Exogenous CO_2 in South American sparkling wine

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Abstract. The sparkling wine production and consumption have increased significantly in the last years. With the increased demand appear the necessity to check the sparkling wine authenticity, because the practice of adding CO_2 in sparkling wine is not allow. A way to control the carbonation process is through the determination of CO_2 $\delta^{13}C$, because the sugar added during the second fermentation define the CO_2 isotopic value, according to elaboration process. For this reason, the aim of this study was to evaluate the relationship between values of $\delta^{13}C$ from still wines and sparkling wines, in order to set up limit values to exogenous carbonation control. Thirty-eight still wines elaborated by microvinification and 59 samples of commercial sparkling wines were analyzed, using an isotope ratio mass spectrometer (IRMS). The most negative value of natural $\delta^{13}C$ from still wine found was −24.7‰, it can be to estimate that lowest values are an indicative of industrial CO_2 addition. Among the commercial sparkling wine from South America evaluated in this study, 10% from the samples showed signs of carbonation. Through this research was possible to establish limits of isotopic values to determine the presence of exogenous CO_2.

1. Introduction

The sparkling wine can be produced by different methods, as traditional (*Champenoise),* Charmat and Asti. In traditional and Charmat methods the second fermentation occurs in two steps; the first step the base wine is produced, and next step the base wine undergoes the second fermentation to elaborate the sparkling wine. In the traditional method, the second fermentation happens in sealed bottles and in the Charmat method in stell tanks [1,2]. The Asti method is similar to the Charmat, however, with a single fermentation, in Brazil this sparkling wine is referred as Moscatel [3,4].

The CO_2 from sparkling wine should be from natural source and formed during the second fermentation. The second fermentation is induced by the added sugar, that can be extracted from plants with C_3-type photosynthetic cycle (Calvin cycle as beet and grape), with $\delta^{13}C$ between −26‰ and −17‰; from C_4 plants with $\delta^{13}C$ close −9‰ (Hatch-Slack cycle as cane sugar and maize). Furthermore, there is the direct carbonation process, with CO_2 from industrial source, $\delta^{13}C$ between −14‰ and 1‰ from carbonate sediments and −75‰ and −22‰ from natural gas combustion [5–8].

The legislation forbids the addition of industrial CO_2 in sparkling wine. The EU legislation not allow the use of industrial CO_2 in semi-sparkling and sparkling wines; Brazil forbids the addition in sparkling wines, but the use in semi-sparkling and gasified wines is allowed [9,10].

Considering that the stable isotopes provide information about the compounds origin, it was identified the necessity to establish a methodology to evaluate the South American sparkling wine. Because the most countries in South America use the cane sugar for the sparkling wine second fermentation, differently of Europa that use beet sugar. For this reason, the objective of this research was to establish a methodology to evaluate the CO_2 origin in sparkling wine from South America, considering the production method. The experiment was realized with an isotope ratio mass spectrometry (IRMS). The CO_2 $\delta^{13}C$ values from still wines were used to found the control limits estimated. The methodology was applied in commercial sparkling wine and assess them according to the found control limits.

2. Material and methods

2.1. Samples

Thirty-eight samples of still wines from the 2014 harvest were analyzed, of the cultivars: Chardonnay, White Muscat, Glera, Italic Riesling and Pinot Noir. Grapes often used to produce sparkling wine and all the samples produced by microvinification. Five samples of industrial CO_2 from different manufacturers and six samples of carbonated water were analyzed to determine the $\delta^{13}C$ of the industrial CO_2. In addition, 59 commercial sparkling wines from several production methods were evaluated, in which 41 Brazilian, five Argentinean and six Chilean.

2.2. Sample preparation

To determine CO_2 $\delta^{13}C$, the wine and sparkling wine samples were refrigerated at temperature between 2 and 4 °C. Next, the liquid was quickly transferred into a 12 mL vial and sealed with plastic cover and Teflon-silicone septum. Then 50 μL of the sample was transferred with a gastight syringe into another vial. This vial was filled with Helium, through the flush and fill system [10]. After preparation, the vial with the sample was placed on the autosampler at 27°C, the sample was analyzed by GasBench II coupled to isotope ratio mass spectrometry – IRMS Delta V Advantage (Thermo Electron, Bremen, Germany).

