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Research Article

Reintroducing Autochthonous Minor Grapevine Varieties to Improve Wine Quality and Viticulture Sustainability in a Climate Change Scenario

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One of the major challenges that global warming poses to viticulture is the maintenance of adequate acidity at maturity in white grapes for sparkling winemaking. This issue arises from three main occurrences: (i) with higher temperatures, degradation of malic acid is significantly enhanced; (ii) with a general advancement in grapevine phenology, grape maturity may occur under suboptimal climatic conditions; and (iii) harvesting grapes at "traditional" dates results in overripe fruits for sparkling destinations. In this biennial work, we compared the fruit and must composition of a local, widely grown white grape variety in the Colli Piacentini area (cv. Ortrugo, ORT) with those of a minor autochthonous variety, namely, Barbesino (BRB). Furthermore, we compared the composition, aromatic, and sensory profiles of wines obtained from ORT and BRB grapes picked on the same date and, in addition, of a second Barbesino wine from late harvest (BRB-LH). ORT and BRB had a similar sugar accumulation dynamic, whereas BRB exhibited a delayed loss of titratable acidity. In more details, BRB had lower malic acid degradation rates when malate concentration was <9 g/L. As a result, with comparable yield and total soluble solid content (TSS) (~20°Brix), BRB had a higher berry titratable acidity and malic acid concentration at harvest than ORT. BRB wines showed the highest titratable acidity (TA), while ORT had the lowest TA and a higher pH, and as expected, BRB-LH had the highest pH and a lower TA than BRB although still higher than those of ORT wine. The aroma profiles of wines were mainly characterized by fermentative aromas, including esters, fatty acids, higher alcohols, and C_6 compounds, and BRB-LH wines showed the highest concentration of higher alcohols, while the fermentative esters were higher in ORT wines. Panelists considered BRB significantly fresher and with bigger aroma intensity than ORT, confirming that the higher acidity detected in BRB musts is well preserved in final wines. Our work demonstrates that local minor varieties can be reconsidered in light of the new climate change-related issues impairing viticulture sustainability today. In particular, currently neglected cultivars could help preserve must acidity as compared to traditional varieties having early ripening, maintaining the links with terroir and local traditions at the same time.

1. Introduction

One of the main strengths of the wine industry is the large availability of grapevine cultivars. Today, more than 13,000 varieties are globally available, and the various wine regions around the world have built their success on the intimate links between one (or more) of those cultivars and the local environment (i.e., pedoclimatic conditions, traditions, and regional wine styles) [1]. For instance, in the Mediterranean area, from 1960 to 1990, many regions created wine appellations to valorize wines obtained within a specific territory, using the cultivars and the technologies that were best suited for the regions at that time [1, 2]. The genotypes were often chosen according to (i) remunerative yield and medium-to-high bud fruitfulness; (ii) early veraison and ripening to minimize the risk of cluster rot occurring late in the

season due to more abundant precipitation; (iii) high sugar and phenolics accumulation; and (iv) absence of undesired aromas (i.e., foxy) or other peculiar traits not matching the wine styles appreciated at that time [2].

Currently, the actors of many wine regions, including new-world countries focused on a few international varieties, have started wondering if cultivars chosen 40/50 years ago are still appropriate [2–5], as climate change is nowadays posing serious issues to viticulture. The average rise in temperatures and evapotranspiration demand, together with the slight reduction in total annual rainfall and a marked change toward a more erratic distribution, lead to multiple negative effects on the final wines [6, 7]. Faster heat accumulation during the season causes the compression of phenological stages, the increase of organic acids degradation rates, and the advancement of veraison and ripening until they fatally overlap with the hottest days of the year. Even a moderate increase in temperatures during berry ripening may cause a dramatic rise in organic acid degradation rates by respiration [8]. The profile of acids and their concentration at harvest are important parameters in relation to the processing of grape juices and wines as well as to the determination of their chemical composition [7]. Moreover, they strongly influence some sensory features such as taste and equilibrium. The advancement of veraison also increases the susceptibility of grapes to sunburn and berry dehydration. Under such conditions, grapes at harvest often present excessive sugars, poor acidity, and low amounts of secondary metabolites and aromatic compounds, which are sometimes also atypical [6, 7]. High grape sugar concentration might cause a stress response in yeast, which can lead to stuck and sluggish fermentations and to unbalanced wines [9, 10]. As a result, wines are likely to present high alcohol content, low acidity, high pH, low color intensity, atypical flavors, and a scarce attitude to aging. The high ethanol concentration in wine increases hotness and bitterness perceptions, decreasing the acidity sensations and the perception of some important aromatic compounds such as higher alcohols, esters, and monoterpenes [11]. Even worse is the case with the production of white and/or sparkling wines, for which grape and must acidity are key drivers of the final products quality [7, 12, 13].

This current scenario clearly contradicts at least two of the four previously mentioned criteria adopted in the past for choosing varieties to be included in the appellations (namely, early veraison and ripening, and high sugar concentration). For this reason, after more than 50 years, the introduction of new varieties or the reconsideration of minor and insofar neglected cultivars or clones is gaining enormous interest and popularity [12, 14]. Many works have described and characterized fruit composition of old local grapevine varieties or accessions under various aims. In northern Spain, for instance, seven old varieties were tested for grape composition under elevated CO_2 and temperature vs. Tempranillo as a reference, and the latter exhibited scarce adaptability to such conditions, with significant loss of sugars, acidity, and anthocyanins. Conversely, some local old varieties showed stable composition [15, 16]. Within the same framework, in Spain and Portugal, several research groups are looking for ancient clones of Tempranillo able to cope with water scarcity, high air temperatures and CO_2, or having delayed ripening tends [17–22]. Other groups in France are actively seeking for quantitative trait loci controlling grape organic acids degradation with the goal of selecting new varieties with reduced or postponed loss of acidity [23]. However, all the mentioned studies were limited to grape composition, and the presence of the promising traits in the final wines was never demonstrated.

In this work, the most cultivated white cultivar in the Colli Piacentini wine region (northern Italy), cv. Ortrugo, was compared to a currently neglected local variety, namely, Barbesino. Ortrugo, despite having renown problems of retaining acidity at harvest, is traditionally used to produce midsparkling and sparkling wines for which good acidity at harvest is mandatory. Conversely, local tradition affirms that Barbesino was historically marginalized due to its late ripening, and the first agronomic trials confirmed better retention of acids at harvest [12].

The aim of this work was to verify if this promising genotype can guarantee a satisfying productivity and improve the acidity and sensory attributes of wines as compared to those obtained from the standard local variety. The general hypothesis was that local biodiversity is hiding the potentialities needed to solve the problems of districts dealing with cultivars having early ripening and poor acidity at harvest and to maintain adequate wine quality in the current climate change scenario.

2. Materials and Methods

2.1. Experimental Site and Layout. The experiment was carried out over two consecutive seasons (2019 and 2020) in a vineyard germplasm collection at Mossi 1558 Estate (Albareto, Ziano Piacentino, Italy, 44° 97′ 93″N, 09° 40′ 99″E, and 270 m asl). The plot consists of several local and international varieties, including Ortrugo, the most widely cultivated white cultivar in the area, and Barbesino, a minor local variety currently cultivated in less than one hectare surface in the area. According to the relative abundance of the propagation material at the time of planting, Ortrugo is present in one row of 27 vines, whereas Barbesino was planted in two rows of 27 vines each. This study was conducted on these three adjacent rows, and Ortrugo (ORT) and Barbesino (BRB) represented the two treatments. In addition, in 2020, one of the two rows of Barbesino was selected and tagged for a delayed harvest (BRB-LH). The rows were divided into three uniform sections to maintain three biological replicates along the study, and all the vines grafted on Kober 5BB rootstock were planted in 2003 at 2.2 m × 2 m spacing (between row and within row distance, respectively) with coupled vines in the row for a resulting density of 4545 plants/hectare. Each year, vines were cane-pruned (VSP, Guyot pruning system) in winter to retain 12 buds per vine, and thinning was applied between BBCH 14-15 to maintain one primary shoot per node, and nine test vines per treatment (three per replicate/section) were randomly chosen along the row(s) and tagged. Furthermore, the tagged vines were used for detailed assessment of vegetative growth,

yield components, and grape composition at harvest, whereas the others were used for veraison-to-harvest berry samplings and winemaking. Phenological stages were determined after Lorenz et al. [24]. Daily maximum temperatures (T_{max}), mean temperatures (T_{mean}), minimum temperatures (T_{min}), and rainfall from 1 April to 31 October of both years were obtained by a weather station located nearby (200 m) the vineyard (Supplementary Figure 1). Other details about site and vineyard features and management can be found in the study by Frioni et al. [12].

