OENO One
VINE AND WINE
OPEN ACCESS JOURNAL
By IVES International Viticulture & Enology Society

DOI: https://doi.org/10.20870/oeno-one.2023.57.2.7239

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

An investigation of vine water status as a major factor in the quality of Merlot wine produced in terraced and non-terraced vineyards in the Vipava Valley, Slovenia

Alenka Mihelčič[1], Andreja Vanzo[1], Paolo Sivilotti[2], Borut Vrščaj[1,3] and Klemen Lisjak[1*]

[1] Agricultural Institute of Slovenia, Department of Fruit Growing, Viticulture, and Oenology and Department of Agroecology and Natural Resources, Hacquetova ulica 17, 1000 Ljubljana, Slovenia
[2] University of Udine, Department of Agricultural, Environmental, and Animal Science via delle Scienze 206, 33100 Udine, Italy
[3] Faculty of Environmental Protection, Trg mladosti 7, 3320 Velenje, Slovenia

*correspondence:
klemen.lisjak@kis.si

Associate editor:
Vivian Zufferey

Received:
29 October 2022

Accepted:
3 April 2023

Published:
19 May 2023

ABSTRACT

Terraced vineyards are cultural landscapes with a special value. The increase in costs and the lack of professional workers make viticulture on terraces difficult to be maintained. Thus, in the face of climate change and production challenges, we aimed to study the impact of slope-wise cultivation on wine quality. The quality of Merlot wines from terraced and slightly lower non-terraced vineyards within a small area characterised by similar mesoclimatic features was compared in the seasons 2019 and 2020. The non-terraced and terraced vineyards differed in both soil profile and morphology. The number of buds, number of clusters, and leaf area were standardised, and the stem water potential (SWP) was measured during wine-growing seasons. Upon reaching maturity, grapes were hand-picked on the same day in all vineyards and microvinified. The wines were analysed chemically and sensorially. In both years, the SWP showed higher water stress in the vines from terraces. The yield, berry weight, and leaf area were lower on terraced than on non-terraced vineyards in both years, and the skin- and seed-to-flesh ratios were higher. The darker seed colour pointed to the advanced ripening on terraces, where the wines had a higher alcohol concentration and a higher total dry extract. The wines from terraces had higher concentrations of total polyphenols, anthocyanins, and proanthocyanidins (PAs) than the wines from non-terraced vineyards in both years, and the PAs in the wines from terraces in 2019 had fewer prodelphinidins and were more galloylated. Higher concentrations of higher alcohols and lower concentrations of esters and methoxypyrazines were found in the wines from terraces. The sensory analysis revealed a preference for wines from terraces with better colour intensity, fruitiness, astringency, midpalate, and overall quality. Under experimental conditions (the same harvest date, standardised viticultural variables), the wines from terraces had both better phenolic potential and better sensory quality than the wines from non-terraced vineyards.

KEYWORDS: water stress, soil, stem water potential, polyphenols, aromatic compounds

INTRODUCTION

Wine composition is determined by a complex interplay between grape variety, viticultural practices, genetic and environmental factors, such as soil, location, and climatic conditions (Atanassov *et al*., 2009). Factors spanning from grape growing location to viticultural practice affecting wine quality are known as the "terroir" (van Leeuwen *et al*., 2018). This term has become a communication tool to differentiate wine production locations (Hira and Swartz, 2014), which can affect consumer decisions in wine purchasing (Famularo *et al*., 2010).

Little has been published about the "terroir" of the Vipava Valley wine-growing region, which is in western Slovenia at the transition from central Slovenia to the Friulian Plain in Italy. In the Vipava Valley, of the approximately 2092 hectares, 58.8 % of vineyards are terraced. One-fifth of the Vipava Valley is covered by flysch (Perko and Orozen Adamic, 2001), a sedimentary geological formation composed of layers of two different rocks: predominantly carbonate marl and quartz sandstone. In general, the soils on the terraced slopes are skeletal, have medium to low organic matter content and low water holding capacity. In contrast, the soils that developed in the alluvial deposits of the flat bottoms contain a negligible amount of skeleton. Overall, the soils in the non-terraced lowland vineyards have a high to very high water holding capacity so water stress in these vineyards rarely occurs.

Because of the different available water content of the soils from the terraced or non-terraced vineyards, during the summer season, water stress conditions appear more easily in the former vineyards (van Leeuwen *et al*., 2004). On the other hand, we know that the grapevines on the terraces are forced to root deeper, but the low available water content of such soils represents the factor mainly accounting for the water stress conditions (Smart *et al*., 2006). Water stress conditions do not always have a negative impact on grape quality, and several experiments carried out around different viticultural areas revealed that moderate water stress conditions are profitable to affect the biosynthesis of phenylpropanoids, abscisic acid, isoprenoids, carotenoids, amino acids, and fatty acids (Deluc *et al*., 2009). Among polyphenols, Castellarin *et al*. (2007) and Palai *et al*. (2022) explained that anthocyanins increased when water stress occurred at both the pre- and post-flowering stage, and there was also a metabolic shift towards tri-substituted and methoxylated forms. As regards proanthocyanidins, Calderan *et al*. (2021) showed that proanthocyanidin concentration was negligibly affected by the limited conditions of water supply in seeds and skins of Refošk grapes; however, the increase in the galloylation of seed and skin proanthocyanidins was shown. As regards aroma compounds in grape berries, there are contrasting results in the literature on the effect of water stress, but Palai *et al*. (2022), Savoi *et al*. (2020), Bindon *et al*. (2007) and Koundouras *et al*. (2009) revealed a positive effect of mild water stress on the concentration of several aroma classes.

Winegrowers describe wines from terraces as sensorially more pleasant, fuller, and richer. The most important compounds for red wines' sensory perception are phenolics, including flavan-3-ols (catechin, epicatechin, gallocatechin, epigallocatechin, and epicatechin-3-O-gallate), proanthocyanidins, and anthocyanins. Proanthocyanidins, or condensed tannins and anthocyanins, are among the most important polyphenols in red grapes, having a decisive influence on wine quality (Chira *et al*., 2009). Proanthocyanidins are responsible for bitterness and astringency (Lisjak *et al*., 2020), while anthocyanins are red pigments responsible for wine colour (Falginella *et al*., 2012). Beside polyphenols, wine aroma is an important factor affecting wine quality itself (King *et al*., 2010). However, aroma compounds are very complex, and the complexity is reflected in the combined effect of hundreds of different volatile compounds (Guadagni *et al*., 1963). Differences in volatile compounds occur due to climatic conditions, soil, grape variety, grape maturity, fermentation conditions, oenological processes, and wine ageing (Carpena *et al*., 2020). The most important aromatic compounds for the aromatic profile of the wine itself are formed during the fermentation process and refer to esters and higher alcohols (Maarse, 1991).

Knowledge of the natural environmental conditions is essential to produce high-quality wines. Only an accurate comprehension of environmental factors provides the important insights needed to plan and improve viticulture and winemaking practices. This study aimed, therefore, to assess the impact of vineyard relief on wine quality in the Vipava Valley, a sub-Mediterranean wine-growing region in Slovenia. Terraced vineyards are agriculture adapted to natural conditions and an erosion protection measure, while terraced landscapes are considered cultural heritage. This is important not only for wine quality but also for the protection of natural resources, wine tourism, and the local economy. The quality of Merlot wines made from grapes grown on the skeletal and dry soils of the terraced flysch vineyards and the deep loamy soils of the non-terraced vineyards in the slightly lower plains of the Vipava Valley was evaluated. The wines from the terraced and non-terraced vineyards were compared in terms of polyphenol content, structural characteristics of proanthocyanidins, aromatic compounds (esters, higher alcohols) and specific sensory characteristics.

MATERIALS AND METHODS

1. Study area and experimental design

The experiment was conducted in the 2019 and 2020 seasons in 10 vineyards planted with the grape *Vitis vinifera* L. cv. Merlot in a 10 km^2 area with a similar mesoclimate in the Vipava Valley in Slovenia (Figure 1). The calculated Huglin Index for 2019 and 2020 was 2408 °C and 2375 °C, respectively (Table 1). Two groups of vineyards were selected for the experiment: a) five of them were planted on terraced slopes with hyper-skeletal soils formed on Flysch terrace slopes and consisting of up to 90 % coarse material and low soil organic matter content, and b) five vineyards planted in alluvial lowlands on dense and largely non-skeletal clayey. Three experimental plots were randomly established in each vineyard. In the case of the non-terraced vineyards, each plot consisted of 15 vines. In the case of the terraced vineyards where the vines are planted in two rows, each plot consisted of both 15 vines in the inner and 15 vines in the outer rows to obtain a representative sample. In the inner rows, which are close to the slope and where the soil is denser and less porous, a lower water holding capacity is expected, while the vines in the outer rows, which are more exposed to the sun and wind, are more likely to suffer from drought. The vineyards were not irrigated, and neither mineral nor organic fertilisers were used. Detailed information about the relief, altitude, slope, row orientation, aspect, soil, rootstock, planting year, and training system for the vineyards in ten locations are shown in Supplementary Table 1. The average daily precipitation and temperature data were collected from the meteorological station Bilje, located within 10 km of the vineyard locations (Environment Agency of the Republic of Slovenia, https://meteo.arso.gov.si/).

2. Soil properties of selected terraced and non-terraced vineyards

The terraces are dominated by Skeletic Calcaric Cambisols according to the WRB soil classification (FAO, 2022), where coarse rock fragments (skeletons) make up > 40 % of the total soil volume, or even by Hyper-skeletic Calcaric Cambisols (with more than 70 % of coarse fragments). In the isolated areas where the soils are affected by water erosion, there are Skeletic Calcaric Regosols (Vrščaj, 2017). In general, the soils on the terraced slopes of the Vipava Valley are skeletal, medium deep, rich to very rich in calcium carbonate ($CaCO_3$), have high pH (> 7), medium to low organic matter content and low water holding capacity (between 40 and 130 mm). The soils are not in contact with groundwater, and the ascending flow of soil moisture is almost non-existent due to the high skeletal content and the predominance of large pores that do not allow capillary ascending water flow in the dry season. In contrast, the other group of soils (Eutric Fluvisols (Loamic, Siltic) according to the WRB soil classification) that developed in the alluvial deposits of the flat bottoms of the Vipava Valley contain a negligible amount of skeleton. The soils here are deep (2–3 m) to very deep (>3 m), silty-loamy, structured, and permeable, moderately rich in soil organic matter and rich in nutrients and calcium, with a high presence of soil biota (earthworms) and, most importantly, they are in contact with groundwater. Overall, the soils in the non-terraced vineyards have a high to very high soil water holding capacity (150–240 mm), so water stress in the vineyards rarely occurs. The soil analysis data were used to calculate the water holding capacity using the software SPAW Hydrology (version 6.0; USDA, US; downloaded at https://spaw-hydrology.software.informer.com/6.0/).

