

Modeling malic acid dynamics to ensure quality, aroma and freshness of Pinot blanc wines in South Tyrol (Italy)

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ABSTRACT

Pinot blanc is a leading grapevine variety in South Tyrol (Italy) for wine production. The high quality of its wines derives from a typical aroma of elegant apple notes and lively acidity. The typicity of the final wine depends on the origin of the vine, the soil, the oenological practices and time of harvest. The South Tyrolean mountainous areas meet the cold climatic requirements of Pinot blanc, which guarantee its sweet-acidic harmony obtained when organic acids are in balance with the other components of the wine. However, increasing temperatures in valley sites during the berry development period boost the activity of malic acid (MA) enzymes, which negatively affect the final sugar/acid ratio. Researchers are currently focused on understanding acid dynamics in wines, and there are no references for the best sugar/acid ratio for Pinot blanc. Moreover, the contribution of individual acids to the sensory profile of this wine has not yet been studied. In this study we address the effect of different climate conditions and site elevations on the sugar/acid ratio in developmental grapevine berries, and we evaluate the effect on wine bouquet. Even if different models and indices have been proposed for predicting sugar content, no predictive models exist for MA in white grapes. In a three-year study (2017, 2018 and 2019) that involved eight vineyards in four different location in South Tyrol at various elevations ranging from 223 to 730 m a.s.l., the relationships between bioclimatic indices, such as growing-degree day (GDD) and grapevine sugar ripeness (GSR) and grapevine berry content were investigated. The analysis reveals that GDD may potentially predict MA dynamics in Pinot blanc; hence, a GDD-based model was used to determine the GDD to reach target MA concentrations (3.5, 3.0, 2.5, 2.0 g/L). This simple model was improved with additional temperature-based parameters by feature selection, and the best three advanced models were selected and evaluated by 5-fold cross-validation. These models could be used to support location and harvest date choice to produce high-quality Pinot blanc wines.

KEYWORDS

Pinot blanc, South Tyrol, sugar, GDD, GSR, organic acids, MA

Supplementary data can be downloaded through: <https://oenone.eu/article/view/4570>

INTRODUCTION

Pinot blanc is an ancient grapevine variety first described in 1868 (Jackson, 2008). It is the result of an independent mutation of Pinot noir and for several years it was confused with Chardonnay, until its relationship with its ancestor was demonstrated (Vezzulli *et al.*, 2012). Pinot blanc has heat requirements compatible with cool climate viticulture regions and vineyard elevations of between 400 and 500 m a.s.l. (Pedri and Pertoll, 2013; Dupas de Matos *et al.*, 2020). South Tyrol is one of the smallest and most important winemaking areas in Italy. White wines represent up to 62 % of the total South Tyrolean wine production; Pinot gris, Gewürztraminer and Pinot blanc are the leading cultivars covering 11.9, 10.8 and 10.2 % respectively of the entire area (Suedtirol Wein, 2020).

Pinot blanc wines produced in South Tyrol express an elegant scent of apples and are characterised by lively acidity and typical aromas of apple, pear, citrus and green notes, and occasionally notes of quince and exotic fruit or spicy and nutty notes (Pedri and Pertoll, 2013). Fruity aroma, acidity and relatively low alcohol content are characteristic of white wines produced in cool climate wine regions (Molitor and Junk, 2019). The specific taste of a wine results from a suitable balance between sugar (sweet), organic acids (sour) and polyphenols (bitter/astringent) (Briones-Labarca *et al.*, 2017). Carbohydrates and organic acids are primary grapevine metabolites, being the most important determinants for berry and wine organoleptic quality and followed by secondary metabolites, such as phenolic compounds and aromatic substances (Rusjan *et al.*, 2008). 95–99 % of carbohydrates in grapevine berries are glucose and fructose (Keller, 2010). The glucose/fructose ratio in the berry is approximately 1:1 at pre-*véraison*, when average temperatures are above 10 °C, and it increases with temperature during ripening (Keller, 2010). This proportion is also dependent on the ability of different cultivars to accumulate sugars. Pinot blanc, like Chardonnay, is classified as a high-fructose variety (Kliewer *et al.*, 1967).

70–90 % of the total amount of organic acids in mature grape berries comprises tartrate and malate (Keller, 2010); a titratable acidity level of 6.5–8.5 g/L is considered an optimal range for well-balance wines (Conde *et al.*, 2007). Organic acid content influences the taste, chemical stability and pH of juices and wines, therefore directly affecting berry and wine quality (Eyduran *et al.*, 2015).

Different acids are responsible for different organoleptic properties (Chidi *et al.*, 2018). Acid-balanced wines will have refreshing or crisp sensory undertones, while wines of high acidity will taste sour and sharp (Volschenk *et al.*, 2006). Moreover, Margalit (1997) found that the organoleptic perception of wines can be significantly influenced by minor changes in wine pH, along with changes in total acidity (0.2–0.5 g/L).

Tartaric acid is the main acid in grapes, followed by malic acid (MA); the former is responsible for wine biological stability, while the latter confers the typical “green tones” to wines (Bakker and Clarke, 2011). The tartaric acid and MA ratio differs according to grapevine variety, thus affecting the final grapevine acidity level (Kliewer *et al.*, 1967). Mature grape can contain 5.0–10.0 g/L of tartaric acid, while MA can range from 2.0 to 6.5 g/L or more in vintages characterised by cool summers in cool-climate viticulture regions (Ribéreau-Gayon *et al.*, 2006). Tartaric acid concentration in berry juice is relatively constant during berry ripening (Cholet *et al.*, 2016; Rösti *et al.*, 2018); conversely, MA content is subject to fluctuation, because it is transformed to fructose and glucose or used as a source of carbon and energy for respiration (Conde *et al.*, 2007). MA is also sensitive to warm temperatures both before and after *véraison*, when process linked to the biosynthetic pathway and the respiration process respectively take place (Kliewer *et al.*, 1967; Ruffner *et al.*, 1984). Many studies reported the negative correlation between high temperatures and MA content after *véraison* (Sweetman *et al.*, 2014; Rienth *et al.*, 2016; Blank *et al.*, 2019). However, the biochemical and molecular mechanisms involved downstream of the MA metabolic pathways are still raveled, and an unclear slower drop in MA content has been observed in cooler regions (Sweetman *et al.*, 2014). MA degradation appears to be slower in cool climates, where it represents up to the 50 % of total acidity in berry juice (Jackson, 2008), and faster in warmer regions, as reported for Pinot noir by Blank *et al.* (2019). This negative correlation between MA content and high temperatures are ascribed to the differences observed in the optimum activity temperatures of enzymes involved in the biosynthesis and catabolism of MA (Conde *et al.*, 2007); therefore, climatic conditions and environmental stressors can physically and biochemically affect grapevine berries during the ripening period, as well as influence the final sugar/organic acid ratio (Volschenk *et al.*, 2006).

After monitoring and analysing the sugar and organic acid composition of 98 grapevine cultivars over two years, Liu *et al.* (2006) found that the acids were sensitive to climate change, while the concentration of sugars appeared to remain relatively stable.

The typicity of a final wine is the result of several factors, such as the origin of a grapevine variety, soil, agricultural and oenological practices and vintage (Maitre *et al.*, 2010). Preserving the typicity and quality of South Tyrolean Pinot blanc is of primary importance for historical, traditional and economic reasons. However, to the best of our knowledge, the literature about Pinot blanc is very scarce, and references for the optimum sugar/acid balance and evaluations of the effect of climate alterations on Pinot blanc wine quality are lacking. For this reason, we aimed to investigate the effect of vineyard microclimatic conditions on the grape berry's development process, with a focus on the sugar/organic acid ratio. Rising temperatures increase sugar concentration and deplete MA in the berries, thus altering the typical fresh aromas of green apple, citrus fruit and floral aromas of Pinot blanc. Therefore, establishing vineyards on cooler winegrowing sites could potentially result in a reduction in MA dynamics and thus preserve wine quality.

MATERIALS AND METHODS

1. Study area

The study area is located in South Tyrol (Italy), a traditional winegrowing region in the Alps, where Pinot blanc is one of the most important varieties (Egarter Vigl *et al.*, 2018). South Tyrolean topography is more complex than most of the typical winegrowing regions worldwide; grapevines are grown at elevations ranging from 206 to 1323 m a.s.l. in different microclimatic zones (Becker *et al.*, 2007; Ferretti, 2020). Eight vineyards homogeneously distributed in the Adige Valley (Autonomous Province of Bolzano) and located in four typical viticultural zones (municipalities), Eppan (Ep), Nals (Na), Terlan (Te) and Tramin (Tr), were selected for the current study (Figure 1). The study was conducted over three consecutive vegetative growing seasons (VS) from 2017 to 2019.