To determine ethanol δ^{13}C, the wine samples were distilled under the cryogenic action at −196°C and under vacuum (10^{-2} mbar), 1.5 mL of wine by 25 min was used; 2 μL of the distilled were injected in the elemental analyzer – Flash EA (Thermo Electron, Bremen, Germany). The sample was combusted inside the elemental analyzer in a vertical quartz tube at 900°C, containing copper oxide and silvered cobaltous-cobaltic oxide under a continuous flow of ultra-pure Helium at 90 mL.min^{-1}. After the produced gases were passed through a reduction column at 680°C containing reduced copper, the water was removed in the trap with magnesium perchlorate, and the gases were separated on a Porapak Q chromatographic column at 43°C. Then, the gases were transferred to the Delta plus XL mass spectrometer, which has a triple ion collector for the simultaneously measures of the signals m/z 44, 45 and 46 of CO2 formed in the combustion [11]. The sparkling wine samples were placed in agitation for 60 min. to remove CO_2 and were analyzed through the methodology described.

2.3. Isotope ratio mass spectrometer and GasBench configuration to determine CO_2 δ^{13}C

The isotope ratio mass spectrometer worked with 10^{-7} mbar pressure and acceleration electrical voltage of 3.05 kV. The $[CO_2]^+$ signal was monitored for three Faraday cups. The autosampler coupled to a GasBench with a transfer needle collected the headspace from the vial and the CO_2 was transferred slowly ($2\,\text{mL} \cdot \text{min}^{-1}$) to the capillary. The gas sample was sent to the diffusion trap (Nafion^R) to remove the water and after, to the Valco valve and into an isothermal gas chromatograph column, where CO_2 is separated from any other gases. The loop injection was set at 100 μL, the gas chromatograph column used to separate gases was a PoraPlot Q 25 m, 0.32 mm (Varian, The United States). The column was kept at a continuous Helium flow at 2 mL.min^{-1}, with pressure at 10–12 psi and 70°C. The gas sample passed through the water removal and it was transferred to the isotope ratio mass spectrometer via an open split.

The software Isodat 2.5 (Thermo Electron) controlled the mass spectrometer, GasBench and data acquisition. Each sample was analyzed with 10 repetitive loop injection. The isotope ratio was achieved relative to Sucrose 8542 NIST, international standard, and the results were reported as absolute isotope values expressed at δ‰.

2.4. Statistical analyses

The results were evaluated using analysis of variance (ANOVA) complemented by Tukey's test, with 95% confidence interval ($p < 0.05$). Furthermore, the Pearson's Correlation was used to check the linear relationship between δ^{13}C from CO_2 and ethanol. All statistical analyses were performed using the SPSS 20.0 for Windows.

Table 1. CO_2 and ethanol δ^{13}C values from experimental still wines and difference between them.

Grape	n	CO_2(‰)	Ethanol (‰)	Dif. (‰)
Chardonnay	9	-23.4^{Aa}	-29.3^{Ba}	5.9
White Muscat	17	22.7^{Aa}	-27.6^{Bb}	5.1
Glera	4	-24.0^{Aa}	-29.2^{Ba}	5.2
Italian Riesling	4	-23.5^{Aa}	-30.3^{Ba}	6.8
Pinot Noir	4	-22.1^{Aa}	-29.5^{Ba}	7.4
Mean		-23.1^{A}	-29.2^{B}	6.1

Means followed by different capital letters in the line and means followed by different lowercase letters in the column differ significantly by ANOVA complemented by Tukey's test of multiple comparisons; significance level of 5%.

3. Results and discussion

The CO_2 δ^{13}C values from still wines harvest of 2014 (Table 1) did not differentiate the grape varieties. On the other hand, these values were efficient to distinguish the CO_2 δ^{13}C from industrial CO_2 (-33.9 ± 1.63‰) and carbonated water (-29.1 ± 2.18‰) in relation to the still wines (-23.1 ± 1.26‰). The δ^{13}C values from natural CO_2 in the wines found in this study are close to the values reported by Calderone et al. [12], which founded an average CO_2 δ^{13}C of −22.0‰ for the wines from C_3 plants.

