alcoholic fermentation behaviour and wine aroma. *Int. J. Food Microbiol.* **2010**, *138*, 19–25. [CrossRef] [PubMed]

38. Uzkuç, N.M.Ç.; ¸Si¸sli, B.; Ay, M.; Togay, S.Ö.; Yüceer, Y.K.; Bayhan, A.; Toklucu, A.K. Effects of spontaneous fermentation on Karalahna and Cabernet Sauvignon young red wines: Volatile compounds, sensory profiles and identification of autochthonous yeasts. *Eur. Food Res. Technol.* **2020**, *246*, 81–92. [CrossRef]
39. Medina, K.; Boido, E.; Fariña, L.; Gioia, O.; Gomez, M.E.; Barquet, M.; Gaggero, C.; Dellacassa, E.; Carrau, F. Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*. *Food Chem.* **2013**, *141*, 2513–2521. [CrossRef] [PubMed]
40. Padilla, B.; Gil, J.V.; Manzanares, P. Past and future of non-*Saccharomyces* yeasts: From spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Front. Microbiol.* **2016**, *7*, 411. [CrossRef]
41. Ivit, N.N.; Longo, R.; Kemp, B. The effect of non-*Saccharomyces* and *Saccharomyces* non-*cerevisiae* yeasts on ethanol and glycerol levels in wine. *Fermentation* **2020**, *6*, 77. [CrossRef]
42. Ciani, M.; Comitini, F. Influence of temperature and oxygen concentration on the fermentation behaviour of *Candida stellata* in mixed fermentation with Saccharomyces cerevisiae. *World J. Microbiol. Biotechnol.* **2006**, *22*, 619–623. [CrossRef]
43. Goold, H.D.; Kroukamp, H.; Williams, T.C.; Paulsen, I.T.; Varela, C.; Pretorius, I.S. Yeast's balancing act between ethanol and glycerol production in low-alcohol wines. *Microb. Biotechnol.* **2017**, *10*, 264–278. [CrossRef] [PubMed]
44. Cliff, M.A.; King, M.C.; Schlosser, J. Anthocyanin, phenolic composition, colour measurement and sensory analysis of BC commercial red wines. *Food Res. Int.* **2007**, *40*, 92–100. [CrossRef]
45. Varela, C.; Siebert, T.; Cozzolino, D.; Rose, L.; Maclean, H.; Henschke, P.A. Discovering a chemical basis for differentiating wines made by fermentationwith 'wild' indigenous inoculated yeasts: Role of yeast volatile compounds. *Austr. J. Grape Wine Res.* **2009**, *15*, 238–248. [CrossRef]
46. Blanco, P.; Mirás-Avalos, J.M.; Pereira, E.; Orriols, I. Fermentative aroma compounds and sensory profles of Godello and Albariño wines as infuenced by *Saccharomyces cerevisiae* yeast strains. *J. Sci. Food Agric.* **2013**, *93*, 2849–2857. [CrossRef]
47. Puertas, B.; Jimenez-Hierro, M.J.; Cantos-Villar, E.; Marrufo-Curtido, A.; Carbú, M.; Cuevas, F.J.; Moreno-Rojas, J.M.; González-Rodríguez, V.E.; Cantoral, J.M.; Ruiz-Moreno, M.J. The influence of yeast on chemical composition and sensory properties of dry white wines. *Food Chem.* **2018**, *253*, 227–235. [CrossRef] [PubMed]
48. Garde-Cerdan, T.; Ancin-Azpilicueta, C. Contribution of wild yeasts to the formation of volatile compounds in inoculated wine fermentations. *Eur. Food Res. Technol.* **2006**, *222*, 15–25. [CrossRef]
49. Carpena, M.; Fraga-Corral, M.; Otero, P.; Nogueira, R.A.; Garcia-Oliveira, P.; Prieto, M.A.; Simal-Gandara, J. Secondary aroma: Influence of wine microorganisms in their aroma profile. *Foods* **2021**, *10*, 51. [CrossRef]
50. Li, H.; Tao, Y.-S.; Wang, H.; Zhang, L. Impact odorants of Chardonnay dry white wine from Changli County (China). *Eur. Food Res. Technol.* **2008**, *227*, 287–292. [CrossRef]
51. Cai, J.; Zhu, B.Q.; Wang, Y.H.; Lu, L.; Lan, Y.B.; Reeves, M.J.; Duan, C.Q. Influence of pre-fermentation cold maceration treatment on aroma compounds of Cabernet Sauvignon wines fermented in different industrial scale fermenters. *Food Chem.* **2014**, *154*, 217–229. [CrossRef]
52. Liu, P.T.; Lu, L.; Duan, C.Q.; Yan, G.L. The contribution of indigenous non-*Saccharomyces* wine yeast to improved aromatic quality of Cabernet Sauvignon wines by spontaneous fermentation. *LWT Food Sci. Technol.* **2016**, *71*, 356–363. [CrossRef]
53. Swiegers, J.H.; Pretorius, I.S. Yeast modulation of wine flavor. *Adv. Appl. Microbiol.* **2005**, *57*, 131–175. [PubMed]
54. Escudero, A.; Campo, E.; Fariña, L.; Cacho, J.; Ferreira, V. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J. Agric. Food Chem.* **2007**, *55*, 4501–4510. [CrossRef] [PubMed]
55. Tomasino, E.; Bolman, S. The potential effect of β-ionone and β-damascenone on sensory perception of Pinot Noir wine aroma. *Molecules* **2021**, *26*, 1288. [CrossRef] [PubMed]
56. Tofalo, R.; Patrignani, F.; Lanciotti, R.; Perpetuini, G.; Schirone, M.; Di Gianvito, P.; Pizzoni, D.; Arfelli, G.; Suzzi, G. Aroma profile of Montepulciano d'Abruzzo wine fermented by single and co-culture starters of autochthonous *Saccharomyces* and non-*Saccharomyces* yeasts. *Front. Microbiol.* **2016**, *7*, 610. [CrossRef]
57. Chen, Y.; Zhang, W.; He, Y.; Wang, B.; Xiao, J.; Zhou, X.Y.; Xu, J.; Jiang, L.; Shi, X. Microbial community composition and its role in volatile compound formation during the spontaneous fermentation of ice wine made from Vidal grapes. *Process Biochem.* **2020**, *92*, 365–377. [CrossRef]
58. Gomez-Miguez, M.J.; Cacho, J.F.; Ferreira, V.; Vicario, I.M.; Heredia, F.J. Volatile components of Zalema white wines. *Food Chem.* **2007**, *100*, 1464–1473. [CrossRef]
59. Welke, J.E.; Zanus, M.; Lazzarotto, M.; Zini, C.A. Quantitative analysis of headspace volatile compounds using comprehensive two-dimensional gas chromatography and their contribution to the aroma of chardonnay wine. *Food Res. Int.* **2014**, *59*, 85–99. [CrossRef]
60. Fracassetti, D.; Camoni, D.; Montresor, L.; Bodon, R.; Limbo, S. Chemical characterization and volatile profile of Trebbiano di Lugana wine: A Case Study. *Foods* **2020**, *9*, 956. [CrossRef]