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Influence of precipitation on the phenolic and isotopic composition of *Vitis Vinifera* red wines

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Abstract. This study investigates how precipitation from November to February during each harvest year, influence the phenolic and isotopic profiles of red wines, particularly focusing on trans-resveratrol, total phenolic compounds, and carbon and oxygen isotopes ($^{13}C/^{12}C$ and $^{18}O/^{16}O$). Wines from Merlot, Cabernet Sauvignon and Cabernet Franc grapes, grown in Rio Grande do Sul (Brazil) and produced between 2020 and 2023 on a laboratory scale, were analyzed. Phenolic compounds, important for wine quality and human health, were quantified using spectrophotometry, with resveratrol levels assessed via high-performance liquid chromatography (HPLC). Isotopic compositions were measured using isotope ratio mass spectrometry (IRMS). Among the varieties, Cabernet Franc showed the strongest correlations: its resveratrol, total phenolic content and 18O/16O were closely linked to January precipitation, while a strong relationship between 13C/12C and resveratrol was also observed. In contrast, Merlot and Cabernet Sauvignon showed minimal climatic influence on these variables. These findings highlight Cabernet Franc's sensitivity to the accumulated precipitation in January, close to its harvest, and its potential as a marker for environmental influences. Future research will incorporate 2024 data and expand to other regions of Rio Grande do Sul.

1. Introduction

The introduction of grapevines in Brazil occurred across several states and regions, including São Paulo, Minas Gerais, Paraná, Pernambuco, the São Francisco Valley, Rio de Janeiro, Santa Catarina, and Rio Grande do Sul. However, the development and consolidation of viticulture in Brazil were significantly shaped by the arrival of Italian immigrants in the late 19th century. These settlers established themselves in the area now known as the Serra Gaúcha, laying the foundation for what would become the country's most prominent wine-producing region [1].

The growing demand for Brazilian wine has driven significant economic development, leading to job creation, the emergence of new businesses, and enhanced regional competitiveness. This expansion has contributed notably to the country's overall economic growth, particularly in key vitivinicultural areas [2].

According to the Brazilian Ministry of Agriculture and Livestock, the state of Rio Grande do Sul—located in southern Brazil—accounts for the largest area under grapevine cultivation, followed by the northeastern states of Pernambuco and Bahia [3]. As a result, Rio Grande do Sul is widely recognized as Brazil's leading vitivinicultural region with the production of 664.9 1000 tonnes in 2023 vintage, according to data from the Sistema de Cadastro Vinícola - Secretaria Estadual da Agricultura, Pecuária, Produção Sustentável e Irrigação (SISDEVIN/SDA)[4].

Wine is composed of a complex mixture of alcohols, sugars, acids, minerals, proteins, and a wide range of compounds, including organic acids, volatile compounds, and polyphenols. Among these constituents, polyphenols are particularly significant due to their strong influence on wine quality—contributing to attributes such as color, flavor, and mouthfeel. In addition to their sensory impact, polyphenols are associated with various health benefits, notably antioxidant and cardioprotective properties [5].

The phenolic compounds present in wine have generated great scientific interest, as studies confirm that moderate consumption of the beverage brings health benefits. This is due to the presence of substances that can help in the prevention of cardiovascular diseases, with anti-inflammatory and anticancer effects, in addition to contributing to increased longevity. Wine stands out as one of the main sources of these bioactive compounds. Among them are stilbenes, a class of non-flavonoid phenolic compounds. Trans-resveratrol (3, 4′, 5-trihydroxy-stilbene) is the most widely researched stilbene, primarily recognized for its antioxidant properties [6].

The combined analysis of isotopes and phenolic compounds provides a powerful tool for assessing terroir variability, wine authenticity, and quality. Isotope ratios not only reflect environmental and physiological factors but also correlate with key phenolic compounds that influence wine's sensory and antioxidant properties [7, 8]. Certain phenolic compounds serve as a varietal or regional marker, and their presence is associated with isotopic signatures, supporting the use of isotopic analysis in wine authenticity studies [7]. The carbon and oxygen isotopes are strongly influenced by environmental factors, such as precipitation, soil, temperature, altitude, longitude, and also vineyard soil properties, vine vigor, leaf area. These factors, in turn, affect grape composition, including phenolic content [9, 8].

