

Research Article

Application of Isotopic Ratio and Elemental Analysis as Tools for Geographical Indication of High-Altitude Wines in Santa Catarina

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Abstract

In 2021, the high-altitude wine-producing region in Santa Catarina was recognized as a Geographical Indication by the National Institute of Industrial Property, highlighting the state's significance in Brazil's wine industry. This recognition not only ensures the authenticity of the wines but is also crucial for detecting fraud, thus protecting both consumers and producers. To guarantee the wines' provenance, one of the main techniques used for verifying their origin is Isotopic Ratio Mass Spectrometry, which measures slight variations between isotopes such as $^2\text{H}/^1\text{H}$, $^{13}\text{C}/^{12}\text{C}$, and $^{18}\text{O}/^{16}\text{O}$. Isotopic analysis can identify the vintage, production location, grape variety, and type of wine produced. For instance, the $^{13}\text{C}/^{12}\text{C}$ isotopic fractionation revealed that wines from the 2023 harvest have more positive $\delta^{13}\text{C}$ values than those from 2024, reflecting climatic conditions like higher rainfall in 2023. When analyzing location, wines from Bom Retiro showed lower $\delta^{13}\text{C}$ values compared to those from Urussanga, confirming the influence of latitude on isotopic values. Additionally, when comparing red varieties (Cabernet Cantor and Cabernet Cortiz) with white ones (Sauvignon Blanc and Chardonnay), it was observed that alcohol content and isotopic values vary depending on grape species, cultivation conditions, and biochemical processes.

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Therefore, this technique allows the identification of grape varieties and production regions, making it an effective method to differentiate between grape species and growing areas of high-altitude wines in Santa Catarina.

Keywords: Geographical indication; High-altitude wines; Isotopic analysis; Stable isotopes

Introduction

Wines were the first products to demonstrate the significant influence of natural factors on their quality. However, the characteristics of these products—closely linked to their origin—are determined not only by natural conditions but also by the human environment and the social relationships surrounding it [1].

For ensuring the provenance of wines, analytical techniques are essential for identifying fraud, which can harm both the consumer and the producer, thus guaranteeing fair and standardized criteria for all parties involved. One way to verify the provenance of high-altitude wines produced in Santa Catarina is through authentication methods and geographical indication. This approach offers several benefits, including the possibility of structuring a control and evaluation protocol for the state's high-altitude wines. Wine authentication involves analytical processes that confirm compliance with legal regulations. This verification not only protects the consumer in a growing and globalized wine market but also safeguards the brand, especially for high-quality wines, which are more susceptible to fraud [2].

To assess authenticity and determine geographical indication, the isotopic ratio technique of some elements present in the wine is used. This technique was adopted by the European Commission in 1990 as the first official analytical method based on the determination of stable isotopes [3]. A few years later, the European Commission began using it for the authentication analyses of wines sold in Europe.

One of the instruments used to determine the isotopic ratio is the Isotope Ratio Mass Spectrometer (IRMS), and for wines, the carbon isotope is typically evaluated. Variations in element composition allow the precise assessment of whether any adulteration occurred during wine production, leading to a geographical indication that identifies illegally added substances.

The carbon isotopic ratio ($^{13}\text{C}/^{12}\text{C}$) is primarily derived from the isotopic fractionation of CO_2 during the photosynthesis process [4]. The diffusion of CO_2 through leaf stomata and the enzymatic reduction of CO_2 by the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) during carboxylation contribute to the fractionation of the ^{13}C isotope, as both processes favoring the lighter isotope ^{12}C . There are three main types of photosynthetic metabolism in plants: C_3 (Calvin Cycle), C_4 (Hatch-Slack Pathway), and CAM (Crassulacean Acid Metabolism). However, environmental conditions can affect these δ values, including temperature, humidity, and plant resource status (such as water use efficiency and sources of water and nitrogen) [5].

