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Use of Isotope Ratio Determination ($^{13}C/^{12}C$) to Assess the Production Method of Sparkling Wine

Joël S. Rossier*, Valérie Maury, Laetitia Gaillard, and Elmar Pfammatter

Abstract: The production of a sparkling wine can be performed with different methods taking from a few weeks to several years, which often justifies a difference in added value for the consumer. This paper presents the use of isotope ratio $\delta^{13}C$ measurements combined with physico-chemical analyses for the determination of mislabelling of sparkling wines produced by 'ancestral', 'traditional', 'closed tank' or 'gasification' methods. This work shows that the isotope composition of CO_2 compared with that of the corresponding dried residue of wine (DRW) can assess whether carbonate CO_2 in a sparkling wine originates from alcohol fermentation or from artificial gas addition. Isotopic ratios expressed as $\delta^{13}C_{CO2}$ and $\delta^{13}C_{DRW}$ measurements have been obtained for each wine by gasbench isotopic ratio mass spectroscopy and cavity ring down infrared spectroscopy, respectively. When the difference between $\delta^{13}C_{CO2}$ and $\delta^{13}C_{DRW}$ is negative, the presence of artificial CO_2 can be undoubtedly inferred, which would exclude the production methods 'ancestral' or 'traditional' for instance. Other parameters such as alcohol content, sugar and acid distributions are also important to complete the analytical panel to aid fraud tracking.

Keywords: Ancestral method · Closed tank · Isotopic ratio · Sparkling wine · Traditional method

1. Introduction

Our laboratory has specialised on food fraud detection by means of isotopic finger printing for the last 15 years. Different methods have been introduced based on isotopic ratio mass spectrometry (IRMS) including a method to detect the geographical origin of fruit and vegetables grown in Valais thanks to the isotopic ratio of ^{18}O in the water fraction of those products.[1] In order to complete the scope of control, other kinds of analysis have been introduced, based on the $^{13}C/^{12}C$ isotope ratio $\delta^{13}C$. This parameter enables the integrity of natural products to be checked in case of suspicion of addition of artificial ingredients; for instance the EA-IRMS method has been used to determine whether a honey has been adulterated by the addition of cane sugar, either as a non-permitted additive or as a feeding solution for the bees. More recently, our laboratory has introduced a method to determine the $\delta^{13}C$ of CO_2 in sparkling drinks, including sparkling water or wine by the gasbench IRMS technique. Other laboratories have used similar methods for $\delta^{13}C$ analysis of CO_2 in ciders.[2] This article shows how this technology has been applied in the field of sparkling wine control and is an addition to other studies already performed on the authenticity of sparkling drinks using the isotope ratio.[3–7] On the other hand, $\delta^{13}C$ of the dried residue of wine (DRW) has also been developed in order to detect whether non-volatile wine components originate from grape or from chaptalisation (sugar addition to harvested grapes to increase the alcohol level) with cane sugar for example. The latter application was implemented by cavity ring down IR spectroscopy (CRDS) instrumentation recently acquired in our laboratory.

Sparkling wines obtained by 'ancestral' or 'traditional' methods are perceived as high value end products whereas industrial sparkling wines, often produced in 'closed tanks' or simply 'gasified' are found at very low prices in supermarkets. These different methods reflect different costs of production as well as a strong differentiation for the consumer. It is clear that the label 'traditional' or 'ancestral' method provides an added value which could lead to mislabelling. For the consumer, it is impossible to detect this kind of fraud by simple degustation; therefore, it is important that food control authorities are able to track whether a producer is misleading the consumer by mislabelling its sparkling wine.

Table 1 briefly summarises what is commonly expected for the different production methods. 'Ancestral' method means that the sparkling wine is produced with minimal intervention; the addition of '*liqueur de tirage*' or '*liqueur d'expédition*' (see Table 1 for an explanation of the terminology; the French expressions are used throughout) is forbidden, which means that the whole wine carbon component should originate exclusively from the grapes; furthermore, after a partial alcoholic fermentation in a tank the cuvée is bottled and further fermented until equilibrium is reached, stopping the fermentation at a relatively low alcoholic content (~7.5 % vol.). Once bottled, this wine is fermented without transfer to a tank, addition or filtration steps; as a consequence the residual sugar should exclusively come from the grapes (no beet or cane sugar addition), and the CO_2 should only originate from the fermentation of the grape sugar; in addition these wines should not have undergone malolactic fermentation. At the end of the fermentation, a disgorging step is performed to remove most of the yeast and other precipitate, but no filtration is performed during the production process. It has to be noted that some exception to these rules have been introduced for the method 'dioise ancestral' for which a transfer to a closed tank together with a filtration is permitted.[8]

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Table 1. Vinification processes of the different production methods.

