Evaluation of Technological and Phenolic Maturation of Grapes and Wine Aromatic Profile under Subtropical Conditions

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Accepted for publication: May 2025

Keywords: viticulture, sensory analysis, gas chromatography, wine aroma, grape maturity

The purpose of this study was to evaluate the technological and phenolic maturation of 'Cabernet Sauvignon', 'Merlot', 'Pinot Noir', 'Fiano' and 'Viognier' grapes, as well as their wines sensory and volatile profiles, under subtropical conditions in southern Brazil. The experiment was conducted during the 2020/2021 and 2021/2022 growing seasons. The must physicochemical parameters analysed were pH, total soluble solids, total titratable acidity, total polyphenols, total anthocyanins, and total tannins. Volatile compounds in the wines were determined using gas chromatography (HS-SPME-GC/MS). Sensory analysis was performed using visual, olfactory and taste parameters, with quantitative assessments based on rating scales. The total soluble solids ranged from 18.1°Brix ('Viognier') to 19.8°Brix ('Pinot Noir'), while titratable acidity varied from 7.40 g/L ('Merlot') to 9.48 g/L ('Pinot Noir'). The grapes from all cultivars exhibited suitable parameters for technological and phenolic maturation, with distinct characteristics such as adequate pH, total soluble solids, and slightly elevated total titratable acidity. Sensory analysis of the red wines revealed profiles characterised by red fruit, earthy/animal and floral notes, with key volatile compounds such as isoamyl alcohol, phenylethyl alcohol, diethyl succinate and isoamyl acetate. The sensory profile of the white wines was related to citrus, sweet fruit and floral aromas, attributed to the presence of citronellol, β-damascenone, ethyl-2-phenylacetate and ethyl octanoate. The results of this study provide valuable insights into the characteristics of wines produced in this emerging wine region of southern Brazil, contributing to a better understanding of the region's viticultural potential.

INTRODUCTION

The global vine and wine sector faces significant challenges, including extreme climatic conditions, declining wine production, and reduced wine consumption, leading to a steady decrease in vineyard surface area worldwide since 2018. In contrast, Brazil has defied this trend, achieving a 12.1% increase in wine production in 2023 compared to the previous year, and a remarkable 31.4% rise above its five-year average. As the second-largest wine market in South America, Brazil has seen wine production emerge as an increasingly attractive venture for its productive sector. This growth is further supported by an 11.6% increase in wine consumption in 2023 (International Organisation of Vine and Wine [OIV], 2024).

New vineyards have been established in high-altitude regions of Brazil, primarily above 800 metres, where climatic conditions are more favourable for producing superior-quality grapes for winemaking (Vianna *et al.*, 2016). This shift in the geographical focus of Brazilian viticulture has led to the discovery of new 'terroirs', enabling the production of distinctive wines and playing a pivotal role in the evolution of the country's wine industry (Wurz *et al.*, 2017).

In the context of increasingly rapid and pervasive climate change, studies on grape varieties already established in traditional wine regions around the world, but now cultivated in different regions with distinct climatic conditions, provide valuable insights into their agronomic performance. Such studies are crucial for understanding how these varieties adapt and thrive in new environments. In addition, understanding and characterising emerging 'terroirs' with potential for fine wine production is essential. These studies should focus on analysing climatic conditions, as well as the physicochemical and organoleptic qualities of grapes and their resulting wines.

As a promising wine market, the high-altitude regions

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Acknowledgements: This work was supported by the National Council for Scientific and Technological Development – CNPq, from the Ministry of Science and Technology of Brazil (grant numbers: 409.156/2021-3 and 307.705/2021-8), and in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001

of southern Brazil offer favourable conditions for expanding wine production. Paraná state is the sixth-largest grape producer in Brazil, and recent government initiatives, such as the Paraná Viticulture Revitalization Program (REVITIS), have been implemented to support the wine sector (Government of Paraná, 2024). Among the cultivars grown in southern Brazil are those of French and Italian origin, such as ‘Cabernet Sauvignon’, ‘Merlot’, ‘Pinot Noir’, ‘Viognier’ and ‘Fiano’ (Brighenti *et al*., 2017; Fowler *et al*., 2020; Meneguzzi *et al*., 2020; Bonin *et al*., 2024). Genetic material is a critical factor in fruit production, influencing both harvest conditions and the physicochemical qualities of the fruit (Kyriacou & Rouphael, 2018). Therefore, selecting the appropriate cultivar is an essential step for winegrowers to ensure the success of their operations.

The production of wine grape cultivars (*Vitis vinifera* L.) is a recent activity in some regions of southern Brazil, and there is a lack of studies in the literature on the sensory profile and aromatic composition of wines under subtropical conditions. Research in this area can assist in cultivar selection and enhance an understanding of the region’s wines based on its unique ‘terroir’. The purpose of this study was to evaluate the sensory profile and volatile compounds of five wine grape cultivars, as well as the characteristics of grape maturation and their respective wines.

MATERIALS AND METHODS

Experimental area

The experiment was carried out in a vineyard in the municipality of Campo Largo, Paraná state, Southern Brazil (25° 23’41’S and 49° 30’12’W), in the 2020/2021 and 2021/2022 seasons. This region is characterised by an altitude of 975 m above sea level and is part of the Cfb climate (subtropical with temperate summer) according to the Köppen classification (Alvares *et al*., 2013), with rainfall distributed throughout the year and the possibility of severe frosts. Climate parameters were assessed according to the World Meteorological Organization (WMO), average monthly data on air temperature (minimum and maximum) and rainfall from 2020-08-31 to 2022-03-31were provided by the Paraná Meteorological System (SIMEPAR) from the closest weather station to the vineyard (Fig. 1). The average annual air temperature was approximately 17.7°C, relative humidity was 83.14%, annual rainfall was 1 187 mm, daily average solar radiation was 320.4 W/m^2, with wind speeds of 2.22 m/s.

Five grape cultivars (*Vitis vinifera* L.) were evaluated: three red (‘Cabernet Sauvignon’, ‘Merlot’ and ‘Pinot Noir’) and two white (‘Fiano’ and ‘Viognier’). All the vineyards were trellised at 1.0 m from the ground, with three wires, and the vines were grafted onto Paulsen 1103 rootstock. The ‘Cabernet Sauvignon’ and ‘Merlot’ were planted in 2004, at a spacing of 2.70 × 1.5 m (2 469 vines/ha). The cultivars ‘Viognier’, ‘Pinot Noir’ and ‘Fiano’ were planted in 2010 with a spacing of 2.70 × 1.25 m (2 963 vines/ha). Pruning was carried out using the double spur cordon system, leaving two buds per spur, for the red cultivars. The white cultivars were pruned using long pruning (Guyot) with three to four rods, arched bilaterally. After pruning, 4% hydrogen cyanamide was applied to induce and standardise sprouting. Fungal disease control, canopy management (weeding, defoliation and pruning) and fertilisation were carried out in accordance with the technical recommendations for the crop.

