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Stable Carbon Isotopic Composition of the Wine and CO_2 Bubbles of Sparkling Wines: Detecting C_4 Sugar Additions

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Sparkling wines have become a popular beverage in recent years, and the production of these wines is subject to adulteration during fermentation. This study investigated the stable carbon isotopic composition (expressed as $\delta^{13}C$) of the wine and of the CO_2 bubbles produced during the second fermentation for a number of sparkling wines produced in different countries around the world. Carbon isotope ratio analyses were used to estimate the addition of sugar obtained from C_4 plants (sugar cane or corn). The average $\delta^{13}C$ values of the Brazilian brut, demi-sec, and doux sparkling wines were −20.5 ± 1.2‰ (n = 18), −18.1 ± 1.3‰ (n = 9), and −15.8‰ (n = 1), respectively. These values were statistically heavier (more positive carbon isotope ratio values) than the average $\delta^{13}C$ of sparkling wines produced in other parts of South America (Argentina and Chile, −26.1 ± 1.6‰, n = 5) and Europe (France, Germany, Italy, Portugal, and Spain, −25.5 ± 1.2‰, n = 12), but not statistically different from sparkling wines produced in the United States or Australia. The most likely explanation for differences in the carbon isotope ratios of wines from these different regions is the addition of C_4 sugar during the production of some sparkling wines from Australia, Brazil, and the United States. The isotopic composition of the CO_2 bubbles ($\delta^{13}C$-CO_2) followed similar trends. The average $\delta^{13}C$-CO_2 of most of the Brazilian and Argentine sparkling wines was −10.8 ± 1.2‰ (n = 23), indicating that the likely source of carbon for the second fermentation was sugar cane. Conversely, the average $\delta^{13}C$-CO_2 of most of the sparkling wines produced in Chile and Europe was −22.0 ± 1.2‰ (n = 13), suggesting that a different sugar (most likely sugar beet) was most used in the second fermentation. It was concluded that in many cases, the carbon isotope ratios of sparkling wine and CO_2 bubbles can provide valuable information about the sugar sources.

KEYWORDS: Sparkling wine; isotopic composition; C_4 sugar; CO_2; bubbles

INTRODUCTION

Sparkling wine is a beverage that originated in the Champagne region of France and that has now become a popular beverage globally, so that it is now produced in other regions of the world. The production of sparkling wines typically consists of two fermentation steps: a first fermentation that results in ethanol synthesis and a second short fermentation to produce CO_2. Normally the second fermentation is achieved by the addition of small amounts of sugar. In efforts to control the quality of sparkling wines, many countries have developed specific legislation to describe the final composition and fermentation steps that are allowable in order to maintain the original characteristics of the product. For instance, Brazilian legislation allows the addition of sugar to the grape must during the initial fermentation in order to increase an ethanol content of up to 3 °GL [Gay-Lussac (GL) is the volumetric percentage of alcohol in a beverage]. Therefore, the addition of sugar in making a Brazilian sparkling wine could occur during either or both of the two fermentation steps. In Brazil, sugar can also be added following the second fermentation in order to adequately sweeten the wine in accordance with its sweetness type. However, in California (United States), where a large number of sparkling wines are produced, legislation allows only the addition of sugar for the second fermentation step.

In Europe, where restrictions are often tighter, the addition of sugar will depend on both the region and method of production of the sparkling wine. There are two main methods

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for producing sparkling wines. If the second fermentation is performed within the bottle, the method is called *Traditionalle* or *Champenoise*. On the other hand, if the second fermentation occurs in large pressurized tanks, the method is called *Charmat*. A third, and less common, method, called direct carbonation, is the injection of carbon dioxide originated from a combustion process to the wine following the first fermentation (*1*).

Stable isotope analyses have been a useful tool for assessing the adulteration of food products (*2*). One approach is based on the fact that photosynthesis in most plants follows one of two main photosynthetic pathway types (C_3 versus C_4) and that plants with these pathways differ in the carbon isotope ratios of their products. Tropical grasses, such sugar cane, have C_4 photosynthesis, with the first organic molecule formed after the uptake of the atmospheric CO_2 composed by four atoms of carbon. On the other hand, grapes have C_3 photosynthesis, in which the first organic molecule of photosynthesis is composed by three atoms of carbon. The stable carbon isotope ratios (expressed as $\delta^{13}C$) of C_4 and C_3 plants are different, with $\delta^{13}C$ values of C_4 plants varying from −11 to −14‰ and those of C_3 plants varying from −24 to −32‰ (*3*). Thus, the stable carbon isotope ratio has been widely used to trace the presence of sugar and alcohol derived from C_4 plants in beverages that are traditionally made from C_3 plants (*2*, *4*−*6*).