	Ancestral		Traditional	Closed tank	Gas addition
		Dioise			
alcoholic fermentation	partial	partial	yes	optional	optional
malolactic fermentation	no	no	optional	optional	optional
'*liqueur de tirage*'[a] addition	no	no	yes	optional	no
'in-bottle' fermentation	yes	yes	yes	no	no
'in closed tank' fermentation	NA[b]	NA	NA	yes	no
authorised artificial CO_2 addition	no	no	no	no	yes
'*liqueur d'expédition*'[c] addition	no	no	optional	optional	optional
disgorging[d]	yes	no	yes	no	no
transfer in '*cuve de tirage*'	no	yes	no	yes	yes
filtration	no	yes	no	yes	yes
isobarometric '*Tirage*'[e]	no	yes	no	yes	yes

[a]*Liqueur de tirage:* solution containing yeast and sugar. Sugar can be C3 or C4. This solution is added when tranquil wine is transferred to the bottle or to the closed tank for the second alcoholic fermentation. [b]Not applicable. [c]*Liqueur d'expédition:* solution containing wine and sugar. Sugar can be C3 or C4. This solution is added after disgorging and determines which type of wine is produced (*brut, sec, demi sec, doux*). Quantity of sugar present in the solution defines the type of sparkling wine. [d]Disgorging describes a process that allows the elimination of a large part of residues present in bottle at the end of fermentation. This process substitutes filtration for some methods. Filtration eliminates all residues and the sparkling wine is totally limpid, disgorged sparkling wines retain some residue. [e]*'tirage isobarique':* This term is used when sparkling wine is transferred from tank to bottle. During this process, pressure is used to transfer wine from a closed tank into the bottle. This pressure is generally done by applying a flux of artificial CO_2.

Most famous sparkling wines such as Champagne or Cremant are produced by the so-called 'traditional' method, which is defined in the EU regulation 607/2009 as follows:[9]

"The expressions 'bottle-fermented by the traditional method' or 'traditional method' or 'classical method' or 'classical traditional method' may be used only to describe sparkling wines with protected designations of origin or with a geographical indication of a third country or quality sparkling wines provided the product:

(a) was made sparkling by a second alcoholic fermentation in the bottle;

(b) stayed without interruption in contact with the lees for at least nine months in the same undertaking from the time when the cuvée was constituted;

(c) was separated from the lees by disgorging."

This definition means that the vinification consists of taking a still wine (typically ~11% vol. alcohol, low residual sugar, filtered, stabilised with or without malolactic fermentation) of a given origin and adding a '*liqueur de tirage*', which means about 30 g/L of sugar and letting it ferment in bottle in a controlled environment with regular manual or industrial bottle rotation. This method excludes transfer in tanks or filtration, but it is permitted after a disgorging step to add an '*liqueur expédition*' (sugar solution and wine) which would lead to a wine of different sweetness and therefore different classification (*brut, sec, demi sec, doux, etc.*); in any case, the CO_2 present in the bottle should arise exclusively from the in-bottle fermentation and not from any artificial source.

In order to decrease both the time and cost of sparkling wine production, industrial methods such as 'closed tank' fermentation are practiced. This consists of a double-coated steel tank which can withstand pressure up to several bars. In these tanks, different sparkling wines can be produced, but in most of the cases, the closed tank is filled with still wines supplemented with a '*liqueur de tirage*' and fermented for two to three weeks. With these industrial techniques, the temperature and pressure can be optimised in the closed tank to maximise the fermentation efficiency; after filtration, these sparkling wines are standardised and bottled with a process called '*tirage isobarique*'. These sparkling wines can be sold at a very competitive price compared to those produced with an 'ancestral' or 'traditional' method.

Finally, gasified wine can be obtained by simply injecting CO_2 into still wine and bottling, which has very little in common with 'ancestral' or 'traditional' methods in terms of cost and prestige.

The $\delta^{13}C$ isotope ratio has previously been used to detect the addition of cane sugar in the production of sparkling wine.[10] Photosynthesis in most plants follows one of two main pathways (C3 or C4) and plants that exhibit these different pathways have been shown to differ in the carbon isotope ratios of their products. Photosynthesis in sugar cane follows the C4 pathway, which means that the first organic molecule formed after uptake of atmospheric CO_2 is composed of four atoms of carbon. Grape vines, however, follow the C3 photosynthetic pathway in which the first organic molecule is composed of three atoms of carbon. The stable carbon isotope ratios (expressed as $\delta^{13}C$) of C4 and C3 plants are significantly different, with $\delta^{13}C$ values for C4 plants varying from –11 to –14 and those of C3 plants varying from –24 to –32.[11]

In order to track fraud and protect the consumer, exhaustive analyses of 30 different sparkling wines produced either by 'ancestral', 'ancestral dioise', 'traditional', 'closed tank' and 'gasified' methods have been performed and reported in this work. Parameters such as $\delta^{13}C$ of CO_2 reveal whether the wine has been produced exclusively in bottle, $\delta^{13}C$ of the dried residue of wine would tell whether cane sugar has been added, whereas other parameters such as alcohol content, distribution of residual sugar (glucose/fructose), distribution of acids (malic/lactic) disclose something about the fermentation process of the wine, and therefore contribute to the plausibility of the production method declared on the label.

2. Material and Methods

The methods of the different analyses are briefly described hereafter.