3. Assessment of vine water status

During the seasons 2019 and 2020, vine water status was assessed four times, from late May/early June to late August (at pre-flowering, berry-set, pre-veraison and post-veraison stages), by means of a Scholander pressure chamber (Soil Moisture Corp., Santa Barbara, CA, USA) (Deloire and Heyns, 2011). For the measurements, fully expanded and mature leaves from different vines on each plot were covered with aluminium foil and cling film one hour before the measurement. From each vineyard, four leaves per plot were randomly sampled on each date. The leaves were collected at midday (i.e., between 12:00 and 14:00) and placed in the pressure chamber with the cut end of the petiole protruding from the chamber. The measurement was taken as soon as the first xylem sap emerged from the end of the petiole.

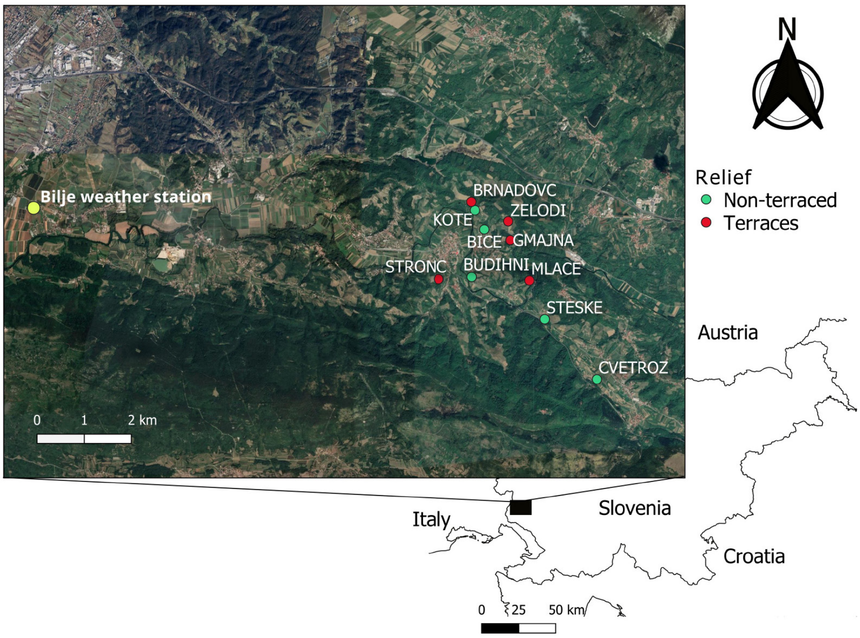


FIGURE 1. Map of the study area and locations of the investigated vineyards in the Vipava Valley.

4. Leaf-area measurements, yield evaluation, grape sampling, and seed colour

The number of buds, number of clusters, and leaf area were standardized to obtain similar conditions in terraced and non-terraced vineyards. In both years, in spring, the number of buds on the canes was standardised, while later, the cluster number was adapted to match a similar crop load based on the actual measured leaf area and on an estimation of the yield. Leaf area on main and lateral shoots was estimated at the onset of veraison (berry colouration < 5 %) in 2019 and 2020. On one vine per plot, the length of the main vein of all leaves was measured while keeping separated the main and lateral leaves and each shoot according to size (vigorous, medium, and small). Using this procedure, the regression between the length of the main veins and the leaf area was evaluated separately for the main and lateral leaves. The number of shoots, according to size (vigorous, medium, small), was counted on three vines per plot to calculate the leaf area of the vines by multiplying the average leaf area of the shoots by the total number of shoots per vine (Sivilotti *et al*., 2020). On September 21, in both 2019 and 2020, the number of clusters per vine was counted, and the yield per vine was evaluated. Average cluster weight was then calculated by rating the yield per vine and the number of clusters. Approximately 5 kg of grapes per plot were randomly harvested in each vineyard, immediately taken to the laboratory and stored at 4 °C for 2 days until microvinification. Seed colour was determined as described previously (Calderan *et al*., 2021; Fredes *et al*., 2010).

5. Microvinification

Microvinification was performed in both 2019 and 2020 in triplicates, once for each experimental plot (10 vineyards, three plots per vineyard). Healthy berries were destemmed and crushed manually at 4–8 °C in an anaerobic atmosphere. After 2 days of cold maceration (at 10 °C), yeasts were inoculated (0.2 g kg^{-1}; F15, Laffort, France), and yeast nutrients were added (20 g hL^{-1} of diammonium phosphate, Laffort, France). Fermentation and maceration lasted 15 days at 20 ± 2 °C, and the pomace was punched down twice a day during active fermentation. After 15 days, free-run wine was decanted and allowed to settle for 24 h at 4 °C. Afterwards, the wines were transferred into glass bottles, where malolactic fermentation was performed by adding lactic acid bacteria *Oenococcus oeni* (1 g hL^{-1}; LACTOENOS® B7 Direct, Laffort, France). At the end of malolactic fermentation, 40 mg L^{-1} of SO_2 was added, and the wines were bottled in 0.75 L dark glass bottles and sealed with screw caps. The bottles were stored at 15 °C for 4 months, at which time chemical and sensorial analyses were performed. In 2020, the non-terraced vineyard Kote and terraced vineyard Stronc were excluded from microvinification due to yield loss and leaf necrosis at a late stage of maturation, respectively. In 2019, there was some hail damage (up to 30 %) in the Brnadovc vineyard just 8 days before harvest; but for this vineyard, at harvest time, only healthy bunches and berries were selected for microvinification.

6. Basic physicochemical analyses of wines

At the time of the sensory characterisation, the basic physicochemical wine variables were determined. Titratable acidity was measured using sodium hydroxide and bromothymol blue as a colorimetric change indicator (Commission Regulation (EEC) (1990)). Wine pH was measured with a MeterLab PHM210 (Radiometer Analytical, Lyon, France), alcohol content was measured with an alcohol meter (Alcolyzer Wine M, Anton Paar, Graz, Austria), while ash and the total dry extract in wines were determined according to the procedures of the International Organization of Vine and Wine (OIV).

7. Analysis of esters, C6 compounds, aldehydes, and lactone

Esters and other compounds were determined by liquid-liquid extraction with dichloromethane using an HP 6890 GC (Hewlett Packard, Waldbronn, Germany) coupled to an HP 5973 MS (Hewlett Packard, Palo Alto, CA, USA) using a capillary column CP-WAX 57CB 50 m × 0.25 mm, film thickness 0.20 µm (Varian, Lake Forest, CA, USA) coupled to a fused-silica-deactivated 2 m × 0.25 mm guard column (Agilent Technologies Palo Alto, CA, USA) under conditions previously described (Bavčar *et al*., 2011; Bavčar and Česnik, 2011). A calibration solution of all analysed compounds in dichloromethane was used for quantification. Retention times and mass spectra (scanning in selective ion monitoring mode—SIM) were used for identification. For quantification, the peak area of the analysed compound in the sample was multiplied by the concentration of the same compound in the calibration solution and divided by the peak area of the same compound in the calibration solution. The calibration solution was scanned for every six samples. The results were corrected according to the concentration factor and recovery of internal standard 4-nonanol.

8. Analysis of higher alcohols

Higher alcohols were analysed with an HP 6890 GC-FID, as previously described (Bavčar *et al*., 2011; Bavčar and Česnik, 2011). The capillary column (Varian, CP-WAX 57CB, 50 m × 0.25 mm, film thickness in micrometer-um 0.20 µ) and liner (Agilent Technologies, part number 5183-4647) were used. To 5 mL of wine distillate, 50 µL of internal standard 4-methyl-2-pentanol (Sigma Aldrich; 2.78 g dissolved in 100 mL absolute ethanol) was added, and the sample was injected directly into the GC-FID under the conditions described. One-point calibration (one concentration level) with all analysed compounds diluted in 12 % by volume absolute ethanol was injected after every five samples, and this was used for the identification and quantification of the analysed compounds.

9. Analysis of methoxypyrazines

Methoxypyrazines in wines were analysed using a GC-MS (Agilent Technologies, Palo Alto, CA, USA) equipped with a Gerstel MPS2 multipurpose sampler (Gerstel, Mülheim an der Ruhr, Germany) as described previously by Šuklje *et al*. (2012).

10. Spectrophotometric analyses of wine polyphenols

Spectrophotometric analyses were performed using an Agilent 8453 spectrophotometer (Agilent Technologies Inc., Palo Alto, CA, USA), as described previously (Rigo *et al*., 2000). Prior to analysis, polar compounds (sugars, free SO_2, amino and organic acids) were removed from the wines using Sep-Pak C-18 columns (0.5 g, Waters). Total anthocyanins (TA) were determined based on the maximum absorbance in the visible range between 536 and 542 nm and evaluated in mg L^{-1}. Total polyphenols (TP), expressed as (+)-catechin in mg L^{-1}, were estimated by Folin–Ciocalteu reagent reduction to blue pigments due to the phenols in the alkaline solution. Proanthocyanidins (PAs) were evaluated by conversion to cyanidin and expressed as mg L^{-1} cyanidin chloride. Monomeric and low-degree polymerised flavanols consisting of two to four units were determined by their reaction with vanillin. The vanillin index (VAN) method provides a good estimation of monomers and a low degree of polymerised flavanols corresponding to two to four units. The chromatic properties, colour, and hue (tonality) of the wines were determined by measurements of absorbance at 420, 520, and 620 nm (A420, A520, and A620) in a cuvette with a 1 mm optical path. The colour intensity (CI) represented the sum of the measured absorbance values multiplied by 10, while the hue of the wine was defined as the ratio A420:A520 (Glories, 1984).