All eight vineyards are cultivated with the same variety (Pinot blanc), clone (Lb-16), rootstock (SO4), training system (guyot), planting system (1.9 m x 0.9 m) and plant density (approximately 7063 plant/ha); additional information is given in Table 1. Two elevation-diverse vineyards from each municipality were selected. Each vineyard was given the municipality's name and

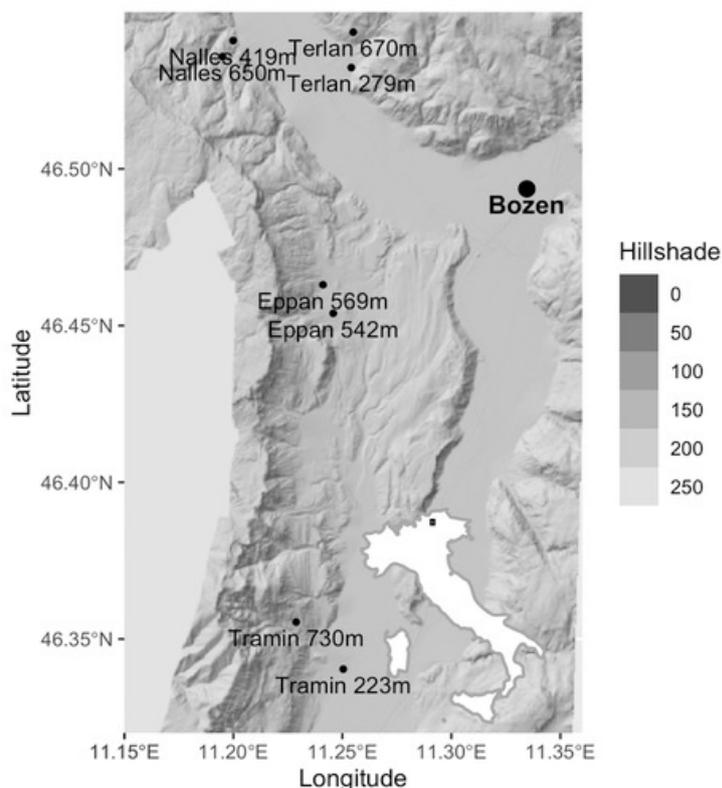


FIGURE 1. Location of the studied vineyards.

TABLE 1. Vineyard information.

Vineyard name	Abbreviation	Planting year	Elevation m a.s.l.	Slope %	Exposition	Row direction
Eppan 1	Ep_1	2008	542	16.5	East-Southeast	North-South
Eppan 2	Ep_2	2002	569	20.0	East-Southeast	North-Southwest
Nals 1	Na_1	2011	419	21.0	East-Southeast	North-Southeast
Nals 2	Na_2	2010	650	23.0	East-Southeast	North-Southwest
Terlan 1	Te_1	2007	279	12.0	South-Southwest	East-West
Terlan 2	Te_2	2006	670	40.0	South-Southwest	East-West
Tramin 1	Tr_1	2010	223	6.5	Southeast-South	North-Southwest
Tramin 2	Tr_2	1997	730	40.0	Southeast-South	Northeast-Southwest

a code number for elevation (1 for lower sites, 2 for higher sites); the vineyards will be hereafter referred to in their abbreviated form (Table 1).

2. Climate data

A temperature sensor was placed 2 m aboveground in each vineyard to measure air temperature over VS. Temperature was recorded hourly and summarised daily by calculating base parameters, such as average, minimum and maximum temperatures. Missing values were replaced with predicted values specifically generated by a forecasting model for the South Tyrol region (Egarter Vigl *et al.*, 2018). Prior to being processed, data were analysed and compared for reliability with the available sensor data. Secondary climate parameters and bioclimatic indices were determined, such as cumulative temperature per phenological period, Growing Degree Days (GDD; Winkler, 1974) and Grapevine Sugar Ripeness (GSR; Parker *et al.*, 2020). Six phenological periods [based on the BBCH scale for grapevines (Lorenz *et al.*, 2008)] were taken into consideration: 1) grapevine vegetative season (VS, 1st April - 31 October); 2) 1st April – budburst (BBCH 7), pre-bd; 3) budburst (BBCH 7) – flowering (BBCH 65), bd-bloom; 4) flowering (BBCH 65) – *véraison* (BBCH 81), bloom-ver; 5) *véraison* (BBCH 81) - ripening (BBCH 89), ver-rip; and 6) post-ripening – 31 October, post-rip. GDD and GSR were calculated using the base temperatures of 10 °C and 0 °C respectively. Negative values were excluded (Equation 1).

$$\frac{T_{max} + T_{min}}{2} - T_{base}$$

$$\text{if } \frac{T_{max} + T_{min}}{2} < T_{base}, \frac{T_{max} + T_{min}}{2} = 0$$

EQUATION 1. Growing degree day equation and grapevine sugar ripeness, $T_{base} = 10$ °C and $T_{base} = 0$ °C respectively.

The model developed by Egarter Vigl *et al.* (2018) also provided an estimation of the monthly potential solar radiation for each site. Moreover, the transpiration efficiency – an indicator of the water content and evaporative demand (drought stress) – was evaluated via the stable carbon isotopic fraction $^{13}\text{C}/^{12}\text{C}$ of ethanol in the wine following the standard method OIV-MA-AS312-06 R2001 (Reg. CE 2676/1990; Reg. CE 440/2003).

3. Phenological data and grape maturity tests

The grapevine phenology stages (budburst, full flowering and *véraison*) were dated according to the international BBCH scale (Lorenz *et al.*, 2008).

The berry ripening process was monitored from *véraison* to technological maturity. In each vineyard, three replicates of 150 berries were periodically sampled and crushed into juice. Specifically, three berries located at the top, in the middle and at the bottom of the grape cluster were collected from 50 bunches per replicate. The samples were immediately filtered and analysed for total soluble solids (TSS, g/L), pH, MA content (g/L), tartaric acid content (g/L), total acidity (g/L), ammonia and amino nitrogen (mg/L) by spectroscopic method (FT-IR WineScan™, FOSS, Denmark). Plant phenological data, sugar- and MA-content were given bioclimatic indices to estimate the potential of those indices as predictors for the ripening process and grape quality in terms of sugar/MA balance.

The production regulation of DOC South Tyrol for Pinot blanc wines stipulates a minimum of 10.5 % volume of alcohol, corresponding to ≈ 205 g/L in grape juice (Provincia Autonoma di Bolzano, 2019); the data collected between 1985 and 2017 by Laimburg Research Centre indicated an average value of 227.9 +/- 1.7 g/L for Pinot blanc

grapes at harvest (Laimburg Research Centre, 2020). Therefore, the standardised technological maturity day for Pinot blanc grapes - defined as day of year (DOY) when a sugar concentration of 220 g/L is reached (DOY220) - was predicted for each site and year from the test grape maturity data generated by linear regression models. The predicted technological DOY220 is related to the grape ripening stage, unless otherwise indicated. The MA content at DOY220 was also estimated using a log-linear regression model. Historical data showed an average MA content of 3.0 +/- 0.4 g/L at harvest. On this basis, the following values were selected as the minimum acceptable values for South Tyrolean Pinot blanc grapes at harvest: 220 g/L TSS, 2.5 g/L MA content and a maximum sugar/MA ratio of 88.

4. Wine production and sensory evaluation

Microvinifications were performed with three biological replicates for each condition (8 vineyards x 2 harvest dates x 3 biological replicates, 48 total wines) for harvest years 2017–2019. The harvest date was chosen on the basis of the maturity test data and the environmental conditions (*e.g.*, rainfall). Each replicate comprised 60 kg of grapes that were harvested, weighed and pressed using a EuroPressT1 press (Scharfenberger GmbH & Co. KG Maschinenbau, Bad Dürkheim, Germany), after which 30 mg/L of sulfite was added. Prior to fermentation, solid matter was removed by spontaneous settling overnight at 4 °C. An inoculum for each must was prepared at 20 °C with 250 mg/L of *Saccharomyces cerevisiae* strain VL2 active dry yeast (Laffort, Bordeaux, France), along with 300 mg/L diammonium phosphate. Clear musts were then racked to 34-litre glass carboys for fermentation with the addition of 200 mg/L diammonium phosphate halfway through fermentation. After fermentation was complete, the wines were cooled for 12–24 hours at 17–18 °C and then their turbid must was static-racked off into nitrogen-saturated glass carboys, with an approximate 2-minute injection of N₂ into each carboy. The carboys were placed in a refrigeration cell for 5–7 days, then amended with 30 mg/L of sulfite (E 224). Until bottling, the carboys were maintained at a temperature of 18 °C, regularly monitored and adjusted for SO₂ with potassium metabisulfite. Wines were further racked whenever necessary.

For the sensory assessment, a panel of a minimum 12 experts (female and male) between the ages of 26 and 59 years old was annually

trained to evaluate Pinot blanc wines using a quantitative descriptive analysis. Seven aromatic descriptors were selected: apple, banana, grapefruit, lemon, peach, pear and pineapple. Five gustatory/hedonistic terms were chosen to evaluate taste/mouth sensation: acidity, bitterness, complexity and overall impression; for further information refer to Kadison (2020). Three tasting sessions were carried out for each vintage, with each session consisting of the entire series of a single replicate; *i.e.*, 16 unique wines. Three of the 16 wines were randomly chosen and tasting was repeated (making a total of 19 wines tasted) in order to identify taster error. From the end of fermentation until testing, wines were aged in carboys (approximately eight months). Data were collected using FIZZ Sensory Analysis Software (version 2.61, Biosystèmes, Couternon, France) in randomised order for each panelist, using random identification numbers for each wine.