2.1 *Isotope Analysis*

Isotope ratio of carbon $^{13}C/^{12}C$ is a fingerprint measurement, which can reveal

the origin of food ingredients. This measurement is expressed as $\delta^{13}C$, as expressed in Eqn. (1):

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} - 1 \right) \times 1000\ ‰ \quad (1)$$

The $^{13}C/^{12}C$ standard established by convention was the Pee Dee Belemnite (PDB) and was based on a Cretaceous marine fossil, Belemnitella americana, which was from the Pee Dee Formation in South Carolina. This material had an anomalously high $^{13}C/^{12}C$ ratio (0.0112372), and was established as the $\delta^{13}C$ value of zero.[12]

In the particular case of sparkling wine, we want to know whether CO_2 present in the bottles is of artificial origin, or the product of the alcoholic fermentation of sugar. Artificial CO_2 is mainly obtained from the combustion of oil with typically $\delta^{13}C_{CO2}$ values lower than –30. On the other hand, CO_2 originating from the fermentation of grape or cane sugar shows a typical value around –25 or –10 respectively. In this paper we also measured the rest of the composition of wine (glycerol, polyphenol, residual sugar, *etc*.) which should mainly originate from grape fermentation; for this reason, a further $^{13}C/^{12}C$ isotope ratio measurement of dried residue of wine ($\delta^{13}C_{DRW}$) was conducted. Comparison between these analyses reveals whether natural or artificial CO_2 is present in the bottle. Therefore, if the difference between $\delta^{13}C_{CO2}$ and of $\delta^{13}C_{DRW}$ is negative, artificial CO_2 has been added. Furthermore, analysis of $\delta^{13}C$ of CO_2 and dried residue of wine reveals whether sugar has been added and in this case, which kind of sugar was used during the production of sparkling wine.

2.1.1 *Analysis of CO_2 by IRMS (Gasbench)*

CO_2 analysis is performed with a protocol similar to that explained earlier.[1] The protocol differs only for the sample preparation. A bottle of sparkling wine is cooled at 2–4 °C for a minimum of one night in order to increase CO_2 solubility. After cooling, 50 µL of the sparkling wine is transferred in a 20 mL tube and sealed with a Teflon/silicon septum; CO_2 present in the tube is pushed in the IRMS with helium and measured. Measurement is repeated three times for each sample.

2.1.2 *Dried Residue of Wine Analysis by CRDS*

Special tin capsules (3.5 × 9 mm, Säntis, SA84992102) are prepared. For each sparkling wine, six capsules are prepared. 20 µL of sparkling wine are deposited with a micropipette and heated at 65 °C overnight. The isotope ratio $^{13}C/^{12}C$ is determined by pyrolising the sample at a temperature of approximately 1600 °C. This technique done by Wavelength-Scanned Cavity Ring Spectroscopy (WS-CRDS) is briefly explained hereafter. The sample is combusted by reaction between pure O_2 (quality 4.7) and tin into its elementary constituents. Chromium oxide is used as a catalyst. At the end of the combustion, excess O_2 is adsorbed with help of copper wire. If sulphur has been produced, it will be eliminated by niobium oxide and silver wool. Vector gas drives gas formed from the dried residue of wine combustion into a hot transfer line composed of steel to WS-CRDS (Picarro ^{13}C CM-CRDS System, B2221-i). Before and after each group of analyses, injection of oil (NBS 22) as a reference analysis is performed. Analysis conditions: nitrogen flow is 100 mL/min, vector gas (N_2, quality 5.0), oxygen flow 30 mL/min, reactor temperature (for combustion) is 980 °C, sample delay is 10 sec, sample stop is 1 sec, oxygen stop is 30 sec and total run time is 300 sec. Standard deviation is 0.3 δ for $\delta\ ^{13}C$ analysis.

2.2 *Other Analyses*

Other parameters such as alcohol, fructose, glucose, malic and lactic acid are analysed by FTIR by the following method.

2.2.1 *FTIR Measurement*

Approximately 20 mL of sample (sparkling wine) are placed in a 50 mL Erlenmeyer and treated in an ultrasonic bath until all CO_2 has disappeared. Samples are directly injected in the FTIR instrument (Bruker optics GmbH, module ATR platinum diamond 1) and each sample is analysed 3 times. The reported values are the mean of the three analyses. All parameters are measured concurrently (sugar concentration, alcohol concentration, *etc.*) and evaluated with proprietary Bruker software.

Malic and lactic acid analysis gives information whether malolactic fermentation has occurred. Since this method gives only a semi-quantitative answer for these two parameters, it will only be reported if malolactic fermentation has been done or not for each wine and not the absolute value of malic and lactic acid.

3. Results

Table 2 summarises all results obtained by the analysis on sparkling wines.

3.1 *Ancestral Method (Wine 1)*

The ancestral method is relatively rare and it was difficult to obtain a sample from normal retail outlets for this study. The results obtained with this wine reflect perfectly the analytical profile (Fig. 1), which can be theoretically expected from an ancestral wine. The principle of ancestral production is recapitulated here. The grapes undergo a first alcoholic fermentation in a standard tank until the alcohol concentration reaches ~5–6 %vol.[13] At this point, partially fermented cuvée is transferred into bottles and fermentation is starting again until equilibrium is reached. This wine has a low alcohol content of 7.5% vol. and a high concentration of residual sugar composed of a larger fraction of fructose over glucose (G/F = 40%). According to Beranger,[14] the content of glucose and fructose is almost equivalent in non-fermented juice; during alcoholic fermentation glucose is more rapidly consumed than fructose so that, if a natural fermentation has occurred, fructose should dominate over glucose.