Maturation variables and microvinification

During the 2020/2021 and 2021/2022 seasons, grapes were harvested at commercial ripeness, based on the physical, chemical and sanitary characteristics of the bunches, following the technical standards established by the winery. A total of 150 berries were randomly collected from each

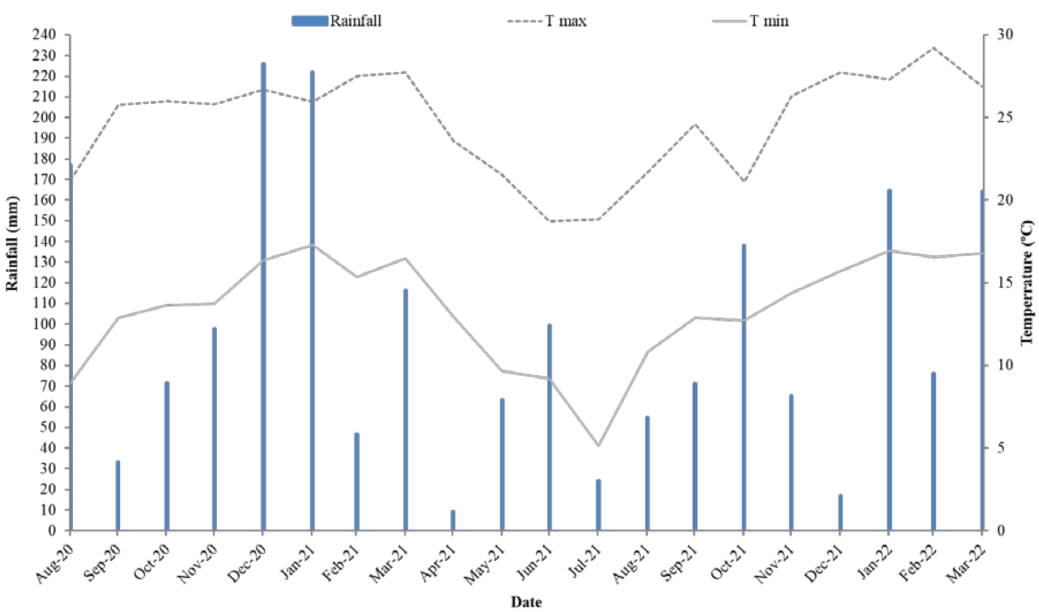

FIGURE 1
Monthly rainfall (mm), average monthly maximum temperature (Tmax, °C) and average monthly minimum temperature (Tmin, °C) in Lapa, PR, Brazil in the 2020/2021 and 2021/2022 seasons.

cultivar. These berries were transported to the laboratory for weighing, skin separation for analysis, and maceration. The must was analysed to determine total soluble solids (TSS), total titratable acidity (TTA) and pH, following the methodologies outlined by the International Organisation of Vine and Wine (OIV, 2020).

Total soluble solids (TSS) were determined using a refractometer (ITREFD 45 model, Instrutemp, São Paulo, SP, Brazil). The device was calibrated with distilled water, after which the must was distributed over the prism and the reading was taken directly in °Brix. Total titratable acidity (TTA) was obtained by titration of a solution of 5 mL of must diluted in 50 mL of distilled water with a standardised alkaline solution of 0.1 N sodium hydroxide (NaOH), using phenolphthalein (1%) as an indicator. The volume of NaOH consumed was used to determine the TTA in mEq/L, and it was then converted into g/L of tartaric acid. The hydrogen potential (pH) was recorded from the samples collected on the day of harvest using a pH-meter (BEL Engineering – model: w3b pH-meter, Monza, Milan, Italy), after calibration in known buffer solutions of pH 4.0 and 7.0.

Bunch samples were collected during harvest to obtain phenolic compounds. To extract anthocyanins, polyphenols and tannins from the peels, the extract was prepared according to the methodology adapted from Pereira *et al*. (2005, 2020). Twenty grams of skin were weighed and mixed with 100 mL of 96% ethanol, and the skins were crushed using a semi-automatic crusher (Britania – model: BMX630PL, Curitiba, Brazil) for 3 min. The extracts were then left to cool (5°C) for one hour. The extracts were centrifuged in an SL700 centrifuge at 3 500 rpm for 15 min. and the supernatant was stored in Falcon tubes.

To determine total anthocyanins (TA), 1 mL of the extract, 1 mL of ethanol and 20 mL of 0.7% hydrochloric acid (HCl) were added to a test tube. Five mL of the mixture was added to two closed tubes (A1 and A2), and 2 mL of distilled water was added to the first (A1). In the second closed tube (A2), 2 mL of 0.7% $NaHSO_3$ were added. After stirring for 10 min., the absorbance of the samples from the two tubes was read at 520 nm in a spectrophotometer (Model WUV - M51, Weblaborsp, São Paulo, Brazil), using 1 cm quartz optical path cuvettes. The spectrophotometer calibrated with distilled water and the concentration of total anthocyanins (TA) was expressed according to the equation: TA = (A1-A2) x 875; where 875 is the molar extinction coefficient of malvidin to express the result directly in mg/L.

The total polyphenol index (TPI) was then assessed according to the methodology proposed by Rizzon (2010). The TPI was determined by diluting the extract, at a ratio of 1% for red cultivars and 5% for white cultivars, with distilled water using a 100 mL volumetric flask. The absorbance was determined on a spectrophotometer at 280 nm, using quartz cuvettes with an optical path of 1 cm, zeroing the apparatus with distilled water between samples. The polyphenol index was obtained using the equation: TPI = absorbance value x dilution factor. To estimate total polyphenols (TP), the corrected TPI values were submitted to a standard curve of gallic acid x absorbance (280 nm), following the equation: $y = 0.0364x + 0.0009$, $r = 0.9989$, with the TP value expressed in mg/L of gallic acid.

To determine total tannins (TT) in closed test tubes, 2 mL of the extract diluted 1:50, 1 mL of distilled water and 6 mL of HCL 12N were added to tubes A1 and A2. Tube A1, closed and protected from light, was placed in a water bath for 30 minutes. Tube A2 was kept at room temperature. After 30 minutes of boiling (100°C), tube A1 was removed. Thereafter, 1 mL of ethanol was added to tubes A1 and A2. The absorbance was then measured at 550 nm. The concentration of total tannins (TT) is given by the expression: TT = (A1 - A2) x 19.33, where 19.33 is the cyanidin molar extinction coefficient obtained by acid hydrolysis of the condensed tannins and corrected to express the result in g/L. A sample of 30 kg of grapes was harvested manually from each treatment. The microvinifications were carried out in triplicate for each cultivar in a commercial winery, according to the protocol adapted from Makhotkina *et al*. (2013) and Pszczolkowski (2014). Subsequently, the sensory analysis of the wines was conducted and the volatile compounds of the wines identified using a headspace solid-phase microextraction/gas chromatography with mass spectrometry detection (HS-SPME-GC-MS).