5. Statistical analysis

Daily temperature measurements were obtained from the average of 24-hourly values. Descriptive statistics related to the main climate features, phenological data and bioclimatic indices are hereafter provided and summarised. The Hierarchical Cluster Analysis (HCA) was used to classify sites based on temperature parameters (average and cumulative minimum, minimum above 5 °C and above 10 °C, maximum, day above 35 °C, mean and range temperature), agroclimatic indices (GDD and GSR), and potential radiation, starting from 1st April to the end of each VS, and for each phenological stage (Supplementary Data). The parameters had previously been normalised in the range 0-1. There are two types of HCA, the agglomerative (also known as AGNES, agglomerative nesting) and the divisive method (DIANA, divisive analysis; Kaufman and Rousseeuw, 1990). Due to the number of observations in this study, the agglomerative approach was preferred, because it aims to partition data into a relatively small number of clusters (Hastie *et al.*, 2013). It was carried out using the *factoextra* R package (Kassambara and Mundt, 2016) and the *agnes* function; the dissimilarity matrix between each observation was calculated using the Euclidean method. The average approach was used as a clustering method (Zepeda-Mendoza and Resendis-Antonio, 2013). The non-parametric Kendall rank correlation between indices, GDD and GSR, and each phenological stage and the VS was carried out to study the relationship between climate parameters and grapevine phenology.

Multiple comparisons for unequal sample size were made with the least square mean by applying Tukey adjustment for group comparison, and using *lsmeans* R package (Lenth, 2016). Pearson's correlation coefficients were computed between climate parameters and grape juice content.

The raw data from the sensory analysis underwent an initial descriptive statistical analysis to identify and remove values for tasters who were unable to consistently repeat scores for a given parameter. One-way ANOVA was applied on each single parameter assessed for the three repeated wines from a single tasting session, in order to compare the repeatability of the taster with the repeatability of the entire panel. Each parameter for which the taster's F-statistic was lower than that of the entire panel was not included in the final statistical analyses.

Climate conditions, stable carbon isotope fraction, must compounds and a selection of sensory descriptors and hedonistic terms for wines of vintage 2017, 2018 and 2019 were also investigated following a two-step multivariate procedure. Firstly, a principal component analysis (PCA) was carried out on standardised values (*z*-score); the PCA method was selected as a multivariate technique that avoids multicollinearity and reduces the dimensionality of a large number of variables (Koufos *et al.*, 2014). Hence, hierarchical cluster analyses of the components (HCPCA) were performed using the *FactoMineR* R package (Lê *et al.*, 2008), and the *factoextra* package was used for extracting and visualising the results (Kassambara and Mundt, 2016).

To target MA in developmental grapevine berries based on GDD accumulation, a model was fitted and evaluated (log-linear fit). First, a data quality assessment was carried out to check for influential observations, Cook's distance, outliers, and standardised residuals with Bonferroni *p*-value < 0.5. The malic model – which was used to estimate the MA content in grapevine berries as a function of the amount of GDD accumulated starting from 1st April – was fitted to MA and its efficiency was assessed by applying a 5-fold cross-validation using the *trainControl* function from the R package *caret* (Kuhn, 2008). The *k*-fold cross-validation is a technique for estimating the predictive capabilities of a model by randomly splitting the original dataset into *k* partitions; the first fold is used for testing and *k*-1 is used for training the model. The procedure is iterated over the same dataset for all folds. This method was preferred to the leave-one-out cross-validation

(LOOCV) usually applied to small datasets, because it provides a more accurate estimate of the test error rate (Gareth *et al.*, 2014) and a lower variance than LOOCV (Efron, 1983). The higher the *k* value, the higher the accuracy in cross-validation (Yadav and Shukla, 2016), but this can lead to overfitting. Therefore, the model metrics [coefficient of determination (R^2), root mean square error (RMSE) and mean absolute error (MAE)] were compared across 10-, 5- and 3-folds (data not shown) and the 5-folds were finally selected. This base model was further improved with an exhaustive feature selection by using the R package *leaps* (Lumley and Miller, 2017). In order for the performance of the algorithms to be evaluated against all possible combinations of the features considered in the dataset [Bayesian Information Criteria, root mean square error (MRSE) and adjusted- R^2 (adj- R^2)]. The three best predictive models were selected, and their efficiency evaluated by a 5-fold cross-validation [RMSE, absolute root mean square error (ARMSE), proportion of variance (R^2), and mean absolute error (MAE)]. The performance of the models was tested on different metrics [R^2 , adj- R^2 , Akaike's Information Criteria (AIC), BIC, average prediction error rate (APE) and the F-statistic *p*-value (*p*-value)]. The predictive capability of the four models was finally tested by comparing the predicted and the real values.

RESULTS

1. Climatic and phenological data

Descriptive statistics for daily temperatures, bioclimatic indices (GDD, GSR) and the sum of the number of days with maximum temperature above 35 °C for the VS per site are summarised in Table 2.

Average air temperature recorded during the grapevine vegetative period over the three years ranged from 16.84 °C (Tr_2; lowest elevation) to 18.84 °C (Tr_1; highest elevation). The average minimum temperatures were lowest in Na_2 and Ep_1 (12.02 and 12.05 °C respectively). Meanwhile, the highest average maximum temperatures were recorded in Te_1 and Tr_1 (25.8 and 25.6 °C respectively), with more than 7 days of temperatures above 35 °C and larger thermal excursions. GDD ranged from 1603.1 to 1972.5 in Tr_2 and Te_1 respectively, while GSR ranged from 3717.7 to 4108.2 Wh/m² in Tr_2 and Te_1 respectively.

TABLE 2. Descriptive statistics.

Period	Site	Tair avg °C	Tair min °C	Tair max °C	Tair range °C	Tmin5 °C	Tmin10 °C	GDD °C	GSR °C	DDover35 nr. Day	Potential solar radiation Wh/m2
VS	Ep_1	17.46	12.05	23.27	11.21	0.19	2.38	1653.93	3779.06	0.67	124893
		+/-0.5	+/-0.5	+/-0.6	+/-0.3	+/-0.1	+/-0.5	+/-116.1	+/-119.7	+/-1.15	
	Ep_2	17.24	12.33	22.9	10.55	0.16	2.3	1641.78	3762.58	0.67	126015
		+/-0.6	+/-0.8	+/-0.6	+/-0.3	+/-0.1	+/-0.6	+/-136.3	+/-145.8	+/-1.2	
	Na_1	18.37	13.62	23.84	10.22	0.05	1.84	1872.59	4007.58	2	114782.7
		+/-0.6	+/-0.6	+/-0.8	+/-0.6	+/-0.0	+/-0.7	+/-136.4	+/-139.2	+/-1.0	
	Na_2	16.96	12.02	22.86	10.84	0.15	2.45	1612.03	3732.43	1	122911.9
		+/-0.5	+/-0.6	+/-0.7	+/-0.4	+/-0.0	+/-0.5	+/-130.2	+/-136.2	+/-1.0	
	Te_1	18.53	12.65	25.75	13.1	0.11	2.11	1971.5	4108.18	8.67	122232.8
		+/-0.5	+/-0.5	+/-0.9	+/-0.7	+/-0.0	+/-0.3	+/-121.8	+/-124.7	+/-0.6	
	Te_2	16.94	12.1	22.98	10.88	0.13	2.49	1631.91	3754.03	1.33	139877.1
		+/-0.6	+/-0.5	+/-1.0	+/-0.5	+/-0.0	+/-0.5	+/-149.9	+/-157.6	+/-1.15	
	Tr_1	18.84	12.5	25.6	13.11	0.16	2.15	1941.45	4076.76	7.33	123906.4
		+/-0.5	+/-0.6	+/-0.7	+/-1.0	+/-0.1	+/-0.4	+/-108.63	+/-111.3	+/-2.1	
	Tr_2	16.84	12.28	22.46	10.18	0.17	2.46	1603.1	3717.72	0.67	136627
		+/-0.6	+/-0.6	+/-1.0	+/-0.5	+/-0.1	+/-0.7	+/-153.6	+/-163.8	+/-1.2	

Average daily temperatures, GDD, GSR and sum of days with average temperature above 35 °C recorded during VS (1st April - 31 October). For each site, the average of the three vintages (2017 to 2019) +/- standard deviation is reported for each parameter. Abbreviations: Tair avg = average air temperature, Tair min = minimum air temperature, Tair max = maximum air temperature, Tair range = thermal excursion, Tmin5 = minimum air temperature below 5 °C, Tmin10 = minimum air temperature below 10 °C, GDD, GSR, DDover35 = days with temperature over 35 °C and Wh/m2 = potential solar radiation.

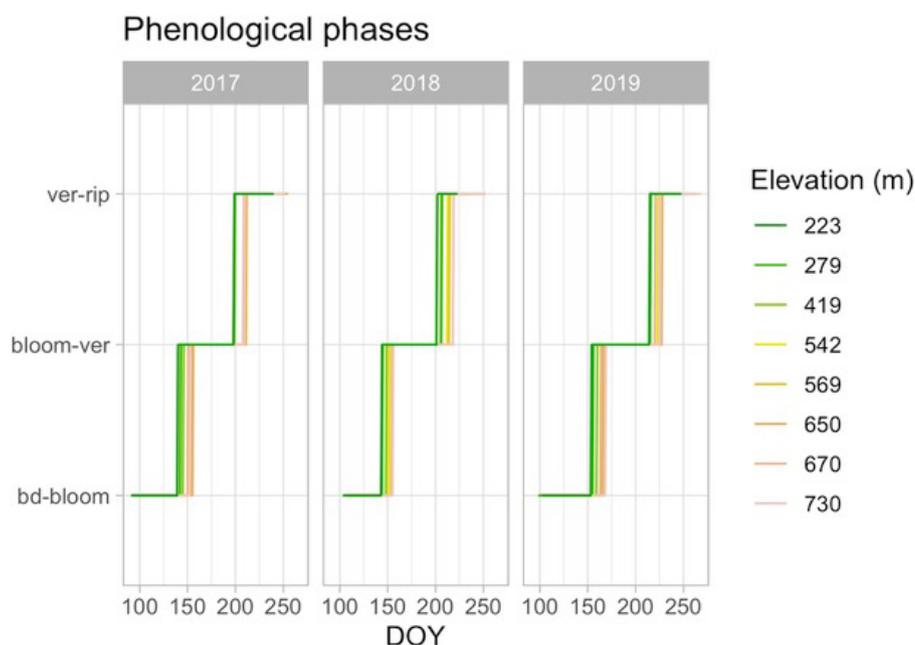


FIGURE 2. Phenological stages.