Last but not least, $\delta^{13}C$ of CO_2 has a value of –24.59 whereas $\delta^{13}C$ of the dried residue of wine has a value of –25.46. This slight positive difference in $\delta^{13}C$ between CO_2 and dried residue of wine means that the CO_2 originates from the in-bottle fermentation of the grape sugar and not from any artificial gas addition. Martinelli *et al*.[10] have already observed and explained the slight positive difference in $\delta^{13}C$ between CO_2 and dried residue of wine; this is due to the fact that during alcoholic fermentation enzymes favour sugar molecules with lighter carbon. Furthermore CO_2 partition between liquid and gas phase favours light CO_2 in the gas phase, leading to this slight positive difference. From Table 2 it is possible to see that this wine has not undergone malolactic fermentation since it could not be initiated within the closed bottle.

For all preceding reasons it is possible to assess that this wine declared as produced by the 'ancestral' method is correctly labelled and the detected parameters are in line with a sparkling wine produced without any bottle opening after the alcoholic fermentation.

It should be noted that in some cases, ancestral method regulations allow the use of a transfer between the end of the alcoholic fermentation and the final bottling in clean bottles. This specific case will be presented in the next section.

3.2 *Ancestral Dioise Method (Wines 2–4)*

The 'dioise ancestral' method derives from the 'ancestral' method with some specific differing features. After fermentation in bottle, the sparkling wine is transferred to a closed tank to enable the bottles to be washed to remove any trace of residue. After washing, sparkling wine is filtered and transferred back into the bottles. The analytical profile of the wine is expected to be similar to the ancestral one described in

Table 2. Analytical parameters measured in the sparkling wines.

Declared method of production	Alcool [% vol.]	Fructose [g/L]	Glucose [g/L]	Ratio Glucose/Fructose (G/F)	Malolactic Fermentation (FML)	$\delta^{13}C$ of CO_2	$\delta^{13}C_{DRW}$	$\delta^{13}C_{CO_2} - \delta^{13}C_{DRW}$	Origin of CO_2	Origin of Wine
1. ancestral	7.46	48.6	19.2	0.4	no	–24.6	–25.5	0.87	natural	France
2. dioise ancestral	6.92	45.3	16.3	0.36	no	–28.1	–26.8	–1.3	artificial	France
3. dioise ancestral	6.5	50.4	20	0.4	no	–27.3	–25.8	–1.54	artificial	France
4. dioise ancestral	8.3	40.9	13.1	0.32	no	–26.9	–26.9	–0.03	artificial	France
5. traditional brut	11.9	5.8	5.3	0.91	yes	–21.5	–26	4.47	natural	France
6. traditional brut	12.1	5.3	4.7	0.89	yes	–23	–26	2.98	natural	France
7. traditional brut	12.2	1.1	<0.1	–	yes	–22.7	–26.9	4.18	natural	Switzerland
8. traditional brut	12.4	5.1	3.5	0.69	–	–22.8	–26.7	3.9	natural	Switzerland
9. traditional brut	12.4	5.52	4.88	0.88	no	–22.6	–26.6	4.04	natural	Switzerland
10. traditional brut	12.2	5.9	4.5	0.76	no	–22.7	–26.6	3.9	natural	Switzerland
11. traditional brut	11.7	4.9	5.3	1.08	no	–23.3	–25.8	2.53	natural	Spain
12. traditional sec	12.1	10.4	11.7	1.13	yes	–22.7	–25.5	2.8	natural	Spain
13. traditional semi-sec	11.7	19.3	20.2	1.05	yes	–23.5	–25.5	2	natural	Spain
14. traditional semi-sec	11.3	18.3	18.5	1.01	yes	–26.9	–25.3	–1.6	artificial	Spain
15. traditional	11.2	18.8	18.4	0.98	yes	–35.1	–26.1	–9	artificial	Spain
16. traditional brut	12	4.4	3.2	0.73	no	–10.2	–24.8	14.6	natural	Switzerland
17.traditional brut	11.8	6.9	5.5	0.8	no	–13.1	–25.6	12.4	natural	Switzerland
18. traditional brut	11.9	0.62	0.6	0.97	no	–10.3	–24.7	14.4	natural	Spain
19. traditional brut	11.3	7.55	4.62	0.61	no	–12	–25.4	13.4	natural	Spain
20. Closed tank doux	6.9	41.9	26.1	0.62	no	–21.9	–25.5	3.63	natural	Italy
21. Closed tank brut	11.1	7.16	6.4	0.89	yes	–22.4	–25.7	3.3	natural	France
22. Closed tank brut	11.3	6.92	7.08	1.02	no	–26.1	–26.4	0.3	natural	France
23. Closed tank	10.1	8.9	5.67	0.64	yes	–30.4	–25.4	–5	artificial	Italy
24. Closed tank	10.2	9.72	6.63	0.68	no	–26	–24.4	–1.6	artificial	Italy
25. Closed tank extra-sec	10.6	11.1	3.9	0.35	no	–11.6	–19.3	7.7	natural	Italy
26. Closed tank brut	10.6	9	5.23	0.58	no	–11.1	–19.9	8.8	natural	Italy
27. Closed tank extra-sec	10.7	11	5.22	0.47	no	–11	–19	8	natural	Italy
28. Closed tank extra-sec	11.1	8.2	4.6	0.56	–	–10.3	–19	8.66	natural	Italy
29. Wine-based gaseous drink	7.74	41.2	41	1	no	–31	–27.2	–3.8	artificial	Italy
30. Wine-based gaseous drink	7.3	39.8	40.1	1.01	–	–28.8	–26.4	–2.42	artificial	France

section 4.1 above, but is only partially the case as presented in Fig. 2.