Grapes belong to the C_3 plant group, making the most common adulteration in wines the addition of sugars derived from C_4 plants during the fermentation process, a procedure known as chaptalization. The ^{13}C values are distinct for the two species and do not overlap when analyzed, making it possible to use these values to determine the carbon source [6] and thus verify the authenticity of the wine.

In this study, the carbon isotopic ratio ($^{13}C/^{12}C$) was evaluated to verify the variations of results for different types of grapes to identify isotopic markers. Additionally, statistical methods were applied to interpret the results of the analyses performed, using Matlab software (R2019a).

Materials and Methods

Standards

To perform the sample analyses, a laboratory standard of White Sugar with the VPDB reference was processed every 10 readings of the sample batch. The calculated deviation of the standards from the reference value resulted in a value of 0.6, which matches the deviation accepted by the laboratory.

Sample Preparation

The wine samples were provided by the Epagri unit in Videira - SC, which is a project partner and responsible for the microvinifications. The vintages analyzed were from 2023 and 2024, totaling 65 samples.

To analyze the carbon isotopes, the samples were distilled to extract ethanol, using a steam distillation system. A total of 10mL of each sample was used, with 2mL of a 20% calcium hydroxide ($Ca(OH)_2$) solution and 5 drops of simethicone added to prevent foaming. The distilled ethanol was then transferred to a 25mL volumetric flask and subsequently placed in a tube, where it was refrigerated until the analyses began.

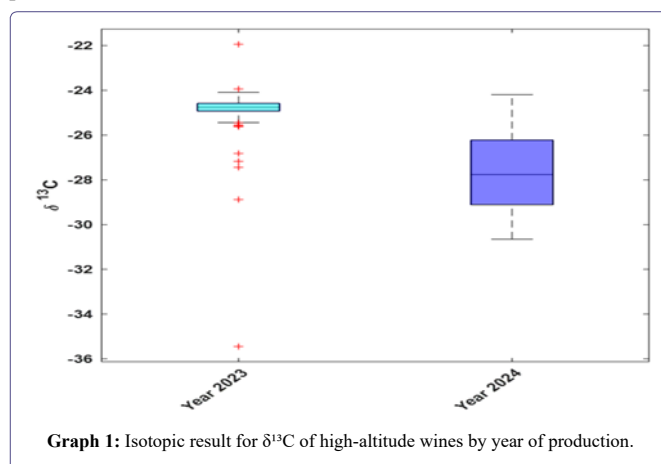
Method Specifications

The equipment used was the Isotope Ratio Mass Spectrometer (Delta V, ThermoFischer), which incorporates a Flash 2000 elemental organic analyzer (ThermoFischer), coupled with a Conflo IV (ThermoFischer) and a mass detector. A 1 μ L distilled sample was injected using a liquid sampler (AI 1310, ThermoFischer), positioned above a quartz reactor. In this reactor sample oxidation occurs, which is filled with chromium oxide, cobalt oxide, silver, and quartz wool, and maintained at a temperature of 1020°C. A reduction reactor, connected immediately after the oxidation one, contains reduced copper and operates at 650°C. The carrier gas used was helium 5.0 (White Martins). The samples are converted into CO_2 , NO_x , and H_2O , and after passing through the reactors, the gasses are reduced to N_2 and CO_2 [7]. These gasses are then separated by a chromatographic column at 60°C, where a magnesium perchlorate cartridge is used to remove the water before passage. After the chromatographic column, the sample is analyzed by a Thermal Conductivity Detector (TCD), which allows for the visualization of the separation between carbon and nitrogen peaks and provides information about the elemental composition of the samples. Subsequently, the samples enter the mass spectrometer, where the isotopic ratios of carbon and nitrogen are measured relative to a reference gas as CO_2 for determining ^{13}C and N_2 for ^{15}N , previously calibrated with international standards of known composition [8]. For carbon analysis, the reference gas used was carbon dioxide 4.5 (CO_2) (White Martins), calibrated with the USGS40 standard.

Results and Discussion

The obtained data were processed in different ways due to the possibility of multiple interpretations. The discussions begin with the evaluation of vintages from different years, followed by production in different cities, and conclude with grape variety and the type of wine produced.