The $\delta^{13}C$ value of a beverage resembles the $\delta^{13}C$ of the fruit or cereal that originated this specific beverage with only small isotope effects associated with fractionation events during synthesis (*2*). Addition of C_4 sugar or C_4-derived alcohol would change the $\delta^{13}C$ values of the original beverage (see, e.g., refs *2*, *4*−*7*, and *9*). In two recent studies, stable carbon isotope ratios were used to detect a high content of C_4-derived compounds in Brazilian brandies (*5*) and in red wine samples (*10*). Sparkling wines are suitable for isotopic analyses, because sugar can be added in one or several steps of its production and because sugar in Brazil and many other countries is derived from sugar cane, a C_4 plant isotopically distinct from grapes.

The origin of the CO_2 bubbles could also be determined by this method. In Europe, generally beet sugar is used to promote the second fermentation. Beet is a C_3 plant with an isotopic composition similar to that of the grape. Therefore, CO_2 with isotopic composition near that of C_3 plants would likely indicate the presence of beet sugar. In many countries it is more common to use sugar from corn or sugar cane (C_4 plants) with much higher $\delta^{13}C$ values for the second fermentation, allowing a distinction between the type of sugar used during the second fermentation. Finally, there is a third possibility to directly add CO_2 from the combustion of fossil fuels, which have $\delta^{13}C$ values varying from approximately −28 to −35‰ (*11*). In such cases, the sparkling wine label must clearly specify this product as a gassified wine and not a sparkling wine.

Dunbar (*1*) had conducted carbon isotope studies on a limited number of sparkling wines from New Zealand, showing that these sparkling wines had carbon isotope ratios consistent with C_4-derived carbon in both the ethanol and CO_2 components. In this paper we further investigated the addition of C_4 sugar in sparkling wines from various places around the world, analyzing the $\delta^{13}C$ of the liquid (wine) and the CO_2 bubbles. This information would be useful to detect adulteration of the wine by extra addition of C_4 sugar and to investigate the botanical origin of the sugar added to the second fermentation.

EXPERIMENTAL METHODS

We analyzed the stable carbon isotopic composition of the wine and CO_2 bubbles in 75 sparkling wine samples. From this total, 33 were sparkling wines made in Brazil, 19 from Europe (7 from Italy, 6 from Spain, 3 from France, 2 from Portugal, and 1 from Germany), 2 from Argentina, 3 from Chile, 16 from the United States, and 2 from Australia (see **Table 1**).

We sampled 30 mL of the CO_2 in the bubbles from the bottle headspace through the cork with a 50 mL syringe. There was no contamination with the air present in the needle or need to purge the syringe with an inert gas because the pressure inside the bottle was far above the atmospheric pressure inside the laboratory room; this overpressure would slightly vent the syringe before the sample could be taken. We used a glass with Teflon embolus 50 mL syringe for sampling. The CO_2 in these air samples was purified in a vacuum line using a dry ice/ethanol slush and trapping of the CO_2 in a liquid nitrogen trap. The pure CO_2 was analyzed in a dual inlet port of a mass spectrometer (IRMS) (ThermoQuest Finnigan-Matt Delta Plus). One milliliter of the wine was also collected for isotope analysis. The wine was not distilled because we were interested not only in the sugar added for fermentation but also in the sugar added to adjust sweetness. A 1 mL volume of wine was placed in a tin capsule and analyzed in continuous flow through an elemental analyzer (Carlo Erba) in line with the mass spectrometer. The stable carbon isotope ratio was expressed by the classical δ (per mil) notation, defined as

$$\delta^{13}C\ (‰) = \left(\frac{R_{sample}}{R_{std}} - 1\right) \times 1000 \qquad (1)$$

where R_{sample} and R_{std} are the $^{13}C/^{12}C$ ratios of the sample and the PDB standard, respectively. The reproducibility for the gas samples, analyzed in the dual inlet port of the Finnigan Delta Plus, was 0.03‰ and for the wine samples in continuous flow analyses, 0.05‰.

Average results are presented with their respective standard deviations and the number of samples. Differences between samples were tested in cases where there were enough data to run ANOVA followed by Tukey's honest significant difference for unequal number of samples at a 5% level of probability.