2.2. Yield Components, Fruit Composition, and Vine Balance. Each year, from veraison to 2 weeks postharvest, three 50-berry samples (one per replicate) were taken weekly from non-tagged vines of each varietal to avoid alterations in natural cluster morphology and berry ripening on tagged vines. During sampling, it was assured that the removed berries were collected from clusters located on both sides of the row and, within each cluster, the top, median, and bottom portions were also represented. Ten berries were used to calculate the berry volume by submerging them in deionized water. Remaining berries were weighed and crushed to obtain juice. Musts were analyzed immediately for total soluble solids (TSS) using a temperature-compensated desk refractometer, whereas pH and titratable acidity (TA) were measured by titration with 0.1 N NaOH to a pH 8.2 end point and expressed as g/L of tartaric acid equivalents. To assess tartaric and malic acid concentrations, an aliquot of the must was diluted four times, then filtered through a 0.22 μm polypropylene syringe for high-performance liquid chromatography (HPLC) analysis and transferred to autosampler vials. All solvents were of HPLC grade. The chromatographic method was developed using an Agilent 1260 Infinity Quaternary LC (Agilent Technology) consisting of a G1311B/C quaternary pump with an inline degassing unit, a G1329B autosampler, a G1330B thermostat, a G1316B thermostated column compartment, and a G4212B diode array detector (DAD) fitted with a 10 mm path, 1 μL volume Max-Light cartridge flow cell. An Allure Organic Acid column, 300 × 4.6 mm and 5 μm (Restek), maintained at 30 ± 0.1°C, was used. Separation was performed in isocratic conditions using water, pH-adjusted to 2.5 using orthophosphoric acid, at a flow rate of 0.8 mL/min. 15 μL of sample was injected. The elution was monitored at 200 to 700 nm and detected by UV-vis absorption with DAD at 210 nm. Organic acids were identified using authentic standards, and quantification was based on peak areas and performed by external calibration with standards. Berry malic acid concentration was estimated by multiplying must malic acid concentration per average berry volume. The TSS/TA and tartaric/malic acid ratios (HT/HM) were then calculated. Must malic acid loss rates were calculated as the difference in malate concentrations between two consecutive sampling dates divided by the number of elapsed days.

In both years, ORT and BRB vines were harvested when ORT scored a TSS of about 20°Brix. In 2020, additionally, BRB-LH vines were harvested at the achievement of TSS of about 24°Brix, and the resulting harvest dates were 5 Sep 2019 (99 days after anthesis-DAA) and 26 Aug 2020 (85 DAA) for ORT and BRB, whereas BRB-LH was harvested on 14 Sep 2020 (104 DAA).

At harvest, test vines were individually picked, the mass of clusters was weighted, and the total cluster number per vine was counted. Concurrently, three representative clusters per vine—usually inserted on basal, median, and apical cane portions—were taken to the laboratory for further subsampling. Fruits were individually weighted and the main rachis length measured to calculate the cluster compactness index, expressed as the cluster mass-to-rachis length ratio [25]. From each of the three clusters, a 50-berry subsample was taken by carefully cutting each berry at the pedicel with small sharp scissors and then crushing, and the obtained must was then used for technological maturity determinations.

Upon completion of leaf fall (end of November), all test vines were pruned, and the removed one-year-old pruning weight was immediately recorded in the field using a portable digital scale, and the yield to total pruning weight ratio (kg/kg), otherwise, known as the Ravaz index, was then calculated.

2.3. Chemicals and Reagents. The methanol, ethanol, acetonitrile, dichloromethane, sulphuric acid, hydrochloric acid, gallic acid, catechin, vanillin, and Folin–Ciocalteu reagent, ethyl acetate, ethyl butyrate, ethyl isobutyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl isovalerate, ethyl lactate, ethyl pyruvate, ethyl succinate, ethyl propionate, butyl acetate, isoamyl acetate, methyl hexanoate, methyl octanoate, diethyl malate, diethyl succinate, 2-phenylethyl acetate, 2-methylbutyl acetate, isoamyl lactate, diethyl malate, propyl acetate, *cis*-3-hexen-1-ol, 1-hexanol, 3-ethyl butanol, 1-pentanol, 2-octanol, isobutyl alcohol, isopentyl alcohol, hexyl alcohol, phenyl ethyl alcohol, methionol, benzyl alcohol, isohexyl alcohol, acetaldehyde, isobutyric acid, 2-methylbutanoic acid, butanoic acid, pentanoic acid, hexanoic acid, octanoic acid, and decanoic acid standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), and the chemicals were all at least of analytical grade. HPLC-grade water was obtained by a Milli-Q system (Millipore Filter Corp., Bedford, MA, USA).

2.4. Winemaking. In 2020, about 30 kg of grapes per replicate for each treatment (ORT, BRB, and BRB-LH, three replicates per three treatments) were hand-harvested, and each grape sample was destemmed and gently pressed at 0.8 bar-1 minute with a hydraulic press (Model W40; Grifo Marchetti, Piadena, CR, Italy) to obtain approximately 20 L of juice for each batch. The juices were moved separately to 30 litre stainless steel vats, 50 mg/L potassium metabisulphite (Sigma-Aldrich, St. Louis, MO, USA) was added, and the juices were inoculated with *Saccharomyces cerevisiae* at 30 g/hL (L'Enotecnica, Nizza Monferrato, Italy). The fermentations were performed at 17 ± 1°C and monitored daily by measuring wine density until the end of the process (constant density for three consecutive days). At the end of the alcoholic fermentations, the wines were racked, added to

potassium metabisulphite at 40 mg/L, bottled in 330 mL glass crown-capped bottles, and stored at 8°C for two months.

2.5. Oenological Analyses. The oenological parameters were determined in each wine sample: ethanol content, pH, titratable acidity (TA), volatile acidity (VA), free, combined, and total SO_2 were measured using OIV methods [26]. A kit K-FRUGL 11/05 for the determination of D-fructose and D-glucose was purchased from Megazyme International Ireland Ltd. (Megazyme International Ltd., Wicklow, Ireland), and all analyses were performed in triplicate. Wine organic acids were analyzed as reported by Izquierdo-Llopart et al. [27], with some modifications. Briefly, 30 mL of each wine were treated with 500 mg of PVPP (L'Enoclar PVPP E, L'Enotecnica, Nizza Monferrato, Italy), filtered on 0.22 μm membranes (Sartorius Stedim Biotech GmbH, Heidelberg, Germany), and analyzed for L- (+) tartaric, L- (−) malic, citric, and acetic acid using the HPLC Perkin-Elmer Series 200 (Perkin Elmer, Shelton, CT, USA) system coupled with a diode array detector (DAD) set to 210 nm and LC-Net II/ADC communication module with ChromNAV Control Center software (Jasco Europe Srl, Cremella, Italy). The analyses were performed isocratically at 0.5 mL/min with a Phenomenex Rezex ROA-organic Acid H^+ (8%) (300 mm × 7.8 mm) column using 0.005 N H_2SO_4 as the mobile phase. The injection volume was 10 μL, and the column was thermostated at 40°C.

2.6. Phenolic Compounds and Chromatic Properties of Wines. Flavans reactive with vanillin (FrV) and total flavonoids (TF) were determined using a spectrophotometer (V-730 UV-Vis, Jasco Europe Srl, Cremella, Italy), as reported by Di Stefano [28], and total phenolic compounds were also evaluated using the Folin–Ciocalteu index (FCI) [29]. The results were expressed as mg/L of gallic acid equivalents (FCI) and as mg/L of catechin equivalents (TF and FrV) by means of calibration curves. The wine colorimetric properties were measured using CIELab, a uniform three-dimensional space defined by the colorimetric coordinates L^*, a^*, b^*, C^*, and H^*, and the total color difference (ΔE) was calculated using the following equation: $\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{0,5}$ where L_0^*, a_0^*, and b_0^* correspond to the ORT wines values, while L^*, a^*, and b^* were the values measured in the BRB and BRB-LH wines [30].

2.7. Wine Aroma Compounds. The determination was carried out following the method reported by Piñeiro et al. [31], with some modification. Aroma compounds were purified using a Visiprep SPE vacuum manifold (12-port model) from Supelco (Supelco Park, Bellefonte, PA) and LiChrolut EN resins and prepacked in 200 mg cartridges obtained from Merck (Darmstadt, Germany). To 50 mL of each wine sample, 400 μL of 2-octanol (100 μL of 1 g/L solution in ethanol) was added as internal standard, and the cartridges were conditioned by rinsing with 4 ml of dichloromethane, 4 mL of methanol, and 4 mL of an ethanol-water mixture (12%, v/v). Then, 50 mL of sample was rinsed through the cartridge at around 2 mL/min by vacuum suction, and a clean-up was obtained by flushing the cartridge with 10 mL of Milli-Q water. The cartridge was then dried under vacuum, and aroma compounds were finally eluted from the solid phase using 2 mL of dichloromethane. Eluted extracts from SPE were stored at −40°C until the chromatography analysis. An aliquot of 2 μL of each extracted wine sample was injected into the GC/MS in split mode (20 : 1) and analyzed for identification and quantification of the volatile compounds contained in the extracted volume. Gas chromatographic analysis was performed using a Trace GC ultra gas chromatograph (Thermo Scientific, San Jose, CA, USA) equipped with an ISQ single-quadrupole mass spectrometer (Thermo Scientific), and the system was controlled using the Excalibur 2.1 software (Thermo Scientific). The carrier gas was helium at a constant flow rate of 1 ml/min. The analysis was carried out using a capillary column Rtx-5Sil MS, 30 m, 0.25 mm i.d., and 0.25 mm film thickness (Restek Corporation, Bellefonte, PA, USA), having a column head pressure of 55 kPa. The oven temperature was from 40°C (held for 6 min) to 200°C at 5°C/min (held for 1 min) and then to 280°C (held for 5 min) at 80°C/min. MS transfer-line and ion source temperatures were 230°C and 250°C, respectively, and electron ionization was set at 70 eV. The MS detector scanned within a mass range of m/z 30–400. The putative identification of volatile compounds was carried out by comparing the mass spectra with those available in the data system library (NIST 08, National Institute of Standards and Technology, Gaithersburg, MD, 2008). A positive characterization was achieved when a volatile compound was identified with a probability of >70% in at least three independent samples. The identity of the compounds was further confirmed by comparison of the retention times with authentic standards here reported: ethyl acetate, ethyl butyrate, ethyl isobutyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl isovalerate, ethyl lactate, ethyl pyruvate, isoamyl acetate, methyl hexanoate, diethyl malate, diethyl succinate, 2-phenylethyl acetate, isoamyl lactate, *cis*-3-hexen-1-ol, 1-hexanol, 3-ethyl butanol, 1-pentanol, 2-octanol, isobutyl alcohol, isopentyl alcohol, phenyl ethyl alcohol, methionol, benzyl alcohol, acetaldehyde, isobutyric acid, butanoic acid, hexanoic acid, octanoic acid, and decanoic acid. All reagents and standards were purchased from Sigma-Aldrich (St. Louis, MO). Data (μg/L) were obtained by measuring the relative peak area of each identified compound in relation to that of the added internal standard.