Sensory analysis

The sensory analysis of the wines was carried out in the Laboratory of the Center for Grape and Wine Studies at the Federal University of Santa Catarina (NEUVIN/UFSC), Florianópolis, Brazil. The sensory analysis was approved by the Human Research Ethics Committee (CEP/SD) of the Health Sciences Sector of the Federal University of Paraná (CAAE number 62582022.0.0000.0102) and by the Research Ethics Committee of the Federal University of Santa Catarina (CAAE number 62582022.0.3001.0121). All panellists signed the informed consent form, as determined by the Research Ethics Committee.

The experimental wines were evaluated using blind tasting by a team of 10 wine professionals (five women and five men). The evaluators were all considered wine professionals as defined by Ballester *et al*. (2008) (i.e., wine researchers, winemakers, wine consultants and wine students with past training in the sensory analysis of wines). The sessions lasted around one hour and 30 minutes, and six to 10 wine samples were analysed at the ideal serving temperature. Evaluators performed an assessment of the visual, olfactory and taste parameters of the wines. For sensory analysis, an adapted descriptive sheet for wines developed by the International Organization of Vine and Wine was used, and the scale was a structured numbered scale with scores ranging from excellent to inadequate (OIV, 2021).

For visual assessment, the criteria of limpidity (clarity) and general appearance were evaluated. For the olfactory component, intensity, genuineness and quality were assessed. For the taste characteristics, intensity, genuineness, quality and persistence were assessed. Finally, a score was given for the overall aspect of the evaluated sample. The sum of all the evaluated scores added to a maximum of 100 points for each wine. The results of the sensory analysis were interpreted on the averages of the evaluators' scores and were presented graphically on a scale from 0 to 10.

Wines were classified into various aroma groups according to the adapted methodology proposed by Sun *et al*. (2018). The aromas were categorised into classic wine descriptors: red fruits, black fruits, citric fruits, sweet fruits, floral, herbaceous, spicy, empyreumatic, chemical, earthy and animal. The aroma profile of each wine was calculated based on the intensity (I%) and frequency (F%) of detection, calculated from the modified frequency (MF%), using the formula: MF%= $\sqrt{(I(\%).F(\%))}$, where F(%) is the frequency of detection of an aroma group and I(%) is the relative intensity of the aroma according to the aroma intensity scores in the sensory analysis, expressed as a percentage. The MF values were obtained from the average of the wines of the 2021 and 2022 seasons.

Analysis of volatile compounds by gas chromatography (HS-SPME - GC/MS)

For the extraction of the volatile components of the samples, an already optimised methodology, previously described by Tao *et al*. (2008), was used. In a 15 mL vial containing a magnetic stirring bar, 7.5 mL of sample, 0.7500 ± 0.005 g of NaCl, and 2 uL of α-pinene solution, used as internal standard, were added. The vial was inserted into a container with a glass jacket which was placed on a magnetic stirring plate and connected to a thermostatic bath with water circulation (SOLAB SL 152, Piracicaba, SP, Brazil). The vial was kept in a water bath at 40 ± 0.2°C, and the contents were stirred for 5 min. The solid-phase microextraction (SPME) fibre was exposed to the headspace of the vial, which was kept at the same temperature for 30 minutes. SPME fibre composed of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) with a coating of 50/30 µm thick and 1 cm long (SUPELCO, Bellefonte, PA, USA) was used. The compound α-pinene was used as an internal standard because it is not a typical volatile compound found in wine. It showed a location-perfect ion peak different from the peaks found in the volatile compounds of wines.

The SPME fibre containing the adsorbed volatile components was manually inserted into the gas chromatography with mass spectrometry detection (GCMS) injector at 250°C (splitless mode, equipped with glass liner, 0.75 mm I.D.) and held for 5 min. The desorbed components were separated on an Agilent 7890A GCMS using a methodology adapted from Tao and Li (2009). An Agilent HP-5MS column (30 m x 0.25 mm x 0.25 µm) composed of dimethyl/diphenyl polysiloxane (95%/5%) was used with a helium gas flow rate of 1.0 mL/min. The oven temperature was maintained at 40°C for 5 min, followed by a heating ramp from 40°C to 260°C with a heating rate of 9°C/min. The interface and ion source temperatures were set at 300°C. Data were acquired in full-scan mode with a range of 30 m/z to 400 m/z. The mass spectrometer was operated with electron impact at 70 eV. The peaks were manually integrated into the G1701EA GC/MSD Chemstation software.

The volatile substances were characterised by comparing the mass spectrum and the experimental Kovats Index (KI) for each component with the respective mass spectra and theoretical Kovats Index of the standards described by Adams (2017). Experimental KI values were obtained by injecting a sample of saturated hydrocarbons (Sigma-Aldrich) under the same conditions used for the samples and calculated according to Van den Dool and Kratz (1963). Each sample was analysed in triplicate for all cultivars and the percentage of the volatile compounds was obtained based on the area of the specific compound compared to the total area of all the measured volatile compounds. Compounds not identified in the literature were identified by characterisation based on NIST software with over 90% similarity.

Experimental design and statistical data analysis

The experiment was laid out in a randomised complete block design, with five blocks and five vines per plot. Wines were evaluated in triplicate and means were subjected to analysis of variance (ANOVA) and then submitted to the F test ($p < 0.05$).

The evaluation of the technological and phenolic maturation and the analysis of the volatile compounds were subjected to ANOVA, and the averages were compared using the Scott-Knott test ($p < 0.05$), using the SISVAR program (Ferreira, 2019). The results of the sensory analysis were based on the averages of the evaluators' scores for each attribute, with the results presented graphically. Subsequently, multivariate analysis of the principal components (PCA) was performed using STATISTICA v10.0.