11. Analysis of the proanthocyanidin structural characteristics

The mean degree of polymerisation (mDP), the percentage of galloylation (% G), and the percentage of prodelphinidins (% P) in the wines were determined after acid-catalysed degradation using phloroglucinol as the nucleophilic reagent (Drinkine *et al*., 2007; Kennedy and Jones, 2001). Prior to analysis, the wine samples were cleaned and concentrated by solid phase extraction, as described previously (Lisjak *et al*., 2020; Calderan *et al*., 2021). Samples were analysed using a 1290 infinity UHPLC system coupled to a DAD detector (G7117B) and a 6460 triple quadrupole MS (Agilent Technologies, Santa Clara, CA, USA) under conditions described (Lisjak *et al*., 2020). The identification of flavan-3-ols and their phloroglucinol adducts was based on the molecular ion $(M-H)^-$, which was m/z 289 for catechin and epicatechin; m/z 305 for gallocatechin and epigallocatechin; m/z 441 for epicatechin gallate; m/z 413 for catechin- and epicatechin-phloroglucinol; m/z 429 for epigallocatechin-phloroglucinol; and m/z 565 for epicatechin gallate-phloroglucinol. mDP, % P, and % G were estimated using the response factors of PA cleavage products at 280 nm and calculated as described previously (Kennedy and Jones, 2001). The mDP value represented the molar ratio between the sum of all flavan-3-ol units generated by phloroglucinolysis and the sum of the terminal units.

12. Wine sensory characterisation

Sensory characterisations of wines from 2019 and 2020 were performed on 4-month-old wines. Sensory analyses were performed by ten professional wine tasters officially certified to evaluate wines at Slovenian-authorised organisations for wine trade certificates. There were six males and four females. Their mean age was 45 years (range 36–58 years), and their primary occupation was the winemaker/oenologist or researcher in the field. According to Slovenian legislation, they must have an education in wine sensory evaluation at least once every two years, they need to attend additional training, and once every four years, their organoleptic abilities are tested at an authorised organisation. They compared seven wines in one ranking test (one ranking test per day, n = 5 days). Colour intensity, fruitiness, midpalate, astringency, and the overall quality of 7 wines were characterised by a ranking test on a 0–7-point scale, from the least to the highest colour intensity, the least to the most fruitiness, the least to the most astringency, midpalate, and overall quality. Wines were then evaluated according to the 20-point Buxbaum scale system of positive ranking, and total Buxbaum scores were used for statistical analysis. Buxbaum model assigns a certain number of points to each of five categories which are then totalled to obtain the overall rating score for a given wine: clarity (2 points), colour (2 points), odour (4 points), taste (6 points), and the balance of odour and taste (6 points). The minor unit of the scale is 0.1. The wines were served in a random order at room temperature (20±2 °C) in OIV tasting glasses in daylight. To avoid carryover effects, at least 30 s between tasting each sample was applied.

13. Data analysis

Before analysis, the Shapiro–Wilk normality test was used to check the normality of the data, while the homogeneity of variances was tested with the Leven test. The significance of stem water potential was tested using the Kruskall–Wallis non-parametric rank sum test, and after statistical significance (p-value < 0.05), the *post-hoc* Dunn's test was applied at $p < 0.016$ (Bonferroni correction) using the agricolae R package (Mendiburu, 2021). The significance between terraced and non-terraced vineyards, separated for vintages, yield variables, basic wine parameters, aromas, phenolic composition, and sensory features, was tested with a t-test. Data were also processed through Principal Component Analysis (PCA), but prior to analysis, the significance of the loadings was determined to reduce the number of variables used in the PCA. To avoid the vintage effect, the data were analysed by individual year. All statistical analyses were performed using R (RStudio Team, 2019) and plotted using the ggplot2 package (Wickham *et al*., 2022).

RESULTS AND DISCUSSION

1. Weather conditions and grapevine water status

The seasonal temperatures had different trends in 2019 and 2020 (Figure 2). In 2019, the temperatures were lower, and precipitation was higher in April and May in comparison to 2020. On the other hand, from June to September 2019, there was less precipitation than in 2020, especially in August. In addition, from late June to late August, a higher number

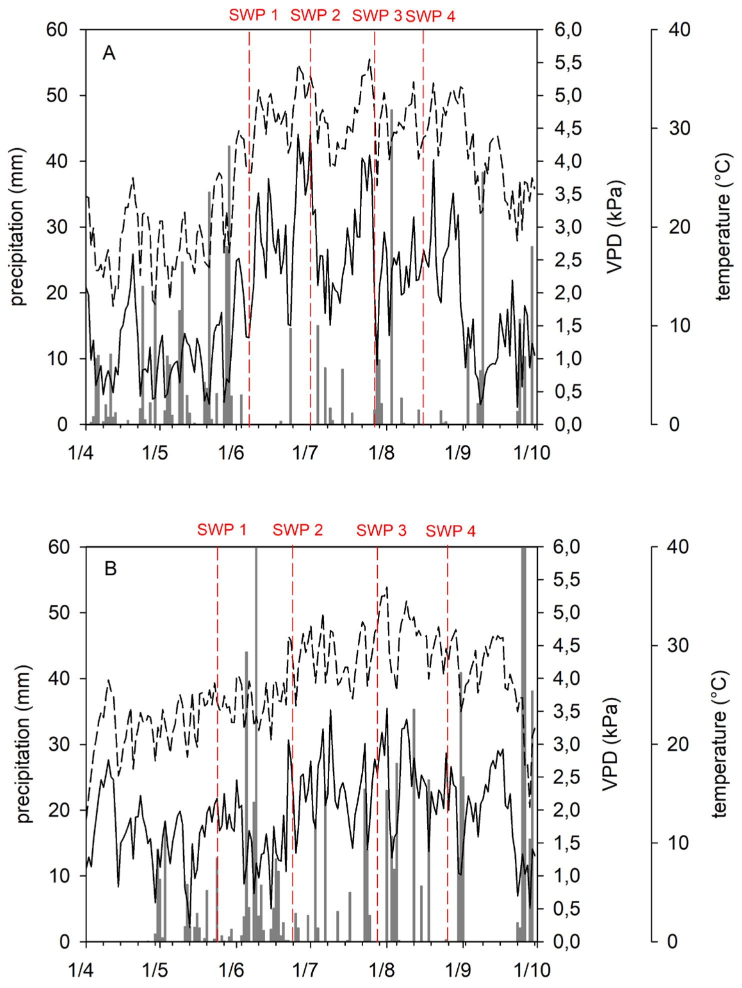


FIGURE 2. Daily average air temperature (dashed line), maximum vapour pressure deficit (VPD, solid line), and precipitation (grey columns) recorded from April to September 2019 and 2020 at the Bilje weather station. Stem water potential (SWP) was measured at four phenological stages (1, pre-flowering; 2, berry-set; 3, pre-veraison; 4, post-veraison).

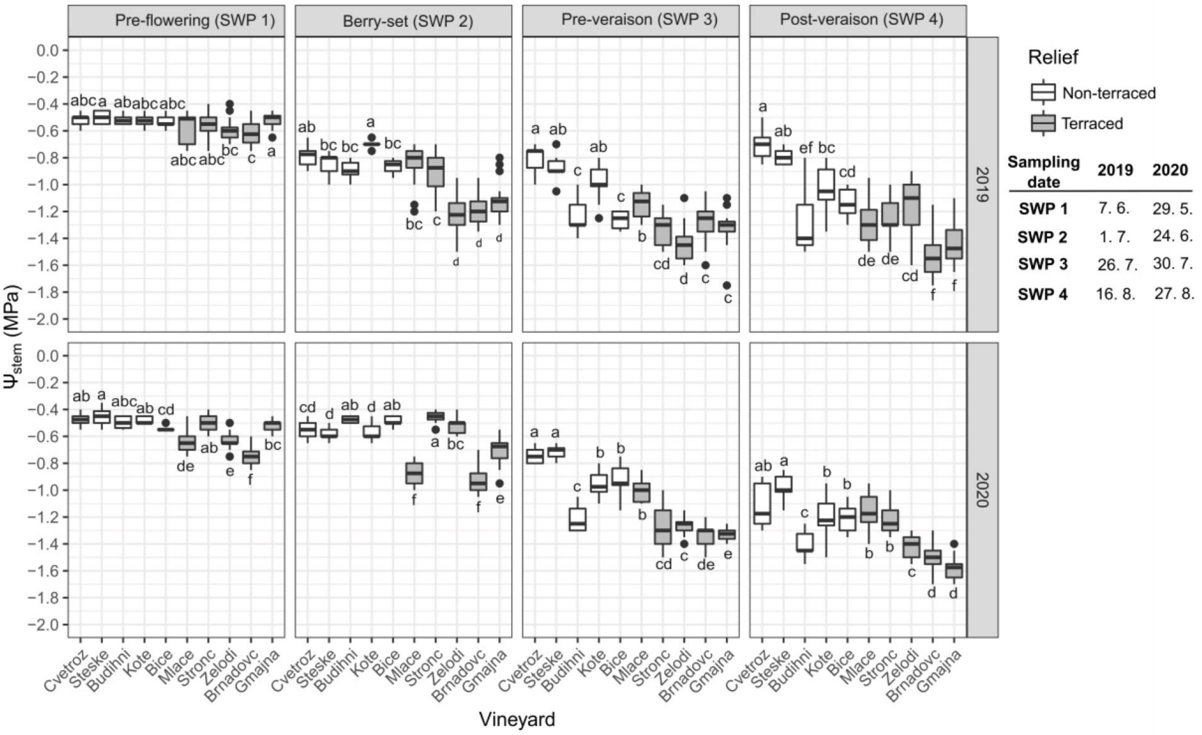
of days with temperatures exceeding 34 °C were registered in 2019 (15 and 4 days in 2019 and 2020, respectively) (Table 1). In 2020, the temperatures were lower from late June to late August than in 2019, with smaller fluctuations.

Plant water status was monitored four times during the season, using the stem water potential (SWP) method (Ψ_{stem}) determined at midday (i.e., between 12:00 and 14:00). Trends of Ψ_{stem} in each vineyard in 2019 and 2020 are summarised in Figure 3. At the first sampling (SWP 1, flowering period), the non-terraced vineyards had a better water status than the terraced vineyards. However, in later sampling dates, the difference in Ψ_{stem} between the non-terraced and terraced vineyards became more pronounced. In agreement with van Leeuwen *et al.* (2009), well-watered grapevines have Ψ_{stem} above –0.6 MPa, while lower values represent conditions of mild ($-0.9 < \Psi_{stem} < -0.6$ MPa), moderate ($-1.1 < \Psi_{stem} < -0.9$ MPa), severe ($-1.4 < \Psi_{stem} < -1.1$ MPa), and excessive water stress ($\Psi_{stem} < -1.4$ MPa). At SWP 1, the vines from the non-terraced vineyards reported no water stress in both years. On the contrary, some on the terraces already showed mild water stress. At berry set (SWP 2), the non-terraced vineyards showed mild water stress in 2019 and no water stress in 2020; differently, terraced vineyards reported mild to severe water stress conditions in 2019, while in 2020, they reported no to mild water stress. At pre-veraison (SWP 3), in both years, the vines from non-terraced vineyards had mild water stress (except Budihni and Bice in 2019 and Budihni in 2020), while the vines from terraced vineyards showed mostly severe water stress (except Mlace vineyard, which showed moderate water stress in both years). The soil from Mlace is dominated by marl, and compared to other vineyards on flysch, the soil is loamier and more compact, providing a greater retention of water. After veraison (SWP 4), non-terraced vineyards reported mild to moderate water stress in both seasons (except Budihni, which had severe water stress). The non-skeletal, clay-loam soil of the non-terraced vineyards in the Vipava Valley exhibit higher matric potential and greater water retention capacity, which strongly influences the water status of the vines (Whalley *et al.*, 2013). On the other hand, terraced vineyards reported severe to excessive

TABLE 1. Mean meteorological variables of the weather station in Bilje ARSO (https://meteo.arso.gov.si/) from April 1st to September 30th in 2019 and 2020.