The productivity and quality of grapes and wine are influenced by the maturation process of the grapes, which is closely linked to climate fluctuations. Being extreme events more frequent, they can affect aspects such as berry size, skin thickness, and the formation of important compounds like phenolics [10].

In this sense, the aim of this study is to determine the levels of trans-resveratrol, total phenolic compounds, $^{13}C/^{12}C$ and $^{18}O/^{16}O$ in red wines from the Merlot, Cabernet Sauvignon, and Cabernet Franc varieties, produced on a laboratory scale (2020 to 2023 harvests) elaborated with grapes from the Rio Grande do Sul region, assessing also the influence of precipitation.

2. Material and methods

2.1. Samples

Merlot (n=20), Cabernet Sauvignon (n=14), and Cabernet Franc (n=4) wines, produced on a laboratory scale through microvinification, from the 2020 to the 2023 harvest, were analysed, totalling 77 samples. The grapes were collected by agricultural inspectors of the Secretary of Agriculture of the State of Rio Grande do Sul (SEAPI) in each harvest, between January and February. These samples have their origins in the most important localities in Rio Grande do Sul. The climate is subtropical, the state is relatively humid, with precipitation distributed throughout the year, but with a higher concentration of rainfall during the summer months (December to March), due to the influence of cold fronts and instability systems.

2.2. Analysis

The analysis were carried out at the Oenological Reference Laboratory (LAREN/SEAPI) of the State Secretariat of Agriculture of Rio Grande do Sul, located in Caxias do Sul, Brazil.

2.2.1. Total phenolic compounds

The total phenolic compounds were analyzed by direct absorbance measurement at 280 nm using a Prove 600 Spectroquant spectrophotometer (Merck Millipore), following the methodology described by [11].

2.2.2. Resveratrol

The trans-resveratrol content was determined by High Performance Liquid Chromatography (HPLC), with diode array detector, following [12].

2.2.3. Carbon

The determination of the carbon isotope ratio ($^{13}C/^{12}C$) in ethanol was carried out according to the international method OIV-MA-AS312-06 [13]. Ethanol was extracted from the samples by cryogenic distillation, following the protocol described by [18]. The ethanol extracted (1μL) was analysed online using an elemental analyser (Flash EA 1112, Thermo Scientific, Bremen, Germany) equipped with an autosampler (AS 1310) and coupled to a Delta V advantage isotope ratio mass spectrometer. The results were calibrated on the V-PDB scale (Vienna Pee Dee Belemnite) in per mil (‰) against the international reference material Sucrose (NIST 8542, value of -10.45 ‰) and beet powder (value of -26.39 ‰) and expressed in $\delta^{13}C$ (delta per mil, ‰).

2.2.4. Oxygen

The oxygen isotopic ratio ($^{18}O/^{16}O$) was determined using a Delta V Advantage isotope ratio mass spectrometer coupled to a Gasbench II gas chromatograph (both from Thermo Scientific, Bremen, Germany). The measurement was based on the equilibrium established through isotopic exchange between CO_2 from the primary standard and the sample according to the method OIV-MA-AS2-12 [13]. The results were expressed in $\delta^{18}O$ (delta per mil, ‰), relative to the international standard VSMOW2.

2.3. Statistical analysis

The Pearson linear correlation coefficient was used to estimate the linear relationship between variables. It ranges from -1 to 1, where a value of 1 indicates a perfect and direct linear correlation, and a value of -1 indicates a perfect and inverse linear correlation. In this study, strong linear correlations were considered for absolute values of 0.8 or higher. For the statistical analysis, the Cabernet Franc variety has n=4 (one per year), while Cabernet Sauvignon has n=14 (at least 3 per year), and Merlot has n=20 (at least 4 per year).

3. Results and discussion

The levels of trans-resveratrol, TPC, $^{18}O/^{16}O$ and $^{13}C/^{12}C$ were analyzed and discussed according to grape variety and correlated with the amount of rainfall in December and January, the months preceding the grape harvest. These findings are presented in the following tables (Table 1, Table 2 and Table 3).