The analysis of results shows a negative difference between the δ^{13} value of CO_2 and dried residue of wine ($\delta^{13}C_{CO2} - \delta^{13}C_{DRW} < 0$ see explanation in section 2.1) which means that CO_2 is partially of artificial origin. After discussion with sparkling wine producers,[15] the presence of this artificial CO_2 could be rationally understood. As described earlier, after fermentation in bottle, this sparkling wine is transferred into closed tanks. When the sparkling wine is returned from the closed tanks to the bottles, artificial CO_2 is used to compensate for the volume decrease in the closed tank as the volume in the tank decreases. During this process called '*tirage isobarique*' artificial CO_2 is dissolved and exchanged into the wine and it becomes measurable. It is interesting to see that the amounts of measurable artificial CO_2 can vary a lot within the same batch. This can be explained by the fact that the first bottle filled will not present any trace of artificial CO_2 but a bottle filled when the tank is almost empty will present a significant concentration of artificial CO_2. So it is expected that concentration of artificial CO_2 will change as a function of when bottles are filled. Finally the question remains: should this addition of artificial CO_2 be declared on the bottle, similar to the requirement of sparkling water labelling 'contains artificial CO_2'? Indeed, regulations state that sparkling wine should contain CO_2 exclusively originating from the alcoholic fermentation if not otherwise labelled.

The other physico-chemical parameters show that the sparkling wines follow the requirements of the ancestral method, described in section 3.1.

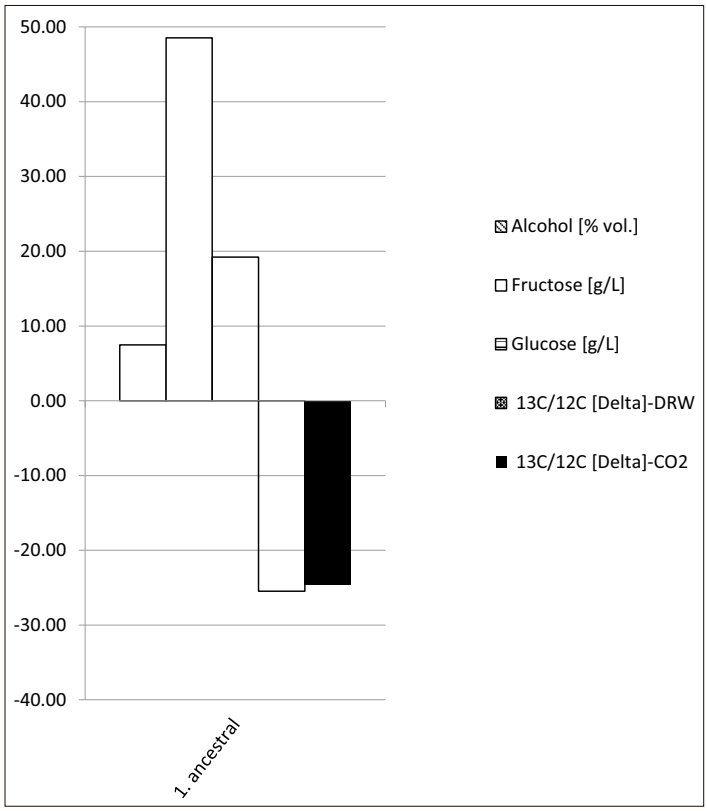

Fig. 1. Results obtained for all tested parameters for sparkling wines, in function of bottle inscription, produced by the 'ancestral' method.