	2019	2020
Average T (°C)	19.7	19.3
Average T_{max} (°C)	26.0	25.9
Average T_{min} (°C)	13.9	13.1
Cumulative rain (mm)	549.6	880.9
Winkler Index (°C)	1772	1718
Huglin Index (°C)	2408	2375
Days with T_{max} > 34 °C	15	4
Solar radiation (MJm^{-2})	3491	3803
Active hours (h)	3846	4189

water stress conditions in both years. Due to the high percentage of very large skeletal particles in flysch soils (up to 80 %) in vineyards with excessive water stress (Brnadovc, Gmajna, and Zelodi), it can be assumed that (hyper) skeletal soil structures impacted their water retention capacity. As regards the rootstocks (Supplementary Table 1), the Merlot grafted on Kober 5BB (non-terraced vineyard Budihni) showed lower values of stem water potential as compared to the other non-terraced vineyards grafted on S.O.4 at pre-veraison and post-veraison stages. The lower tolerance of Kober 5BB to water stress agrees with what was reported by Carbonneau (1985). As regards the Merlot grafted on 1103 Paulsen (terraced vineyard Stronc), the stem water potential was the highest among the terraced vineyards till berry-set, and thereafter, the values were reduced, reaching similar values as the other terraced vineyards. There was not always a clear trend of stem water potential as related to rootstock and rooting depth, and probably the combination of effects due also to soil texture, percentage of the skeleton and, thus, available water content is responsible for the differences ascertained in two seasons investigated. In summary, the trends of Ψstem highlighted differences in soils between and within non-terraced and terraced vineyards.



Sampling date	2019	2020
SWP 1	7. 6.	29. 5.
SWP 2	1. 7.	24. 6.
SWP 3	26. 7.	30. 7.
SWP 4	16. 8.	27. 8.

FIGURE 3. Trends of stem water potential (Ψ_{stem}) in five non-terraced and five terraced vineyards at different phenological stages in the seasons 2019 and 2020. Boxplots represent median values, 25th and 75th percentiles, 95 % confidence interval and outliers of the data set. The data were analysed with the Kruskall–Wallis non-parametric rank sum test and means were separated with a *post-hoc* Dunn's test (Bonferroni correction). Different letters indicate statistically significant differences between the vineyards at each sampling date.

2. Leaf area, yield parameters, and seed colour

In both years, lower yield and berry weight were found in the vineyards on terraces (Table 2 and Supplementary Table 2). Moreover, higher skin- and seed-to-flesh ratios and darker seed colours were found in the grapes from terraces in both years. Several studies on the effect of water status on berry composition at harvest have shown that its impact depends to a large extent on the changes caused to berry weight (Ojeda *et al.,* 2001). As regards the leaf area, it was higher in non-terraced vineyards than in terraced; however, the difference was not significant in 2020. The higher and more evenly distributed precipitation in 2020 resulted in higher leaf area, higher yield, and higher berry weight compared to the 2019 vintage.

3. Basic physicochemical variables of wines

Higher values of the alcohol content, total dry extract (significant only in 2019), and titratable acidity (significant only in 2020) were found in the wines from terraces (Table 2, Supplementary Table 2). As opposed, there were no differences in the content of ash or pH between the wines from the non-terraced and terraced vineyards in both years. All wines were fermented to dryness (glucose + fructose < 1 g L^{-1}); therefore, the sugar content was not reported.

4. Aromatic compounds in wines

Higher concentrations of all analysed higher alcohols were found in wines from terraces in both seasons (Table 3; Supplementary Table 3). Differences were observed in the case of propan-1-ol in both seasons, while for butan-1-ol, 2-methylbutan-1-ol, and 3-methylbutan-1-ol only in 2019.

The concentration of analysed esters was generally lower in the wines from terraces. Lower values of ethyl butanoate, ethyl hexanoate, and ethyl octanoate were determined in the wines from terraces in both years. In the present experiment, a negative impact of water stress on a concentration of esters in Merlot wines from terraces was found. These results agree with Talaverano *et al*. (2018), that reported a negative effect of water stress on the concentration of esters in Cabernet-Sauvignon wines, while Qian *et al*. (2009) showed that esters in Merlot wines were not affected by irrigation. Among the other aromatic compounds, hexan-1-ol, (3Z)-hex-3-en-1-ol, benzaldehyde, and phenylmethanol were lower in the wines from terraces (however, non-significant in 2019), and the oxolan-2-one concentration was higher in the wines from terraces in 2020.

The concentrations of methoxypyrazines in wines from terraced and non-terraced vineyards were determined only in 2019 (Supplementary Table 3). The wines from non-terraced vineyards contained, on average, 2.3 n gL^{-1} of 3-isobutyl-2-methoxypyrazine (IBMP), whereas in 4 out of 5 locations on terraces, the concentration of IBMP was below the limit of detection. 3-isopropyl-2-methoxypyrazine (IPMP) concentration was below the limit of detection in all analysed wines. High methoxypyrazine concentration in grapes at harvest is generally associated with a lack of maturity, negatively impacting the final wine quality (Chapman *et al*., 2004). Lower concentrations of IBMP in wines from terraces could be impacted by the advanced ripening on terraces and

TABLE 2. Leaf area, grape variables, and seed colour of grape cv. Merlot and the basic wine variables of the non-terraced and terraced vineyards in the 2019 and 2020 vintages in the Vipava Valley.

		2019			2020		
		Non-terraced (n = 5)	Terraced (n = 5)	Sign. t	Non-terraced (n = 5)	Terraced (n = 5)	Sign. t
Grape variables	Yield (kg)	1.92 ± 0.55	0.75 ± 0.28	0.000***	3.04 ± 0.72	1.56 ± 0.63	0.000***
	Leaf area (m^2 vine^{-1})	3.5 ± 1.25	2.01 ± 0.33	0.001***	2.96 ± 0.99	2.63 ± 0.61	0.348
	Berry weight (g)	1.52 ± 0.11	1.15 ± 0.12	0.000***	1.73 ± 0.09	1.48 ± 0.15	0.000***
	Skin weight (mg berry^{-1})	175.2 ± 13.0	215.1 ± 27.9	0.000***	160.9 ± 16.4	174.2 ± 20.5	0.06
	Seed weight (mg berry^{-1})	54.0 ± 65	66.6 ± 6.0	0.000***	46.8 ± 4.0	54.3 ± 5.3	0.000***
	Skin-to-flesh ratio (mg g^{-1} flesh)	147.7 ± 24.8	267.5 ± 57.6	0.000***	118.8 ± 20.4	150.7 ± 31.8	0.003**
	Seed-to-flesh ratio mg g^{-1} flesh)	46.8 ± 9.7	80.3 ± 10.3	0.000****	35.6 ± 6.1	48.6 ± 7.4	0.000***
	Seed colour	4.77 ± 0.51	5.18 ± 0.42	0.024*	4.03 ± 0.52	5.72 ± 0.6	0.000***
Basic wine variables	Alcohol (vol.%)	12.34 ± 0.28	12.78 ± 0.65	0.030*	11.63 ± 0.45	12.65 ± 0.28	0.000***
	Total dry extract (g L^{-1})	25.6 ± 1.4	28.2 ± 1.3	0.000***	24. 7 ± 1.5	26.3 ± 2.5	0.070
	Titratable acidity (g L^{-1})	5.2 ± 0.26	5.3 ± 0.2	0.070	5.5 ± 0.42	5.9 ± 0.3	0.024*
	Ash (g L^{-1})	2.88 ± 0.34	3.07 ± 0.29	0.130	3.59 ± 0.39	3.32 ± 0.39	0.136
	pH value	3.75 ± 0.16	3.77 ± 0.09	0.566	3.81 ± 0.16	3.7 ± 0.19	0.169

The data represent mean ± standard deviation and were analysed with a *t*-test (* $p < 0.05$;** $p < 0.01$; *** $p < 0.001$; otherwise not significant).

higher bunch light exposure (lower leaf area), as reported by Šuklje *et al*. (2012).

The effect of the relief on the fermentative aromatic compounds is quite complex since there is the combined effect of the water stress, which modifies the grape composition of aroma precursors and fermentation behaviour. Due to the high percentage of soil skeleton and, consequently, the high water permeability of flysch terraces, the soil warms faster, resulting in an accelerated speed of the phenological stages and grape ripening (Bodin and Morlat, 2006). The grapevines grown on the terraces had a smaller leaf area compared to the grapevines in the non-terraced vineyards in both years but were only significant in 2019. Reduced vigour increases the cluster sunlight exposure and, thus, also the berry temperature. The same result could be obtained by leaf removal, and several studies proved a positive effect of such viticultural techniques on the aromatic volatile content of the wines (Bubola *et al*., 2019; Cincotta *et al*., 2022, Moreno *et al*., 2017). Studies on Pinot Noir indicated that sunlight and UV radiation contributed to fruity and rose aromas and increased the concentration of some higher alcohols (Song *et al*., 2015). However, the authors of the same study suggested that sunlight and UV radiation could also be responsible for a reduction in the concentration of some esters. In a study carried out in Friuli Venezia Giulia on Ribolla Gialla, Škrab *et al*. (2021) discussed the complexity of factors affecting the occurrence of esters in wines, and they reported how the equilibrium between canopy and yield was crucial to obtain a higher concentration of this class of aromatics in wines. Thus, differences in soils, vine water stress, meteorological conditions, maturation degree, and fermentation course represent the factors that interplay and, thus, unpredictably affect the concentration of aroma compounds in red wines (Falqué *et al*., 2001).