Table 1. Levels of trans-resveratrol, TPC, $^{18}O/^{16}O$ and $^{13}C/^{12}C$ in Cabernet Franc samples.

Year of Harvest	trans-Resveratrol level (mg/L)	Total Phenolic Content (mg/g)	$^{18}O/^{16}O$	$^{13}C/^{12}C$
2020	4.21	250	2.61	-27.95
2021	4.13	210	-1.80	-27.24
2022	0.89	260	3.61	-26.66
2023	1.80	310	1.62	-26.80

For resveratrol, it was identified that the Cabernet Franc variety has the highest linear correlation with the total precipitation in January before harvest (r = 0.84). The Merlot (r = 0.62) and Cabernet Sauvignon (r = 0.83) varieties show the greatest linear dependence of resveratrol related to the rainfall in December. Resveratrol is produced by plants as a defense mechanism in response to adverse conditions like mechanical injury, bacterial or fungal infections, and ultraviolet radiation. Its presence is influenced by various factors, including the grape variety, environmental conditions (such as climate, soil, and location), and winemaking techniques [14].

Only for the Cabernet Franc variety was it possible to identify a significant linear correlation between total phenolic compounds and total precipitation in the month of January (r = -0.84).. Polyphenols constitute key compounds in wine. They significantly influence its sensory characteristics, most notably astringency, coloration, and bitterness, and also contribute to its capacity for aging [16]. This result is in line with the study reported by [15], in which phenolic compounds showed a strong positive correlation with January precipitation in wines from the *Vitis labrusca* species

In the case of this study, the storage influence on the resveratrol and TPC contents can also be considered. Studies investigating the degradation of trans-resveratrol during the storage and aging of red wines suggest that freshly bottled wines may not exhibit the same rate of resveratrol loss as aged wines, likely due to variations in residual enzymatic activity over time [17].

It was identified that the Cabernet Franc variety has the highest linear correlation of Oxygen related to the total precipitation in January before harvest (r = -0.86) (Figure 1). Similarly, the Merlot variety shows the strongest relationship in January (r = -0.73). Meanwhile, the Cabernet Sauvignon variety has the highest linear correlation of Oxygen related to the total precipitation in December, before harvest.

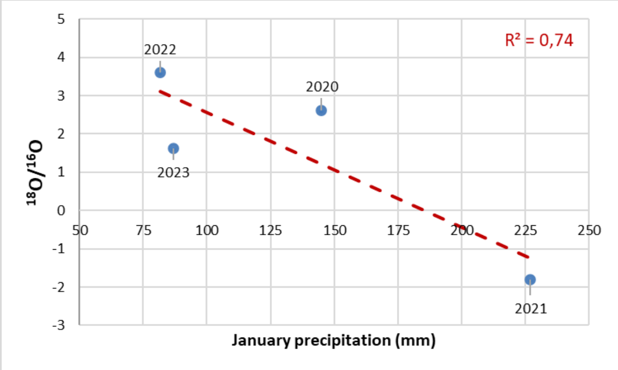

Figure 1. Cabernet Franc $^{18}O/^{16}O$ as a function of the January precipitation with linear fit (R^2 = 0.74).

Several factors, including precipitation, temperature, and soil composition, can influence the phenolic and isotopic profile of wines. Each harvest is marked by distinct characteristics shaped by environmental conditions, while each cultivar exhibits unique behaviors based on its physiological traits.

Table 2. Levels of trans-resveratrol, TPC, $^{18}O/^{16}O$ and $^{13}C/^{12}C$ in Cabernet Sauvignon samples.

Year of Harvest	Trans-Resveratrol level (mg/L)	Total Phenolic Content (mg/g)	$^{18}O/^{16}O$	$^{13}C/^{12}C$
2020	0.85	201	1.06	-28.2
2020	0.86	325	3.74	-28.3
2020	0.00	200	1.70	-28.2
2021	2.67	202	-0.35	-27.6
2021	2.33	300	0.02	-26.9
2021	1.89	197	-2.08	-27.3
2021	2.15	201	1.29	-29.1
2022	1.03	288	3.26	-28.4
2022	1.11	195	3.48	-27.0
2022	0.96	209	2.59	-26.9
2023	4.50	310	0.21	-26.6
2023	2.40	313	0.25	-27.8
2023	2.79	237	-1.45	-27.3
2023	2.05	369	-1.90	-27.6

Table 3. Levels of trans-resveratrol, TPC, $^{18}O/^{16}O$ and $^{13}C/^{12}C$ in Merlot samples.