RESULTS AND DISCUSSION

Technological maturation and phenolic compounds

The cultivars exhibited significant differences in total soluble solids (TSS), total titratable acidity (TTA) and pH. The average TSS content across all cultivars was 19.14°Brix. 'Pinot Noir', 'Fiano' and 'Merlot' displayed the highest sugar content in the must, with values of 19.8°Brix, 19.6°Brix and 19.4°Brix, respectively (Table 1); followed by 'Cabernet Sauvignon' (18.8°Brix) and 'Viognier' (18.1°Brix). In comparison, Assefa *et al*. (2024), evaluating 'Pinot Noir' in Australia, reported TSS values ranging from 20.7°Brix to 22.4°Brix. Similarly, Somkuwar *et al*. (2024), studying 'Viognier' in India, observed an average TSS value of 23.32°Brix over two seasons. Rocha *et al*. (2022), analysing 'Merlot' in Brazil's most traditional wine region (Serra Gaúcha, Rio Grande do Sul), reported TSS values of 19.5°Brix over three seasons. Sugar content in grapes can vary depending on factors such as climatic conditions, cultivar, ripeness level and berry health. For the production of quality wines, the TSS should ideally exceed 18°Brix, with the recommended range being between 19°Brix and 25°Brix (Jackson, 2020). All cultivars in this study reached levels above 18°Brix, demonstrating suitable conditions for winemaking.

The average pH value across the cultivars was 3.13. The highest pH values were observed in 'Merlot' (3.20) and 'Viognier' (3.19), which did not differ statistically from each other (Table 1). In contrast, the lowest pH values were recorded in 'Pinot Noir' (3.05) and 'Fiano' (3.07). For white wine cultivars, must pH values should ideally remain below 3.4 to ensure quality winemaking (Jackson, 2020). For red wine cultivars, a pH range of 3.0 to 3.35 is considered optimal, reflecting good maturity levels and suitability for producing premium red wines (Gombau *et al*., 2020). All evaluated cultivars exhibited pH values within the recommended ranges, indicating appropriate grape maturity. For TTA, 'Merlot', 'Viognier' and 'Fiano' displayed the

TABLE 1
Mean values and coefficients of variation (%) for total soluble solids content (TSS), total titratable acidity (TTA), pH, total polyphenol index (TPI), total polyphenols (TP), total anthocyanin content (TA) and total tannins (TT) of 'Cabernet Sauvignon', 'Fiano', 'Merlot', 'Pinot Noir' and 'Viognier' cultivars in Campo Largo, Brazil.

Cultivar	TSS (°Brix)	TTA (g/L)	pH	TPI	TP (mg/L)	TA (mg/L)	TT (g/L)
Cabernet Sauvignon	18.8 b	9.01 b	3.12 b	51.81 a	1421.72 a	516.50 a	2.03 a
Fiano	19.6 a	8.18 c	3.07 c	6.52 d	178.57 d	nd	0.97 c
Merlot	19.4 a	7.40 c	3.20 a	46.75 b	1284.33 b	297.14b	1.51 b
Pinot Noir	19.8 a	9.48 a	3.05 c	35.78 c	982.14 c	193.57 c	1.05 c
Viognier	18.1 b	8.02 c	3.19 a	4.05 d	111.26 d	nd	0.26 d
CV (%)	3.82	4.82	2.00	9.98	9.88	1.84	20.48

Notes: The data is the average for the 2020/2021 and 2021/2022 harvests. Values followed by different letters in a column differ significantly (Scott-Knott test. $p < 0.05$). nd, not detected.

lowest average TTA values, with 7.40 g/L, 8.02 g/L and 8.18 g/L, respectively (Table 1). In contrast, 'Pinot Noir' had the highest TTA, at 9.48 g/L, which was statistically superior to the other cultivars.

In another study in the traditional Brazilian wine region (Serra Gaúcha, RS), the authors reported average values over three seasons for 'Merlot' as follows: TSS (19.5°Brix), TTA (5.18 g/L) and pH (3.4). For 'Cabernet Sauvignon', the averages were 17.9°Brix, 6.19 g/L TTA and pH 3.25 (Rocha *et al*., 2022). The mean TSS (19.4°Brix) of 'Merlot' in this study was comparable, although it exhibited a higher TTA value (7.40 g/L). For 'Cabernet Sauvignon', TSS (18.8°Brix) and TTA (9.01 g/L) were higher in Campo Largo, PR than in Serra Gaúcha. Both cultivars showed lower pH values in Campo Largo, yet these values remained within the desirable range for winemaking in both studies. These results suggest that the region of Campo Largo, Paraná exhibits promising technological maturation parameters for red cultivars, with adequate levels of TSS and greater TTA values, comparable to those of Serra Gaúcha, which is one of the most traditional and well-established regions for 'Merlot' and 'Cabernet Sauvignon' wine production in Brazil.

In other studies, Costa *et al*. (2015) in Portugal and Bindon *et al*. (2012) in Australia reported TTA values for 'Cabernet Sauvignon' of 6.7 g/L and 5.3 g/L, with corresponding pH values of 3.25 and 3.48, respectively. These values represent adequate levels of TTA and pH for winemaking in these globally recognised wine regions. However, a study conducted in Southern Brazil (Dois Vizinhos, Paraná state) during the 2008/2009 harvest found significantly lower TTA values for 'Cabernet Sauvignon' and 'Merlot'. at 3.69 g/L and 2.71 g/L, respectively. These levels were insufficient to ensure the desired stability for long-term wine storage (Penso *et al*., 2014). In contrast, the TTA values observed in this study for 'Merlot' (7.40 g/L) and 'Cabernet Sauvignon' (9.01 g/L) were considerably higher.

In general, suitable parameters for winemaking include a pH range of approximately 3.0 to 3.6, TTA between 4.5 and 9.0 g/L, and TSS levels above 18°Brix. Overall, the TSS levels observed in this study were lower than those reported in traditional wine regions worldwide. However, this aligns with a growing trend in wine consumption, where there is increasing demand for wines with lower alcohol levels (Silva, 2024). Achieving such wines requires lower TSS levels to produce the desired alcohol content. The total soluble solids (TSS) and pH values obtained from the cultivars in this study indicate that 'Cabernet Sauvignon', 'Fiano', 'Merlot', 'Pinot Noir' and 'Viognier' reached suitable maturity levels, making them adequate for winemaking. These results further confirm that the climate and soil conditions of Campo Largo, Southern Brazil favour the production of grapes with higher acidity (mostly exceeding 7.5 g/L). This characteristic is particularly advantageous for producing still wines and, especially, sparkling wines.

Regarding phenolic compounds, the highest total polyphenol (TP) values were found in the red cultivars, as expected (Table 1). 'Cabernet Sauvignon' exhibited the highest TP content (1 421 mg/L), followed by 'Merlot' (1 284 mg/L). The lowest TP values were observed in the white cultivars 'Fiano' (178 mg/L) and 'Viognier' (111 mg/L), which did not differ significantly from each other. Edaphoclimatic conditions and agronomic management practices significantly influence the phenolic composition of grapes, which in turn strongly affects sensory quality parameters of wines, such as colour intensity and stability, ageing potential, bitterness and astringency (Burin *et al*., 2010).