The length of the different phenological periods (bd-bloom = budburst-flowering, bloom-ver = flowering-*véraison*, ver-rip = *véraison*-technological ripening) for each vintage (2017, 2018 and 2019), reported as the first and last Day of the Year (DOY) on which phenological events took place. The different colours indicate each site with increasing elevation.

The DOY of the main grapevine phenological stages (budburst, flowering, *véraison* and technological maturity) in the different vineyards depended on site elevation. Indeed, a clear gradient due to site elevation can be distinguished for each vintage (Figure 2 and Table S1). However, annual variations related to seasonal temperature alterations seem to be more relevant in the regulation of phenological stages (Table 1S). Budburst occurred much earlier in 2017 for all sites (\approx 1st April), and around five days earlier than in 2018 and 2019. The 2018 vintage was characterised by lower temperatures in early spring and a delayed budburst, then higher temperatures in the vegetative season compared to 2017 (according to the recorded DD_{over35} values). Meanwhile, flowering, *véraison* and technological ripening dates in 2018 were similar to those in 2017 or even earlier (by approximately 5 days), but in 2019 flowering and *véraison* were delayed by 10 days and ripening by 20 days, because of cooler conditions before flowering (May). Therefore, the warmest vintage was 2018, with an average VS temperature of 18.3 °C, compared to the cooler 2017 and 2019 vintages with temperatures of 17.3 and 17.4 °C respectively.

2. Site classification based on climatic and phenological features

Zoning and climate suitability for winegrowing areas are commonly determined by carrying out bioclimatic measurements, which can also provide information about the impact of climate change on viticulture (Malheiro *et al.*, 2010). One of the most used heat unit indices is the Winkler index (WI), which measures GDD calculated over the vegetative growing period, 1st April - 31 October (Winkler, 1974), and GSR, which was recently proposed for sugar ripening (Parker *et al.*, 2020). Both the indices were analysed in this study in order to carry out zoning in the South Tyrolean Pinot blanc winegrowing region: Ep_1, Ep_2, Na_2, Te_2 and Tr_2 were found to belong to the second Winkler region, Na_1 and Tr_1 to the third, and Te_1 to the fourth (the warmest region). The vineyards were divided into two statistically different groups for both GDD and GSR. The first group was represented by Tr_1, which had the lowest elevation, being located at 223 m a.s.l., while the second group comprised sites located above 550 m a.s.l. (Ep_2, Na_2, Te_2, Tr_2). Vineyards located at middle elevation range, from 279 to 542 m a.s.l. (Te_1, Na_1, Ep_1), were not statistically different from the others.

The Kendall's correlation revealed significant relationships (p -value < 0.01) between phenological DOY and both indices; however, GDD was more significant ($p = 1.76 \times 10^{-5}$) than GSR ($p = 1.17 \times 10^{-4}$). Thus, GDD was the most reliable index for estimating grapevine phenological stages for the Pinot blanc variety. The sum of heat units, expressed as GDD, is lowest between bud break and flowering (average of 289.0 \pm 24.4 °C), and is considerably elevated during the flowering-*véraison* period (May to end of July; 725.3 \pm 57.2 °C, with a maximum value of 849.38 °C and a minimum of 628.1 °C). Major variability can be observed in the final stage of berry ripening, when cumulated GDD ranged between 274.9 °C (Te_1 2018) and 544.8 °C (Tr_2 2017), with a mean value of 407.9 \pm 76.2 °C.

Due to the lower discrimination obtained by the analysis of GDD and GSR, and in order to better classify the vineyards, a further HCA analysis was conducted. Four groups that resembled the vineyard elevations were observed (Figure S1). The first group comprised Tr_1 and Te_1 (both under 300 m a.s.l.), the second Na_1 (at 419 m a.s.l.), the third Ep_2, Ep_1, Na_2 (between 500 m a.s.l. and 650 m a.s.l.), and lastly, Te_2 and Tr_2 the fourth (the two highest sites). Regarding the vineyards above 500 m a.s.l., it should be noted that the two sites at the highest elevations have higher temperatures during the flowering and ripening periods than the other three sites located above 500 m a.s.l.

3. Grape ripening

Grapevine maturity tests were performed throughout the ripening period until harvest (Table S2). Moreover, all ripening parameters were predicted by linear regression models to DOY220 (TSS 220 g/L) (Table S3), except for MA, which was estimated by a log-linear regression model.

3.1. Vineyard location and final grape sugar/MA content

Close to harvest time, a different trend between berry juice components, sugar and MA was observed with respect to increasing site elevation (Figure 3a). In general, valley sites with warmer environmental conditions were associated with a lower amount of MA than the cooler, higher sites; for instance, the average MA concentrations recorded in Tr_1 and Tr_2 were 1.53 \pm 0.38 g/L and 2.37 \pm 0.40 g/L respectively. On the other hand, the average TSS values were 235.06 \pm 4.3 g/L and 232.74 \pm 10.12 g/L for Tr_1 and Tr_2 respectively.

Maturity analyses of grape berries were conducted before ripening at different time points as already discussed in Materials and Methods, and on the day of the harvest the grapes showed a different sugar content as the harvest date was chosen taking into account both the grape juice composition and the environmental conditions at harvest (*i.e.*, rainfall). Hence, for standardising purposes,

all berry compounds were predicted by linear regression analysis on the technological ripening day (DOY220). The predicted MA content at DOY220 resembled the malic content-elevation trend observed at harvest, with the lower MA content generally recorded in the lower sites in comparison to the higher and cooler elevation (Figure 3b).

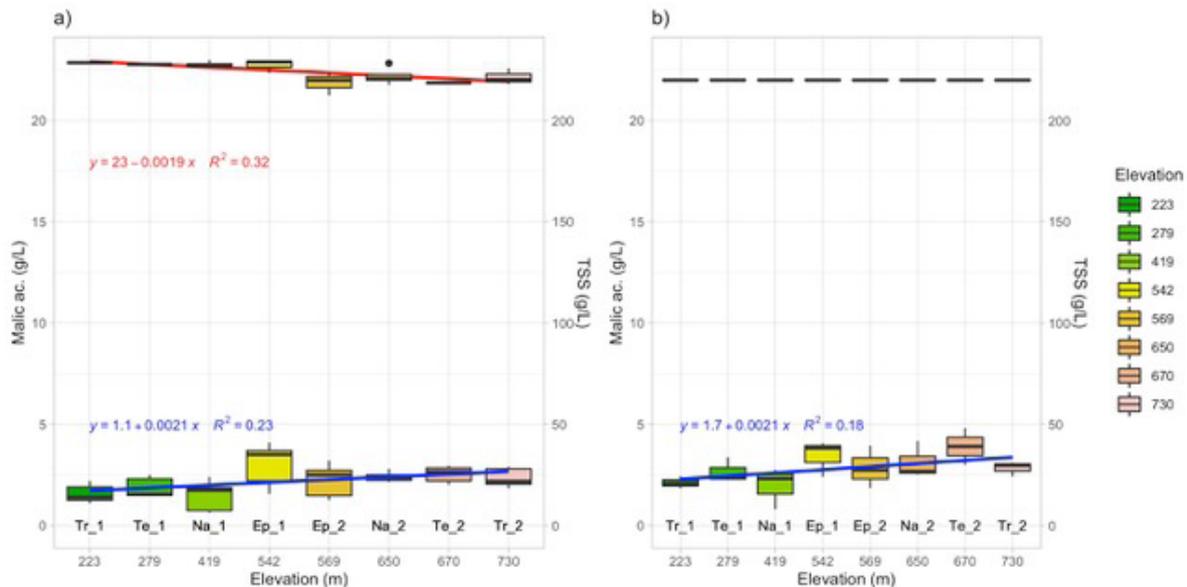


FIGURE 3. Box plot of MA (g/L) and sugar (TSS g/L) content.

^aBox plot of MA (g/L) and sugar content (TSS g/L) in grapevine juice close to harvest time. For each field, the mean of the three years and three replicates per year was measured. The range, median and distribution density of each maturity parameter for each site, according to a specific elevation, are displayed. The red and the blue lines represent the trend line for sugar and MA respectively.

^bBox plot of predicted MA (g/L) was calculated at standardised sugar content (technological maturity = TSS 220 g/L). In blue the trend line calculated for the MA content.