2.8. Sensorial Analysis. Sensory profiling of wines was carried out on stables wines 10 weeks postbottling through the quantitative descriptive analysis (QDA), a technique encompassing the following stages: (i) a lexicon generation process; (ii) a set of sensory tests designed to quantify on a rating scale the intensity of the sensory terms established in the lexicon generation phase; and (iii) the statistical processing of the results and their interpretation to obtain the sensory profiles of the products [32, 33]. Sensory analysis was performed at the SensoryLab (Università Cattolica del

Sacro Cuore, Piacenza, Italy), a laboratory complying with the ISO 8589:2007 standard.

The wines were evaluated by a panel of nine assessors with a broad experience in wine sensory evaluation as well as interest and availability, and the panelists were asked to evaluate the visual, taste, and aroma attributes on a 9-point line scale (anchored at both extremes as "not perceived at all" and "extremely intense"); descriptors were selected by the panelists with support from the existing literature in the field [34].

The wine samples were monadically served to panelists, and three-digit random numbers were assigned to each sample for tracking purposes prior to service. The wine samples were evaluated in duplicate on two sessions on the same day, and the order of presentation was balanced and randomized across samples, panelists, and replicates, according to a rotated tasting plan [35]. The panelists were provided with still mineral water and unsalted breadsticks to cleanse their palates between samples.

No approval from the Human Ethics Committee was required by our institution to perform the sensory analysis in this research.

2.9. Statistical Analyses. Field and grape composition data (ORT vs BRB) collected over 2 years were subjected to a two-way ANOVA (treatment, year), and the evolution of parameters assessed over multiple samplings during the season was analyzed using the function repeated measures ANOVA in IBM SPSS Statistics 24.0 (SPSS Inc., Chicago, IL, USA). Furthermore, comparison of musts and wine composition (ORT vs BRB vs BRB-LH) was subjected to a one-way ANOVA, and statistically significant differences between samples were tested using a post hoc comparison test (SNK test at $P < 0.05$).

The wine sensory dataset was first processed as reported by Romanini et al. [36] by assessing the validation and replicability power of the panel via analytical replicate, and data were recorded with the ADS system using the Horizon Design and "Centro Studi Assaggiatori Brescia" as already applied by Vezzulli et al. [37]. Data were then processed with Microsoft Excel 2021 to obtain spider graphs, and statistically significant differences between samples were tested according to Vercesi et al. [38, 39]: after the application of the Levene test per each descriptor, the nonparametric Friedman T test (with judges as blocks) was used, and the significant differences among the wines were assessed through a minimally significant difference evaluated on the sum of the ranks as reported by Freund et al. [40]. Statistical elaboration was carried out by IBM SPSS Statistics 27.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. TSS Accumulation and Organic Acids Depletion in Ortrugo and Barbesino Grapes. In both years, ripening kinetics showed that BRB had a notably different pattern of organic acid loss as compared to ORT, whereas the sugar accumulation dynamics were similar (Figures 1(a) and 1(b)). Although, especially in 2020, ORT showed slightly higher TSS than BRB right after veraison, beyond the 14-15°Brix threshold, no differences were found between the two cultivars until the end of the season (Figures 1(a) and 1(b)). Conversely, BRB had a constantly higher TA either pre- and postveraison as compared to ORT (in 2020, it ranged from +10.8 g/L at 70 DAA to +2.9 at 96 DAA). Grapes pH tracked the pattern observed for TA, with BRB showing a lower pH than ORT during the entire ripening process, in both years (Supplementary Figure 2).

In a recent paper, Shahood et al. [41] showed that different cultivars could exhibit, other than different timing of the occurrence of phenological and ripening progression, a significantly variable duration of the veraison period, with a considerable genotype-dependent heterogeneity in berry growth, sugars accumulation, and malic acid breakdown within a single cluster. In such a context, the remarkable difference in TA with a very similar TSS pattern supports the conclusion that BRB and ORT have a concomitant onset of veraison occurrence, but we cannot exclude that BRB and ORT display different veraison durations or time extensions. The slower rise of pH exhibited by BRB in 2020 can be a hint for such a hypothesis (Supplementary Figure 2B). However, if this is the case, effects are limited to the very early stages of ripening; otherwise, TSS should be diverging in the two varieties after 15°Brix, and this did not occur.

Interestingly, differences between varieties were unaffected by the seasonal weather course and were consistent also in 2020, when due to higher heat summation early in the season (+117 cumulated GDD between May 1 (day of year 121) and May 31 (DOY 151), Supplementary Figure 1), veraison of both varietals was anticipated and TA decrease was steeper (Figure 1(b)). The tight correlations found between TA and TSS during ripening in the two seasons confirm that ORT exhibits far lower acidity than BRB at varying TSS. Actually, the TA gap between the two treatments slightly widens, moving from the lowest Brix levels recorded at the onset of veraison to full maturity (Figure 2).

Figure 3(a) shows the malic acid concentration trend in ORT and BRB during the entire 2020 season. BRB had a much higher preveraison malic acid pool (+4.3 g/L), a delayed onset of malic acid degradation, and a higher concentration at harvest. Single-berry malic acid content followed a slightly different pattern preveraison, when no difference was found between varieties (Figure 3(b)). This was related to the fact that when the first grape sampling was performed at 59 DAA, malic acid in BRB was not yet at the peak; that was reached a few days later than ORT, and this is reflected in the must malic acid loss rates (Figure 3(c)), which were close to zero for BRB at the first sampling. Later, BRB preserved a significantly higher malic acid concentration until the last sampling stage. On a berry content basis, treatments were following the same pattern, but differences had a lower magnitude, likely a consequence of the lower ORT berry size (Supplementary Figure 3) and eventual dilution/concentration interactive effects. However, although the undoubtedly higher malic acid pool in BRB, in this cultivar, malic loss rates were much higher than those calculated for ORT. The capability of BRB to show higher acidity at harvest seemed related to a different minimum

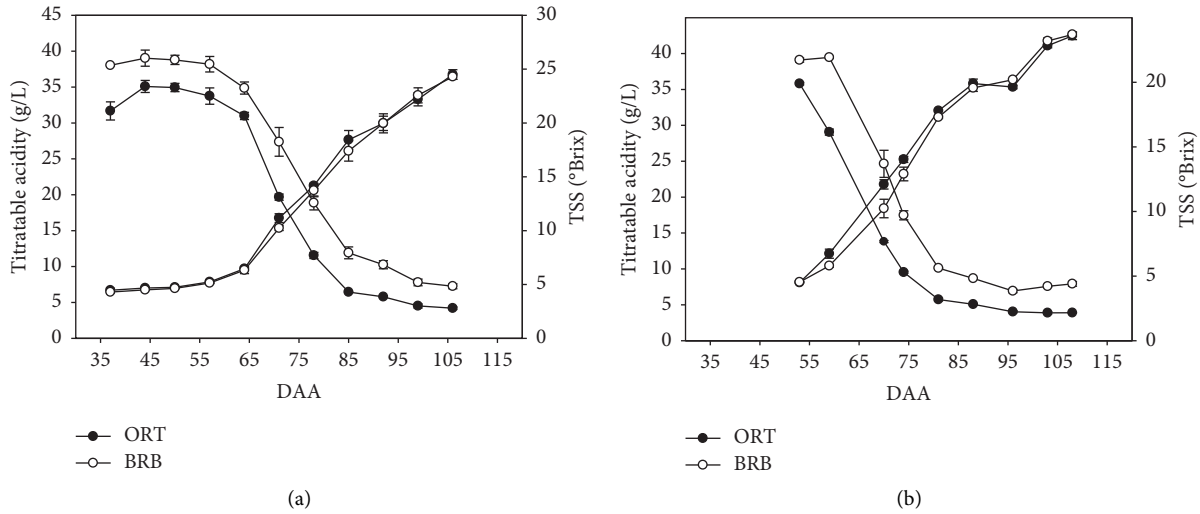

Figure 1: Seasonal dynamics total soluble solids (TSS) and titratable acidity in grapes from cv. Barbesino (BRB) and cv. Ortrugo (ORT) grapevines in 2019 (a) and 2020 (b) (mean values ± standard error; $n = 3$). DAA = days after anthesis.