To calculate the proportion of C_4 and C_3 carbon in a wine, we use the isotopic mass balance

$$C_4\ (\%) = 100 \times \frac{\delta^{13}C_{sample} - \delta^{13}C_{std}}{\delta^{13}C_{C_4 ethanol} - \delta^{13}C_{std}} \qquad (2)$$

where $\delta^{13}C_{sample}$ is the isotope ratio of a wine sample, $\delta^{13}C_{std}$ is the isotope ratio of a standard C_3 wine, without any addition of C_4 carbon, and $\delta^{13}C_{C4ethanol}$ is the isotope ratio of an ethanol obtained from C_4 sugar. We calculated this value using the equation proposed by Rossman et al. (*12*):

$$\delta^{113}C_{C_4 ethanol} = 1.07\delta^{13}C_{C_4-sugar} + 0.472 \qquad (3)$$

We used as the isotope ratio of the C_4 sugar the average $\delta^{13}C$ of five sugar samples derived from sugar cane (−11.5 ± 0.3‰). Using this last value in eq 3, we obtained a $\delta^{13}C_{C_4 ethanol}$ equal to −11.8‰. For comparison, we measured five samples of sugar beet from Europe, which had an average $\delta^{13}C$ of −26.3 ± 0.8‰ and would produce a wine with a $\delta^{13}C$ value equal to −27.7‰.

We grouped samples by country of origin— Brazil, the United States, and Austrália—or by continent—South America (Argentina and Chile) and Europe (France, Germany, Italy, Portugal, and Spain) and according to the sweetness types of wines. Three categories were considered in increasing order of sweetness: brut, demi-sec, and doux. A fourth category, asti, was also included. Asti is a city located in the northwest region of Italy, which is famous for its sparkling wine made with Moscatel grapes. The Brazilian legislation defines asti as a sparkling wine made with Moscatel grapes, with an alcohol content varying from 7 to 10 °GL, derived from only a single fermentation. In this category we included an asti sparkling wine made by a federal company (Embrapa) following the Brazilian legislation. This sample was labeled certified Brazilian asti. From 33 samples of Brazilian sparkling wines, 18 samples were brut, 9 demi-sec, 5 asti, and 1 doux. Most of the

Table 1. $\delta^{13}C$ Values (‰ Units) of Sparkling Wines and of CO_2 Produced during Fermentation