3.2. Grapevine juice – climate relationships

In order to explore the relationship between berry maturity parameters and climatic variables, pairwise correlations using the Pearson method were conducted. The significance of the correlation coefficient (R) was evaluated using the Holm adjustment p -value at three significance levels ($*** < 0.001$, $** < 0.01$ and $* < 0.05$); correlation coefficients greater than 0.75 or lower than -0.75 for negative relationships are shown in bold in Table 3. The increase in berry sugar content was mainly related to high temperatures (T_{air_avg} and T_{air_max} , $R = 0.88***$) experienced during the grape vegetative period (budburst – ripening), and GSR (T_{air_GSR} , $R = 0.88***$), which was confirmed as a good predictor for sugar ripening. However, this index seemed to fall for organic acid content (g/L) in comparison to GDD, which showed a significant high correlation, $R = -0.80***$ compared to $R = -0.73***$. The negative correlations observed between GDD

and total grape juice acidity was mainly due to the strong relationship shown by GDD with MA content ($R = -0.78***$), rather than tartaric acid content ($R = -0.57***$). Indeed, tartaric acid content is relative stable during berry ripening; nevertheless, its loss ($R = -0.71***$) could be explained by an increase in GSR due to climatic conditions. Therefore, the composition of grape berry may be affected by high temperatures due to a concentration effect possibly being triggered by water losses.

3.3. Sugar content, MA kinetics and bioclimatic indices

The correlations between sugar, MA and the two bioclimatic indices were further explored by linear and non-linear regression analysis. Sugar and MA concentrations in grape juice were correlated with “day after *véraison*”, and GDD and GSR over the VS (Figure 4). These analyses highlighted two different dynamics occurring between sugar concentration and MA degradation.

TABLE 3. Pearson correlation between grape parameters and climatic variables.

Parameter	Elevation (m)	Mean Potential Radiation (Wh/m ²)	Tair avg °C	Tair min °C	Tair max °C	Tair range °C	GDD °C	GSR °C	Tmin5 °C	Tmin10 °C	DDover35 nr. Day
TSS (g/L)	-0.07	0.03	0.88***	0.75***	0.88***	0.66***	0.75***	0.88***	0.00	0.22	0.16
pH	-0.28	-0.13	0.74***	0.58***	0.76***	0.63***	0.75***	0.73***	-0.08	0.11	0.35
Tot. ac. (g/L)	0.20	0.17	-0.73***	-0.69***	-0.69***	-0.42*	-0.80***	-0.73***	0.24	0.10	-0.19
YAN (mg/L)	-0.02	0.20	-0.20	-0.32	-0.17	0.05	-0.30	-0.24	0.30	0.21	0.00
Amino N (mg/L)	-0.01	0.19	0.01	-0.14	0.04	0.19	-0.14	-0.03	0.33	0.24	-0.01
Ammonium N (mg/L)	-0.03	0.16	-0.46**	-0.52***	-0.44**	-0.19	-0.48**	-0.49***	0.21	0.08	-0.02
Malic ac. (g/L)	0.24	0.19	-0.66***	-0.65***	-0.61***	-0.34	-0.78***	-0.66***	0.32	0.20	-0.19
Tartaric ac. (g/L)	-0.07	0.06	-0.69***	-0.70***	-0.66***	-0.37	-0.57***	-0.71***	0.02	-0.22	-0.03
Berry weight (g)	0.56***	0.42*	0.41*	0.40*	0.41*	0.25	0.03	0.43*	0.38	0.38	-0.37

YAN = yeast assimilable nitrogen. *** *p*-value < 0.001; ** *p*-value < 0.01; * *p*-value < 0.05.

After *véraison* the rate of increase in sugar proceeded smoothly ($R^2 = 0.69$) and it seemed to be independent of vineyard elevation, while MA degradation was influenced by site altitude. Indeed, two data patterns, under and above the trend line, can be observed for vineyards located below and above 500 m a.s.l. respectively (Figure 4a). Moreover, the longer ripening periods of grapevine located in sites at high elevation resulted in a constant increase in the sugar concentration and the retention of MA. These vineyards showed MA values generally above 2.5 g/L, while at lower elevations those values were slightly lower being not more than 1.5 g/L (Figure 4a). The use of GDD seemed to be more effective for predicting

MA content after *véraison* (log-linear correlation, $R^2 = 0.66$) compared to GSR ($R^2 = 0.44$), while in the case of sugar prediction using GDD, a segregation effect based on site elevation was observed (Figure 4b). GSR should therefore be preferred for predicting sugar concentration ($R^2 = 0.77$) over GDD ($R^2 = 0.57$) (Figure 4c). In terms of vineyard grouping according to the HCA classification, an increase in the R^2 values of almost all the regression lines was observed (Figure 4d). Interestingly, with the same amount of GDD, sites located at higher elevations showed a more efficient sugar content increase compared to those located at lower elevations.

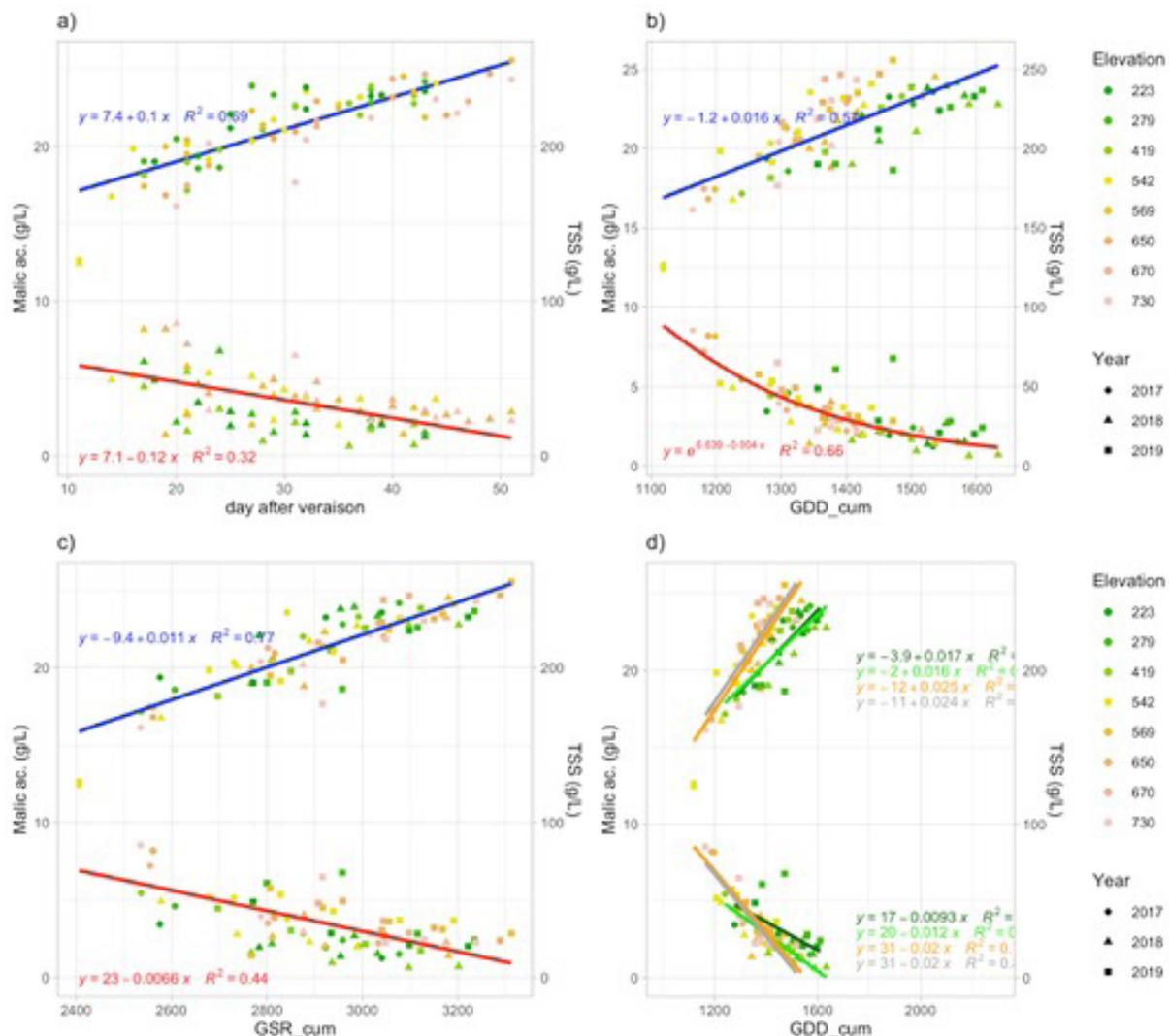


FIGURE 4. MA and sugar dynamics in Pinot blanc berries.

Scatterplot and regression lines of TSS and MA dynamics over the days after *véraison*: ^bGDD and ^cGSR. The red and the blue lines and formula represent trend lines and the equations, for sugar and MA content respectively. ^dScatterplot and regression lines of the sugar (TSS g/L) and the MA dynamics over GDD for vineyard groups classified in accordance with HCA. Circles, triangles and squares represent vintages 2017, 2018 and 2019 respectively. Trend lines: dark-green = group 1 (≤ 300 m a.s.l.), green = group 2 (> 300 and < 500 m a.s.l.), orange = group 3 (> 500 and ≤ 650 m a.s.l.) and dark-grey = group 4 (> 650 m a.s.l.).