PCA was applied to the volatiles for each year separately to avoid the influence of the vintage effect (Figure 4). In 2019, the first two eigenvalues of the PCA explained 61.0 % of the variance of the dataset, separating the wines of the terraced and non-terraced vineyards, noticeable along the second component (PC2). Wines from the terraces of Zelodi, Mlace, and Gmajna were associated with higher alcohols, whereas wines from the non-terraced vineyards of Cvetroz, Steske, and Kote were more associated with esters. In 2020, the PCA explained 41.3 % and 16.5 % of the total variance on the PC1 and PC2 axis, respectively. Similarly to 2019, the wines from terraces in 2020 correlated with a higher content of higher alcohols, and the wines from non-terraced vineyards with higher concentrations of esters. The only exception was the wine from the non-terraced vineyard Budihni, which is positioned on the lower left side of PC1 next to the wines from the terraces and, thus, more associated with a higher content of higher alcohols. When the soil of the Budihni vineyard was formed, the sediment from the hinterland of the Vipava Valley was well and densely packed, with a good ratio of clay and sandstone, which is also indicated by its grey colour or pseudo-gley marbling. Such soils begin to crack in the summer, during drought and are quickly saturated with water when it rains. Due to extremely low rainfall throughout the season, the vines in the Budihni vineyard were under greater water stress compared to other non-terraced vineyards, similar to what happens on the vineyards in terraces.

5. Wine colour properties, the concentration of phenols, and structural characteristics of proanthocyanidins

Colour intensity and the concentrations of TA, TP, and PAs were higher in the wines from terraces in both years, whereas the low molecular weight fraction of proanthocyanidins (VAN) did not report significance between terraces and non-terraced vineyards (Table 4, and the data of individual vineyards in Supplementary Table 4). Colour hue was instead lower in wines from terraces, and this agrees with other experiments dealing with water stress (Gamero *et al*., 2014). Higher concentrations of monomeric anthocyanins in wines from terraces analysed 4 months after fermentation might negatively correlate with colour hue.

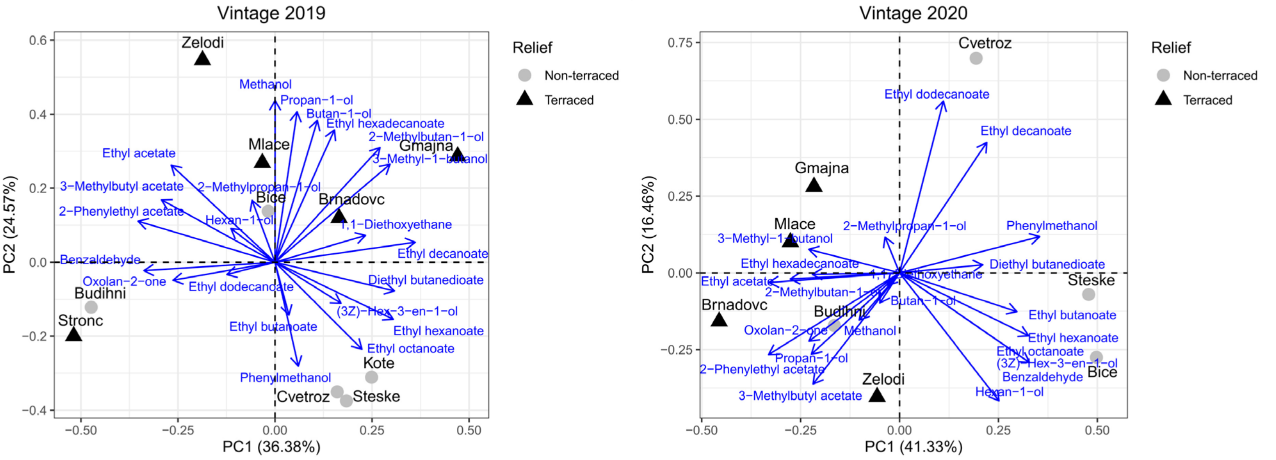


FIGURE 4. Principal Component Analysis (PCA), showing the projection of the data set in the PC1 × PC2 plot for all measured volatile components of Merlot wines from the seasons 2019 and 2020.

TABLE 3. Average concentrations of aromatic compounds in Merlot wines from non-terraced and terraced vineyards in the 2019 and 2020 vintages in the Vipava Valley.

	2019			2020		
	Non-terraced (n = 5)	Terraced (n = 5)	Sign. t	Non-terraced (n = 4)	Terraced (n = 4)	Sign. t
Higher alcohols (m g L^{-1})						
1,1-Diethoxyethane	22.6 ± 9. 9	29.6 ± 10.4	0.097	12.5 ± 4.9	15.5 ± 9.4	0.347
Propan-1-ol	29.5 ± 3.3	43.5 ± 10.0	0.000***	37.3 ± 3.7	43.2 ± 4.5	0.003**
2-Methylpropan-1-ol	52.3 ± 7.5	53.4 ± 43.3	0.627	36.1 ± 4.0	63.1 ± 4.2	0.975
Butan-1-ol	1.0 ± 0.0	1.3 ± 0.3	0.003**	1.3 ± 0.2	1.4 ± 0.2	0.101
2-Methylbutan-1-ol	79.3 ± 6.5	88.5 ± 7.9	0.003**	83.6 ± 7.6	87.9 ± 4.4	0.138
3-Methybutan-1-ol	231.5 ± 11.6	249.2 ± 17.4	0.005**	270.1 ± 21.4	272.8 ± 11.1	0.717
SUM	416.2	465.5		440.9	483.9	
Ethyl acetate (m g L^{-1})	40.9 ± 8.9	49.5 ± 12.1	0.050*	39.1 ± 16.5	60.7 ± 8.5	0.001***
Methanol (m g L^{-1})	84.3 ± 7.1	102.0 ± 10.9	0.000***	81.4 ± 14.8	88.4 ± 25.2	0.423
***Esters* (µg L^{-1})**						
Ethyl butanoate	85 ± 10	72 ±13	0.005**	45 ± 6	38 ± 6	0.028*
3-Methylbutyl acetate	331 ± 55	363 ± 52	0.143	118 ± 8	127 ± 1949	0.129
Ethyl hexanoate	191 ± 17	171 ± 15	0.004**	91 ± 5	79 ± 8	0.004***
Ethyl octanoate	161 ± 18	136 ± 15	0.001***	90 ± 9	74 ± 8	0.048*
Ethyl decanoate	47 ± 3	46 ± 6	0.573	66 ± 29	50 ± 23	0.205
Diethyl butanedioate	652 ± 126	601 ± 248	0.500	2629 ± 431	52560 ± 216	0.667
2-Phenylethyl acetate	29 ± 9	33 ± 7	0.207	10 ± 1	13 ± 41	0.000***
Ethyl dodecanoate	3162 ± 507	2974 ± 279	0.252	31 ± 22	23 ± 24	0.169
Ethyl hexadecanoate	23 ± 10	39 ± 18	0.007**	62 ± 28	67 ± 19	0.160
SUM	4681	4435		3141	3031	
Other compounds (µg L^{-1})						
Hexan-1-ol	1677 ± 176	1622 ± 256	0.546	718 ± 69	639 ± 76	0.044*
(3Z)-Hex-3-en-1-ol	13 ± 2	13 ± 2	0.973	11 ± 3	8 ± 1	0.029*
Benzaldehyde	9 ± 6	8 ± 7	0.673	2 ± 1	2 ± 1	0.857
Phenylmethanol	479 ± 384	334 ± 137	0.202	704 ± 182	382 ± 173	0.001***
Oxolan-2-one	9535 ± 845	9117 ± 1308	0.324	5159 ± 1131	6133 ± 314	0.050*

The data represent mean ± standard deviation and were analysed with a t-test (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; otherwise not-significant)

Higher seed- and skin-to-flesh ratios, changes in phenol extractability from grapes due to advanced maturation, and changes in the phenol biosynthesis due to water stress are among reasons for higher phenol concentrations in wines from terraces. The impact of water deficit on phenol biosynthesis was reported by Bucchetti *et al*. (2011), who found that water deficits consistently increased anthocyanin concentration in Merlot grapes by increasing content per berry and reducing fruit growth, whereas skin tannin concentration increased less and mostly just by reducing fruit growth. Chacón-Vozmediano *et al*. (2021) found that under water stress conditions, the concentrations of anthocyanins, catechins, tannins, and total polyphenols in the skin of Merlot grapes in semi-arid Mediterranean climate scarcely varied, whereas an increase in the concentration of all the phenolic compounds took place in seeds at higher stress levels. On the other hand, Peterlunger *et al*. (2005) found that total polyphenol and anthocyanin concentrations were higher in Merlot grapes at maturity in the water-stressed treatment compared to the control, whereas proanthocyanidins were higher in the skins of stressed berries, but water stress had no effect on their concentration in the seeds. It is generally considered that water stress stimulates the biosynthesis of polyphenols, but there are many factors which influence the final quality of grapes and wine, which may result in discrepancies between obtained results (Deloire *et al.,* 2004). The timing of water stress (i.e., before or after veraison), the water stress levels and the duration of the water stress will affect the concentration of major phenols (Deloire *et al.,* 2004). Regarding the phenol extractability into wine, advanced grape maturation on terraces and higher levels of alcohol might have enhanced the extractability of PAs from seeds in comparison to non-terraced vineyards. This would agree with Pastor del Rio and Kennedy (2006), who reported that wine made from increasingly mature grapes results in an increase in the proportion of seed-derived tannins.

Besides the phenolic concentration, wine proanthocyanidin structural characteristics strongly impact the sensorial properties of wines. In relation to proanthocyanidin structural characteristics (Table 4), higher galloylation (% G) was found in the wines from terraces in 2019, while negligible differences were found in 2020. Interestingly, the prodelphinidins (% P) were lower in the wines from terraces in 2019, and similarly to galloylation, no differences were found in 2020. Moreover, the mean degree of polymerisation did not report differences between non-terraced vineyards and terraces, and slight incoherent trends were ascertained between the seasons for both % G and % P. As reported by Calderan *et al*. (2021), more severe water stress in Refošk grapes resulted in darker seed colour, a higher anthocyanin concentration, and a higher degree of galloylation of seed and skin proanthocyanidins, while there was no effect on proanthocyanidin concentration in seeds and skins.