In a study conducted in the high-altitude plateau of Santa Catarina, Brazil, higher TP levels were reported for 'Merlot' and 'Cabernet Sauvignon' (ranging from 1 444 mg/L to 2 152 mg/L at 950 metres, and 3 246 mg/L to 3 579 mg/L at 1 400 meters, respectively) (Muniz *et al*., 2015). The TP values observed in this study were intermediate compared to those from higher-altitude regions, which is an expected result, as the growing cycles are longer and the conditions at higher altitudes (1 200 metres to 1 400 metres) are more favourable for the accumulation of polyphenols and anthocyanins (Muniz *et al*., 2015; Brighenti *et al*., 2017; Marcon Filho *et al*., 2019).

Regarding total anthocyanin content (TA), the highest value was in 'Cabernet Sauvignon' (516.50 mg/L), followed by 'Merlot' (297.14 mg/L) and 'Pinot Noir' (193.57 mg/L), with statistically significant differences among them (Table 1). The TA value for 'Pinot Noir' in Campo Largo

(193.57 mg/L) was higher than that reported in a study conducted in the Northeast region of Brazil, where TA levels were 100.36 mg/L (Souza Nascimento *et al*., 2016). The climatic conditions in the Northeast region, characterised by higher temperatures and lower altitudes, contrast with those of Southern Brazil, where cooler, high-altitude conditions favour the accumulation of higher concentrations of TA (Da Silva Padilha *et al*., 2016).

Fanzone *et al*. (2011), studying red cultivars in Argentina, reported TA values of 681.83 mg/L and 644.13 mg/L for 'Cabernet Sauvignon' and 'Merlot', respectively. These values were higher than those observed in Campo Largo, where TA levels were 516.50 mg/L for 'Cabernet Sauvignon' and 297.14 mg/L for 'Merlot'. Similarly, the TA levels in this study were lower than those reported by Marcon Filho *et al*. (2019) for different clones of 'Cabernet Sauvignon' grown in São Joaquim (Santa Catarina plateau, Southern Brazil), where TA values ranged from 835 mg/L to 2 065 mg/L over four seasons. The lower TA values observed in Campo Largo are likely due to the shorter growing cycle compared to São Joaquim (1 300 metre altitude), where cooler temperatures extend the vine's developmental period. These results suggest that the red wines produced in Campo Largo, Brazil may have shorter storage potential than those from high-altitude regions such as Santa Catarina, Brazil and Argentina. 'Cabernet Sauvignon' in Campo Largo presented distinct characteristics. This wine was noted for its lower tannin astringency, which contrasts with the typically high astringency of young 'Cabernet Sauvignon' wines from Southern Brazil (Marcon Filho *et al*., 2019; Miele, 2021). The synthesis and accumulation of phenolic compounds are heavily influenced by environmental factors, including light, temperature, altitude, soil type, water availability, nutritional status, and other developmental processes (Downey *et al*., 2006; Dai *et al*., 2011).

Tannins and anthocyanins are among the most important polyphenols in wine, contributing to sensory characteristics such as astringency and colour, as well as influencing the wine's longevity and evolution. Tannins interact with proteins and other polymers, such as polysaccharides, leading to the sensation of astringency (Kuhlman *et al*., 2024). In terms of total tannins (TT) extracted from the skins, 'Cabernet Sauvignon' exhibited the highest concentration (2.03 g/L), followed by 'Merlot' (1.51 g/L). 'Pinot Noir' and 'Fiano' showed similar TT levels, while 'Viognier' had the lowest concentration (0.26 g/L). The TT and TPI values for 'Merlot' (1.51 g/L and 46.7, respectively) in Campo Largo were comparable to those reported by Miele (2021) in a traditional wine region of Brazil (Serra Gaúcha, RS), with values of 1.62 g/L and 47.9, respectively. These results suggest a good adaptation of the 'Merlot' cultivar in Campo Largo, as 'Merlot' in Rio Grande do Sul state (RS) is widely recognised for its adaptation and quality. Rio Grande do Sul is the leading producer of 'Merlot' grapes and wines in Brazil.

Wine volatile compounds

Twenty-six volatile compounds were identified in the studied wines. These compounds, along with their corresponding aroma descriptors as documented in the literature, are presented in Table 2. Isoamyl alcohol was the most abundant compound across all evaluated wines, with its concentration being statistically higher in red wines, accounting for over 40% of the total chromatogram area. This compound is a higher alcohol, associated with fruity aromas, and was also found in significant proportions in the white wines analysed. These findings align with a study conducted by Lu *et al*. (2022) on 'Cabernet Sauvignon' wines in China, where isoamyl alcohol was identified as the most prevalent higher alcohol among the 12 types detected. In wines, higher alcohols play a crucial role in enhancing aromatic complexity and mouthfeel. Higher alcohols are produced through the catabolism of amino acids via the Ehrlich pathway, which directly or indirectly influences the synthesis of aromatic compounds. Higher alcohols furthermore serve as precursors for esters, which are key contributors to wine aroma (Styger *et al*., 2011).

In the analysis of 'Merlot' wines, isoamyl alcohol (47.93%) and isoamyl acetate (5.58%) were associated with fruity notes, particularly red and black fruits. Phenylethyl alcohol was present in higher concentrations in 'Merlot' wines (18.66%) compared to other cultivars, and was statistically more abundant than in white wines ('Fiano' and 'Viognier'). This compound is directly linked to floral aromas. Hexanol, associated with herbaceous aromas, was found in lower quantities in 'Merlot' compared to 'Cabernet Sauvignon', 'Pinot Noir' and 'Viognier'. Diethyl succinate (4.44%) was significantly higher in 'Merlot' wines and is primarily associated with pleasant grape and wine aromas, as well as earthy notes. This compound is characteristic of malolactic fermentation in young wines, but its concentration also increases during storage and ageing (Cortés-Diéguez et al., 2015).

For 'Cabernet Sauvignon', volatile compounds similar to those found in 'Merlot' wines include isoamyl alcohol (42.58%), isoamyl acetate (8.62%), ethyl hexanoate (9.84%), and phenylethyl alcohol (13.35%), contributing primarily to fruity (red and black fruits), floral and spicy aromas. Notably, hexanol (7.23%) was found in significantly higher concentrations in 'Cabernet Sauvignon' than in 'Merlot' and 'Fiano' wines. Simpson (1979) suggests that hexanol levels depend largely on grape variety, with minimal influence from fermentation conditions. Descriptors for hexanol include 'grassy', 'herbaceous', 'woody', 'green' and 'bitter' (Moyano *et al*., 2002). In addition, 2-phenyl ethyl acetate (2.04%) was also statistically higher in 'Cabernet Sauvignon', contributing to floral and sweet aromas.