3.4. Effect of climatic parameters, grape berry juice at harvest and sensory data

The effect of climatic parameters on the appropriate balance between sugar and acidity, and the relationship between these parameters and must compounds, which determines the aromatic profile of South Tyrolean Pinot blanc wines, were studied by carrying out a PCA (Figure 5 and Table S4). The proportion of sugar and MA predicted at DOY220 was also included as ripeness degree indicator, and the stable isotopic carbon fraction of wines, $^{13}\text{C}/^{12}\text{C}$ (Table S5), was included as a measure of the transpiration efficiency of grapevines (thus as a drought stress marker). The first dimension retained 38.1 % of the explained variance and the second 16.0 %, and five PCs accounted for 77.8 % of the total variance. PC1 mostly correlated with climatic parameters, such as GDD and Tair_avg (0.92 and 0.89 respectively), as well as Tair_max (0.81), but it was negatively associated with MA (-0.92), total acidity and Tmin10 (the latter two both -0.87) (Figure 5a). In the same direction of GDD component, the sensory attributes of banana and pear were observed. Moreover, it is worth noting that vineyard elevation along with high number of days with temperatures below 10 °C enhance MA accumulation and decrease the TSS/MA ratio resulting in a stronger sourness perception. In Figure 5b, observations were grouped according to the elevation range (</> 500 m a.s.l.); the two groups are clearly distinguished, and segregation seems mainly driven by climatic features, such as DDOVER35, Tair_max and Tair_avg. PC2 was positively influenced by lemon (0.75), elevation (0.66) and sourness (0.65), while DDOVER35 (-0.73) and pH (-0.60) were negatively associated variables (Figure 5a). Peach, apple and pineapple flavours were found to be the most closely related to high-quality scores (overall impression), which, from our results, appear to be indirectly affected by tartaric acid and green aromas (Figure S2b). The most appreciated wines were Ep_2 2019, Te_2 2017 and Na_2 2018, with a mean overall score of 5.8 +/- 0.1 and a predicted (DOY220) TSS/MA ratio of 72.2 +/- 15.8; meanwhile, the lowest scores (< 4.5) were associated with a higher mean ratio of 96.61 +/- 19.86. Regarding MA content, the predicted mean values were 3.15 +/- 0.73 g/L and 2.34 +/- 0.45 g/L for the most and least appreciated wines respectively. Figure S2 provides further information on variable and individual contribution to PCs. In the loading and score plots of PC2 and PC3 (Figure S2b,e), a high variation in the overall impression of wines produced at higher elevations compared to < 500 m a.s.l can

be seen. In particular, higher overall impression scores were generally given to the wines produced in the upper sites which had been exposed to mild temperatures during the vegetative period, thus balancing the fruit-herbaceous notes and sourness; meanwhile, the hot seasons, which were associated with water deficit, resulted in wines which received considerably lower final scores.

Based on the PCA, agglomerative hierarchical clustering was carried out and observations were categorised into four clusters (factor map in Figure 5c). Wines from the 2018 vintage were clearly separated from those from the 2017 and 2019 vintages, which were clustered. Moreover, a further grouping internal to the vintage classification and based on the site elevation range was observed, except for Na_2 2018 (> / < 500 m a.s.l.).

3.5. Predictive modelling of MA concentration in ripening berries

As previously described, the correlation found between MA and GDD is stronger than the correlation with GSR. If GSR is useful for predicting sugar content, GDD is preferable for MA content. Therefore, a model to estimate MA concentration in Pinot blanc berries as a function of GDD was proposed.

The model was evaluated for influential observation, Cook's distance, and outliers (*i.e.*, the standardised residuals with Bonferroni *p-value* < 0.5). The goodness of the fit (RMSE) was 0.256 (absolute of 0.091) and the proportion of variance explained (R^2) was 0.798 (Table 4). The model's MA predictive capacity was also evaluated (Figure S3). The difference between the observed and predicted values ranged from -1.343 to 4.031 g/L, the mean of the absolute difference was 0.623 +/- 0.629; however, after removing the extreme upper values (12.43 g/L), the maximum difference dropped to 2.848 g/L and the mean decreased to 0.582 +/- 0.508. The model predicted the number of GDD at which the amount of MA is around 3.5, 3.0 and 2.5 g/L to be 1358, 1400 and 1450 respectively. With more than 1510 GDD, MA content can fall to below 2.0 g/L.

The PCA results suggested that other parameters affected the MA content in berries, such as average, maximum and below 10 °C temperatures, which may be not recognised by the linear correlation analysis. Therefore, multiple linear regression and model reduction were carried out on an exhaustive selection of predictor variables to improve the predictivity of the model.

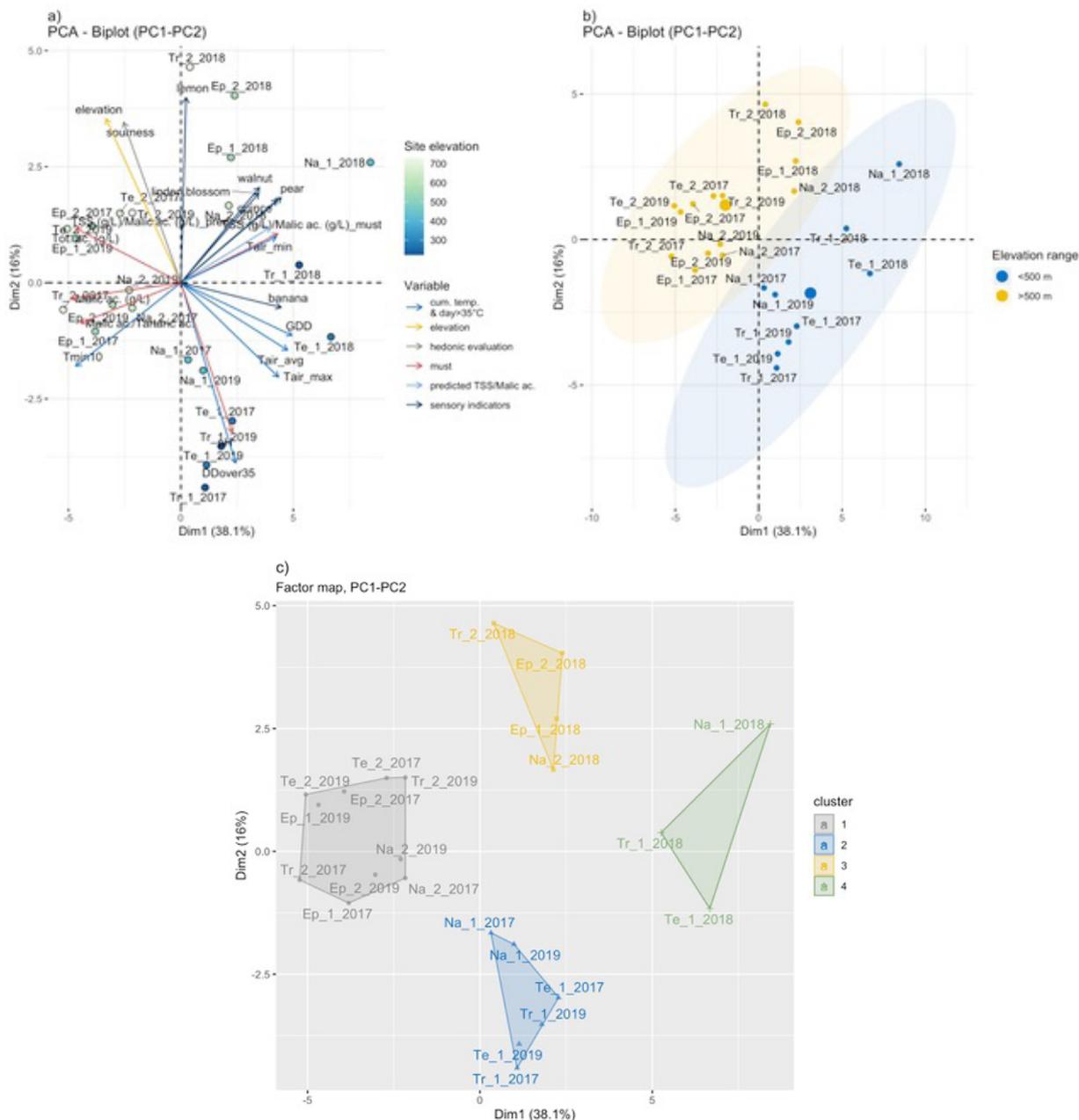


FIGURE 5. PCA and HCPCA of climatic and ambient variables, must compounds and sensory attributes.

^aBiplot of the first two components: individual dots (colours according to elevation) represent wines from different locations and production year; the different coloured arrows represent different variables (stable carbon isotopic fraction, temperature, bioclimatic indices and days with temperature above 35 °C, elevation, sensory descriptors, final sensory scores and must compounds).

^bBiplot of the first two components with individuals grouped according to elevation range.

^cFactor map of the HCA computed in the first and second dimension.

For simplicity, only models with at least three predictors were taken into account and selected on the base of metrics, such as BIC, MRSE and adjusted- R^2 (data not shown). The three most reliable models were: i) GDD and minimum temperature and < 10 °C temperature as predictor variables, ii) GDD, < 10 °C and sum of the days with temperatures above 35 °C, and iii) GDD, maximum temperature and sum of the days

with temperatures above 35 °C. They were all further evaluated using a 5-fold cross-validation approach; the statistical metrics are available in Table 4. The first model that included the sum of minimum temperatures and minimum temperatures below 10 °C showed the best performance (the lowest RMSE, 0.252, ARMSE, 0.091, and MAE, 0.194, and the highest R^2 , 0.832).

