Contribution to the Study of the Origin of CO_2 in Spanish Sparkling Wines by Determination of the $^{13}C/^{12}C$ Isotope Ratio

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The $^{13}C/^{12}C$ isotope ratio was determined in sparkling wines, granvás, and gassified wines of different Spanish commercial brands with a view to establishing the origin of the CO_2 contained in them. This technique permits a distinction between the CO_2 originating from C_3 and C_4 plants and that coming from industrial sources.

Keywords: *Sparkling wines; $^{13}C/^{12}C$ isotope ratio; characterization of CO_2*

INTRODUCTION

The development of industrialized societies and the consequent rise in living standards have led to increasing demands concerning product quality and typification. With respect to sparkling wines, especially the cavas, we thought the characterization of CO_2 interesting. It is crucial to have available a reliable analytical method that will permit the establishment of the origin of CO_2 since this may come exclusively from the fermentation process or may have been increased artificially owing to the addition of industrial CO_2. It is also interesting to know, or confirm, if it has been formed owing to the addition of sugar to the base wine before production of the secondary fermentation, which is in fact a usual practice in the making of certain wines and cavas.

Depending on the elaboration process, Spanish legislation distinguishes the following.

Cavas: wines whose second fermentation occurs in the bottle, in which the whole process of elaboration and aging must take place until the lees have been eliminated. Depending on their residual sugar contents, the different types of cava are classified as extra brut (brut nature), brut, extra dry, dry, semidry, and sweet, with residual sugar contents ranging from <6 to 50 g/L.

Granvás (large containers): wines whose second fermentation occurs in large sealed containers, from where they are decanted into bottles for commercialization.

Gassified wines: wines in which part or all of the carbon dioxide contained in them has been added.

VEC: sparkling wines of quality whose geographical zones of production differ from those of cavas.

It is well-known that the constitutive molecules of plants are synthesized, under the action of light radiation, from water and carbon dioxide, atmospheric CO_2 penetrating the plants through the stomata of the leaves. The process of photosynthesis may follow two different routes (Martin et al., 1988). In C_3 plants (Calvin cycle) 3-ribulose diphosphate is used to fix the CO_2, and in this, the $\delta^{13}C$ values lie in the −22‰ to −33‰ range. C_4 plants (dicarboxylic acid route or the Hatch−Slack pathway) use oxaloacetate as a CO_2 acceptor and have $\delta^{13}C$ values between −10‰ and −20‰. Among the latter type (C_4) is sugar cane while the grapevine is a C_3 plant such that it is possible to distinguish between the CO_2 produced from the fermentation of the sugar present in the grape must and that originating from added cane sugar.

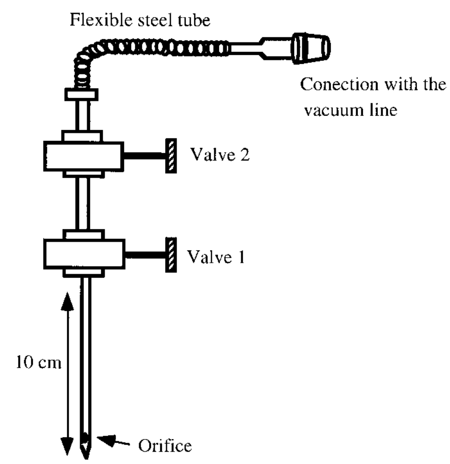

Figure 1. Device for sample collection.

In gassified wines, the origin of the added CO_2 can often be established since, if it comes from the air, the $\delta^{13}C$ ranges from −6.7‰ to −7.4‰. However, it is more likely that "*food quality*" CO_2 will be used, in many cases coming from the combustion of fossil fuels, in which case the $\delta^{13}C$ values will lie in the following ranges (Winkler, 1984): petroleum (−25‰ to −31‰); carbon (−22‰ to −33‰); natural gas (−15‰ to −75‰). Accordingly, it is only possible to identify the CO_2 as coming from a thermal source in cases in which the $\delta^{13}C$ value is less than −33‰.