code	country	winery	name	type	$\delta^{13}C$-wine	$\delta^{13}C$-CO_2
1	Brazil	George Aubert	Fétiche	brut	−17.8	−10.5
2	Brazil	George Aubert	Leggera	brut	−19.5	−10.6
3	Brazil	Salton	Carrefour	brut	−19.6	−9.8
4	Brazil	Salton	Salton	brut	−19.7	−10.6
5	Brazil	Salton	Salton Reserva Ouro	brut	−19.7	−14.4
6	Brazil	Geroge Aubert	George Aubert	brut	−19.8	−10.1
7	Brazil	George Aubert	George Aubert	brut	−20.2	−10.4
8	Brazil	Armando Peterlongo	Peterlongo	brut	−20.2	−24.3
9	Brazil	Salton	Salton	brut	−20.3	−10.2
10	Brazil	Bacardi-Martini	De Greville	brut	−20.4	−9.5
11	Brazil	Salton	Salton	brut	−20.4	−9.9
12	Brazil	Salton	Salton Volpi	brut	−20.4	−12.8
13	Brazil	Miolo	Miolo	brut	−20.6	−10.8
14	Brazil	Aurora	Conde de Foucauld	brut	−21.1	−24.3
15	Brazil	Aurora	Marcus James	brut	−21.9	−18.0
16	Brazil	Chandon do Brasil	Chandon	brut	−22.0	−11.3
17	Brazil	Luiz Valduga e Filhos	Valduga	brut	−22.4	−9.3
18	Brazil	Chandon do Brasil	Chandon Excellence	brut	−23.0	−10.6
19	Brazil	Armando Peterlongo	Peterlongo	demi-sec	−16.5	−28.1
20	Brazil	Salton	Salton	demi-sec	−16.8	−27.7
21	Brazil	George Aubert	Georges Aubert	demi-sec	−17.3	−10.1
22	Brazil	George Aubert	Georges Aubert	demi-sec	−17.4	−9.9
23	Brazil	Garibaldi	Garibaldi	demi-sec	−17.5	−22.8
24	Brazil	Aurora	Conde de Foucauld	demi-sec	−18.2	−29.1
25	Brazil	Salton	Salton	demi-sec	−18.7	−11.5
26	Brazil	Chandon do Brasil	Chandon	demi-sec	−20.2	−10.5
27	Brazil	Chandon do Brasil	Chandon Passion	demi-sec	−20.2	−11.2
28	Brazil	George Aubert	Georges Aubert	doux	−15.8	−10.4
29	Brazil	Aurora	Aurora	asti	−17.6	−12.8
30	Brazil	Salton	Salton	asti	−18.5	−13.3
31	Brazil	Luiz Valduga e Filhos	Casa Valduga	asti	−17.7	−20.9
32	Brazil	Miolo	Terranova	asti	−23.8	−21.1
33	Brazil	Embrapa	Embrapa Asti	asti	−27.5	−24.3
34	Argentina	El Algarro Bal	La Sierra	brut	−26.3	−10.1
35	Argentina	Allied Domecq	Petigny	brut	−26.3	−10.2
36	Australia	Banrock Station	Sparkling Chardonnay	brut	−18.9	−8.4
37	Australia	Taltarni	Brut Tache	brut	−20.7	−9.2
38	Chile	Vina Undurraga	Undurraga	brut	−23.4	−22.4
39	Chile	Alberto Valdivieso	Valdivieso	brut	−24.6	−22.0
40	Chile	Viña Santa Carolina	Santa Carolina	brut	−26.9	−20.5
41	Spain	Jaume Serra	Cristalino	brut	−25.7	−22.6
42	Spain	Freixenet	Cordon Negro	brut	−26.2	−22.5
43	Spain	Freixenet	Carta Nevada	demi-sec	−25.8	−21.4
44	Spain	Xenius	Brut Reserva	brut	−24.9	−21.4
45	Spain	Segura Viudas	Aria Brut Natura	brut	−25.2	−20.4
46	Spain	Segura Viudas	Brut Reserva	brut	−25.1	−21.4
47	France	Nicolas Feuilliate	Nicolas Feuilliate	brut	−24.1	−21.9
48	France	François Montad	François Montad	brut	−26.4	−9.6
49	France	Bouvet	Signature Brut	brut	−26.1	−21.2
50	Germany	Henkell CO	Henkell	brut	−27.4	−24.7
51	Italy	C.S.S.C.scar.l.	Asti	asti	−25.9	−22.3
52	Italy	Martini & Rossi	Asti Spumante	asti	−25.8	−22.6
53	Italy	Zonin	Portacomaro D'Asti	asti	−25.3	−20.5
54	Italy	Zonin	Zonin	brut	−27.0	−23.0
55	Italy	Abbazia di S. Galvenzio	Piemonte Brachetto	doux	−25.0	−22.7
56	Italy	Abbazia di S. Galvenzio	Moscato	doux	−25.5	−21.7
57	Italy	Abbazia di S. Galvenzio	Malvasia	doux	−27.4	−20.5
58	Portugal	Caves Aliança	Aliança Danubio	brut	−25.7	−19.0
59	Portugal	Caves Aliança	Aliança Reserva	brut	−25.9	−7.7
60	USA	Korbel	Chardonnay	brut	−18.3	−9.5
61	USA	Korbel	Natural	brut	−19.9	−9.2
62	USA	Korbel	Korbel Brut	brut	−19.3	−8.9
63	USA	Korbel	Rouge	brut	−21.1	−8.3
64	USA	Korbel	Korbel Extra Dry	brut	−18.2	−8.8
65	USA	Domaine Ste. Michelle	Extra Dry	brut	−17.9	−10.6
66	USA	Domaine Ste. Michelle	Cuvée Brut	brut	−20.1	−10.0
67	USA	Domaine Ste. Michelle	Blanc de Blanc	brut	−23.8	−28.0
68	USA	Argyle	Argyle Brut	brut	−25.7	−21.7
69	USA	Gloria Ferrer	Sonoma Brut	brut	−18.8	−9.8
70	USA	Gloria Ferrer	Royal Cuvée	brut	−19.3	−9.8
71	USA	Roederer Estate	Anderson Valley Brut	brut	−19.6	−9.9
72	USA	Ballatore	Gran Spumante	brut	−17.9	−18.0
73	USA	Kenwood	Yulupa Cuvée Brut	brut	−19.4	−8.9
74	USA	Banrock Station	Sparkling Chardonnay	brut	−18.9	−8.4
75	USA	Taltarni	Brut Tache	brut	−20.7	−9.2

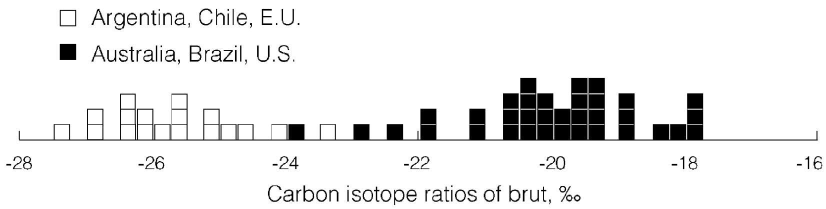

Figure 1. Frequency distribution of the carbon isotope ratios of brut sparkling wines.

