Use of $^{13}\text{C}/^{12}\text{C}$ Ratios for Studying the Origin of CO$_2$ in Sparkling Wines

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Introduction

The methods used for the production of a sparkling wine can be divided into three general categories. These are a) the Champagne process, b) the tank fermentation (or Charmat) process or c) direct carbonation. The cost of producing wines therefore be desirable to have an analytical method that could determine the method by which a sparkling wine was made.

A brief description of the methods of sparkling wine production follows: a) Champagne. This is a two-stage fermentation process with step one being the fermentation of the must (grape juice) to dryness as is done in still wine production. All but a small portion of the yeast cells are removed and the wine is then bottled along with a small quantity of sugar solution. The fermentation then restarts in the bottle and continues until the sugar has been used up. During fermentation the bottles are placed approximately 30°C above horizontal and turned daily so that the yeast growth is slowly transferred to the neck of the bottle. When all of the yeast is resting against the cork, the wine is chilled and the cork quickly removed. A small quantity of liquer (wine with approximately 20 % alcohol and 50 % sugar or a brandy and sugar solution) is added to replace the lost wine. In addition, up to 150 ppm sulphur dioxide is added to prevent further fermentation. The bottle is then recorked.

b) Tank Fermentation (Charmat Process). There are many variations possible with this process but basically it involves fermenting the wine in bulk in large tanks under pressure. The wine is then cleared of yeast and residual sweetening is added. Sulphur dioxide is added to prevent further fermentation and the already sparkling wine is bottled under pressure.

The advantages of a system such as this that it can be done on a large scale without the high labour content that is required with the bottle fermentation method to rack bottles and remove corks. However, the quality of sparkling wine produced by this method is not considered to be of the same standard as that produced by the traditional champagne method.

c) Carbonation. Carbonated wines are produced by directly injecting "food grade" cylinder CO$_2$ into a still wine prior to bottling. This system is the cheapest of all and produces a wine that is generally considered to be of inferior quality [1]. It is known that two basic pathways exist by which a plant is able to fix CO$_2$, and that the isotope discrimination against $^{13}\text{CO}_2$ by each of these pathways is different. The C-3 (or Calvin) pathway uses ribulose-1,5-diphosphate as the CO$_2$ acceptor molecule and the carbon of plants fixed via this pathway have $\delta^{13}\text{C}$ values (definition of $\delta^{13}\text{C}$ given in "Methods" section) in the range of $-22^{0/00}$ to $-33^{0/00}$ (parts per thousand) [2]. The alternative pathway called the C-4 or Hatch-Slack pathway uses oxaloacetate as the CO$_2$ acceptor molecule, and plants utilising this method of photosynthesis have $\delta^{13}\text{C}$ values in the range of $-10^{0/00}$ to $-20^{0/00}$ [2]. The C-4 pathway is however present in only a minority of plants such as the tropical grasses e.g. corn and sugar cane. As grapes use the C-3 method of CO$_2$ fixation it is thus possible to distinguish CO$_2$ produced from the fermentation of grape sugar from that produced from the fermentation of cane sugar.

As stated previously it is also possible that the CO$_2$ in a sparkling wine can originate from a cylinder, i.e. carbonated wines, so it is necessary to determine the gas's origin. If the CO$_2$ has come from the air then it should have a $\delta^{13}\text{C}$ value in the range $-6.7^{0/00}$ to $-7.4^{0/00}$ as was found by Keeling [3] for rural air. However if this CO$_2$ came from city air which
has been contaminated by the burning of fossil fuels (C-3), then this range could be changed considerably. The most probable source for cylinder “food grade” CO2 is however still the burning of fossil fuels (C-3), with a range of -22% to -33%. The most likely source has been contaminated by the burning of fossil fuels (C-3), with a range of -22% to -33%. The resultant $\delta^{13}C$ value of the CO2 produced then depends upon the type of fossil fuel used. For coal, oil and natural gas the following range of $\delta^{13}C$ values have been found:

- **Coal**: Distribution as for modern plants i.e. -22% to -33%. The resultant $\delta^{13}C$ value has been determined as $13C/12C = 0.0112372$ [5].
- **Oil**: Distribution as for modern plants i.e. -22% to -33%. The resultant $\delta^{13}C$ value has been determined as $13C/12C = 0.0112372$ [5].
- **Natural gas**: Distribution as for modern plants i.e. -22% to -33%. The resultant $\delta^{13}C$ value has been determined as $13C/12C = 0.0112372$ [5].

Therefore unless the $\delta^{13}C$ value is less than -33% it will be impossible to distinguish it from the CO2 produced naturally from the fermentation of grape sugars.

As an additional comparison the $\delta^{13}C$ value of the ethanol in the wine was also measured. By using both the ethanol and CO2 values it should then be possible to gain further information as to the wine making process used.