TABLE 4. Model evaluation, performance comparison and summary of the prediction power.

Model evaluation							
	Nr. obs	RMSE	ARMSE	R^2	MAE		
MA ~ GDD	85	0.256	0.091	0.798	0.206		
1. GDD-Tair_min-Tmin10	86	0.252	0.091	0.832	0.194		
2. GDD-Tmin10-DDover35	86	0.258	0.093	0.818	0.202		
3. GDD-Tair_max- DDover35	86	0.259	0.093	0.816	0.201		
Model performance							
	Nr. obs	R^2	Adj- R^2	AIC	BIC	APE	F-statistic <i>p-value</i>
MA ~ GDD	85	0.785	0.782	12.400	19.700	0.246	2.07x10 ⁻²⁹
1. GDD-Tair_min-Tmin10	86	0.820	0.813	4.160	16.400	0.232	2.12x10 ⁻³⁰
2. GDD-Tmin10-DDover35	86	0.812	0.805	7.870	20.100	0.237	1.24x10 ⁻²⁹
3. GDD-Tair_max- DDover35	86	0.810	0.803	8.560	20.800	0.238	1.72x10 ⁻²⁹
Model prediction							
	MA ~ GDD	Model.1	Model.2	Model.3			
Mean	0.623	0.567	0.584	0.589			
Standard deviation	0.629	0.683	0.696	0.679			
Min	-1.343	-1.756	-1.740	-2.002			
Max	4.031	4.428	4.688	4.445			

The metrics for model evaluation were: root mean square error (RMSE), absolute root mean square error (ARMSE), proportion of variance (R^2), and mean absolute error (MAE).

The metrics for assessing the model performance were: R^2 , adjusted- R^2 (adj- R^2), Akaike’s Information Criteria (AIC), Bayesian Information Criteria (BIC), average prediction error rate (APE) and the F-statistic *p-value* (*p-value*). Nr. obs = the number of observations for the statistics.

For each model, the mean of absolute difference between predicted and real MA values, the standard deviation and the minimum and maximum are also reported.

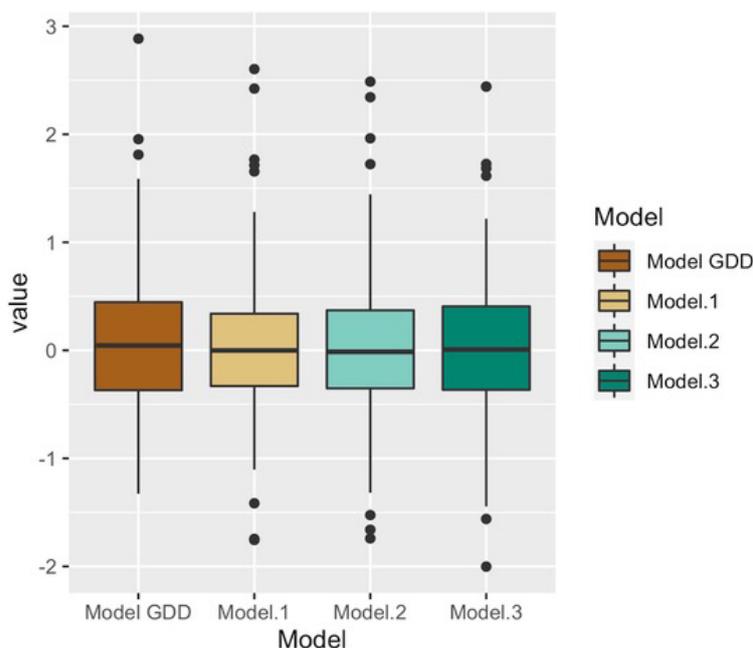


FIGURE 6. Boxplot of the spread of the difference between predicted and measured MA values.

For each model, summary statistics are the median, hinges, whiskers and all outlying points. Model.1 = GDD - Tair_min-Tmin10, Model.2 = GDD - Tmin10 - DDover35, Model.3 = GDD - Tair_max - DDover35.

The model with GDD, minimum temperatures and day with temperature above 35 °C also provided good performance, while the third model produced the worst scores. The boxplot in Figure 6 shows the statistics for the difference between real and predicted values (Table S6).

DISCUSSION

Wine style, aroma and flavour are the most important factors to take into account when determining the harvest date, and they have recently become more relevant for winemakers than standard physiochemical parameters, such as sugar content. In particular, the typical fresh notes of the South Tyrolean Pinot blanc wines are associated with high acidity; indeed in accordance with the production regulations of DOC South Tyrol Pinot blanc, this dry wine must be straw yellow in colour with green hues, and have a pleasant typical aroma and noticeable acidity in the mouth. Using this information as a basis and after analysing historical data collected on South Tyrol, the threshold values used in this study for producing typical Pinot blanc wines were 220 g/L TSS, 2.5 g/L MA content and a maximum sugar/MA ratio of 88. Thus, achieving a balance between sugar and organic acid appeared to be crucial in order to preserve the traditional aroma of this wine.

Currently, Pinot blanc is cultivated within a broad elevation range of approximately 200 to 750 m a.s.l. in South Tyrol, an Alpine region with very variable environmental conditions that can affect individual vineyard microclimate, ripening processes and final quality of the wine (Ferretti, 2020). Interestingly, in the present study, the vineyard average temperatures recorded during the grape vegetative period varied depending on site elevation within a range of 0 to 2 °C, and they significantly affected the phenological timing of Pinot blanc. This result corresponds to the findings of previous studies by Failla *et al.* (2004) on the Nebbiolo grape in the Alpine environment, and by Valtellina and Rienth *et al.* (2020) on Chasselas in the AOC-Lavaux region in Switzerland, in which the vertical temperature gradient, due to elevation, was found to have a direct influence on bud break, flowering and technological maturity dates. Indeed, air temperature is considered one of the most important factors which drive the growth and development of grapevines (Winkler, 1974; Fraga *et al.*, 2019). It should be noted that during the short period of analysis (2017–2019) a high variability in weather conditions between vintages was recorded; the temperature gradient

linked to site elevation was thus often exceeded, which led to large differences in the phenological timing between vintages. In a long-term study, 2012–2018, by de Rességuier *et al.* (2020) on Merlot in the Bordeaux area, the advanced timing of phenological stages was associated with warmer years, while cooler years caused a delay. Moreover, an intra-annual variability in the duration of each phenological stage was observed, indeed meteorological conditions experienced during the vegetative season influenced the duration of each stage, especially budbreak and maturity (de Rességuier *et al.*, 2020). On the other hand, in the present study, phenological timing was found to be clearly driven by the elevation gradient when each vegetative season was considered separately. Our findings are in accordance with those reported by Rienth *et al.* (2020). In our study, a relevant vintage effect that influenced both vine and berry physiology was observed; however, elevation was found to be the leading driver of precocity in all the years of the study (2017–2019). On the basis of this information, as well as the bioclimatic indices and derived climatic parameters, the Pinot blanc grape growing sites were classified using HCA.

The relationship between phenology and temperature found in this research confirms that the classical GDD index can still be used as a good predictor for the timing of phenological events, even if over time improved, sophisticated, mathematical models have also been developed to this end (Fraga *et al.*, 2016; Reis Pereira *et al.*, 2018). It is worth noting that more complex indices could be tested in the future; *e.g.*, the biologically effective degree days (BEDD; Gladstones, 1992), for which the potential plant growth is not considered to be linear at all temperatures (Anderson *et al.*, 2012). By using GDD, it was possible to initially distinguish two south Tyrolean Pinot blanc growing areas. However, to better investigate the effect of temperature on the phenological timing and grape ripening processes, the influence of warm extreme events, minimum and maximum temperature should be considered (Malheiro *et al.*, 2010; Koufos *et al.*, 2014; Martinez de Toda and Ramos, 2019). Therefore, by analysing different bioclimatic variables, four main vineyard groups were identified as being easily distinguishable depending on site elevation. These groups were composed of i) the warmest sites with average temperatures over 18.5 °C (< 300 m a.s.l.) and 7 to 8 DDover35 per vegetative season, early bud break and flowering,

ii) sites with average temperatures of around 18 °C and 2 DDover35 with Tair_avg that exceeds the other groups by 1 °C (from 300 to 450 m a.s.l.), iii) sites with average temperatures ranging from 17 to 17.5 °C and located from 500 to 650 m a.s.l., and iv) (the coolest group) sites with average temperatures of < 17 °C, located over 700 m a.s.l. and with later dates for the phenological stages. It should be noted that a simplified analysis based on temperature parameters and potential solar radiation was applied, and this classification could be further improved in the future by taking into account other factors which influence cultivar behaviour and performance (Ferretti, 2020).