The first interpretation is based on the vintage of high-altitude wines, divided between the years 2023 and 2024. Thus, in graph 1, the wine samples analyzed are organized according to their year of production.



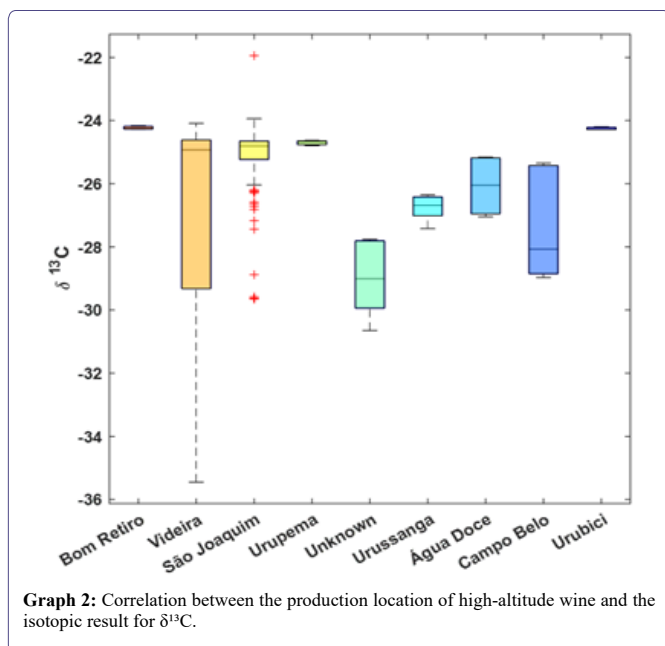
Graph 1: Isotopic result for $\delta^{13}C$ of high-altitude wines by year of production.

In 2023, the Southern region of Brazil, particularly in Santa Catarina, experienced extreme rainfall, with levels above the national historical average. In the tributary basin of the Itaipu Hydroelectric Plant, the natural average flow in October was 91% relative to the historical average [9]. The situation of rivers monitored by the hydrological measurement station in 2023 showed water excess, categorized as “above” or “well above” the climatological average for the state [9].

In 2024, so far, rainfall in the Southern region remains high compared to the national average, but lower than the levels recorded in the previous year [10]. The climatology of the hydrological measurement station indicates that many rivers in the Southern region are above the climatological average, although, compared to 2023, there has been a decrease from “well above average” to “above average” levels [10]. Meteorological data reveal that the isotopic fractionation values of $^{13}C/^{12}C$ in 2023 are more positive than in 2024, reflecting higher rainfall in the previous harvest. This happens because conditions of water stress or abundance alter the $\delta^{13}C$ value, resulting in an imbalance between the plant’s stomatal conductance and carboxylation during the photosynthesis process [5]. CO_2 hydration results in more negative $\delta^{13}C$ values, while CO_2 solubilization tends to bring the values closer to zero.

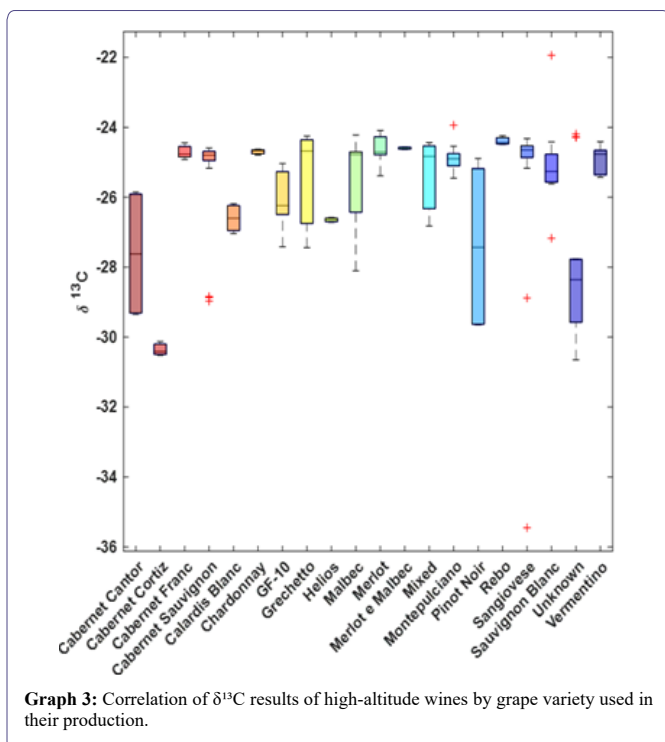
Regarding the wine production locations, graph 2 shows the variation in carbon isotopic values as a function of the grape planting location.