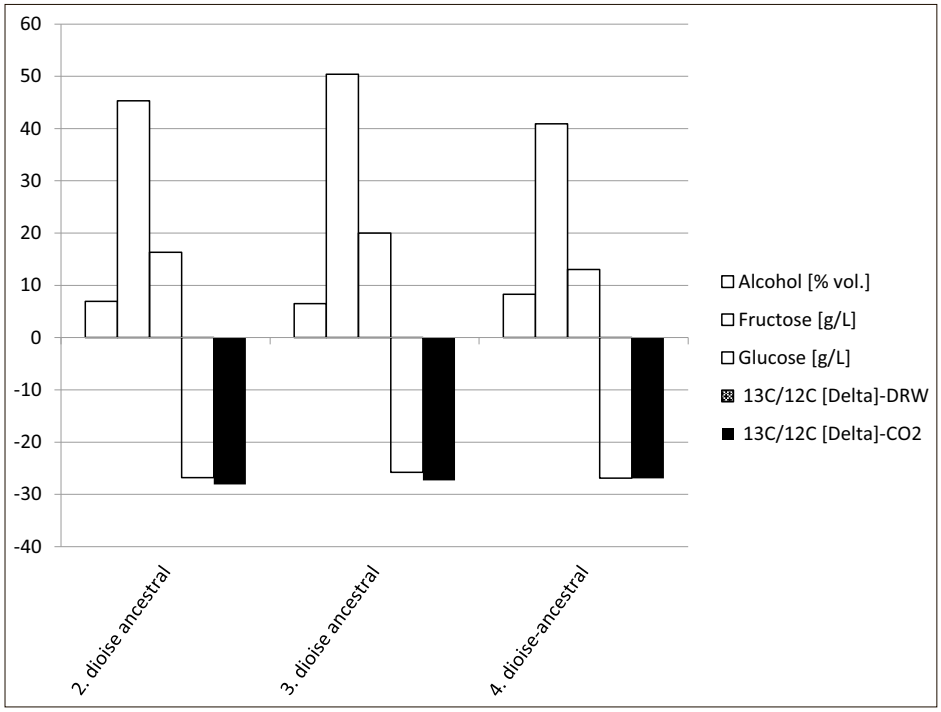

Fig. 2. Results obtained for all tested parameters for sparkling wines, in function of bottle inscription, produced by the 'dioise-ancestral method'. $\delta^{13}C_{CO2} - \delta^{13}C_{DRW}$ exhibits a negative value, revealing the presence of artificial CO_2.

3.3 *Traditional Method (Wines 5–19)*

Sparkling wines 5–19 (Table 2) labelled with 'traditional method' have been analysed and the results are reported in Fig. 3. Wines 5–11 present a typical profile of sparkling wine produced by the 'traditional method': alcohol concentration is around 12+/–1% vol., sugar concentration is low and the fructose content is higher than the glucose content, meaning that the '*liqueur de tirage*' added to the still wine has correctly fermented to generate CO_2. Finally the difference between $\delta^{13}C$ of CO_2 and of the dried residue of wine is positive, showing that no artificial gas has been added, which is expected with the in-bottle fermentation. Half of these wines have undergone malolactic fermentation in order to reduce the acidity in the still wine which is not surprising. First of all, all these wines are '*brut*', meaning that the sugar concentration must lie between 0 g/L and 15g/L, which is respected in each case. The analyses of wines 12 and 13 are very similar to those previously discussed, except that the sugar content is higher and that fructose and glucose exhibit similar concentrations. This sugar originates therefore from the addition of the '*liqueur d'expédition*' added after all fermentation steps in order to sweeten the wine, which is declared with the labels '*sec*' and '*semi sec*' respectively. Those two wines are also considered as conform.

Wines 14 and 15 show profiles similar to wines 12 and 13 concerning the sugar, but the difference of $\delta^{13}C_{CO2} - \delta^{13}C_{DRW}$ is negative, which definitely shows the presence of artificial CO_2. This fact clearly proves that these wines are mislabelled since the presence of this CO_2 cannot be explained if no transfer in tank is done. It is noted that bottles 12 and 13 are half-bottles (37.5 cL), and that a transfer is authorised for technical reasons even for sparkling wines obtained by the traditional method,[16] for this volume. Therefore, the presence of artificial CO_2 is explained for the same reasons as in the 'Ancestral-Dioise'.

Finally, the analyses of $\delta^{13}C$ values of CO_2 of wines 16–19 lay around –11+/–1.5 which originate from the addition of cane sugar (C4) in the '*liqueur de tirage*' which also conforms to the rules.

3.4 *Closed Tank*

The closed tank method enables sparkling wines to be obtained by various vinification steps. A closed tank is hermetically sealed and can withstand the pressure induced by the production of CO_2 of alcoholic fermentation. Furthermore, the tanks are equipped with a double mantel, which enables the wine to be cooled or warmed in order to optimise the work of yeast. With this equipment it is possible to produce sparkling wine directly from partially fermented juice similarly to the ancestral method vinification or from still wine to which yeast and '*liqueur de tirage*' is added. The wine produced with this technology will not undergo fermentation in bottle, which renders it much less costly and risky to produce. At the end of the in-tank fermentation, the wines undergo a filtration step, which efficiently removes the remaining yeast and other suspended material; finally the sparkling wine will be filled in the step called '*tirage*' which necessitates the use of CO_2 pressure in the closed tank to compensate for the volume loss during the '*tirage*' step.