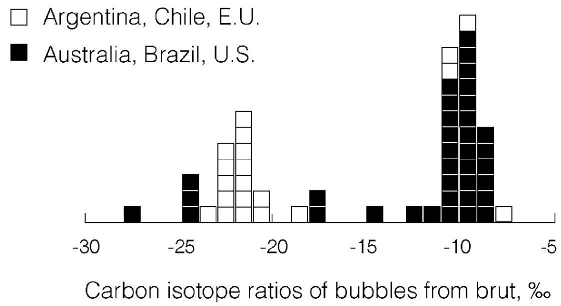

Figure 2. Frequency distribution of the carbon isotope ratios values of the CO_2 bubbles.

non-Brazilian beverages were brut sparkling wines, with the exception of three asti and three doux Italian sparkling wines.

RESULTS

The average $\delta^{13}C$ value of Brazilian brut sparkling wines was −20.5 ± 1.2‰ ($n = 18$) with individual values ranging from −23.0 to −17.8‰ (**Table 1**). The average $\delta^{13}C$ values of other South American (−26.1 ± 1.6‰, $n = 5$) and European (−25.4 ± 1.2 ‰, $n = 12$) brut sparkling wines were statistically lighter ($P < 0.01$) than brut wines made in Brazil (**Figure 1**). On the other hand, the average $\delta^{13}C$ values of United States (−20.0 ± 2.2‰, $n = 16$) and Australian (−19.8 ± 1.3‰, $n = 2$) brut sparkling wines were not different from that of brut sparkling wines made in Brazil (**Figure 1**). The average $\delta^{13}C$ value of Brazilian demi-sec sparkling wines (−18.1 ± 1.3‰, $n = 9$, range from −20.2 to −16.5‰) was significantly ^{13}C heavier ($P < 0.01$) than that of Brazilian brut wines. The average $\delta^{13}C$ value of asti sparkling wines produced in Brazil was not statistically distinct from those of either brut or demi-sec wines (−19.4 ± 2.9‰, $n = 4$, range from −23.8 to −17.6‰). On the other hand, the $\delta^{13}C$ values of the genuine Italian asti had an average $\delta^{13}C$ value of −25.7 ± 0.3‰ ($n = 3$), and the Brazilian certified asti had a $\delta^{13}C$ value of −27.5‰, which is ∼8‰ lighter than the average $\delta^{13}C$ value of commercial Brazilians asti. As the doux sparkling wines are not common in the Brazilian market, we analyzed only one sample, which had a $\delta^{13}C$ value of −15.8 ‰ (**Table 1**). For comparison, the average $\delta^{13}C$ value of three Italian doux sparkling wines was −25.6 ± 0.6‰ ($n = 3$), almost 10‰ lighter than the $\delta^{13}C$ value of the Brazilian doux sparkling wine.

The $\delta^{13}C$ value of the CO_2 bubbles ($\delta^{13}C$-CO_2) of the Brazilian brut sparkling wines can be divided in two groups. In the first grouping, the $\delta^{13}C$-CO_2 value falls between −14.4 and −9.3‰ ($n = 15$). The second group, formed by only samples 8, 14, and 15, had lighter $\delta^{13}C$ values equal to −24.3, −24.3, and −18.0‰, respectively (**Table 1**). Overall, brut sparkling wines produced in South America and Europe had $\delta^{13}C$-CO_2 values lighter than those of the Brazilian, American, and Australian beverages (**Figure 2**). From 17 samples, 13 had isotopic values ranging from −19.9 to −24.7‰, and only 4 samples had isotopic values between −7.7 and −10.2‰ (**Table 1**). Most of the American and Australian brut sparkling wines had $\delta^{13}C$-CO_2 values similar to those of the Brazilian brut wines. The exceptions were American samples 67, 68, and 72, which had values of −28, −21.7, and −18.0‰, respectively (**Table 1**). The $\delta^{13}C$-CO_2 values of nine Brazilian demi-sec sparkling wines could also be separated into two distinct groups, one in which the isotopic composition varied from −9.9 to −11.5‰ ($n = 5$) and other with lighter $\delta^{13}C$-CO_2, varying from −22.8 to −29.1‰ ($n = 4$) (**Table 1**). The same was true for the $\delta^{13}C$-CO_2 of Brazilian asti. Of four samples, two had values between −13.3 and −12.8‰ and two had values between −21.1 and −20.9‰ (**Table 2**). The Brazilian certified asti (−24.3‰) had a lighter $\delta^{13}C$-CO_2 value than the commercial Brazilian sparkling wines, and the Italian asti had an average $\delta^{13}C$-CO_2 value of −21.7‰, similar to the isotopically lightest Brazilian samples. Finally, the $\delta^{13}C$-CO_2 value of the only Brazilian doux sparkling wine sampled was −10‰, a value much heavier than the average $\delta^{13}C$-CO_2 of the Italian doux sparkling wines (−21.6 ± 1.1‰).