For 'Pinot Noir', ethyl hexanoate was present at 14.33%, a higher concentration than in the other red varieties. Esters play a crucial role in the aromatic complexity of wines, with over 160 identified compounds contributing primarily to honey, fruity and floral notes (Noguerol-Pato *et al*., 2009; Chen *et al*., 2013; Gambetta *et al*., 2014; Pereira *et al*., 2014). Other significant compounds in 'Pinot Noir' include isoamyl acetate (8.45%) and isoamyl alcohol (41.17%), which are associated with red and black fruit aromas, as well as hexanol (6.22%), which contributes to herbaceous notes. Ethyl butanoate (1.00%) was found in higher concentrations in 'Pinot Noir' compared to other cultivars, adding nuances of red fruit. In addition, 2-ethyl hexanol was more prevalent

TABLE 2
Volatile compounds (%) in 'Cabernet Sauvignon', 'Fiano', 'Merlot', 'Pinot Noir' and 'Viognier' wines from Campo Largo, Paraná, Brazil.

Compound	Cabernet Sauvignon	Fiano	Merlot	Pinot Noir	Viognier	p-value	Aroma descriptor
Isoamyl alcohol	42.58 a	34.50 b	47.93 a	41.17 a	32.87 b	0.001	Floral, honey, fruit [a]
Ethyl butanoate	0.46 b	0.48 b	0.52 b	1.00 a	0.89 a	< 0.001	Banana, pineapple, strawberry [b]
Ethyl lactate	0.64 b	1.61 a	1.10 a	0.30 b	0.20 b	< 0.001	Solvent, acetone [s]
4-methyl pentanol	0.12 ns	0.19	0.15	0.10	0.12	0.164	No descriptor [d]
Ethyl-2-methylbutanoate	0.12 b	0.95 a	0.24 b	0.10 b	0.13 b	< 0.001	Apple, strawberry, candy [e, c]
Ethyl isovalerate	0.39 b	0.94 a	0.43 b	0.18 c	0.41 b	< 0.001	Fruit, apple [e]
Hexanol	7.23 a	5.12 b	4.33 b	6.22 a	7.34 a	< 0.001	Flower, cut grass, resin, herbaceous, pepper [b, s]
Isoamyl acetate	8.62 a	1.52 b	5.58 a	8.45 a	5.58 a	0.012	Banana, pineapple, strawberry [l]
2-Methyl butyl acetate	1.92 a	0.39 b	1.48 a	1.07 a	1.02 a	0.026	Pineapple, banana, fruit [f, g]
Ethyl hexanoate	9.84 b	9.67 b	6.77 b	14.33 a	17.90 a	< 0.001	Fruit, green apple, strawberry, spices [h, i]
Hexyl acetate	0.45 b	0.07 b	0.16 b	1.15 a	1.29 a	0.025	Apple, pear, fruit, cherry [i, j, l]
2-Ethyl hexanol	0.72 b	0.59 b	0.60 b	1.32 a	0.60 b	< 0.001	Citrus, herbaceous [t]
Ethyl-2-hexanoate	0.01 ns	0.17	0.14	0.15	0.25	0.260	Pepper, sweet, earth [s]
2-Nonanone	0.07 b	0.57 a	0.07 b	0.08 b	0.43 a	< 0.001	Herbaceous, fruit [r]
Linalool	0.02 ns	0.03	0.02	0.01	0.09	0.132	Floral, lavender [e, q]
Nonanal	0.15 ns	0.13	0.14	0.12	0.24	0.380	Citrus, herbaceous [t]
Phenylethyl alcohol	13.35 a	17.32 a	18.66a	9.68 b	6.68 b	0.013	Floral, rose flowers, perfume [b, n]
Diethyl succinate	2.53 b	2.78 b	4.44 a	2.53 b	3.69 b	< 0.001	Wine, fruit, caramel, earthy [t, i]
Ethyl-octanoate	0.32 b	7.49 a	0.28 b	0.50 b	6.02 a	0.002	Melon, wood [s]
Citronellol	4.55 b	3.18 b	4.79 b	4.84 b	13.57 a	0.004	Clove, spice, lime [p, q]
Ethyl-2-phenylacetate	0.15 b	6.99 a	0.12 b	0.48 b	0.27 b	0.001	Fruit, pineapple [p]
2-Phenyl ethyl acetate	2.04 a	0.32 b	0.55 b	0.68 b	0.75 b	0.046	Floral, sweet [o]
4-Ethylguaiacol	0.20 b	0.61 a	0.35 a	0.42 a	0.12 b	0.012	Animal, defect [u]
Caprylic acid	0.20 ns	0.45	0.14	0.39	0.68	0.116	Animal, solvent [n]
β-Damascenone	0.35 b	0.58 a	0.28 b	0.38 b	0.72 a	0.024	Floral, apple pie, honey [l, m]
Ethyl decanoate	0.40 ns	0.57	0.39	0.35	0.49	0.667	Fruit, grapes [v]

Note: The percentage is obtained based on the area of the specific compound compared to the total area of all the volatile compounds identified. The values are the means of the 2020/2021 and 2021/2022 seasons. Values followed by different letters in the same row differ significantly (Scott-Knott test; $p < 0.05$). a Carpena *et al*. (2020), b Wu *et al*. (2019), c Molina *et al*. (2009), d Nan *et al*. (2021), e Pereira *et al*. (2014), f Jiang & Sun (2019), g Yu *et al*. (2019), h Chen *et al*. (2013), i Gambetta *et al*. (2014), j Noguerol-Pato *et al*. (2009), l Peng *et al*. (2013), m Escudero *et al*. (2007), n Feng *et al*. (2017), o Zhao *et al*. (2020), p Lu *et al*. (2022), q Tao *et al*. (2008), r Li *et al*. (2008), s Mayr *et al*. (2014), t Wang *et al*. (2016), u Milheiro *et al*. (2019), v Welke *et al*. (2014).

in 'Pinot Noir', further enhancing its herbaceous flavour profile. These analytical results for the red varieties corroborate the findings from subsequent sensory analyses.
'Fiano' wines exhibited a high concentration of isoamyl alcohol (34.5%), although less than in red varieties. Ethyl lactate (1.61%) was more pronounced in 'Fiano', associated with chemical notes (petrol, solvent and acetone), which were noted in sensory evaluations. 'Fiano' also had elevated levels of ethyl-2-methylbutanoate (0.95%), linked to apple and sweet aromas, and ethyl isovalerate (0.94%), which further enhanced fruity notes, particularly that of apples. Phenylethyl alcohol (17.32%) was more concentrated in 'Fiano' than in 'Viognier' and 'Pinot Noir', adding floral notes. Ethyl octanoate was significantly higher in 'Fiano' (7.49%), relating to sweet fruit (melon) aroma.