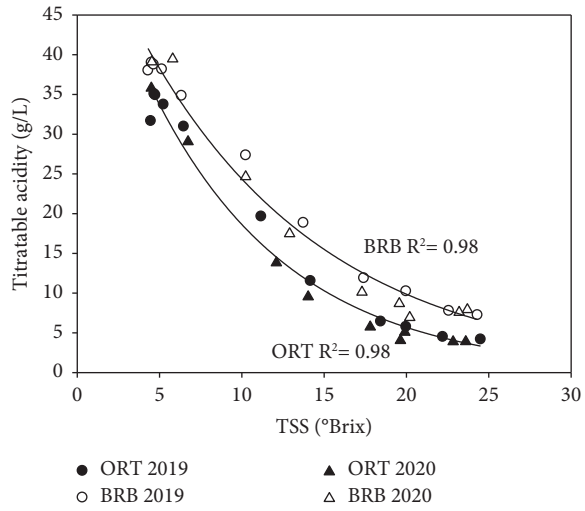

Figure 2: Correlation between total soluble solids (TSS) and titratable acidity in grapes from cv. Ortrugo (ORT, $y = 60.18 * \exp(-0.09x)$, $P < 0.001$) and cv. Barbesino (BRB, $y = 61.02 * \exp(-0.12x)$, $P < 0.001$) grapevines in 2019 and 2020.

malic acid concentration. In fact, BRB achieved a minimum malic acid concentration (about 1.2 g/L) and berry content (0.75 g/berry) only 103 DAA, with no further malate losses onwards. Air temperatures later occurring (21°C Tmean from 103 to 116 DAA) were compatible with malic acid degradation [8]; however, despite this, BRB malic acid concentration was never below the 1.2 g/L threshold. Conversely, in ORT, a very low malic acid concentration (~0.35 g/L, 0.3 g/berry) was already achieved 88 DAA, demonstrating that there is a strict varietal control over grapes minimum malic acid concentration.

Overall, the scenario depicted from the data reported in Figures 1–3 highlights the earlier ripening of ORT varieties as compared to BRB, and according to the available literature, malic acid breakdown during grape ripening is mainly due to oxidation catalyzed by malate dehydrogenase and malic enzyme in the first metabolic stages of the berry respiration process [8]. Temperature is considered the main driver of malate oxidation, and high temperatures post-veraison foster malic acid loss [8, 42]. Another factor affecting malate degradation rates via respiration is the availability of substrate, namely, malic acid abundance [43]. In our conditions, ORT showed an early peak of malate loss (0.86 g/L per day between 59 and 70 DAA), whereas maximum malic acid loss rates in BRB were recorded between 70 and 74 DAA (Figure 3(c)). Noteworthy, the maximum loss rates found in BRB (1.27 g/L per day) were significantly higher than those in ORT. Thereafter, BRB maintained higher malic acid loss rates until 103 DAA beyond which no differences occurred between the two cultivars. These data prompt some considerations as follows: first, because ORT never scored more than 0.9 g/L per day (vs 1.27 g/L per day in BRB), the abundance of the substrate seems to be the main driver of malic acid loss rates; second, lower degradation rates do not correspond to higher acidity at harvest, since ORT was showing the lowest acidity in both years; and third, a key role in final acidity is played by a sort of genotype-dependent minimum acidity. In our case, the lowest malic acid concentration recorded in ORT was 0.35–0.60 g/L, whereas in BRB, the minimum concentration (1.16– 1.26 g/L) never went so low. Even more interesting was the moment when this condition (i.e., flat malic acid degradation rates, <0.05 g/L per day) was achieved: in ORT, loss of acidity was substantially over 88 DAA, whereas in BRB, null malic acid loss rates were reported only after 103 DAA.

The evolution of must malic acid loss rates as a function of TSS achieved within the same period was fitted to two different Gaussian models (Figure 4(a)). In ORT, maximum loss rates were lower in magnitude and were achieved at lower TSS (10.5° Brix vs 13.7°Brix in BRB). Moreover, in ORT, malic acid loss rates were zeroed at TSS levels of about 17.8°Brix, whereas in BRB, null malate losses were recorded only from a TSS concentration of 23.2°Brix. However, Figure 4(b) reveals that

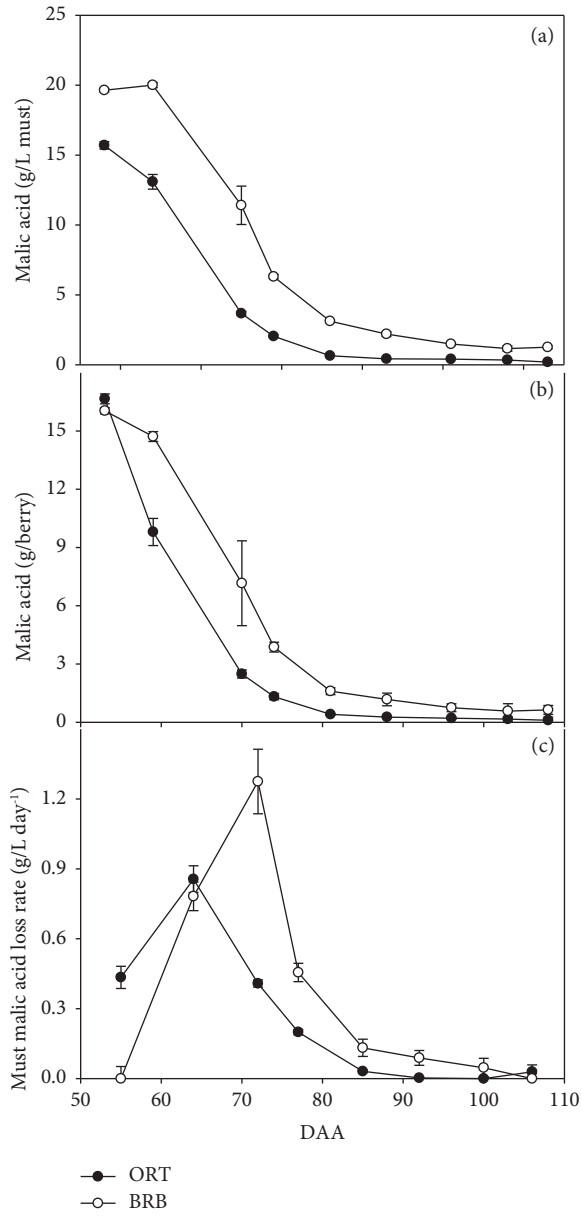

Figure 3: Seasonal dynamic of must malic acid concentration (a), berry malic acid concentration (b), and must malic acid loss rates (c) in grapes from cv. Barbesino (BRB) and cv. Ortrugo (ORT) grapevines in 2020 (mean values ± standard error; $n = 3$). DAA = days after anthesis.

the higher malic acid loss rates in BRB seen in Figure 3(b) are essentially a smokescreen, at least after full veraison. In fact, looking at the malate daily loss as a function of instantaneous malate concentration (Figure 4(b)), the built correlations highlight that ORT is the genotype exhibiting notably higher malic acid loss rates as long as malic acid concentration is lower than 9 g/L, whereas BRB prevails for any malic acid concentration above 10 g/L. In addition, within clusters ripening heterogeneity can be dramatically high between the berry softening stage and the end of veraison (i.e., at TSS < 15°Brix) [41] and variable between cultivars, meaning that the duration of the veraison stage could interact with malic acid loss when malic concentration is >9 g/L. In summary, data hint that (i) in varieties like ORT, the entire stock of malic acid is lost well before the achievement of adequate TSS; (ii) malic acid degradation rates should not be evaluated over chronological time, rather versus the seasonal dynamic malate concentration; and (iii) even in the presence of faster degradation rates as recorded in BRB vs ORT, in some varieties, a fraction of malate can be preserved until late at high TSS, no matter what the air temperatures or the abundance of the substrate are (Figures 4(a) and 4(b)). In this regard, we hypothesize that this fraction of malate is unavailable for respiration. Some hypotheses have been made in the literature (i.e., links with plant K^+ uptakes and translocation to berries) [23] although the reasons still need to be clarified.

In short, our data demonstrate that total acidity at harvest can be quite independent by net malic acid degradation rates during ripening. In the current warming trend scenario, the positive attitudes of a genotype at retaining adequate TA mainly relate to the following: (i) minimum malic acid concentration retained at full ripening, rather independent from canopy management, heat summation, and harvest date, which assumes dignity to be a varietal trait (Figure 3(a)); (ii) specific relationships between total malic acid concentration and derived degradation rates; and (iii) the TSS threshold at which minimum malate is achieved and malic acid degradation is arrested (Figure 4). Displaying a suitable balance between malate (i.e., ≥1.5 g/L) and TSS (≥20°Brix) concentration when null malic acid loss is first observed enables picking grapes with an optimal TSS/TA ratio for white/sparkling winemaking, also in hot vintages and regardless of harvest date, and combinations of these parameters could serve as new benchmarks for spotting those minor or neglected cultivars that can be reintroduced to maintain the quality of musts for white and sparkling wines production.