Petruzzellis *et al*. (2022) hypothesised that the timing of maximum drought periods affects grapes' physical and chemical composition; thus, polyphenol concentration and structural characteristics might differ between the monitored years. In our study, the wines from the drier and hotter season of 2019, when the grapes experienced more water stress at fruit development and before veraison, showed higher phenolic concentrations, higher alcohols, and higher total extract compared to the wines in 2020. Higher temperatures and lower precipitation during the 2019 season could be associated with the faster ripening of grapes (Kuhn *et al*., 2014). In addition, there were nine heat waves in 2019 with temperatures exceeding 30 °C for more than 3 days, which possibly affected phenolic biosynthesis, while in 2020, only five heatwaves were registered. Furthermore, the temperatures were higher at the time of berry set and at pre-veraison in 2019 as compared to 2020.

6. Sensory evaluation of wines

The wines were evaluated by a panel of experts after 4 months from the end of fermentation, and several differences were found comparing the wines from non-terraced and terraced vineyards in both years (Table 5). The wines from the terraces received higher scores for all observed descriptors, colour intensity, fruitiness, astringency, midpalate, overall quality, and higher total Buxbaum score (Table 5 and Supplementary Table 5). The higher total Buxbaum score of terraced wines is an important outcome of the study because wine tasters were trained to use this model. The 20-point scale Buxbaum scale, which comprises wine sensory characteristics such as clarity, colour, odour, taste, and balance, is similar, but less complex than the widely used 100-point scale method. Results are consistent with Peterlunger *et al*. (2005), who found that Merlot wines from water-stressed fruit had a better aroma, more astringency, a more intense colour, and greater harmony in structure than the control wines. Higher colour intensity correlates with higher concentrations of anthocyanins in wines from terraces. Higher astringency positively correlates with a tannin concentration (Gonzalo-Diago *et al*., 2013, Landon *et al*., 2008, Lisjak *et al*., 2020), mDP, and %G (Chira *et al*., 2012; Lisjak *et al*., 2020) and negatively with %P (Lisjak *et al*., 2020, Vidal *et al*., 2003). The wines were tasted at the age of 4 months, and it might be expected that astringency perception would change with ageing. Higher galloylation of PAs has been found to

TABLE 4. Chromatic properties, phenol concentrations and the structural characteristics of the proanthocyanidins (PAs) in Merlot wines from non-terraced and terraced vineyards (CI—colour intensity, TA—total anthocyanins, TP—total polyphenols, VAN—vanillin index, mDP—mean degree of polymerisation, % G—percentage of galloylation, % P—percentage of prodelphinidins).

		2019			2020		
		Non-terraced (n = 5)	Terraced (n = 5)	Sign. t	Non-terraced (n = 4)	Terraced (n = 4)	Sign. t
Chromatic properties	CI	11.46 ± 0.79	12.83 ± 1.08	0.001***	7.96 ± 2.09	12.43 ± 2.28	0.000***
	Hue	0.75 ± 0.06	0.69 ± 0.04	0.007**	0.79 ± 0.10	0.65 ± 0.05	0.001***
Phenol concentration (mg/L^{-1})	TA	602 ± 136	744 ± 75	0.001***	427 ± 63	716 ± 123	0.000***
	TP	1241 ± 82	1393 ± 119	0.001***	1071 ± 169	1332 ± 145	0.001***
	VAN	1522 ± 212	1485 ± 208	0.651	1028 ± 156	982 ± 173	0.539
	PAs	1930 ± 702	2648 ± 505	0.005**	1925 ± 295	2499 ± 234	0.000***
Structural characteristics of PAs	mDP	4.19 ± 0.32	3.99 ± 0.37	0.111	4.57 ± 0.64	4.77 ± 0.65	0.472
	% G	12.21 ± 0.91	15.73 ± 2.47	0.000***	17.13 ± 2.31	16.31 ± 1.55	0.356
	% P	21.45 ± 2.77	18.47 ± 2.78	0.001***	18.82 ± 1.12	20.53 ± 3.29	0.128

The data represent mean ± standard deviation and were analysed with a t-test (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; otherwise not-significant).

TABLE 5. Ranking totals (n = 10 tasters, intensity scale 0–7) for sensory attributes regarding colour intensity, fruitiness, astringency, midpalate, overall quality, and total Buxbaum score of Merlot wines from the non-terraced and terraced vineyards in 2019 and 2020.

	2019			2020		
	Non-terraced (n = 5)	Terraced (n = 5)	Sign. F	Non-terraced (n = 4)	Terraced (n = 4)	Sign. F
Intensity	17.6 ± 8.5	32.4 ± 6.4	0.000***	19.4 ± 10.4	38.2 ± 8.3	0.000***
Fruitiness	16.4 ± 6.3	30.7 ± 6.9	0.000***	24.1 ± 6.1	34.9 ± 6.5	0.001***
Astringency	21.6 ± 5.6	28.2 ± 3.4	0.001***	25.4 ± 7.0	34.3 ± 6.2	0.005**
Midpalate	20.8 ± 5.7	27.9 ± 5.0	0.002**	20.2 ± 8.0	36.2 ± 6	0.000***
Overall quality	18.9 ± 6.3	28.4 ± 6.2	0.000***	20.7 ± 6.8	36.09 ± 7.6	0.000***
Buxbaum total score	16.8 ± 0.3	17.2 ± 0.2	0.004**	16.4 ± 0.3	17.0 ± 0.2	0.000***

The data represent mean ± standard deviation and were analysed with a t-test (**, $p < 0.01$; ***, $p < 0.001$).

correlate positively with the perception of midpalate and the wine's overall sensorial quality (non-published data). The better polyphenol potential in the wines from terraces; however, entails both the better overall sensorial quality and the better ageing potential of these wines.

With the aim of identifying the overall influence of vineyard location on both sensory properties and phenolic content and structural characteristics of PAs, a PCA was carried out, again separated by seasons (Figure 5). In 2019, the first two PCs accounted for 66.6 % of the total explained variance, while in 2020 was much higher, with a value of 80.4 %. In both years, the terraced vineyards were grouped and separated from the non-terraced, except for the Brnadovc vineyard in 2019 and the Budihni vineyard in 2020. The wines from the terraces in 2019 were associated with higher TP, PAs, TA, CI, % G, and sensory ratings. In 2020 the trend of phenolic content and sensory analysis was like in 2019, also with a higher % P and lower colour hue in the wines from the terraces.

6. The relative importance of the yield, basic wine variables, aroma compounds, phenolic content, and sensory attributes according to the relief of vineyards

To understand the relative importance of the examined dataset, the data on the yield, the basic wine variables, and the aroma compounds, the phenolic content and sensory properties were processed by PCA analysis (Figure 6). The first two factors analysed accounted for 81.6 % of the explained variance in 2019 and 81.8 % in 2020. Due to the different distributions and intensity of precipitation during the seasons, the significant loadings varied from year to year. This shows contrasting behaviour in the two seasons, indicating that a more complex interaction with other environmental variables occurred. Still, even though in 2020 the concentration of phenolic and aroma compounds was lower, the trend of differences between the non-terraced and

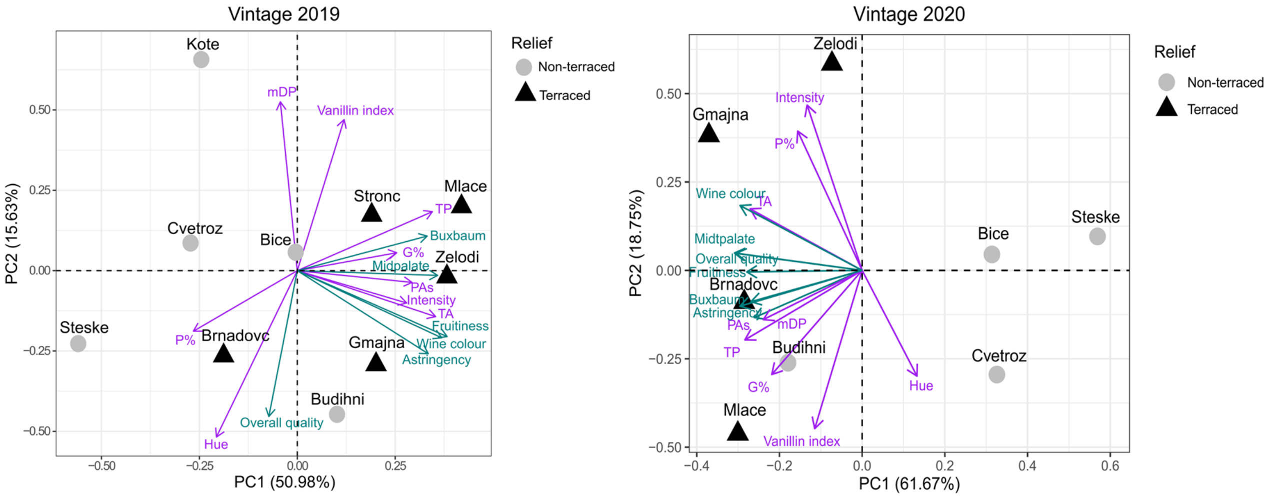


FIGURE 5. Principal Component Analysis (PCA), showing a projection of the data set in the PC1 × PC2 plot of Merlot wines from the seasons 2019 and 2020 according to proanthocyanidins (PAs), the vanillin index, the mean degree of polymerisation (mDP), the percentage of galloylation (% G), the percentage of prodelphinidins (% P), total anthocyanins (TA), colour intensity and hue, total polyphenols (TP), and sensorial evaluations as variables.

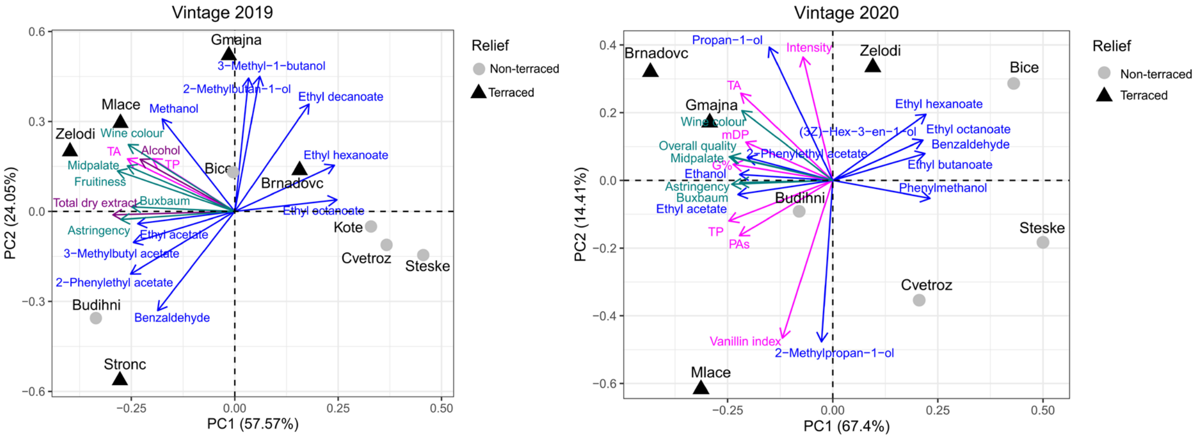


FIGURE 6. Principal Component Analysis (PCA), showing the projection of the data set in the PC1 × PC2 plot on the measured vine basic variables, colour, phenolic content, the structural characteristics of the proanthocyanidins, volatile compounds, and sensorial evaluations of Merlot wines from the seasons 2019 and 2020 as affected by the vineyards' relief.

terraced vineyards was similar between seasons, indicating the importance of the "terroir".