Determination of the $^{13}C/^{12}C$ isotope ratio (Martin et al., 1986; Cahn, 1989) has also sometimes been used to establish the origin of the ethanol, as has isotopic analysis of $^2H/^1H$ and of $^{18}O/^{16}O$ in combination with other techniques, such as NMR, to determine the geographic origin of wines and other limits of interest. Dunbar (1982) uses the relationship $^{13}C/^{12}C$ to determine the origin of the CO_2 in commercial sparkling wines of New Zealand, distinguishing if it proceeds from C_3 or C_4 plants or industrial sources.

EXPERIMENTAL PROCEDURES

Sample collection. CO_2 samples (about 1 mL) were obtained directly from the gas contained in the bottle using the device depicted (designed in the laboratory) in Figure 1. With valve 1 (Nupro SS-6BG-MM) closed, the air contained

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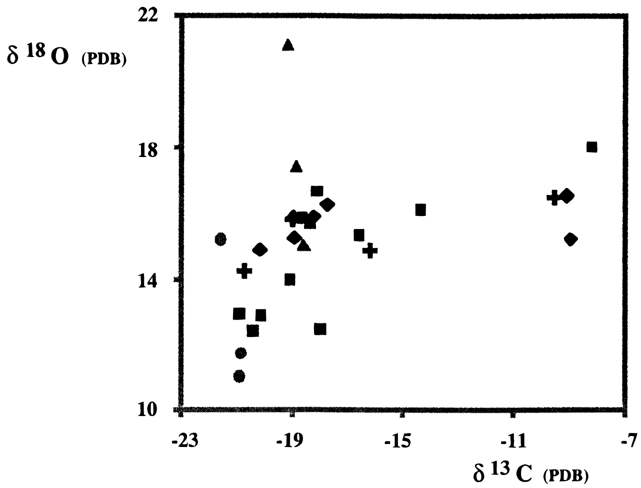

Figure 2. $\delta^{13}C‰_{PDB}$ from CO_2 of must, VEC, and cavas, (brut nature, brut, semidry): ●, must; ◆, brut nature; ■, brut; +, semidry; ▲, VEC.

Table 1. $^{13}C/^{12}C$ and $^{18}O/^{16}O$ Isotope Ratios of the CO_2 from the Fermentation of Grape Must and Cane Sugar

sample	origin	$\delta^{18}O‰_{PDB}$	$\delta^{13}C‰_{PDB}$
grape must	Burgos	11.13	−21.02
	Burgos	11.79	−20.90
	Malaga	15.29	−21.69
cane sugar		27.9	−9.48

Table 2. $^{13}C/^{12}C$ and $^{18}O/^{16}O$ Isotope Ratios of the CO_2 from Cavas

type	sample	anal no.	$\delta^{18}O‰_{PDB}$	$\delta^{13}C‰_{PDB}$
brut classic	1	2	15.94	−19.95
brut nature	2	2	17.67	−9.31
brut nature	3	4	15.27	−9.05
brut nature	4	2	15.96	−19.45
brut nature	5	2	17.43	−18.93
brut	6	2	15.49	−18.43
reserve	7	2	15.07	−18.63
brut nature	8	2	15.33	−18.99
brut nature	9	3	16.34	−20.28
brut nature	10	2	15.96	−18.29
brut	11	3	12.49	−20.41
brut	12	2	16.18	−14.47
brut	13	3	12.80	−18.04
brut	14	3	12.97	−20.19
brut	15	3	13.01	−20.98
brut	16	2	18.07	−8.29
brut	17	2	14.32	−19.17
brut	18	2	15.94	−18.74
brut	19	2	16.70	−18.21
brut	20	2	21.17	−19.19
semidry	21	2	14.95	−16.32
semidry	22	2	14.48	−20.85
semidry	23	2	16.56	−9.63
semidry	24	2	15.89	−19.09

in the installation is removed. Once a vacuum has been created in the system, the cork of the bottle is pierced with the steel needle, which has a lateral orifice through which the gas enters. Valve 2 is closed, valve 1 is opened, and the gas is collected in a steel tube inserted between both valves over a period of 5 min. The CO_2 thus collected is dried and purified in the vacuum line, constructed according to a design afforded by the Scottish Universities Research and Reactor Center (SURRC), with additional modifications performed at the Stable Isotopes Laboratory of the University of Salamanca.