Different studies have highlighted how temperature can actually affect grape composition (*i.e.*, sugar, acidity and phenolic compounds), wine quality and the aesthetic aspects of the same variety cultivated in different locations, due to differences in the timing and dynamics of the grape ripening process (Ramos *et al.*, 2015; Martinez de Toda and Ramos, 2019; Ferretti, 2020). For instance, Ferretti (2020) observed that the elevation and solar radiation of vineyards in South Tyrol may lead to longer or slower ripening periods resulting in the development of different Sauvignon blanc wine bouquets. In the same way, the aroma of Pinot blanc wines depends on the environmental conditions under which the grapevine is grown: the predominant aromas of wines from warmer climatic conditions were pear, quince and exotic fruits; moreover, warmer climatic conditions can enhance the perception of banana, but high fermentation temperature can affect it as well (Molina *et al.*, 2008), while water stress is here associated with increases in concentration of tartaric acid in grape juice, which in turn is associated with the presence of green and hawthorn aromas in the final wine. The observed tartaric acid and water deficit relationship may partly be explained by a concentration effect, as berry weight increased less during the ripening stage (García-Escudero *et al.*, 1995). On the other hand, the intensity of green aroma in wine was associated with vine growth factors, such as water irrigation and nitrogen fertilisation (Mendez-Costabel *et al.*, 2014). Pinot blanc wines produced from grapes grown in cooler conditions (*i.e.*, high elevation during a hot season or cooler vintages) were characterised by fresh notes of lemon, grapefruit, apple and distinctive acidity. In addition, high overall scores seemed to be associated with wines produced in cool environments, exposed to mild temperatures during the VS (with an average

temperature of 17.05 +/- 0.49 °C) and without water stress. It should be noted that a marked wine acidity does not always lead to better scores. Even though wine freshness is mainly determined by low alcohol content and high acidity (Morata *et al.*, 2019), excess acidity leaves a tart or sour taste (Volschenk *et al.*, 2006). The taste of MA has been described as harsh and metallic (Vilela, 2019), and it is commonly associated with unripe or green-apple notes (Volschenk *et al.*, 2006; Bayraktar, 2013; Vilela, 2019); however, different flavours can arise depending on the proportion of other important compounds (*e.g.*, ethanol, tannic acid, sugar, aromatic and mineral substances; Bayraktar, 2013). Therefore, in order to produce quality wine, controlling the sugar/MA ratio is of primary importance. In this study, the wines with higher scores overall were those with a predicted sugar/MA ratio ranging from 55.8 to 87.4, while lower scores were associated with the higher ratio of 96.61, thus confirming the assumption that a well appreciated South Tyrolean Pinot blanc wine should not exceed a sugar/MA ratio of 88. This value may be suitable for producing Pinot blanc wines with a relatively acidic taste and from light to moderate body (Dupas de Matos *et al.*, 2020). That said, it is worth noting the difficulty in defining wine quality due to its “quasi-aesthetic characteristics” and the subjectivity of personal taste (Stone *et al.*, 2008).

Monitoring the ripening of Pinot blanc grapes, it was noticed that when berry development had taken place in warmer conditions, the increase in sugar concentration (g/L) was slightly less efficient compared to berry development in cooler environments, in which slower and smoother grape ripening was observed. A general good correlation between sugar concentration and temperature parameters was observed, in particular with Tair_avg and Tair_max. Despite this, the temperature range at which sugar metabolism enzymes are active vary from 8 to 33 °C (Iland *et al.*, 2002). The lower sugar concentration of Pinot blanc grapes ripened in warmer environments is confirmed by Hochberg *et al.* (2015), who observed important metabolic changes in vines grown at 35 °C, which resulted in reduced photosynthetic activity, an accumulation of secondary sugars (raffinose, fucose and ribulose) and a reduction in primary sugar concentrations (glucose, fructose and sucrose). Moreover, under heat stress conditions with temperatures ranging from 34 to 42 °C, the sugar metabolism of the grape cell suspension displayed a switch from the classical invertase

pathway to an alternative sucrose breakdown pathway leading to glycolysis (George *et al.*, 2015). In addition, our results supported the use of the novel GSR temperature-based model used to determine sugar concentration in ripening berries (Parker *et al.*, 2020). Indeed, a high correlation value of $R = 0.88$ between TSS and GSR was observed for Pinot blanc grapes; this index is thus a promising tool for assisting winemakers in the decision-making process during harvest.

Besides sugar content, the acid content of grape juice is a critical parameter which affects wine quality (Conde *et al.*, 2007), especially the organoleptic and aesthetic characters of Pinot blanc wines as revealed by the sensory data; for this reason it was extensively investigated in the present study. A general positive relationship between MA content in Pinot blanc grapes and vineyard elevation was observed, as previously reported in the cases of grapes from the Alpine winegrowing region Trentino, Chasselas from Lavaux region in Switzerland, and Tempranillo grapes from the Rioja Spanish province (Martinez de Toda and Ramos, 2019; Rienth *et al.*, 2020). In particular, grape acidity is a function of various exogenous factors, one of the most important being berry temperature (associated with air temperature and sunlight exposure), which can directly influence both sugar/organic acid and malic/tartaric acid ratios in grape juice, as widely reported by the literature (Ribéreau-Gayon *et al.*, 2006; Conde *et al.*, 2007; Jackson, 2008). As is known, the tartaric acid content of grapes is generally unaffected by temperature (Rienth *et al.*, 2016; Oliveira *et al.*, 2019); therefore, any differences in the total acidity of grape juice at harvest can be linked to variations in MA content. Malic acid can account for up to half of the total acidity at harvest time and tends to decrease as the grapes ripen, especially during warm periods at the end of the season (Jackson, 2008). Interestingly, when investigating the relationship between climatic variables and Pinot blanc grape juice composition, good negative correlation values were observed between the acidity parameters (total acidity, tartaric- and malic-acid content) and temperature variables, with a corresponding positive correlation with grape juice pH. Therefore, cooler conditions, related specifically to higher elevations or cooler vintages, are associated with higher total acidity and MA content and lower pH, compared to warmer environmental conditions. The negative correlation of temperature with MA content is due to its effect on the balance

between MA biosynthesis and the enhanced MA degradation. Indeed, the optimum temperature for MA accumulation is between 20 and 25 °C, while above 38 °C synthesis greatly declines. Moreover, warm environmental conditions are coupled by the increasing activity of mitochondrial malate dehydrogenase enzyme responsible for the malate breakdown into oxaloacetate (Conde *et al.*, 2007; Keller, 2010). Martinez de Toda and Ramos (2019) showed how MA concentration decreased in Tempranillo grapes within a range of 0.43–0.62 g/L per 1 °C of increase during the VS; moreover, a correlation between the number of days with extreme temperatures above 35 °C and MA decrease was described. However, in cooler climatic conditions (12–22 °C), the uncoupling of MA respiration and the import of photo-assimilates has been observed at the onset of ripening, thus grapevine does not necessarily consume MA in lieu of sugar (Rienth *et al.*, 2016).

In order to access organic acid dynamics in Pinot blanc grapes, an initial screening of the climatic parameters revealed that GDD showed the best negative correlation with total acidity ($R = -0.80^{***}$) and MA content ($R = -0.78^{***}$). In our study, 1450 GDD may be the threshold at which a must MA concentration of above 2.5 g/L can be obtained; this is the minimum value required for producing good quality South Tyrolean Pinot blanc wines. Subsequently, the performance of different predictive models that take into account at least three predictive parameters were investigated. Here, the three most reliable models are presented. Interestingly, the best model incorporates GDD, T_{air_min} and T_{min10} from 1st April as predictor variables, which may target the malate synthase process. The other two models incorporate T_{air_max} and DDover35 instead, and may serve as reduced malate synthesis target in combination with the accelerated catabolic rate. Recently, Olego *et al.* (2015) developed a model for measuring MA concentration in red grapes in order to determine the *optimum* harvest time under Mediterranean conditions. The study reported a negative correlation between MA content and temperature variables; *i.e.*, the daily thermic integral and the Huglin index. However, a weak negative correlation with the daily thermal range from *véraison* to the sampling time ($R = 0.18$) was observed. Consequently, in a study by Olego *et al.* (2015), none of the temperature parameters was selected as a significant predictor for modelling MA. Indeed, the model uses some grape juice parameters resulting from the destructive analysis of ripening grapes, such as pH

and tartaric acid, together with precipitation from harvest time to sampling time. In our research, it is worth noting that the proposed models can be considered to be oversimplified, since other environmental variables are responsible for MA dynamics, such as canopy management, vine water status and solar radiation (Beauchet *et al.*, 2020). Overall, the present study is a first attempt at describing the Pinot blanc berry compound dynamics and the prediction of MA content in grapes using a GDD-based model.

CONCLUSIONS

The acidity of grapes and wines is one of the main factors to affect the winemaking process at different production steps, and it determines wine quality by influencing the perceived organoleptic and aesthetic characters, as well as the stability and aging potential of wines. In particular, MA concentration is considered to be a central component of berry juice at harvest. In this study, a model for monitoring the MA content in Pinot blanc grapes was developed for the first time for the South Tyrol winegrowing region. The climatic variables GDD, Tair_min and Tmin10 were significant predictors for investigating MA concentration. Therefore, our model is an initial step in the development of an efficient decision-making tool for managing the quality of Pinot blanc wines.

It is recommended to perform analyses of the proposed models, as well as to extend the modeling period, in order to ensure their prediction capability and to enhance the model accuracy in view of the complex topography of the South Tyrol region. Climate variability can limit estimation ability; therefore, model responses can differ in other regions and in extreme climate situations. The model is an early-step proposal that in the future could be easily adapted to other grape varieties and winemaking regions. Overall, the results obtained provide new insights into MA prediction modelling for white grapes. The model to estimate MA as a function of GDD for Pinot blanc grapes in South Tyrol could be a useful tool for harvest date choice to produce high-quality Pinot blanc wines. By combining this information with that provided by the GSR, it may be possible to help winegrowers and winemakers in monitoring the sugar/organic acid dynamics in developmental grapes, and/or in finding alternative suitable wine areas for Pinot blanc in the light of the future climate change scenario.

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