Of all the abiotic stresses to which plants growing in fields are exposed, the most influential is water stress (Chacón-Vozmediano *et al.*, 2021). The difference in water stress due to differences in soil properties strongly influenced the quality of the Merlot wines, even if all vineyards were located within a small area with a similar mesoclimate. The higher water stress on terraces resulted in lower yield, lower leaf area, and lower berry mass in comparison to non-terraced vineyards. In addition, higher skin- and seed-to-flesh ratios were found in grapes from terraces. The Merlot wines from terraces had higher concentrations of anthocyanins, proanthocyanidins, total phenols, and proanthocyanidins in 2019 were more galloylated when compared to wines from non-terraced vineyards. The concentration of phenolic compounds in wines was the most evident difference between vineyards located on terraces and those non-terraced in lowlands. Regarding aromatic compounds, wines from terraced vineyards contained higher alcohols, fewer esters, and fewer methoxypyrazines than wines from non-terraced. Finally, Merlot wines from terraced vineyards exerted better overall sensorial quality than Merlot wines from non-terraced vineyards in both investigating years. The differences between the two ecologically distinct vineyard sites confirmed the importance of relief and soil on wine quality. Specific knowledge of the vineyard location and the soil characteristics is crucial for understanding the effect of "terroir" as one of the most important attributes of both wine origin and quality.

ACKNOWLEDGEMENTS

We would like to thank P. Zabukovec and V. Čopi for their help with grape sampling, V. Čopi and L. Bariviera for their help with microvinification, I. Kmetič Ceglar and N. Bizjak from the Central Laboratory of the Agricultural Institute of Slovenia for their valuable help with the basic wine physico-chemical analysis, Dr H. Baša Česnik and T. Sket for their help with the GC-MS and GC-FID analyses, and the University of Nova Gorica for loaning a Scholander pressure chamber. Dr A. Čebulj provided valuable comments and critical reading of the manuscript. The greatest thanks go to the winemakers who made the experiment possible.

FUNDING SOURCES

The work was funded by the Slovenian Research Agency (ARRS): Young Researcher Grant 51919, research projects L4-1841, V4-2263, Research Programme P4-0133 and the Acquavitis (EU Interreg) project implemented as part of the Interreg V-A Italy-Slovenia 2014-2020 programme, funded by the European Regional Development Fund.

REFERENCES

Atanassov, I., Hvarleva, Tz., Rusanov, K., Tsvetkov, I., & Atanassov, A. (2009). Wine metabolite profiling: Possible application in winemaking and grapevine breeding in Bulgaria. *Biotechnology and Biotechnological Equipment*, *23*(4), 1449–1452. Scopus. https://doi.org/10.2478/V10133-009-0011-9

Bavčar, D., Baša Česnik, H., Čuš, F., & Košmerl, T. (2011). The influence of skin contact during alcoholic fermentation on the aroma composition of Ribolla Gialla and Malvasia Istriana Vitis vinifera (L.) grape wines. *International Journal of Food Science & Technology*, *46*(9), 1801–1808. https://doi.org/10.1111/j.1365-2621.2011.02679.x

Bavčar, D., & Česnik, H. (2011). Validation of the method for the determination of some wine volatile compounds. *Acta Agriculturae Slovenica*, *97*(3). https://doi.org/10.2478/v10014-011-0023-7

Bindon, K.A., Dry, P.R., & Loveys, B.R. (2007). Influence of plant water status on the production of C13-norisoprenoid precursors in

Vitis vinifera L. cv. Cabernet sauvignon grape berries. *Journal of Agricultural and Food Chemistry*, 55 (11), 4493-4500. https://doi.org/10.1021/jf063331p

Bodin, F., & Morlat, R. (2006). Characterization of viticultural terroirs using a simple field model based on soil depth I. Validation of the water supply regime, phenology and vine vigour, in the Anjou vineyard (France). *Plant and Soil*, *281*(1), 37–54. https://doi.org/10.1007/s11104-005-3768-0

Bubola, M., Lukić, I., Radeka, S., Sivilotti, P., Grozić, K., Vanzo, A., Bavčar, D. & Lisjak, K. (2019). Enhancement of Istrian Malvasia wine aroma and hydroxycinnamate composition by hand and mechanical leaf removal. *Journal of the Science of Food and Agriculture*, *99*, 904-914. https://onlinelibrary.wiley.com/doi/abs/10.1002/jsfa.9262

Bucchetti, B., Matthews, M. A., Falginella, L., Peterlunger, E., & Castellarin, S. D. (2011). Effect of water deficit on Merlot grape tannins and anthocyanins across four seasons. *Scientia Horticulturae*, *128*(3), 297–305. https://doi.org/10.1016/j.scienta.2011.02.003

Calderan, A., Sivilotti, P., Braidotti, R., Mihelčič, A., Lisjak, K., & Vanzo, A. (2021). Managing moderate water deficit increased anthocyanin concentration and proanthocyanidin galloylation in "Refošk" grapes in Northeast Italy. *Agricultural Water Management*, *246*, 106684. https://doi.org/10.1016/j.agwat.2020.106684

Carbonneau, A. (1985). The early selection of grapevine rootstocks for resistance to drought conditions. *American Journal of Enology and Viticulture, 36*, 195. https://doi.org/10.5344/ajev.1985.36.3.195

Carpena, M., Fraga-Corral, M., Otero, P., Nogueira, R.A., Garcia-Oliveira, P., Prieto, M.A. & Simal-Gandara, J. (2020). Secondary aroma: influence of wine microorganisms in their aroma profile. *Foods*, *10*, 51. https://doi.org/10.3390/foods10010051

Castellarin, S.D., Pfeiffer, A., Sivilotti, P., Degan, M., Peterlunger, E., & di Gaspero, G. (2007). Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant, Cell & Environment*, *30*, 1381–1399. https://doi.org/10.1111/j.1365-3040.2007.01716.x

Chacón-Vozmediano, J. L., Martínez-Gascueña, J., García-Romero, E., Gómez-Alonso, S., García-Navarro, F. J., & Jiménez-Ballesta, R. (2021). Effects of Water Stress on the Phenolic Compounds of 'Merlot' Grapes in a Semi-Arid Mediterranean Climate. Horticulturae, *7*, 161. https://doi.org/10.3390/horticulturae7070161

Chapman, D. M., Thorngate, J. H., Matthews, M. A., Guinard, J.-X., & Ebeler, S. E. (2004). Yield effects on 2-methoxy-3-isobutylpyrazine concentration in Cabernet Sauvignon using a solid phase microextraction gas chromatography/mass spectrometry method. *Journal of Agricultural and Food Chemistry*, *52*(17), 5431–5435. https://doi.org/10.1021/jf0400617

Chira, K., Schmauch, G., Saucier, C., Fabre, S., & Teissedre, P.-L. (2009). Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). *Journal of Agricultural and Food Chemistry*, *57*(2), 545–553. https://doi.org/10.1021/jf802301g

Chira, K., Jourdes, M., & Teissedre, P.-L. (2012). Cabernet sauvignon red wine astringency quality control by tannin characterization and polymerization during storage. *European Food Research and Technology*, *234*(2), 253–261. https://doi.org/10.1007/s00217-011-1627-1

Cincotta, F., Verzera, A., Prestia, O., Tripodi, G., Lechhab, W., Sparacio, A., & Condurso, C. (2022). Influence of leaf removal on grape, wine and aroma compounds of Vitis vinifera L. cv. Merlot under Mediterranean climate. *European Food Research and Technology*, *248*(2), 403–413. https://doi.org/10.1007/s00217-021-03885-w

Commission Regulation (EEC) (1990). Commission Regulation (EEC) No 2676/90 of 17 September 1990 determining Community methods for the analysis of wines, 272 OJ L (1990). http://data.europa.eu/eli/reg/1990/2676/oj/eng

Deloire, A., Carbonneau, A., Wang, Z., & Ojeda, H. (2004). Vine and water: a short review. *OENO One*, *38*(1), 1–13. https://doi.org/10.20870/oeno-one.2004.38.1.932

Deloire, A., & Heyns, D. (2011). The leaf water potentials: Principles, method and thresholds. *Wineland Magazine*, *technical yearbook*, 129–131.

Deluc, L.G., Quilici, D.R., Decendit, A., Grimplet J., Wheatley M., Schlauch, K.A., Merillon, J.-M., Cushman, J., & Cramer, G.R. (2009). Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics, 10*, 212. https://doi.org/10.1186/1471-2164-10-212

Drinkine, J., Lopes, P., Kennedy, J. A., Teissedre, P.-L., & Saucier, C. (2007). Analysis of Ethylidene-Bridged Flavan-3-ols in Wine. *Journal of Agricultural and Food Chemistry*, *55*(4), 1109–1116. https://doi.org/10.1021/jf0626258

Falginella, L., Di Gaspero, G., & Castellarin, S. D. (2012). Expression of flavonoid genes in the red grape berry of „Alicante Bouschet" varies with the histological distribution of anthocyanins and their chemical composition. *Planta*, *236*(4), 1037–1051. https://doi.org/10.1007/s00425-012-1658-2

Falqué, E., Fernández, E., & Dubourdieu, D. (2001). Differentiation of white wines by their aromatic index. *Talanta*, *54*(2), 271–281. https://doi.org/10.1016/S0039-9140(00)00641-X

Famularo, B., Bruwer, J., & Li, E. (2010). Region of origin as choice factor: Wine knowledge and wine tourism involvement influence. *International Journal of Wine Business Research*, *22*(4), 362–385. https://doi.org/10.1108/17511061011092410

FAO (2022). World reference base for soil resources 2022, World soil resources report. International Union of Soil Sciences (IUSS), Vienna.