It is observed that the lower the latitude, the higher the region’s temperature, and consequently, the lower the $\delta^{13}C$ isotopic value. This occurs because temperature directly influences carbon isotopic fractionation, as extreme temperatures reduce the activity of the RuBisCo enzyme, which is essential for plant carboxylation [11].



The samples from Bom Retiro and Urussanga, two cities in Santa Catarina, show significant isotopic diversity. Bom Retiro is located at coordinates -27.7983, -49.4938, while Urussanga is at -28.5237, -49.3303. From graph 2, it is possible to observe that wines from Bom Retiro have a $\delta^{13}\text{C}$ value of -24.50‰, while those from Urussanga reach -27.89‰. These results confirm the relationship between geographic coordinates and isotopic values, where lower latitudes correspond to lower $\delta^{13}\text{C}$ values.

Regarding the grape varieties and types of wines produced, graph 3 presents the isotopic results of high-altitude wines organized by the grape used.



Just as variation in production location and differences between vintages affect the isotopic fractionation of CO_2 , the metabolic cycle of the raw material also plays a role. Studies [12], indicate that red wines have a different composition than white wines, with rosé wines being intermediate between these two. The main difference lies in the sugar content of the grapes, which is higher in red varieties, resulting in a higher alcohol content in red wines compared to white wines.

Analyzing graph 3, the comparison between the red varieties Cabernet Cantor and Cabernet Cortiz with the white varieties Sauvignon Blanc and Chardonnay highlights this difference in alcohol content. The data allow us to affirm that the variation in grape species impacts the $^{13}\text{C}/^{12}\text{C}$ isotopic values, which fluctuate depending on climatic conditions, planting practices, and biochemical processes associated with each variety [13,14].

Thus, through the IRMS technique, it is possible to determine the grape variety used and the production location of high-altitude wines from Santa Catarina. The $\delta^{13}\text{C}$ values are sufficient to differentiate grape species and their planting and production regions. This mapping of regions and $\delta^{13}\text{C}$ values does not yet exist in the state and aims to assist in the creation of a database for regulating wine production. However, much work remains to be done. To enhance the discussion and statistical treatment, new samples from future vintages should be analyzed and compared with the results obtained.

Conclusion

To perform the isotopic analysis of high-altitude wines, it is necessary to obtain information about the plant's water cycle from planting to harvest, the geographic coordinates of the production location, and the grape variety used in the wine production.

With this data, it is possible to trace the origin and verify the authenticity of high-altitude wines, confirming whether they were indeed produced in the declared region. This process helps identify fraud and ensures the delivery of a quality product to the consumer. In future studies, the isotopic ratios of Strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) and Oxygen ($^{18}\text{O}/^{16}\text{O}$) will be evaluated to verify consistency with the results obtained so far.

It is expected that, in the long term, the results will contribute to the establishment of a control and validation protocol for high-altitude wines produced in the controlled provenance regions of the state of Santa Catarina.

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Author's Contribution

Brenda F. R. Crespi: Investigation, Formal Analysis, Writing: Original Draft, Writing: Review & Editing; Nathália C. Andrade: Validation, Supervision, Writing: Review & Editing; Frederico L. F. Soares: Formal Analysis; Vinicius Caliar: Resources; Jocinei Dognini: Project Administration, Funding Acquisition, Writing: Review & Editing.

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