Terpenes and norisoprenoids play a crucial role due to

 DOI: https://doi.org/10.21548/46-2-7285

their low sensory thresholds. Key terpenes in wines include linalool, α-terpineol, nerol, geraniol and citronellol, while significant norisoprenoids are β-damascenone and β-ionone (Ribéreau-Gayon *et al*., 2006). In 'Fiano', β-damascenone constitutes 0.58% of volatile compounds, evoking aromas of florals, ripe fruit, apple pie and honey. This compound was also highlighted in Italian studies comparing 'Aglianico' and 'Fiano' wines, where it was one of the principal terpenes identified (Calabretti *et al*., 2012). Norisoprenoids are vital for their contribution to fruity and floral notes in wine (Alem *et al*., 2019; Marín-San Román *et al*., 2022).

Regarding the 'Viognier' cultivar, one compound that notably distinguished it from others was the terpene citronellol. In wines from other cultivars, this compound accounted for less than 5% of the volatile profile, whereas in 'Viognier' it reached 13.57%, representing a statistically significant difference. Citronellol, a secondary metabolite alcohol, is biosynthesised from acetyl-coenzyme A (Morales *et al*., 2017; Çelebi Uzkuç *et al*., 2020). This monoterpene, primarily influenced by berry and peel composition, imparts pleasant lemon and citrus aromas. The alcohols present in the greatest amounts in 'Viognier' wines were hexanol (7.34%) and isoamyl alcohol (32.87%). Hexyl acetate (1.29%) was found in higher quantities in 'Viognier' and 'Pinot Noir,' contributing to fruity notes. Ethyl hexanoate (17.9%) was significantly more prevalent in 'Viognier' compared to 'Cabernet Sauvignon' (9.84%), 'Fiano' (9.67%) and 'Merlot' (6.77%), adding fruity (green apple) and spicy aromas. These findings in the white wine samples align with the subsequent sensory analysis.

Wine sensory analysis and principal component analysis (PCA)

Regarding the sensory analysis of the wines, 'Merlot' predominantly exhibited fruity aromas, complemented by earthy/animal notes and empyreumatic undertones. The 2022 'Merlot' sample showed superior clarity (8.9) and visual quality (8.9) compared to the 2021 vintage, although both vintages shared similar olfactory and taste profiles (Fig. 2.A). 'Cabernet Sauvignon' wines were characterised by red fruit, floral and herbaceous notes. The 2022 vintage received the highest overall rating (8.8) among all the evaluated wines (Fig. 2.B). The 2021 vintage, however, showed reduced sensory quality with more herbaceous notes. For 'Pinot Noir', key aromas included red fruits and floral notes with spices, and empyreumatic undertones. The 2021 vintage exhibited comparable characteristics, with lower olfactory and taste quality compared to the 2022 vintage, although the visual aspect remained the same in both vintages (Fig. 2.C). For white cultivars, 'Viognier' presented a predominantly fruity aroma profile, with citric and sweet fruit notes, floral hints and herbaceous undertones. The overall score of the 2022 and 2021 vintages was 8.4 and 8.2, respectively (Fig. 3.A). 'Viognier' wines from both vintages showed comparable aroma profiles, with slightly superior results in 2022. In contrast, 'Fiano' displayed aromas of sweet fruit, herbaceous and chemical notes, with citrus fruit undertones (Fig. 3B). The 2022 vintage of 'Fiano' achieved an overall score of 7.7, whereas the 2021 vintage, which scored lower in olfactory and gustatory attributes, recorded the lowest overall score, of 7.1. In white wines, ester-derived aromas, which contribute to fruity and floral notes, typically diminish over time due to natural chemical or enzymatic hydrolysis, a process accelerated by elevated temperatures. As wines

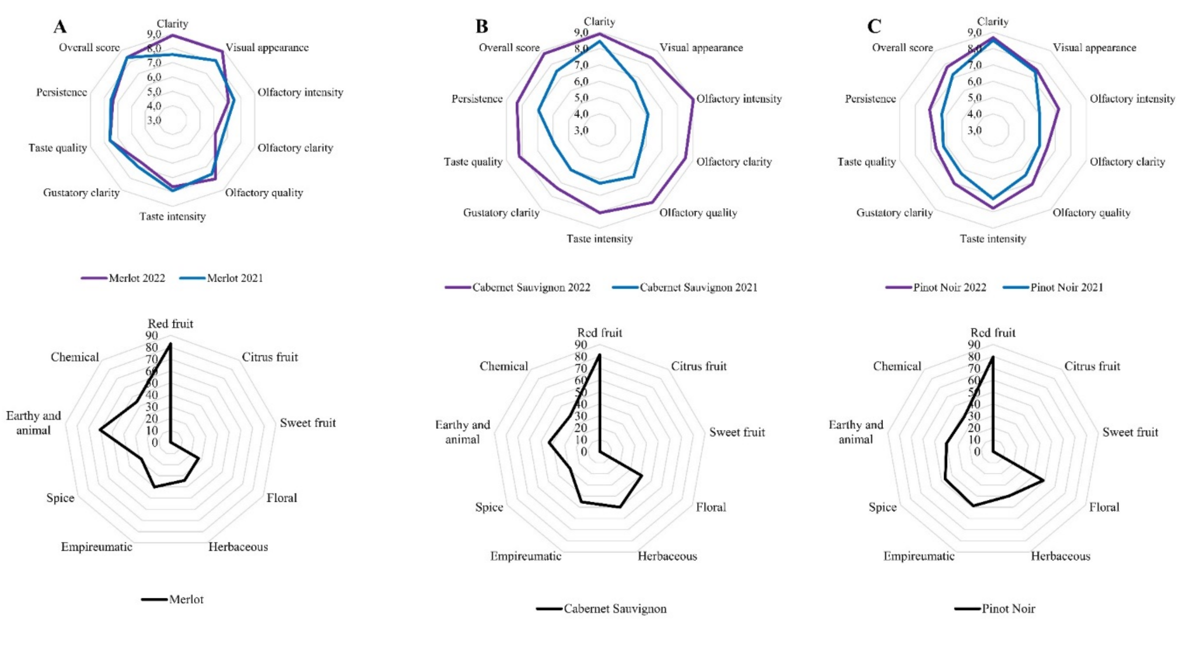

FIGURE 2
Sensory analysis and average aromatic profile of (A) 'Merlot', (B) 'Cabernet Sauvignon' and (C) 'Pinot Noir' wines in Campo Largo, Paraná, Brazil.