DISCUSSION

$\delta^{13}C$ Values of Sparkling Wines. The $\delta^{13}C$ values of Brazilian, American, and Australian sparkling wines were significantly heavier ($P < 0.01$) than the isotopic composition found in sparkling wines from South America and Europe (**Figure 1**). The $\delta^{13}C$ values of these sparkling wines were also heavier than those of European nonsparkling wines (*12*). For instance, the average $\delta^{13}C$ value of 1631 samples of Italian wines was −25.8‰, whereas the average $\delta^{13}C$ values of 576 German and 654 French wines were −26.8 and −27.2‰, respectively (*12*). Humidity and water-stress effects on stomatal aperture are well-known to cause changes in $\delta^{13}C$ values among C_3-photosynthesis plants such as grapes (*3*, *13*) and are thus the likely explanation for the observed differences in $\delta^{13}C$ values of the grapes and also of the resulting $\delta^{13}C$ of wines. However, such $\delta^{13}C$ changes are likely to be <2−3‰ across the entire range of grape distributions. This stomatal aperture effect cannot explain the observed variations in $\delta^{13}C$ values of sparkling wines from several countries. The most probable cause for the heavier $\delta^{13}C$ values of Brazilian, American, and Australian sparkling wines was the addition of C_4-derived sugar (average $\delta^{13}C$ of sugar cane was −11.5 ± 0.8‰). The contribution of C_4 sugar within the two distinct groups of brut sparkling wines can be seen in **Figure 3**. It is clear that there may be at times a 50% or more contribution of C_4 sugar to the overall isotopic signature of the wine.

In Brazil, the addition of sugar is allowable during the fermentation of the base wine to produce a maximum increase of 3 °GL in the alcohol content of the wine. Each 17 g/L of additional sugar during fermentation will contribute to a 1 °GL increase in alcohol content of the wine (*14*). Thus, to a first approximation a maximum of 51 g/L of sugar could be allowable for the first fermentation. To induce the second fermentation, another 24 g/L of sugar is required. In this process CO_2 will be formed and the alcohol content of the wine will be increased only by ∼1.4 °GL.

Table 2. Amount of Sugar (Grams per Liter) Added in Several Steps during the Elaboration of Brazilian Sparkling Wines According to Each Type and the Estimated δ^{13}C Values Obtanied from Equation 2 Using Two Different Values for δ^{13}C-Wine

	1st ferm	2nd ferm	final	total	estimated $\delta^{13}C^a$	estimated $\delta^{13}C^b$	Brazil $\delta^{13}C^c$	USA $\delta^{13}C^c$	Australia $\delta^{13}C^c$	South America $\delta^{13}C^c$	Europe $\delta^{13}C^c$
extra-brut	51	24	6	81	–24.67	–22.09					
brut	51	24	6–15	81–90	–24.67 to –24.54	–22.09 to –21.99	–20.5	–20.0	–19.8	–25.4	–25.5
sec	51	24	15–20	90–95	–24.54 to –24.47	–21.99 to –21.94					
demi-sec	51	24	20–60	95–135	–24.47 to –23.91	–21.94 to –21.49	–18.1				
doux	51	24	>60	>135	>–23.91	>–21.49	–15.8				

[a] Assuming δ^{13}Cstd = –25.8‰ (see text for details). [b] Assuming δ^{13}Cstd = –23‰ (see text for details). [c] The observed average δ^{13}C values for Brazilian, American, South American (Argentina and Chile) and European wines are presented for comparison.

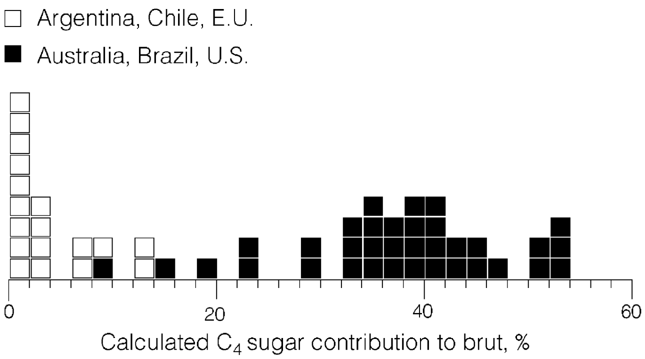

Figure 3. Calculated C_4 sugar contribution to brut sparkling wines.