**Methods**

**Gas Sampling.** The CO2 samples (2 ml) were obtained directly with a gas syringe from the headspace of an unopened bottle of sparkling wine. As all of the bottles sampled had plastic stoppers this method presented no great problems. The gas sample was then injected via a rubber septum into a U-tube in a vacuum line, cooled to liquid nitrogen temperature and the air present pumped away. The CO2 was dried by three distillations using an ethanol slush bath at -110°C, then the $\delta^{13}C$ values were measured using a Micromass 602C isotope ratio mass spectrometer.

In addition to sampling the headspace, samples of the CO2 dissolved in the wine were also taken. This was done by quickly removing 1 ml of wine from a freshly opened bottle and transferring it to a 20 ml flask which was then attached to a vacuum line. Using a process of successive cycles of freezing with an ethanol slush bath and then thawing, the CO2 was removed from the wine. It was then transferred to another section of the line and dried as described previously.

**Ethanol $13C/12C$ Analysis.** The ethanol present in 15 µl of completely degassed wine was distilled into a standard combustion line where it was combusted over a platinum catalyst at 500°C. The gases produced were then passed through a CuO and silver wool furnace to ensure complete oxidation to CO2 which was subsequently dried by distillation using an ethanol slush bath at -110°C. The resultant $\delta^{13}C$ value was then measured.

$\delta^{13}C$ is calculated from the 13C/12C sample and the international standard PDB (Pee Dee Belemnite) absolute isotope ratio using the following equation:

$$\delta^{13}C = \frac{13C/12C_{\text{sample}}}{13C/12C_{\text{PDB}}} - 1 \times 1000$$

Results

**Comparison Between Sampling Methods**

As stated previously, CO2 from the wine was sampled either in the gas phase (in the headspace) or from the solution (dissolved). Mook et al. [6] found that a fractionation existed between dissolved CO2 and gaseous CO2 and at 25°C this was -1.0% with the gas being more enriched. In contrast Deuser and Degens [7] found this fractionation to be negligible. In the case of wine however the situation is more complicated because a) it contains many dissolved solutes e.g. sugars, and b) it is acidic, hence no appreciable amount of bicarbonate exists.

Both of these sampling methods were carried out on 3 wines, the results of which are shown in Table 1.

Also shown in Table 1 is a column headed $\delta$ (gas-solution) which expresses the difference in $\delta^{13}C$ ratio between the gaseous CO2 and that in solution. Errors are also listed. For wine 1 there is no difference in $\delta^{13}C$ values between the gaseous and dissolved CO2 whereas for wines 2 and 3 there are differences of +0.5 and +0.4%, respectively. In each case the gas is heavier. These differences are lower than those found by Mook et al. [6], however because of the relatively crude sampling technique, the combined errors are large so it is difficult to draw any significant conclusions. The error is such that the fractionation could range between 0 and +0.8%.

Because both the gaseous and dissolved CO2 have similar $\delta^{13}C$ values it was decided to use the headspace sampling method because of its relative simplicity. Ten commercial sparkling wines were surveyed in addition to a bottle of fermented wine which had been produced by the Wine Research Unit of the Ministry of Agriculture and Fisheries at Ruakura (New Zealand). This control wine had been produced by the traditional champagne process i.e. the CO2 in the wine had been produced naturally and secondary fermentation within the bottle. The initial fermentation was done without the addition of sugar however cane sugar was added to allow the secondary fermentation to take place. This sugar served then only as a source of carbonation CO2.

The results of these determinations are shown in Table 2. As can be seen from Table 2, a wide range of values were obtained for the carbonation CO2 samples measured. The results of these determinations are shown in Table 2. As can be seen from Table 2, a wide range of values were obtained for the carbonation CO2 samples measured. The results of these determinations are shown in Table 2. As can be seen from Table 2, a wide range of values were obtained for the carbonation CO2 samples measured.

The $\delta^{13}C$ values of the carbonation CO2 for sample Nos. 7, 8, 9 and 10 show that the CO2 has originated from a C-4 source which was most probably cane sugar. Nos. 3, 4, 5, and 6 all appear to have had their CO2 derived from a C-3 source e.g. pure grape sugar. This would then imply fermentation by the Charmat process. However, this CO2 was not derived from the wine, it was used to carbonate as is obvious when the $\delta^{13}C$ values of the ethanol of these wines are considered. In all 4 cases the ethanol $\delta^{13}C$ ratios show that the wine was made with large additions of cane sugar (>60%). This sugar addition would then be reflected in the CO2 produced during the fermentation, so if this CO2 was used to produce the sparkle in the wines then both the alcohol and CO2 should have similar $\delta^{13}C$ values.

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