Fredes, C., Von Bennewitz, E., Holzapfel, E., & Saavedra, F. (2010). Relation between seed appearance and phenolic maturity: a case study using grapes cv. Carménère. *Chilean Journal of Agricultural Research*, *70*(3), 381-389. https://doi.org/10.4067/S0718-58392010000300005

Gamero, E., Moreno, D., Talaverano, I., Prieto, M.H., Guerra, M.T., & Valdés, M.E. (2014). Effects of irrigation and cluster thinning on Tempranillo grape and wine composition. *South African Journal of Enology and Viticulture*, *35*(2), 196-204. https://doi.org/10.21548/35-2-1006

Glories, Y. (1984). La couleur des vins rouges. 2e partie: Mesure, origine et interprétation. *OENO One*, *18*(4), 253. https://doi.org/10.20870/oeno-one.1984.18.4.1744

Gonzalo-Diago, A., Dizy, M., & Fernández-Zurbano, P. (2013). Taste and mouthfeel properties of red wines proanthocyanidins and their relation to the chemical composition. *Journal of Agricultural and Food Chemistry*, *61*(37), 8861–8870. https://doi.org/10.1021/jf401041q

Guadagni, D. G., Buttery, R. G., & Okano, S. (1963). Odour thresholds of some organic compounds associated with food flavours. *Journal of the Science of Food and Agriculture*, *14*(10), 761–765. https://doi.org/10.1002/jsfa.2740141014

Hira, A., & Swartz, T. (2014). What makes Napa Napa? The roots of success in the wine industry. *Wine Economics and Policy*, *3*(1), 37-53. https://doi.org/10.1016/j.wep.2014.02.001

Kennedy, J. A., & Jones, G. P. (2001). Analysis of Proanthocyanidin Cleavage Products Following Acid-Catalysis in the Presence of Excess Phloroglucinol. *Journal of Agricultural and Food Chemistry*, *49*(4), 1740–1746. https://doi.org/10.1021/jf001030o

King, E. S., Kievit, R. L., Curtin, C., Swiegers, J. H., Pretorius, I. S., Bastian, S. E. P., & Leigh Francis, I. (2010). The effect of multiple yeasts co-inoculations on Sauvignon Blanc wine aroma composition, sensory properties and consumer preference. *Food Chemistry*, *122*(3), 618–626. https://doi.org/10.1016/j.foodchem.2010.03.021

Kuhn, N., Guan, L., Dai, Z. W., Wu, B.-H., Lauvergeat, V., Gomès, E., Li, S.-H., Godoy, F., Arce-Johnson, P., & Delrot, S. (2014). Berry ripening: Recently heard through the grapevine. *Journal of Experimental Botany*, *65*(16), 4543–4559. https://doi.org/10.1093/jxb/ert395

Koundouras, S., Zidimitriou, E., Karamolegkou, M., Eimopoulou, E., Kallithraka, S., Tsialtas, J.T., Zioziou, E., Nikolaou, N., & Kotseridis, Y. (2009). Irrigation and rootstock effects on the phenolic concentration and aroma potential of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes. *Journal of Agricultural and Food Chemistry*, *57*(17), 7805-7813. (2009) https://doi.org/10.1021/jf901063a

Landon, J. L., Weller, K., Harbertson, J. F., & Ross, C. F. (2008). Chemical and Sensory Evaluation of Astringency in Washington State Red Wines. *American Journal of Enology and Viticulture*, *59*(2), 153–158. https://www.ajevonline.org/content/59/2/153

Lisjak, K., Lelova, Z., Žigon, U., Bolta, Š. V., Teissedre, P.-L., & Vanzo, A. (2020). Effect of extraction time on content, composition and sensory perception of proanthocyanidins in wine-like medium and during industrial fermentation of Cabernet Sauvignon. *Journal of the Science of Food and Agriculture*, *100*(5), 1887–1896. https://doi.org/10.1002/jsfa.10189

Maarse, H. (1991). *Volatile Compounds in Foods and Beverages*. CRC Press.

Mendiburu, F. de. (2021). *Agricolae: Statistical Procedures for Agricultural Research* (Različica 1.3-5) [Computer software]. https://CRAN.R-project.org/package=agricolae

Moreno, D., Valdés, E., Uriarte, D., Gamero, E., Talaverano, I., & Vilanova, M. (2017). Early leaf removal applied in warm climatic conditions: Impact on Tempranillo wine volatiles. *Food Research International*, *98*, 50–58. https://doi.org/10.1016/j.foodres.2016.09.017

Ojeda, H., Deloire, A., & Carbonneau, A. (2001). Influence of water deficits on grape berry growth. *Vitis*, 40, 141-145. https://doi.org/10.5073/vitis.2001.40.141-145

Palai, G., Caruso, G., Gucci, R., & D'Onofrio, C. (2022). Berry flavonoids are differently modulated by timing and intensities of water deficit in *Vitis vinifera* L. cv. Sangiovese. *Frontiers in Plant Science,* 13. https://doi.org/10.3389/fpls.2022.1040899

Pastor del Rio, J., & Kennedy, J. A. (2006). Development of Proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir Grapes and Extraction into Wine. *American Journal of Enology and Viticulture*, *57*(2), 125.

Perko, D. & Orozen Adamic, M. (2001). *Slovenija: Pokrajine in ljudje*. Zal. Mladinska knj. http://catalog.hathitrust.org/api/volumes/oclc/50304543.html

Peterlunger, E., Sivilotti, P., & Colussi, V. (2005). Water stress increased polyphenolic quality in ´Merlot´ grapes. *Acta Horticulturae*, 689. https://doi.org/10.17660/ActaHortic.2005.689.34

Petruzzellis, F., Natale, S., Bariviera, L., Calderan, A., Mihelčič, A., Reščič, J., Sivilotti, P., Šuklje, K., Lisjak, K., Vanzo, A., & Nardini, A. (2022). High spatial heterogeneity of water stress levels in Refošk grapevines cultivated in Classical Karst. *Agricultural Water Management*, *260*, 107288. https://doi.org/10.1016/j.agwat.2021.107288

Qian, M.C. Fang, Y. & Shellie, K. (2009). Volatile Composition of Merlot Wine from Different Vine Water Status. *Journal of Agricultural and Food Chemistry, 57*(16), 7459–7463. https://pubs.acs.org/doi/abs/10.1021/jf9009558

Rigo, A., Vianello, F., Clementi, G., Rossetto, M., Scarpa, M., Vrhovsek, U., & Mattivi, F. (2000). Contribution of proanthocyanidins to the peroxy radical scavenging capacity of some Italian red wines. *Journal of Agricultural and Food Chemistry*, *48*(6), 1996–2002. https://doi.org/10.1021/jf991203d

Savoi, S., Herrera, J. C., Carlin, S., Lotti, C., Bucchetti, B., Peterlunger, E., Castellarin, S. D., & Mattivi, F. (2020). From grape berries to wines: drought impacts on key secondary metabolites. *OENO One*, *54*(3), 569–582. https://doi.org/10.20870/oeno-one.2020.54.3.3093

Sivilotti, P., Falchi, R., Vanderweide, J., Sabbatini, P., Bubola, M., Vanzo, A., Lisjak, K., Peterlunger, E., & Herrera, J. C. (2020). Yield reduction through cluster or selective berry thinning similarly modulates anthocyanins and proanthocyanidins composition in Refosco dal peduncolo rosso (Vitis vinifera L.) grapes. *Scientia Horticulturae*, *264*, 109176. https://doi.org/10.1016/j.scienta.2019.109166

Smart, D.R., Schwass, E., Lakso, A., & Morano, L. (2006). Grapevine Rooting Patterns: A Comprehensive Analysis and a Review. *American Journal of Enology and Viticulture, 57,* 89. https://doi.org/10.5344/ajev.2006.57.1.89

Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A., & Qian, M. C. (2015). Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of Vitis vinifera L. cv. Pinot noir wine. *Food Chemistry*, *173*, 424–431. https://doi.org/10.1016/j.foodchem.2014.09.150

Škrab, D., Sivilotti, P., Comuzzo, P., Voce, S., Degano, F., Carlin, S., Arapitsas, P., Masuero, D. & Vrhovšek, U. (2021). Cluster thinning and vineyard site modulate the metabolomic profile of Ribolla Gialla base and sparkling wines. *Metabolites, 11*(5), 331. https://doi.org/10.3390/metabo11050331

Šuklje, K., Lisjak, K., Baša Česnik, H., Janeš, L., Du Toit, W. J., Coetzee, Z., Vanzo, A., Deloire, A. (2012). Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazine, glutathione, and hydroxycinnamate evolution during ripening of Sauvignon Blanc (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry,* 60, 37, 9454-9461. http://dx.doi.org/10.1021/jf3020766

Talaverano, I., Ubeda, C., Cáceres-Mella, A., Valdés, M. E., Pastenes, C., & Peña-Neira, Á. (2018). Water stress and ripeness effects on the volatile composition of Cabernet Sauvignon wines. *Journal of the Science of Food and Agriculture*, *98*(3), 1140–1152. https://doi.org/10.1002/jsfa.8565

van Leeuwen, C., Friant, P., Choné, X., Tregoat, O., Koundouras, S., Dubourdieu, D. (2004). Influence of climate, soil, and cultivar on terroir. *American Journal of Enology and. Viticulture,* 55, 207–217. https://doi.org/10.5344/ajev.2004.55.3.207

van Leeuwen, C., Trégoat, O., Choné, X., Bois, B., Pernet, D., & Gaudillère, J.-P. (2009). Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? *OENO One*, *43*(3), 121–134. https://doi.org/10.20870/oeno-one.2009.43.3.798

van Leeuwen, C., Roby, J.-P., & De Rességuier, L. (2018). Soil-related terroir factors: A review. *OENO One*, *52*(2), 173–188. https://doi.org/10.20870/oeno-one.2018.52.2.2208

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E. J. (2003). The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *Journal of the Science of Food and Agriculture*, *83*(6), 564–573. https://doi.org/10.1002/jsfa.1394

Vrščaj, B. (2017). An overview of soils of Slovenia (The Soils of Slovenia). In: B. Vrščaj, B. Repe, & P. Simončič. *The Soils of Slovenia,* 77–133. Springer Netherlands. https://doi.org/10.1007/978-94-017-8585-3_5

Whalley, W. R., Ober, E. S., & Jenkins, M. (2013). Measurement of the matric potential of soil water in the rhizosphere. *Journal of Experimental Botany*, *64*(13), 3951–3963. https://doi.org/10.1093/jxb/ert044

Wickham, H., Chang, W., Henry, L., Pedersen, T. L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., & RStudio. (2022). *ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics* (Različica 3.3.6) [Computer software]. https://CRAN.R-project.org/package=ggplot2.