In study with French ciders, Gaillard et al. [5] observed that there is no significant variation of CO_2 δ^{13}C ratio from the beginning to the end fermentation. The Moscatel sparkling wine undergoes a single fermentation, and the CO_2 is produced from the grape natural sugar, cane sugar is not added. Therefore, the wine produced with the Muscat grape is the reference to establish a limit to the Moscatel sparkling wine. If the lowest value of δ^{13}C from natural CO_2 of the Muscat wine is −24.7‰, it can be estimated that value below than −25‰ is an indication of industrial CO_2 addition. This limit is near to the limits established by Cabañero and Rupérez for sparkling wines (−26‰) and Gaillard et al. for ciders (−25.4‰) [5,13]. However, when values are over −20‰ shall be checked the ethanol δ^{13}C too, once can be an indicative of sugar cane addition beyond the limit that is permitted by the Brazilian law.

The CO_2 and ethanol δ^{13}C results found in this study from still wines showed a weak Pearson correlation, in other words, only 12% of the ethanol δ^{13}C is explained by CO_2 δ^{13}C; the chemical reaction to produce CO_2 and ethanol, during the fermentation, causes an isotope fractionation. This fractionation is due to thermodynamic and kinetic effects, where the difference is based at the isotope free energy, the heavier isotope tends to accumulate on the compounds with stronger binding energy [14,15]. The mean difference between ethanol δ^{13}C and natural CO_2 δ^{13}C of the wines from all grapes studied was 6.1‰. The Muscat grape showed the lowest difference (5.1‰), however, this difference is upper than 2‰ proposal by Martinelli et al. [16], estimated for a theoretical calculation.

The ethanol δ^{13}C mean values found in this study ranging from −30.7 to −26‰ remained near to previous studies conducted on Brazilian wines from grapes Merlot and Cabernet Sauvignon, 2005 to 2008 harvest. A wide variation of the values in both studies was observed [17,18] this can explain the biochemical reactions, causing different isotope fractionation during carbon fixation of

Table 2. δ^{13}C values of commercial sparkling wine produced by the traditional, tank and Moscatel sparkling wine method from different countries.

	CO_2 δ^{13}C (‰)		
Country	Brazil	Argentina	Chile
Method	Mean	Mean	Mean
Traditional	-11.6^{Aa}	-11.0^{Aa}	-18.2^{Ba}
Tank	-16.9^{Ab}	-12.6^{Aa}	-23.8^{Ba}
Moscatel	-21.8^{c}	–	–
	Ethanol δ^{13}C (‰)		
Traditional	-26.0^{Aa}	-25.9^{Aa}	-25.9^{Aa}
Tank	-24.8^{Aab}	-25.7^{Aa}	-26.1^{A}
Moscatel	-24.6^{b}	–	–

Means followed by different capital letters in the line and means followed by different lowercase letters in the column differ significantly by ANOVA complemented by Tukey's test of multiple comparisons; significance level of 5%. The value −45.6‰ found in the Chilean sparkling wine is a discrepant result and it was not considered at the mean and standard deviation (SD).

CO_2 of the plant, due to the influence of genetic variability for each grape [19].

It was observed that CO_2 δ^{13}C is selective to the sparkling wine produced method, showed significant difference among traditional, tank and Moscatel sparkling wine (Table 2).

The Moscatel sparkling wine showed the lowest values for CO_2 δ^{13}C, these results corroborate with the values found by Martinelli et al. [17], in Italian Asti and Brazilian Moscatel and with the study of Cabañero et al. [13] in cava sparkling wine from Spain.

The CO_2 from the sparkling wine produced by the traditional method showed isotope values less negatives than Moscatel. The higher values of CO_2 δ^{13}C found in the traditional method indicates the influence of the cane sugar (C_4 plant) in the CO_2 formation. The values found are lower than proposal at the methodology by OIV, where the CO_2 from C_4 plants range of −10‰ to −7‰ [8].

The values found in ethanol δ^{13}C distinguished the sparkling wine produced by the traditional method to the Moscatel, however, the sparkling wine produced by the tank method not distinguished from the others.