3.2. Yield Components and Fruit Composition at Harvest. ORT and BRB showed similar vine yield in both seasons (Table 1) although ORT had a significantly lower cluster number per vine (10 vs 18 in BRB). This difference was counteracted by the notably higher size of the ORT clusters (300 g vs 152 g in BRB). Interestingly, BRB had looser clusters than ORT (−45%). Loose clusters prevent or minimize rot during wet seasons (Tello and Ibanez 2005), and higher shoot fruitfulness allows for training vines into easily mechanizable systems based on short pruning [44]. Harvesting BRB vines 19 days later (BRB-LH) at a TSS of about 24° Brix did not lead to changes in yield, berry size, or cluster size and compactness (Supplementary Table 2), a sign that the genotype achieves the final berry growth at a relatively low TSS. Overall, given the sufficient, yet not exceptional, average yield of cv. Ortrugo, steering toward less productive varieties is not an option for the local industry, although BRB seems to guarantee a comparable yield, with lower susceptibility to rots.

For white varieties meant for sparkling winemaking, a desirable grape composition at harvest is often as follows: sugars concentration between lower than 22°Brix, a titratable acidity (TA) ≥7.5 g/L, and a pH ≤ 3.2 [7, 13, 45]. Data pooled over two seasons demonstrate that cv. Ortrugo is barely

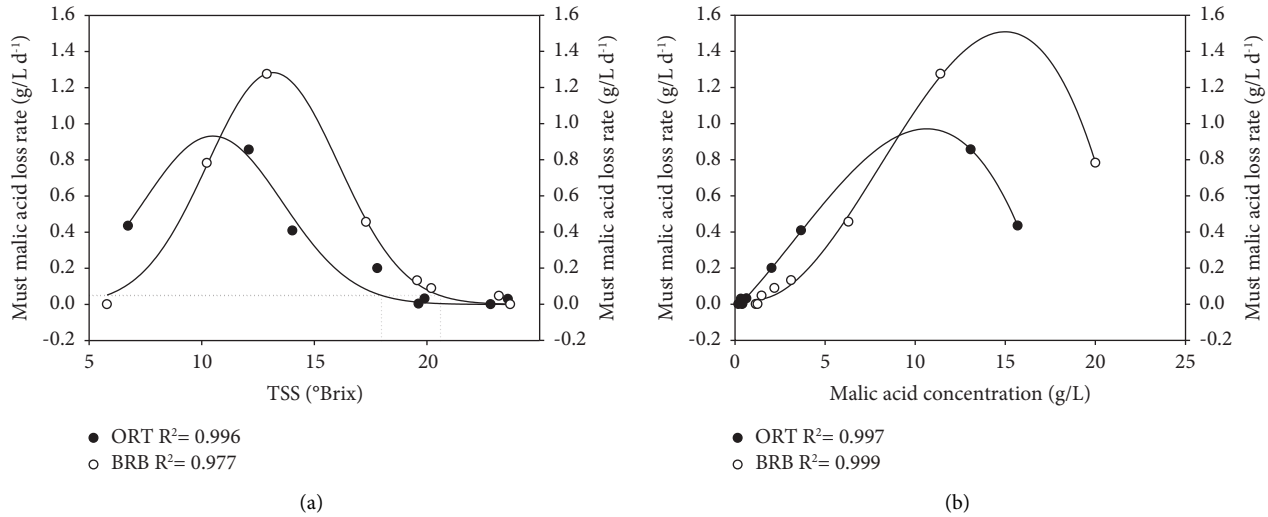

Figure 4: Correlation fitted between total soluble solids (TSS) (a) or must malic acid concentration (b) and must malic acid loss rates in grapes from cv. Ortrugo (ORT) and cv. Barbesino (BRB) in 2020. (a) ORT: $y = 1.28 * \exp(-5 * ((x - 13.16)/2.91)^2)$ $P < 0.001$, BRB: $y = 0.93 * \exp(-5 * ((x - 10.50)/3.09)^2)$ $P < 0.001$. (b) ORT: $y = 0.057 - 0.056 * x + 0.027 * x^2 + 0.001 * x^3 P < 0.001$, BRB: $y = -0.028 + 0.100 * x + 0.008 * x^2 + 0.001 * x^3 P < 0.001$.

Table 1: Yield components, vine balance, and fruit composition at harvest of cv. Ortrugo (ORT) and cv. Barbesino (BRB) grapevines in 2019 and 2020.

	Variety (V)[a]			Year (Y)[a]			F ($V \times Y$)[a]
	ORT	BRB	F (V)[a]	2019	2020	F (Y)[a]	
Vine yield (kg/vine)	2.96	2.83	0.120 ns	2.20	3.82	41.540***[b]	1.179 ns
Cluster weight (g)	300	152	126.564***	199	262	21.714***	3.739 ns
Clusters per vine (n)	10	18	84.173***	12	17	24.536***	10.436**
Cluster compactness (g/cm)	14.79	8.02	30.319***	10.81	12.23	1.314 ns	0.006 ns
Berry weight (g)	1.79	2.18	5.373*	1.80	2.22	6.815*	0.521 ns
Pruning weight (kg/vine)	0.276	0.401	5.724*	0.371	0.296	2.516 ns	2.743 ns
Ravaz index (kg/kg)	11.1	8.2	1.674 ns	6.8	13.6	12.044**	1.667 ns
TSS[c] (°Brix)	19.7	20.6	1.509 ns	20.4	19.8	0.321 ns	3.418 ns
Titratable acidity (g/L)	5.09	9.53	33.572***	7.93	6.49	3.753 ns	1.878 ns
Tartaric acid (g/L)	7.51	7.04	0.294 ns	7.13	7.45	0.175 ns	0.199 ns
Malic acid (g/L)	0.57	2.59	21.215***	2.03	0.98	6.222*	2.401 ns
HT/HM[c]	15.24	3.36	235.084***	6.24	13.37	76.678***	27.440***
TSS/TA[c]	3.87	2.10	49.152***	2.57	3.05	1.129 ns	1.129 ns

[a]V = variety; Y = year, $V \times Y$ =variety × year interaction, and $F = F$ values. [b]*, **, and *** denote significant difference per $P < 0.05$, $P < 0.01$, and $P < 0.005$, respectively. ns means no difference. [c]TSS = total soluble solids; HT = tartaric acid; HM = malic acid; TA = titratable acidity.

capable of reaching such a compositional combination. In both 2019 and 2020, when grapes achieved optimal harvest TSS thresholds (20°Brix), they exhibited inadequate TA (5.09 g/L, as a mean of the two seasons) (Table 1), in agreement with the data shown in Figures 1 and 2 and with other previous works [12, 46]. Conversely, BRB showed optimal acidity for the same TSS levels (9.53 g/L, +67% than ORT) due to a high concentration of malic acid (2.59 g/L vs 0.59 g/L in ORT). Tartaric acid at harvest was substantially unaffected by the genotype. Even if its abundance in grapes is a relevant parameter for the production of sparkling wines, tartaric acid is not a substrate of respiration like malic acid, and therefore changes during ripening are lower and mainly due to dilution and K^+ salt formation [8] and less to air temperature. Often, varieties showing very low malic acid at harvest have high TSS/TA and HT/HM, and their acidity only depends by tartaric acid [7]. In our work, BRB had a considerably lower TSS/TA (2.10 vs 3.87) and HT/HM ratio (3.36 vs 15.24) than ORT. For white/sparkling wines, the TSS/TA ratio should be 2–2.5 and HT/HM ratio should be close to 3 [7, 13]. In summary, our data confirm that while cv. Ortrugo is dramatically affected by global warming, becoming progressively inadequate to produce high-quality white sparkling wines in traditional wine districts, the fruit composition of cv. Barbesino seems to be optimal for the same purpose.

In 2020 (Table 2), BRB grapes harvested at a TSS of about 24°Brix (BRB-LH) showed a quite expected lower must acidity than BRB grapes harvested at 20°Brix (−24%); yet, BRB-LH TA was still higher than that of must obtained from

Table 2: Grapes composition of cv. Ortrugo (ORT) and cv. Barbesino (BRB) grapevines harvested 85 days after anthesis (DAA) and in grapevines cv. Barbesino harvested 104 DAA (BRB-LH), in 2020.