Only samples of the CO_2 present in the gas phase were collected since it has been observed (Dunbar, 1982; Deuser et al., 1975) that the fractionation occurring between gaseous CO_2 and that dissolved in the liquid phase is lower than the errors involved in sample collection of the latter.

$^{13}C/^{12}C$ Measurements. Measurements of isotope abundances were performed with a VG Isogas Mod SIRA Series II mass spectrometer equipped with an IBM PS/2 Mod 50 computer. The results are expressed using the δ notation

Table 3. $^{13}C/^{12}C$ Isotope Ratios of the CO_2 from Granvás

type	sample	anal. no.	$\delta^{18}O‰_{PDB}$	$\delta^{13}C‰_{PDB}$
brut	25	2	15.57	−19.16
semidry	26	3	15.97	−20.45
semidry	27	2	15.88	−18.76
semidry	28	2	14.64	−18.28

Table 4. $^{13}C/^{12}C$ and $^{18}O/^{16}O$ Isotope Ratios of the CO_2 from Gassified Wines

type	sample	anal. no.	$\delta^{18}O‰_{PDB}$	$\delta^{13}C_{PDB}$
semidry	29	2	17.61	−35.32
semidry	30	2	15.03	−37.59
semidry	31	2	17.30	−30.03
semidry	32	2	17.66	−29.73
semidry'	33	2	18.18	−30.48
semidry	34	2	17.68	−35.61
semidry	35	2	12.90	−39.71
semidry	36	2	14.82	−28.24
semidry	37	2	14.98	−38.17
semidry	38	2	19.16	−33.55
semidry	39	2	19.15	−30.97
aguja[a]	40	2	17.95	−25.74
aguja	41	2	19.56	−19.98
aguja	42	2	17.14	−30.54

[a] *Aguja* is the wine that, because of an elaboration process, keeps some of the CO_2 from the fermentation process of grape sugars when it is bottled, and this CO_2 makes bubbles without forming froth when the bottle is opened.

$$\delta^{13}C‰_{PDB} = \frac{^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{PDB}}{^{13}C/^{12}C_{PDB}} \times 1000$$

where $^{13}C/^{12}C_{PDB}$ is the $^{13}C/^{12}C$ ratio for the international PDB (pee dee belemnite) standard, which has been determined as $^{13}C/^{12}C = 0.011\,237\,2$ and $^{18}O/^{16}O = 0.002\,067\,1$ (Hayes, 1983).

RESULTS AND DISCUSSION

Firstly, in order to obtain reference values, a series of determinations was made on must and cane sugar samples subjected to laboratory fermentation in the presence of the yeast *Saccharomyces cerevisiae*. In the first case (must samples) fermentation was performed on about 100 mL of must, obtained from grapes coming from Burgos (1993 crop) and from Malaga (1994 crop). The receptacles used were 1 L bottles sealed with a cork. CO_2 samples were taken at the end of 1 week with the device indicated in the experimental part of this work. In the case of cane sugar, a similar operation was used on a solution of some 100 mL.

The results obtained (Table 1) the measurements of $\delta^{13}C$ in CO_2 coming from the fermentation of grape must (−21.17‰) and cane sugar (−9.48‰) are slightly higher than those found according to the literature (Bricout et al., 1975; Hillaire-Marcel, 1986) for unfermented must (−23.5‰) and that corresponding to the carbon from sugar cane (−11.4‰), indicating that the CO_2 coming from the fermentation is enriched in ^{13}C.