For all reasons explained above, it is possible to obtain diverse physico-chemical parameter profiles for the sparkling

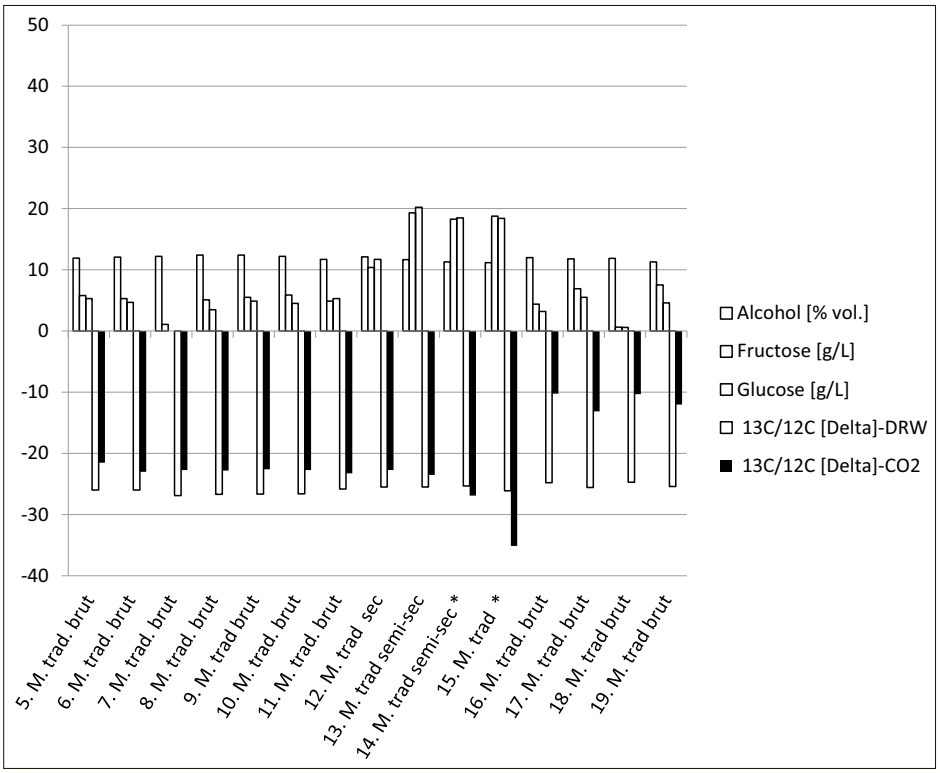

Fig. 3. Results obtained for all tested parameters for sparkling wines, in function of bottle inscription, produced by 'traditional' methods.

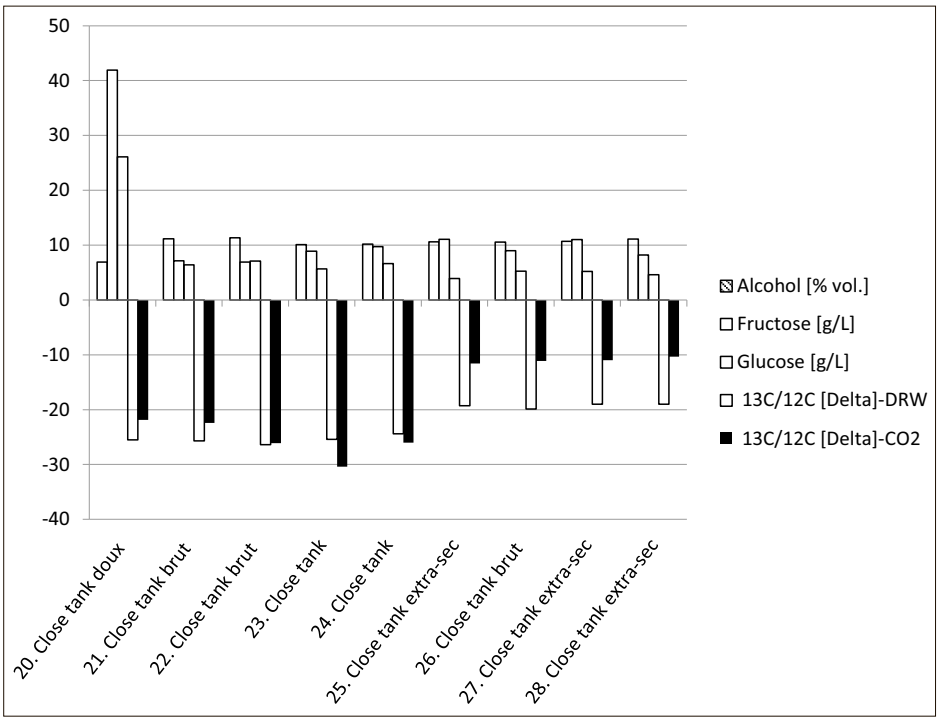

Fig. 4. Results obtained for all tested parameters for sparkling wines, in function of bottle inscription, produced by the closed tank method.