	ORT	BRB	BRB-LH	*F* sign.
TSS[a] (°Brix)	19.6b[b]	20.2b	24.4a	29.607**[c]
Titratable acidity (g/L)	4.87c	8.06a	6.00b	539.16***
Tartaric acid (g/L)	5.91	6.81	6.33	1.785 ns
Malic acid (g/L)	0.35c	1.62a	1.26b	10.969*
HT/HM[a]	21.62a	5.00b	4.70b	194.676***
TSS/TA[a]	4.02b	2.51a	4.07b	45.867***

[a]TSS = total soluble solids; HT = tartaric acid; HM = malic acid; TA = titratable acidity. [b]Different letters within rows indicate significant difference per $P < 0.05$ (SNK test). [c]*, **, and ***denote significant difference per $P < 0.05$, $P < 0.01$, and $P < 0.005$, respectively. ns means no difference.

the ORT harvested at 20°Brix (+25%). This was linked to a very low malic acid concentration in ORT (0.35 g/L) and higher contents in both BRB (1.62 g/L) and BRB-LH (1.26 g/L). Interestingly, BRB-LH had a similar TSS/TA ratio than ORT and a HT/HM comparable to BRB, meaning that postponing harvest in cv. Barbesino leads to an increase in potential alcohol with a slight reduction in acidity that is associated with an unaltered ratio between the two main organic acids. Overall, grapes of BRB-LH seem less suitable than BRB for producing sparkling wines but are more suitable than ORT and BRB for target full-bodied white wines [7].

3.3. Physicochemical Parameters and Chromatic Characteristics of Wines. Table 3 shows the chemical composition of ORT, BRB, and BRB-LH wines produced in 2020. All of the alcoholic fermentations were regularly completed within 12–14 days (residual sugars <0.96 g/L). Volatile acidity ranged from 0.26 to 0.44 g/L, falling within the legal limit [26]. All the wines showed comparable levels of free and total SO_2, well below the limit established by EU Regulation No. 606/2009. ORT wines had an ethanol content of 10.82% v/v and BRB of a 12.10% v/v, while BRB-LH showed the highest alcohol content of 14.85% v/v, reflecting the highest sugar accumulation. As reported in Table 3, BRB wines showed the highest TA (7.61 g/L) and a pH value of 3.06, while ORT wines had the lowest TA (5.71 g/L) and a significantly higher pH (3.15). As expected, BRB-LH had the highest pH (3.29) and a lower TA than BRB (6.60 g/L) although still higher than that of ORT wine. Although TA along with the pH are of great importance for grape wine quality, all individual organic acids play an important role in the organoleptic qualities, and their preservative properties enhance the microbiological and the physicochemical stability of wine [13, 47]. No significant differences resulted in tartaric acid concentration between ORT and BRB wines, while BRB-LH showed a lower tartrate concentration. The differences in TA were mainly due to malic acid: ORT wine had the lowest level (0.86 g/L) compared to BRB (2.70 g/L) and BRB-LH (2.10 g/L). Despite the postponed harvest time of BRB-LH, low reductions in malic acid were observed as compared to BRB wines, in agreement with the organic acid profile recorded in must samples at harvest. This confirms that if malo-lactic fermentation is not undertaken, wines tend to maintain the relative differences in TA previously measured on grapes, resulting in white wines with higher TA and less problems related to too low acidity at harvest.

Analyzing the wine phenolic profiles, BRB-LH showed significantly higher values for all the considered parameters, followed by BRB and ORT (Table 3). The concentration of total flavonoids ranged between 66 and 139 mg/L, while there were no significant differences in flavans reactive to vanillin. The longer ripening period of BRB-LH grapes might have favored a more prolonged synthesis of polyphenolic compounds and, consequently, higher concentrations in final wines [48].

Regarding wine color, BRB-LH showed higher values of a^*, b^*, and C^* than ORT and BRB wines. This was indicated by a gold-yellow color, reflecting the higher polyphenols content detected in BRB-LH [49]. Conversely, BRB was significantly more yellow than ORT (namely, higher b^*), while no difference was detected in a^* and H^* parameters. BRB and ORT wines were characterized by a pale-yellow color with more greenish shades, compared to BRB-LH, and notably, the ΔE^* value for BRB and BRB-LH was >2.7 CIELAB units, indicating that the color differences between wines could be perceived by the human eye [50]. However, the distance to ORT in terms of wine color was much higher for BRB-LH ($\Delta E^* = 10.08$) than for BRB ($\Delta E^* = 4.25$).

3.4. Aroma Composition. To the best of our knowledge, no work in the available literature focused specifically on the differences between the wine aroma profile of a minor variety capable of preserving high acidity versus a common cultivar with low TA. A pool of forty volatile compounds was quantified in experimental wines such as esters (15), higher alcohols (6), fatty acids (5), C_6 compounds (2), and aldehydes (1). As shown in Figure 5, higher alcohols were the largest group of compounds (78.15–82.18%), followed by esters (13.32–17.85%), volatile fatty acids (2.13–3.23%), C_6 volatile compounds (0.89–1.60%), and aldehydes (0.15–1.07%). All the identified aromatic compounds were mainly fermentative aromas [51, 52]. To assess the possible contribution of different components to wine aroma, the detection threshold and the descriptor for each compound are included in Table 4 and in Supplementary Table 3.

3.4.1. Higher Alcohols. Higher alcohols were quantitatively the largest group of volatile compounds detected in experimental wines (48.3–63.5 mg/L), and this result is in line with that from previous studies by Styger et al. [53] and

Table 3: Biochemical composition, phenolic compounds, color, and organic acids concentration in wines obtained by cv. Ortrugo (ORT), cv. Barbesino (BRB) grapes harvested 85 days after anthesis (DAA), and in grapevines cv. Barbesino harvested 104 DAA (BRB-LH), in 2020.

Attributes	ORT	BRB	BRB-LH	*F* sign.
General parameters				
Ethanol (%v/v)	10.82c[a]	12.10b	14.85a	723.736**[b]
Sugars (g/L)	0.77	0.77	0.96	1.200 ns
pH	3.15b	3.06c	3.29a	269.202**
Titratable acidity (g/L of tartaric acid)	5.71c	7.61a	6.60b	48.250**
Volatile acidity (g/L of acetic acid)	0.26c	0.39b	0.44a	131.769**
Free SO_2 (mg/L)	15a	11b	8b	12.036**
Total SO_2 (mg/L)	87a	91a	72b	42.257**
Phenolics				
Total flavonoids (mg/L of catechin)	66c	95b	139a	65.005**
Folin–Ciocalteu index	4.23c	4.79b	5.44a	16.549**
Flavanol reactive to vanillin (mg/L of catechin)	49	60	67	1.876 ns
CIELab				
L^*	94.28b	97.12a	94.07b	15.006**
a^*	−1.21b	−1.02b	0.40a	24.273**
b^*	12.25c	14.60b	22.01a	298.662**
C^*	12.32c	14.64b	22.02a	302.484**
H^*	95.61a	94.12a	88.97b	25.450**
ΔE	—	4.25b	10.08a	85.250**
Color[c]				
Organic acids				
L-(+) tartaric acid (g/L)	3.77a	3.66a	2.95b	38.858**
L-(−) malic acid (g/L)	0.86c	2.70a	2.10b	161.341**
Citric acid (g/L)	0.12b	0.31a	0.25a	59.471**
Acetic acid (g/L)	0.20b	0.44a	0.45a	169.243**

[a]Different letters within rows indicate significant difference per $P < 0.05$ according to Student–Newman–Keuls (SNK test). [b]* and ** denote significant difference per $P < 0.05$ and 0.01, respectively. ns means no difference. [c]Color of wine reproduced on the basis of CIELab coordinates by using the colorizer software (https://colorizer.org, accessed on 15 July 2022).

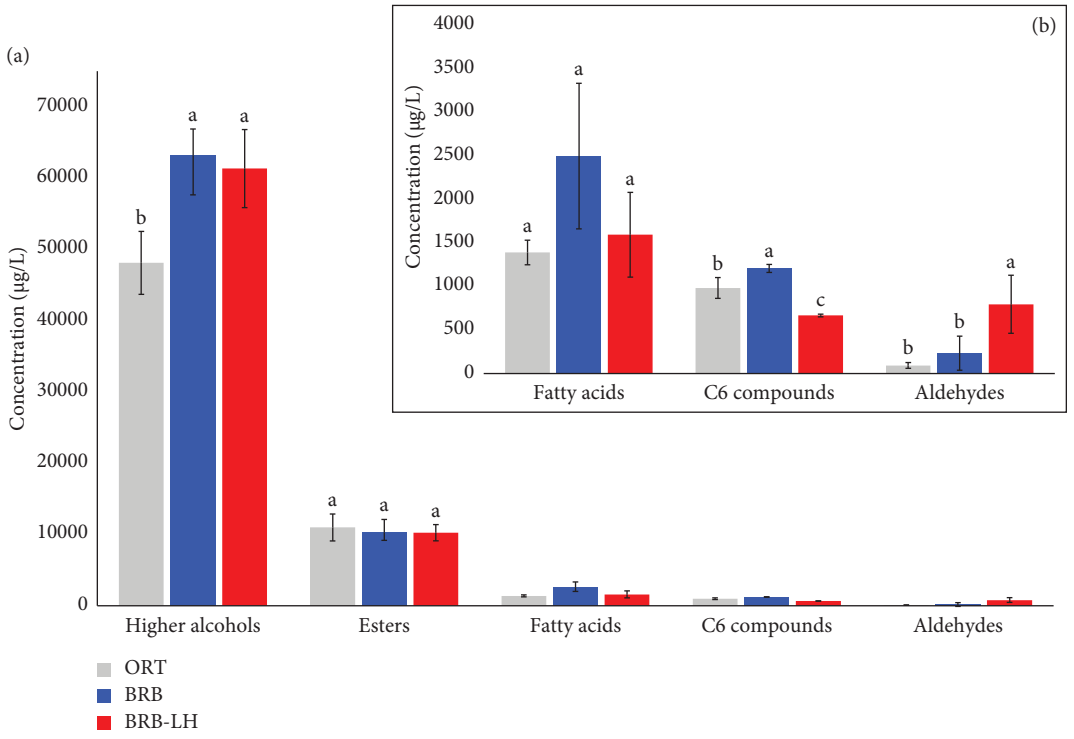

Figure 5: Concentration (μg/L) of quantified volatile compounds of ORT, BRB, and BRB-LH wines grouped by chemical classes. (a) Alcohols and esters. (b) Fatty acids, C_6 compounds, and aldehydes (shown in the inset for sake of clarity, due to low amounts). For each chemical class, different letters indicate significant difference according to Student–Newman–Keuls (SNK test); $n = 3$.