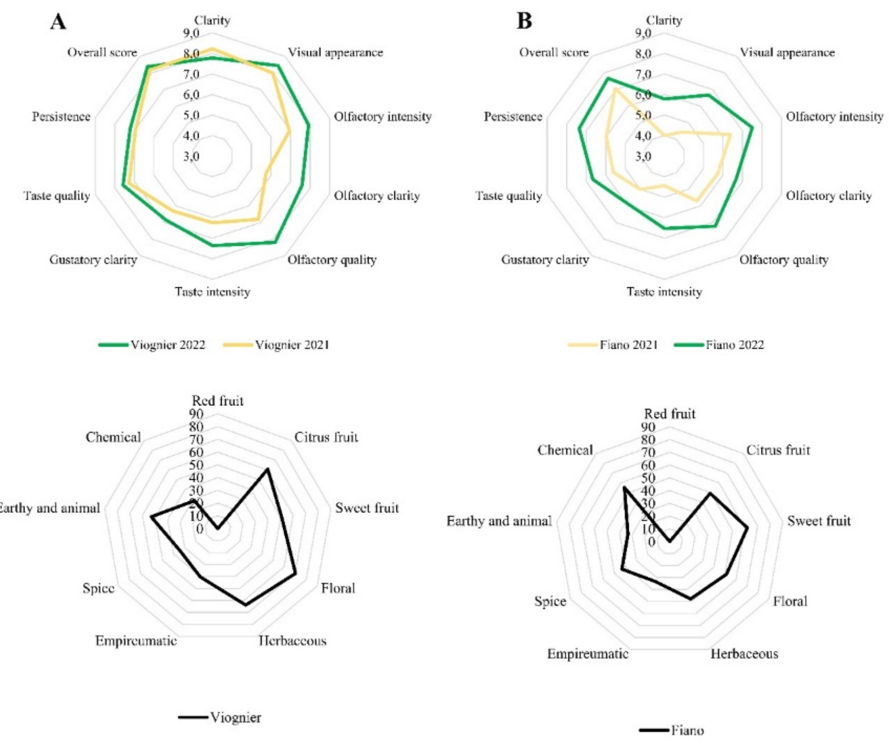

FIGURE 3
Sensory analysis and average aromatic profile of (A) 'Viognier' and (B) 'Fiano' wines in Campo Largo, Paraná, Brazil.

age, certain esters may become nearly undetectable in the olfactory profile (Peynaud & Blouin, 2010).

In the principal component analysis (PCA) of the volatile compounds present in the wines, the two main components explained 82.87% of the variability in the data (Fig. 4). The first component (PC1) was primarily represented by diethyl succinate, ethyl-2-methylbutanoate, ethyl isovalerate, isoamyl acetate and 2-methyl butyl acetate, accounting for 46.35% of the variation in the data. The second component (PC2) was associated with ethyl hexanoate, citronellol, ethyl 2-hexanoate and linalool, explaining 36.52% of the variation. Upon analysing the PCA, red cultivars were grouped on the right side of the X-axis, reflecting their shared high levels of isoamyl alcohol and isoamyl acetate. These compounds are known for contributing to fruity aromas, particularly red fruit notes, which are commonly identified in the sensory profiles of red wines. 'Fiano' was characterised by compounds such as ethyl lactate and ethyl-2-phenylacetate, which are associated with sweet fruit and chemical aromas in these samples. 'Viognier' stood out due to its strong association with citronellol and ethyl hexanoate, both of which are descriptors of fruit aromas, mainly citrus and sweet fruit aromas – the hallmark characteristics of 'Viognier' wines in this study.

CONCLUSIONS

The cultivars 'Cabernet Sauvignon', 'Fiano', 'Merlot', 'Pinot Noir' and 'Viognier' achieved adequate technological and phenolic maturity, demonstrating their suitability for wine production under the conditions of this study. Sensory analysis revealed that the red wines predominantly exhibited profiles characterised by red fruit, earthy/animal and floral notes. Key volatile compounds, such as isoamyl alcohol, phenylethyl alcohol, diethyl succinate and isoamyl acetate, contributed to these aromas. In contrast, the white wines were distinguished by floral, citrus and sweet fruit profiles, primarily driven by compounds such as citronellol, β-damascenone, ethyl-2-phenylacetate and ethyl octanoate.
The alignment between sensory analysis and gas chromatography data enhances our understanding of the wines produced in this terroir of Southern Brazil. These results highlight the potential of this region under subtropical conditions as a promising wine region capable of yielding quality wines with distinct characteristics.

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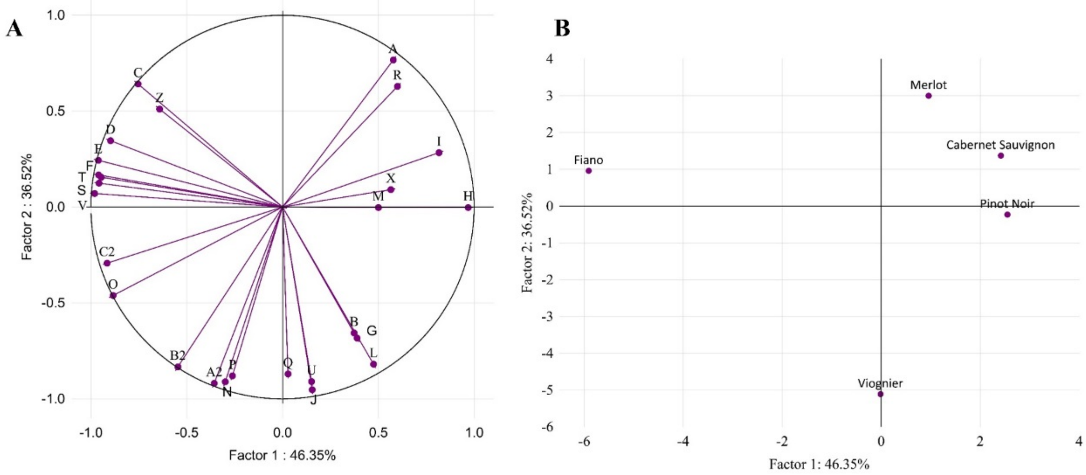

FIGURE 4

Principal component analysis (PCA), variables (A) and factors (B) of the volatile compounds isoamyl alcohol (A), ethyl butanoate (B), ethyl lactate (C), 4-methyl-pentanol (D), ethyl-2- methylbutanoate (E), ethyl isovalerate (F), hexanol (G), isoamyl acetate (H), 2-methyl butyl acetate (I), ethyl hexanoate (J), hexyl acetate (L), 2-ethyl hexanol (M), ethyl 2- hexanoate (N), 2-nonanone (O), linalool (P), nonanal (Q), phenylethyl alcohol (R), diethyl succinate (S), ethyl octanoate (T), citronellol (U), ethyl-2-phenylacetate (V), 2-phenyl ethyl acetate (X), p-ethylguaiacol (Z), caprylic acid (A2), beta-damascenone (B2) and ethyl decanoate (C2) from 'Cabernet Sauvignon', 'Fiano', 'Merlot', 'Pinot Noir' and 'Viognier' wines, in the 2020/2021 and 2021/2022 seasons in Campo Largo, Paraná, Brazil.

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