The $CO_2\delta^{13}$C values, considering the origin country for the sparkling wine and the method traditional and tank, distinguish significantly from Chileans to Argentineans and to Brazilian (Table 2). The difference found can be due to the sugar used for the second fermentation, according to Draycott [20] on South America, Chile and Uruguay are producers of beet sugar. In our study, we also found a Chilean sparkling wine with sugar from C_4 plant, suggesting the use of cane sugar for the second fermentation. These results indicate that Chile use sugar from C_3 and C_4 plants, being it more difficult the control, except when CO_2 δ^{13}C to show values lower than the C_3 plants, hampering the application of the characterized methodology in our study and the use of estimated limits.

Two samples showed CO_2 δ^{13}C lower than results expected considering the production process, one Brazilian (−30.8‰) and other Chilean (−45.6‰) sparkling wine. The Brazilian sparkling wine showed values close to industrial CO_2 found in Brazil, the Chilean sparkling wine has lower result, signaling the use of industrial CO_2 with values all the more negatives. The CO_2 δ^{13}C values from the sparkling wines mentioned are an indication of

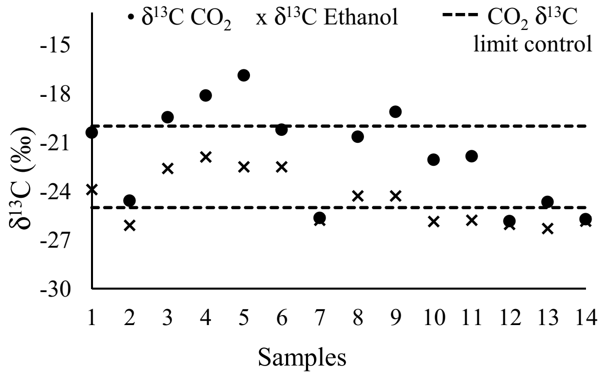

Figure 1. CO_2 and Ethanol δ^{13}C from commercial Moscatel sparkling and CO_2 δ^{13}C estimated control limits representation.

carbonation (gasification) when compared to the CO_2 δ^{13}C found in the still wines.

One Brazilian sparkling wine produced by the tank method showed CO_2 δ^{13}C −22.3‰ and ethanolδ^{13}C −24.5‰. The value of CO_2 δ^{13}C found in this sample is closer than CO_2 δ^{13}C for the still wine, no added sugar; however, the cane sugar addition was proven through the ethanol δ^{13}C that showed 79.23% of C_3. In this case, prove the cane sugar addition for the fermentation, therefore the CO_2 δ^{13}C value produced by the tank method should be close to −16‰. Consequently, it is concluded that the result obtained denote addition of industrial CO_2.

The commercial Moscatel sparkling (Fig. 1) verified showed half of the CO_2 δ^{13}C results within the estimated control limits. This is a warning for this product, because 50% of the samples indicated irregularities.

The samples number 7, 12 and 14 showed CO_2 δ^{13}C lower than the control limit for the Moscatel sparkling, furthermore this results are very close to ethanol δ^{13}C, suggesting industrial CO_2 addition. The samples 3, 4 and 5 showed results above the estimated limit; in this case, the C_3 percentage found for these sparkling wine were 68, 63.7 and 64.7%, respectively, denoting the cane sugar addition beyond that permitted in Brazil. The Brazilian law allows an increase in alcohol up to 1.5% (v/v) for sparkling wine and 2% for Moscatel, from added sugars. The sample number 9 also showed CO_2 δ^{13}C above the control limit, however, the ethanol δ^{13}C remained within the limits, in this case, could be a harvest influence.

The results of ethanol δ^{13}C are less selective than CO_2 δ^{13}C, in relation to produced method; however, it appears as an important tool to help the confirmation of the unlawful addition of industrial CO_2.

4. Conclusion

The CO_2 δ^{13}C values for industrial CO_2 are more negative than the still and sparkling wines. And, based on values from still wines, it was possible to establish a methodology to evaluate the CO_2 source, with control limits of CO_2 δ^{13}C estimated for Moscatel sparkling wines. Applying the methodology set out in this study to the commercial sparkling wines, it was noted that, at least, 15% of the samples analyzed showed evidences of adulteration, being 10% by CO_2 industrial addition.

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