Table 4: Aromatic compounds in wines obtained by cv. Ortrugo (ORT), cv. Barbesino (BRB) grapes harvested 85 days after anthesis (DAA), and in grapevines cv. Barbesino harvested 104 BRB-LH), in 2020.

Compounds	ORT	BRB	BRB-LH	OPT[a]	Odor description[b]	*F* sign.
Esters						
Ethyl acetate	6483a[c]	3018b	2810b	12000	Solvent, fruity, and balsamic	22.954**[d]
Ethyl butyrate	497	670	639	20	Kiwifruit, strawberry, and cheese	0.830 ns
Ethyl isobutyrate	46b	157a	131a	15	Kiwifruit, strawberry, and solvent	8.569*
Ethyl hexanoate	993	1440	935	14	Fruity, green apple, strawberry, spicy, and anise	1.032 ns
Ethyl octanoate	699	936	910	580	Fruity, candy, pineapple, pear, and floral	1.249 ns
Ethyl decanoate	120b	226a	248a	200	Fruity and grape	**
Ethyl isovalerate	n.d.c	5b	30a	1	Fruity and apple	155.484***
Ethyl lactate	891b	1777a	1825a	150000	Acid, medicinal, strawberry, and raspberry	14.849*
Ethyl pyruvate	n.d	42	76	5000	Caramel, ethereal, fruit, vegetable, and sweet	1.346 ns
Isoamyl acetate	203c	395b	520a	160	Banana, fruity, apple, and sweet	26.792***
Methyl hexanoate	44a	n.d.b	n.d.b	14	Fruity, apricot, and pineapple	7.031*
Diethyl malate	158	233	155	760000	Green	1.462 ns
Diethyl succinate	206b	537ba	709a	100000	Wine, caramel, and fruity	5.654*
2-Phenylethyl acetate	595b	736b	1044a	250	Floral and roses	8.521*
Isobutyl acetate	53b	145a	151a	1600	Sweet, fruity, apple, and banana	41.350***
C_6 compounds						
cis-3-Hexen-1-ol	316a	501a	nd	400	Green and fat	14.887*
1-Hexanol	666	704	666	110	Herbaceous, fatty, resinous, floral, green, and cut grass	0.698 ns
Alcohols						
1-Pentanol	n.d.b	30b	83a	80000	Fruity and balsamic	18.588***
Isobutyl alcohol	887c	2301b	3954a	40000	Alcohol	491.199***
Isopentyl alcohol	42975a	42835a	32200b	30000	Alcohol	10.743**
2-Phenyl ethyl alcohol	4070c	17668b	24851a	10000	Floral and rose	119.146***
Methionol	34c	138b	241a	1500	Meat and onion	23.290***
Benzyl alcohol	135a	141a	n.d.b	200000	Floral, rose, phenolic, and balsamic	30.254***
Aldehydes						
Acetaldehyde	93b	233b	795a	100000	Sherry, nutty, and bruised apple	8.291*
Fatty acids						
Isobutyric acid	127	191	229	270	Cheese	2.563 ns
Butanoic acid	n.d.b	n.d.b	147a	170	Cheese and rancid	203.997***
Hexanoic acid	439	1287	743	420	Cheese and greasy	2.123 ns
Octanoic acid	520a	566a	124b	500	Rancidity, candy, cheese, animal, and spicy	32.965***
Decanoic acid	302	452	350	1000	Unpleasant, rancid fat, and animal	2.149 ns

[a]OPT = odor perception threshold. [b]References are provided in Supplementary Table 3. [c]Different letters within rows indicate significant difference per $P < 0.05$ according to Student–Newman–Keuls (SNK test). [d]*, **, and ***denote significant difference per $P < 0.05$, $P < 0.01$, and $P < 0.005$, respectively. ns means no difference. Values are expressed as μg/L.

Ribéreau-Gayon et al. [54]. They are produced by yeast during the alcoholic fermentation through either the anabolic pathway of glucose or the catabolic pathway of amino acids [55]. Their contributions to wine aroma vary from honey, rose, and floral character (2-phenylethyl alcohol and benzyl alcohol) to pungent and solvent-like smells (1-propanol, 1-butanol, 2-, and 3-methyl-1-butanol) [52], and the effects depend on their concentration. The total concentration of higher alcohols found in this study was below (48.1–63.1 mg/L) the spoilage threshold fixed at 300 mg/L [56]. As shown in Table 4, BRB (63.1 mg/L) had the highest concentrations of alcohols, followed by BRB-LH (61.3 mg/L) and ORT wines (48.1 mg/L). Furthermore, the higher alcohols were generally detected at lower concentrations than their perception threshold, except for isopentyl alcohol and phenyl ethyl alcohol. BRB-LH wines contained the highest concentrations of isobutyl alcohol and 2-phenyl ethyl alcohol (rose aroma), while isopentyl alcohol was detected at a highest concentration in ORT and BRB wines.

Alcohols with six carbon atoms contribute to the "leafy" and "herbaceous" odors; however, they usually provide undesirable flavors at high concentrations, having a negative impact on wine quality [54, 56, 57].

3.4.2. Ethyl and Acetate Esters. Fermentative esters represent the largest group of volatiles including 15 individual compounds, as reported in Table 4. The total concentration of fermentative esters ranged from 10.2 to 11.0 mg/l, with no significant differences between the experimental wines (Figure 5). Among the active esters, ethyl isovalerate (apple aroma) was detected only in BRB and BRB-LH wines, while methyl hexanoate (apricot and pineapple aroma) and methyl octanoate (citric aroma) were found in ORT wines only. Conversely, ethyl butyrate, ethyl isobutyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, phenylethyl, isoamyl, and ethyl acetate were found in all experimental wines. The concentration of ethyl acetate in wines ranged from 2.8 mg/L to

6.5 mg/L, and ORT showed the highest value compared to BRB and BRB-LH wines. Notably, a low abundance of this ester (below 70 mg/L) has favorable effects on wine aroma and complexity, since it is related to fruity and balsamic descriptors [58, 59]. The concentrations of medium-chain fatty acid ethyl esters, including ethyl decanoate (grape aroma) and ethyl isobutyrate (kiwifruit and strawberry aroma), were significantly higher in BRB or BRB-LH compared to the ORT wines. In addition, BRB-LH wines showed the higher concentration of 2-phenylethyl acetate (rose aroma) and isoamyl acetate (banana and apple aroma), followed by BRB and ORT wines. Esters, both ethyl and acetate, can have a significant effect on wine aroma by contributing to fruity and floral notes, and they are mainly produced by yeast metabolism through fatty acid acyl- and acetyl-Coenzyme A pathways [60]. Their formation depends on several factors such as sugar content, aeration degree, yeast strain, fermentation temperature, and availability of assimilable nitrogen [61].

3.4.3. Volatile Fatty Acids. Five different fatty acids were identified and quantified in this study, as reported in Table 4. Among them hexanoic acid, octanoic acid, and decanoic acid were present at higher concentration, according to the previous studies of Álvarez-Pérez et al. [62] and Carpena et al. [63]. The fatty acids were generally detected at lower concentrations than their perception threshold, except for hexanoic acid and octanoic acid. No difference in hexanoic acid and decanoic acid was found between the experimental wines, while octanoic acid was detected at the higher concentration in ORT and BRB wines. These compounds are characterized by an unpleasant odor with cheese, rancidity, greasy, and animal descriptors [64]. They are produced during alcoholic fermentation and can have a positive or negative effect, depending on their concentration [65]. However, these compounds were detected in wines well below the spoilage threshold (10 mg/L) [66].

3.4.4. Aldehydes. BRB-LH had significantly higher concentrations of acetaldehyde compared to BRB, while ORT showed the lowest concentration. Acetaldehyde is one of the most important sensory carbonyl compounds formed during vinification and constitutes more than 90% of the total aldehyde content in wine [64]. For all the three different wines, this compound resulted in a perception below its threshold.