wine produced in closed tanks. Results obtained for sparkling wine 20 show a profile which is similar to the ancestral one (Fig. 4), with a low alcohol concentration (~7% vol., residual sugar >65 g/L with fructose dominating glucose and $\delta^{13}C_{CO2} - \delta^{13}C_{DRW}$ >0). Analysis of wines 21 and 22 show the same profile as found for the 'traditional method' so it is possible that these bottles have been filled at the beginning of the process of transfer. The main parameter which indubitably reveals the closed tank production of these wines is the filtration step; indeed, those wines were much clearer than the one produced by the ancestral method. By eye it is generally possible to distinguish residual particles (yeast, protein cluster, *etc.*) in both 'ancestral' and 'traditional method' produced wines; if disgorging efficiently removes yeasts after the in-bottle fermentation the sparkling wine still contains some turbidity. When a sparkling wine has been produced in a closed tank, it will undergo a filtration rendering it very clear. Sparkling wines 23 and 24 exhibit a negative $\delta^{13}C_{CO2} - \delta^{13}C_{DRW}$ value, which reveals the use of artificial CO_2 during 'isobaric *tirage*'. Finally, sparkling wines 25 to 28 show profiles which reveal the use of cane sugar (C4) in the '*liqueur de tirage*' as well as in the '*liqueur d'expédition*'. Since these wines have been filtered, the remaining carbon source of the dried residue of wine is substantially composed of dissolved sugar (C4), which causes a shift in the $\delta^{13}C_{DRW}$ towards higher values (19 +/–1). In contrast, 'traditional wines' (wines 16–19 in Table 2) to which C4 sugar has been added for alcohol fermentation exhibit a $\delta^{13}CO_2$ value around –10 but the $\delta^{13}C_{DRW}$ stays around a C3 value (25+/–1) probably due to the presence of non-filtered residues which remain after disgorging. This last parameter represents a good differentiator between sparkling wines produced with either closed tank or traditional methods.

3.5 *Gasification Method*

A simple gasification method can be used as well and it conforms to regulations if it is correctly labelled. This method only needs the addition of artificial gas to a still wine with or without the addition '*liqueur d'expédition*'. Sparkling drinks obtained by this method are named wine-based gaseous drinks, which can be further diluted by the addition of water. Drinks have been analysed and their profile is shown in Fig. 5.

As expected, sugar has been added and has undertaken no fermentation (fructose and glucose at a similar concentration); the presence of artificial CO_2 is revealed by the isotopic ratio $\delta^{13}C_{CO2} - \delta^{13}C_{DRW}$ <1. It is interesting to note that the presence of added carbonate was included on the labels on these bottles, which means that they conform to legislation.

4. Conclusion and Perspectives

As explained in this article, it is mostly possible to determine if a sparkling wine was produced by the 'ancestral method', 'dioise ancestral method' or by 'traditional methods'. Different parameters analysed allow the exclusion of some production methods as a function of the analytical profile. Depending on the case, one single parameter permits to definitively conclude that the label is non-conform, as for instance if artificial CO_2 is found in 'ancestral' or 'traditional' method labelled

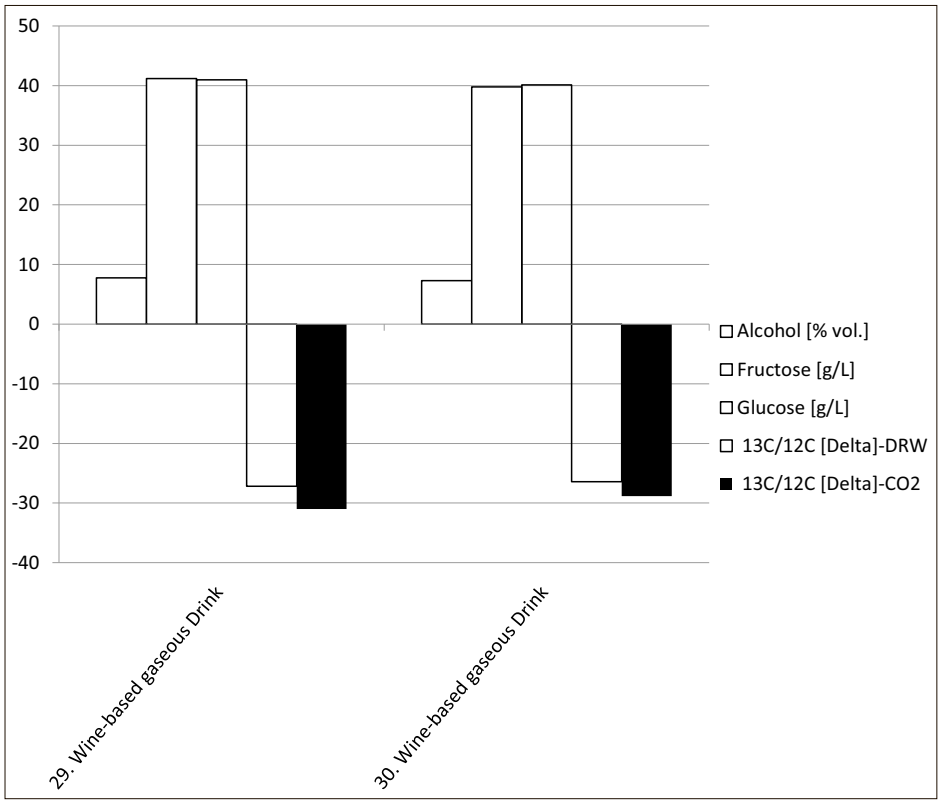

Fig. 5. Results obtained for all tested parameters for wine-based gaseous drinks.

sparkling wines (for bottles of 75 cL). In other cases, a combination of different analytical parameters is necessary, which necessitates a clear understanding of the specificity of the different elaboration methods, *i.e.* which vinification process can be expected for the different methods indicated (labelled). This article is a modest contribution to allow authenticity in sparkling wines to be controlled in order to protect consumers and producers from dishonest providers.

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