Another possibility of sugar addition is as a sweetener, thereby defining the product to a specific category of sparkling wine. The following categories and sugar contents are described: extra-brut (sugar content = 6 g/L); brut (sugar content = 6−15 g/L); sec (sugar content = 15−20 g/L); demi-sec (sugar content = 20−60 g/L), and doux (sugar content >60 g/L). In Brazil, the total amount of allowable sugar addition in sparkling wines varied from 81 to 135 mg/L, depending on the wine type (**Table 2**). Assuming that the density of wine was roughly similar to the density of water (1 g/mL), these additional amounts of sugar would be equivalent to a residual 8.1−13.5% sugar content. Using these proportions and eq 2, and assuming that the added sugar was made from a C_4 plant, one can calculate the allowable potential percentage of C_4 sugar for each type of sparkling wine (**Table 2**). We first assumed as the $\delta^{13}C_{std}$ value the highest average value for wines in Europe, which was the Italian average (−25.8‰) (*12*), and for the $\delta^{13}C_{sugar}$ value we used our measured average value of −11.5‰ (**Table 2**). In this first case, the δ^{13}C values of Brazilian sparkling wines were ^{13}C heavier than the calculated value for every wine type, indicating that the addition of sugar was greater than expected. Even if we consider a $\delta^{13}C_{std}$ with an isotopic composition of −23‰, which is the highest value found in Europe among ~2900 wine samples (*12*), the average δ^{13}C values of Brazilian sparkling wines would still be heavier, indicating that a considerable amount of C_4 sugar was added (**Table 2**). It is also clear from our analyses that δ^{13}C values of American and Australian sparkling wines are also the result of C_4 sugar in their production (**Figure 3**; **Table 2**).

The carbon isotope ratios of European and South American sparkling wines were significantly ^{13}C lighter than those of the Brazilian sparkling wines. There are several possible causes for such differences. In Europe the use of sugar beet (−26.3 ± 0.8‰) instead of sugar cane could result in the lighter isotopic composition found, because the δ^{13}C value of sugar beet is similar to that of grapes. On the other hand, even if other countries besides Brazil use sugar from sugar cane, the amount used would not significantly affect the carbon isotope ratio of their sparkling wines as in Brazil. In most countries, the use of additional sugar to increase the alcohol content of base wines is discouraged or prohibited. Therefore, this limits the addition of exogenous sugar to two steps: (a) sugar added to the base wine to produce a second fermentation and (b) sugar added after the second fermentation to sweeten the wine to its sweetness type. Taking Champagne, the most famous sparkling wine, as an example, the amount of sugar added in each type is shown in **Table 3**. Assuming a carbon isotope ratio for a traditional, nonsupplemented wine (without any addition of C_4 sugar) of −27.2‰ (average δ^{13}C of 654 samples of French wines) and using eq 2, one can estimate the changes in δ^{13}C composition of a sparkling wine after the addition of C_4 cane sugar to promote the second fermentation and to sweeten it to the sweetness type (**Table 3**). It appears that even the sweetest sparkling wine (doux) would have a δ^{13}C value change of only ~1.1‰, resulting in a sparkling wine with a δ^{13}C value of −26.1‰. Such a limited shift in the absolute δ^{13}C values falls within the range of observed year-to-year δ^{13}C variations because of changes in drought and humidity (*12*). Therefore, an isotopic approach cannot detect small sugar additions from a database background that includes natural site-to-site or year-to-year δ^{13}C variations. The C_4 sugar additions are most useful in detecting δ^{13}C shifts exceeding 2−3‰ from their expected values.

Table 3. Amount of Sugar Added (Grams per Liter) and Estimated δ^{13}C of Sparkling Wines from Champagne (France) by Assuming a δ^{13}Cstd Equal to – 27.2‰ (See Text for Details)

	1st ferm	2nd ferm	final	total	estimated δ^{13}C
extra-brut	0	24	0	24	–26.83
brut	0	24	0–15	24–39	–26.83 to –26.60
sec	0	24	15–20	39–44	–26.60 to –26.52
demi-sec	0	24	20–50	44–74	–26.52 to –26.06
doux	0	24	>50	>74	<–26.06