3.5. Wine Sensory Analysis. Table 5 shows the results of the sensory analysis of the 2020 wines. Panelists considered the color of BRB-LH as more yellow and less green when compared to the other wines from earlier picking. BRB showed a fair intensity of the straw-yellow color, while ORT had a high intensity in the greenish-yellow hue. BRB-LH and BRB were considered the most colored wines, confirming the analytical values obtained from the CIELab method. The higher yellow color and b^* value in BRB-LH could be linked to the higher concentration of phenolic compounds, as reported in Table 3.

The olfactory intensity of BRB and BRB-LH was significantly higher than that of ORT, in agreement with the higher concentration of many aroma compounds (Figure 5). The panel perceived a more intense rose aroma in BRB than in ORT, while higher hints of pome fruits (apple and pear) and banana were recognized at the retronasal step. This could be mainly related to the contents in phenyl ethyl alcohol (rose), 2-phenylethyl acetate (floral and rose), and ethyl isovalerate (fruity and apple), in agreement with the results reported in Table 4.

Table 5: Sum of ranks for each attribute assessed by sensory analysis of wines derived from cv. Ortrugo (ORT) and cv. Barbesino (BRB) grapes harvested 85 days after anthesis (DAA) and in grapevines cv. Barbesino harvested 104 DAA (BRB-LH), in 2020.

Descriptors	ORT	BRB	BRB-LH	T (Friedman)
Visual				
Straw yellow	12.5a[a]	18.0ab	23.5b	6.91*
Greenish yellow	21.5b	19.5b	13.0a	3.65*
Olfactory				
Olfactory intensity	12.5a	20.5b	21.0b	4.07*
Fruits	17.5	19.0	17.5	0.12 ns
Apple	19.5	19.5	15.0	0.86 ns
Kiwifruit	21.5b	20.0b	12.5a	4.96*
Tropical fruits	15.5	17.5	21.0	1.00 ns
Flowers	16.5	19.5	18.0	0.29 ns
Rose	16.0a	24.0b	14.0a	6.01*
Vegetables	18.5	21.0	14.5	2.51 ns
Cut grass	16.0	21.0	17.0	0.93 ns
Hay	17.5	14.5	22.0	2.54 ns
Spicy	18.0	15.0	21.0	1.60 ns
Taste				
Body	16.5	17.5	20.0	0.40 ns
Alcoholicity	13.0a	15.0a	26.0b	17.04*
Acidity	13.0a	20.5b	20.5b	3.77*
Bitterness	14.0a	18.0b	22.0ab	5.31*
Minerality	16.5	17.5	20.0	0.47 ns
Softness	20.0	14.5	19.5	1.32 ns
Retro-olfactory				
Persistence	17.0	17.0	20.0	0.36 ns
White fruits	13.5a	22.5b	18.5ab	3.57*
Banana	11.5a	20.5b	22.0b	6.88*
Vegetables	18.0	20.5	15.5	0.96 ns
Spicy	17.0	18.5	18.5	0.11 ns

[a]Values followed by different letters within rows were significantly different according to least significant difference (LSD) with $P < 0.05$. *means significant difference according to least significant difference (LSD) per $P < 0.05$. ns means no difference.

At the taste level, BRB and BRB-LH were considered the sourest wines, whereas acidity in ORT was scarcely perceived (Table 5), in agreement with the TA values and organic acids concentration; even the bitterness—although moderate—was significantly higher in the wines from Barbesino (Table 5) according to the higher total phenolic contents (Table 3). Therefore, sensory analysis confirmed that the higher acidity found in BRB grapes at harvest was maintained in wines and can be distinctly perceived by final consumers. BRB-LH was perceived to be significantly more alcoholic than BRB and ORT, in line with its higher ethanol content [54]. Interestingly, the slight difference in ethanol concentration between ORT and BRB was not perceived by the panelists.

In summary (Figure 6), BRB was considered the most floral, fruity, and the freshest of the three wines, whereas BRB-LH was considered more intense, full-bodied, and

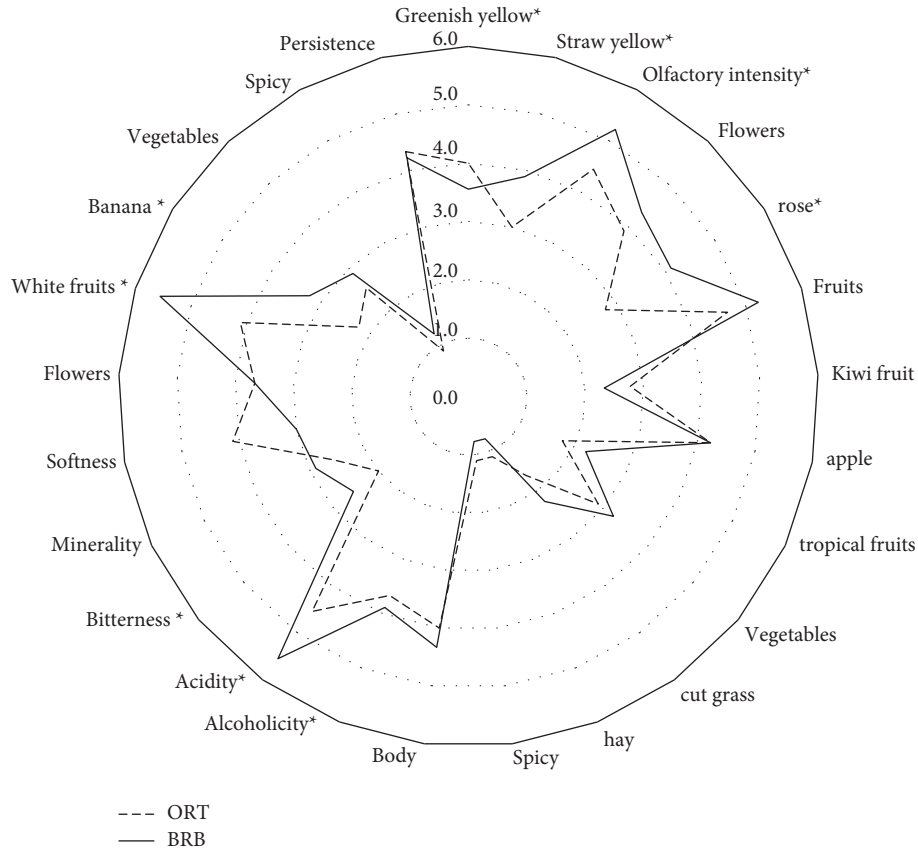

Figure 6: Spider-web representation of the sensory profile of wines obtained cv. Ortugo (ORT) and cv. Barbesino (BRB) grapes. Per each quantitative descriptor and wine sample, the mean score is reported ($n = 10$). * = Significant differences according to Friedman test ($P < 0.05$).

alcoholic. Therefore, cv. Barbesino emerged suitable for making distinctive varietal wines as well as for improving the more flat and low-intensity wines derived from cv. Ortrugo grapes.

4. Conclusions

In the Colli Piacentini wine district, production of high-quality white and sparkling wines is severely impaired by the incapability of the most grown cultivar, Ortrugo, to retain adequate acidity at concurrent satisfying sugar levels. This work showed that, under the same environmental conditions, a minor local variety (today cultivated in less than 1 ha) has instead the potential to retain optimal acidity due to an abundant preveraison organic acids pool, a high final minimum malic acid concentration, and a postponed malate degradation. Data from this study highlighted that varietal choice should be re-interpreted today either at the farm or at regional scale, by favoring those genotypes exhibiting a low postveraison malate degradation rates, expressed as a function of instantaneous malic acid concentration progression. Guaranteeing higher TA and lower TSS/TA ratio at harvest yields fresher white wines with higher acidity and malic acid and a more complex aroma profile, which are more appreciated by modern consumers.

Overall, our data demonstrated that neglected local varieties could hide the potential to improve viticulture resilience to warming trends and should be re-evaluated considering the new environmental pressures jeopardizing wine quality and the sustainability of the industry.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Supplementary Materials

Supplementary Table 1: Date of occurrence of main phenological stages in vines cv. Ortrugo (ORT) and cv. Barbesino (BRB) in 2019 and 2020. Supplementary Table 2: Vine yield, vegetative growth, and vine balance for cv. Ortrugo (ORT) and cv. Barbesino (BRB) grapevines harvested 85 days after anthesis (DAA) and in grapevines cv. Barbesino harvested 104 DAA (BRB-LH), in 2020. Supplementary Table 3: Aromatic compounds analyzed. List of odor perception threshold, odor description, and respective literature references for the aroma compounds detected in wines as part of the study. OPT values are in mg/L. Supplementary Figure 1: Seasonal daily trends of minimum temperature (T_{min}), mean temperature (T_{mean}), maximum temperature (T_{max}), rainfall, and heat accumulation (cumulative GDDs) in 2019 (a) and 2020 (b). DOY = day of the year. Supplementary Figure 2: Seasonal dynamics of pH in grapes from cv. Barbesino (BRB) and cv. Ortrugo (ORT) grapevines in 2019 (a) and 2020 (b) (mean values ± standard error; $n = 3$). DAA = days after anthesis. Supplementary Figure 3: Seasonal dynamics of berry weight in grapes from cv. Barbesino (BRB) and cv. Ortrugo (ORT) grapevines in 2019 (a) and 2020 (b) (mean values ± standard error; $n = 3$). DAA = days after anthesis. (*Supplementary Materials*)

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