Year of Harvest	Trans-Resveratrol level (mg/L)	Total Phenolic Content (mg/g)	$^{18}O/^{16}O$	$^{13}C/^{12}C$
2020	0.00	346	2.38	-27.0
2020	1.36	295	2.57	-27.6
2020	6.50	254	2.59	-27.1
2020	2.65	278	2.34	-26.9
2020	5.41	200	2.45	-27.9
2020	1.16	242	1.10	-26.6
2021	4.19	223	0.01	-27.7
2021	3.92	203	-3.16	-27.2
2021	1.73	217	-2.03	-28.0
2021	2.66	214	-1.87	-28.0
2022	0.91	176	3.39	-26.9
2022	0.98	255	2.91	-26.5
2022	1.17	195	4.73	-25.1
2022	1.42	257	3.75	-27.0
2022	1.89	297	3.06	-27.8
2023	7.41	311	4.88	-26.4
2023	7.64	262	5.4,	-27.0
2023	7.47	242	1.22	-28.6
2023	15.11	263	2.39	-27.4
2023	5.88	242	-1.76	-27.9

It can be observed that, except for the 2023 vintage, the month of January generally shows higher precipitation levels compared to December (Table 4). The months of November and February were also analyzed, however, they showed no relationship with the compounds studied in this work.

Table 4. December and January precipitation levels from 2020 to 2023 vintage.

Year of Harvest	December precipitation (mm)	January precipitation (mm)
2020	37.0	145
2021	125.7	227
2022	31.2	81.6
2023	135	86.8

The $\delta^{13}C$ values ranged from -29.58 to -25.03 ‰, and no significant correlation was observed between the carbon isotopic composition and precipitation levels. These results are consistent with those previously reported by other authors for wines [9,15]. Additionally, studies conducted with grapes from the same region have shown similar $\delta^{13}C$ ranges [18]. This lack of correlation may be explained by the fact that $\delta^{13}C$ values are primarily influenced by plant physiological responses, such as photosynthetic activity and the fractionation process occurring during CO_2 uptake and assimilation on leaf photosynthesis [19].

It is worth noting that the precipitation data are from the Bento Gonçalves Meteorological Station. That is, we do not have access to the exact amounts of precipitation at the precise locations of each sample. Nonetheless, the results indicate some relationship of dependence with Oxygen and Resveratrol, as well as with the rainfall in the two months prior to harvest.

4. Conclusions

This study highlights the interactions between grape variety, rainfall precipitation and key wine constituents such as trans-resveratrol, total phenolic compounds, $^{18}O/^{16}O$ and $^{13}C/^{12}C$ isotopic composition. Among the evaluated cultivars, Cabernet Franc demonstrated the strongest linear correlation between resveratrol content and January precipitation prior to harvest, as well as a significant negative correlation with total phenolics and oxygen concentration in the same period. In contrast, Cabernet Sauvignon showed higher correlation between resveratrol and December precipitation, suggesting varietal differences in phenolic synthesis in response to environmental stimuli. Additionally, although $\delta^{13}C$ values fell within expected ranges and aligned with prior literature, no significant correlation with precipitation was observed, likely due to the physiological nature of isotopic fractionation during photosynthesis.

Despite limitations in the spatial resolution of meteorological data—restricted to the Bento Gonçalves station—the study identified meaningful patterns linking rainfall in the months preceding harvest with both oxygen levels and resveratrol content. These results reinforce the importance of vintage-specific and varietal analyses in understanding wine composition, and they contribute to a broader comprehension of how climatic factors influence the chemical and sensory profiles of wines from Southern Brazil. Additional studies, with more samples and vintages, will be conducted to investigate the relation between the grape cultivar and precipitation on wine composition.

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