δ^{13}C Values of CO_2 Bubbles of Sparkling Wines (δ^{13}C-CO_2). Before we consider the sources of CO_2 in sparkling wines, it is appropriate to investigate potential isotopic fractionation processes that may occur between the dissolved inorganic carbon in the liquid and its gaseous form. The speciation of the dissolved inorganic carbon depends on the pH of the solution (*15*). We measured the pH of three of our samples (samples 12, 22, and 28 in **Table 1**); the values ranged from 3.16 to 3.26, which is similar to pH values found in a large survey of Brazilian white wines (*16*). In these three samples we also measured the dissolved inorganic carbon concentration and calculated the concentration of each carbon species. More than 95% of the dissolved inorganic carbon was in the form of dissolved CO_2. According to Zhang et al. (*17*), in acidified solutions with a pH of 2, the fractionation between the dissolved inorganic carbon and the CO_2 varies from −1.3 to −1.2‰ from 5 to 25 °C, respectively. Assuming an isotopic fractionation of −1‰ for the carbon released during fermentation and a phase-change fractionation of −1.2‰ for CO_2 between liquid and gas phases, the difference in δ^{13}C values between bubbles and wine should be ~2.2‰. Therefore, if C_4 sugars (−11.5 ± 0.3‰) were used in the second fermentation, the bubbles would have an average δ^{13}C of −9.3‰. A comparison of δ^{13}C values of CO_2 bubbles from brut shows that the majority of the samples from Australia, Brazil, and the United States clustered around this value, strongly suggesting that C_4 sugars were the main source of the CO_2 (**Figure 2**). A sparkling wine from Portugal (sample 59) had an unusual δ^{13}C-CO_2 value of −7.7‰. This value is almost 3‰ heavier than the average of C_4 sugars that we have measured (−11.5 ± 0.3‰), making it difficult to determine the source of its CO_2. One possibility is the use of CO_2 from the air, which has an isotopic composition of −7 to −8‰ (*1*, *11*).

On the other hand, the average δ^{13}C-CO_2 of 11 European and 2 Chilean sparkling wines was −22.0 ± 1.4‰ (n = 13). Given that the δ^{13}C value of sugar beet that we analyzed was −26.3 ± 0.8‰ (n = 5), we would expect the bubble to have a δ^{13}C value of −24.1‰. This expected δ^{13}C value was indeed close to that of the bubbles from European and Chilean sparkling wines, suggesting that sugar beet was used in the second fermentation of these wines. It is also possible to consider a small addition of sugar cane during the second fermentation, which would increase the δ^{13}C value of the CO_2 bubble.

Because the asti sparkling wines do not experience a second fermentation, the δ^{13}C value of CO_2 is produced from the fermentation of natural sugar of the grapes during the first fermentation, yielding a smaller concentration of alcohol (~7.5 °GL). Therefore, it would be expected that the δ^{13}C value of the wine and the δ^{13}C-CO_2 value would differ by ~2‰. This is in fact close to what we observed for the Brazilian certified asti and the genuine Italian asti (**Table 1**). Finally, there are three demi-sec Brazilian sparkling wines (samples 19, 20, and 24) that had an average δ^{13}C-CO_2 of −28.3±0.7‰. There was also one American sparkling wine (sample 67) that had the same δ^{13}C-CO_2 value. In these cases the isotopic composition of the CO_2 bubbles was significantly lighter than that of the wines (**Table 1**). This trend is unique, because in the majority of the other samples, the δ^{13}C-CO_2 values were heavier than the δ^{13}C values of the wine (**Table 1**). One possible explanation for such a difference is the use of a sugar of C_3 origin during the second fermentation, because the δ^{13}C-CO_2 values of these three wines were similar to the values found for sugar from beet (average = −26.3 ± 0.8‰). In Brazil the use of sugar from a C_3 plant is rare, and considering that sugar from sugar cane is the cheapest type of sugar in the Brazilian market, we concluded that the use of a different type of sugar is unlikely.

An alternative explanation would be the addition of CO_2 not produced by the wine fermentation. We measured the δ^{13}C-CO_2 of some Brazilian beverages in which commercial CO_2 has been added. The average of these measurements was −33.9 ± 3.1‰ (n = 5). Therefore, the low values of δ^{13}C-CO_2 observed in some sparkling wine samples could be interpreted as being a mixture between CO_2 produced by natural fermentation from C_4 sugar and commercial CO_2. Using eq 2, we estimated the proportion of the commercial CO_2 as 72, 74, and 78% in samples 20, 19, and 24, respectively (**Table 1**). On the other hand, González-Martin et al. (*11*) found in Spain commercial CO_2 and gassified wines with values near −28‰, which indicates that only commercial gases could be present in samples 19, 20, and 24.

The results of this study indicate that the measurement of δ^{13}C values in sparkling wines can be used as an assay for the fractionation of carbon derived from grape versus C_4 sugar sources. Indeed, it appears that a number of commercially produced sparkling wines have δ^{13}C values consistent with a combination of grape and sugar sources during the fermentation process. Finally, the δ^{13}C values of bubbles in sparkling wines are consistent with C_4 sugars such as corn and sugar cane as a significant source of carbon during the second fermentation step.

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