The values of the $\delta^{18}O$ in CO_2 indicate the difference between the geographic origins of the proofs.

Following this, the $^{13}C/^{12}C$ ratio of CO_2 was determined in a series of samples of cava, granvás, and gassified wines supplied by the Regulatory Council of Spanish Sparkling Wines.

The results found for 24 samples of cavas are shown in Table 2; in almost all cases, as expected, values (intermediate between −9.48‰ and −21.17‰) were obtained indicating the presence of CO_2 from two

Table 5. $\delta^{13}C$ and $\delta^{18}O$ in Commercial Gases

sample	anal. no.	origin	$\delta^{18}O‰_{PDB}$	$\delta^{13}C‰_{PDB}$	std. dev
1	4	combustion products ($CO_2 + CH_4$)	−12.97	−36.11	0.02
2	5	fabrication NH_3	−11.02	−32.40	0.06
3	5	purification gases	−12.97	−36.22	0.04
4	4	cogeneration (natural gas)	−13.00	−29.53	0.03
5	4	fabrication carbide	+5.47	−20.37	0.02
6	11	unknown	−13.60	−28.56	0.12

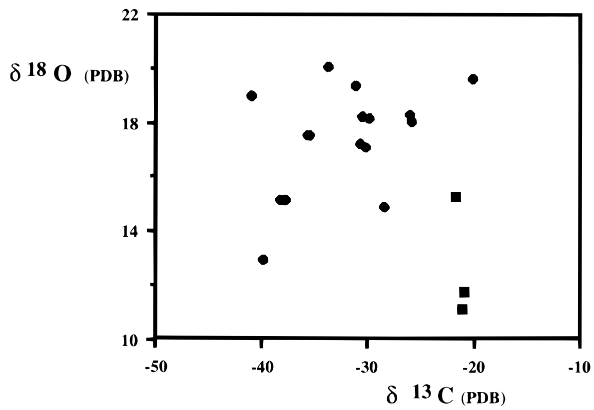

Figure 3. $\delta^{13}C‰_{PDB}$ from CO_2 of must and gassified sparkling wines: ■, grape must; ●, gassified wines.

sources: the sugar contained in the base wine and cane sugar added for the second fermentation to take place in the bottle, Figure 2. In only four cases were excessively high values obtained (between −8.29‰ and −9.63‰), suggesting the presence of atmospheric CO_2.

Additionally, no significant differences were found corresponding to the types brut nature, brut, and semidry.

With respect the samples of granvás, the results obtained (Table 3) reflect an enrichment in ^{13}C, as occurs with the cavas. Industrial CO_2 used as counterpressure gas would not have been employed in any of the cases since, had this been so, the $\delta^{13}C$ values would have been much more negative than those found for CO_2 coming from the fermentation of must.

In view of the low $\delta^{13}C$ values obtained, the results corresponding to 14 samples of gassified sparkling wines (Table 4) indicate that to a greater or lesser extent direct carbonation had been implemented.

In order to determine the origin of the CO_2 present in these bottles, the $\delta^{13}C$ values for "*food quality*" CO_2 were measured in a series of commercially available samples provided by the Spanish Oxygen and Metal Carbides Company. The results are shown in Table 5, which also includes the origin according to the manufacturer's specifications.

In the case of certain gassified sparkling wines, the $\delta^{13}C$ values are clearly different from those obtained for other types of sparkling wine and for must (Figure 3) with much lower ^{13}C contents. This permits one to distinguish between gassified sparkling wines and the other sparkling wines. From the $\delta^{13}C$ values it can be deduced that as under pressure gas most of them use CO_2 from combustion products or products deriving from the purification of gases rich in CO_2 ($\delta^{13}C \approx -36‰$), although to state this unequivocally it would have been necessary to have available all the "*food quality*" types of CO_2 existing on the market.

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