

CONSIDERATION ON STABLE ISOTOPIC DETERMINATION IN ROMANIAN WINES

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ABSTRACT - In the last two years, the Oenology Research Centre of Iași and the National Institute for Research and Improvement of Cryogenics and Isotopic Techniques of Râmnicu Vâlcea, together with the National Institute for Research and Improvement of the Molecular and Isotopic Techniques of Cluj-Napoca, have begun the implementation of the discriminative isotopic determination techniques for wines, at national level. The paper presented isotopic data regarding the wine made from the native grape variety Fetească albă, cultivated in different vine regions of Romania. The results pointed out the specific characteristics of the region, according to climate and precipitation.

Key words: isotopic analysis, wine, vineyards

REZUMAT: *Considerații privind analiza unor izotopi stabili din vinuri românești. În ultimii doi ani, Centrul de Cercetări pentru Oenologie și Institutul Național de Cercetare-Dezvoltare pentru Tehnologii Criogenice și Izotopice – ICSI Rm. Vâlcea, împreună cu Institutul Național de Cercetare-Dezvoltare pentru Tehnologii Izotopice și Moleculare Cluj-Napoca, au început implementarea la nivel național a tehnicilor de analiză discriminativă a izotopilor stabili din vinuri. Lucrarea de față prezintă unele date despre compoziția izotopică a vinurilor obținute din diverse zone ale țării din soiul Fetească albă. Datele sunt caracteristice reprezentative ale soiului, existând o strânsă dependență de zona geografică: solul, clima și, implicit, cantitatea de precipitații acumulată pe perioada de vegetație a strugurelui.*

Cuvinte cheie: analiză izotopică, vin, podgorii

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INTRODUCTION

The economic value is tightly connected, in case of wine selling, to the history of wine and the specific mode of obtaining it. Therefore, it is necessary to carry out accurate and correct analytical determinations for determining quality parameters (geographic origin, grape variety, and vintage year) and other significant parameters for justifying the economic aspect (maturation in oak barrels for a certain period of time).

Authenticity is an important factor in establishing the cost of food and food constituents or additives.

Besides the control of correct product labeling, checking the observation of good oenological practices should be also taken into consideration. Analytical methods can be used to identify if wines are blended or if their production is modified by adding water, alcohol, sugar, etc.

European Union is the leader in the field of food control by the help of stable isotopes. These methods are used to verify the authenticity of beverages and foods, being considered today usual methods in many control laboratories. The correct implementation of these methods is important for avoiding the disputes between the member states and ensuring an unitary control of food and beverage importation.

Grace to modern investigation techniques, variety, vintage year, and wine geographical origin or wine constituents are pointed out. In these cases, special analytical methods determining the differences between identical molecules, but with different geographical origins, are necessary (Christoph, 2004).

For determining the wine geographic origin, data concerning the isotope distribution in biosphere and biomass must be known. The correlation of these data to the climatic ones, as well as the determination of stable isotopes from wine may create an image concerning the origin of the product. The isotopic composition of a product may give information about its origin, because certain biological and physical processes differentiate between heavy and light isotopes, resulting in a change of their ratio. Analysis methods are based on measuring isotopic ratios (carbon, hydrogen, oxygen, etc.) of the product or a component of the analysed product. The obtained values may give information about geographical and botanical origin. In case of wines and distillates, the control laboratories of EU use nuclear magnetic resonance (NMR) and isotopic mass spectroscopy (IMS), for finding out sucrose addition or exogenous water dilution (EEC/2676/90).

MATERIALS AND METHODS

Sample taking over and conditioning. The so-called authentic wines were used for experiments (wines obtained according to EC 2120/2004). Such wines may represent isotopic models, to which other commercial wines are compared (***, 2004).

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For obtaining authentic wines, we have sampled grapes from different vineyards of Romania (*Table 1*). In choosing the regions, the importance of the vineyard was taken into account, in order to cover efficiently the viticultural area of Romania. Each grape sample weighed minimum 10 kg and was representative for that region. Grapes were harvested in a rainy period, or after the rain stopped. For each grape sample, a register card was made for main geographical characteristics (*Table 1*).

Table 1
Geographical characteristics of authentic wines from 2002 harvest

Variety	Vineyard	Coordinates GPS	Soil type	Height (m)	Local climate type
Fetescă Albă	Cotești	45°38'N/27°04'E	clay	206	dry/wet
	Tohani	45°06'N/26°10'E	clay	488	dry/wet
	Ștefănești	44°52'N/24°57'E	clay	342	dry/wet
	Aiud	46°19'N/23°45'E	clay	292	dry/wet
	Cotnari	47°21'N/26°55'E	clay	152	dry/wet
	Bucium	47°05'N/27°39'E	clay	338	dry/wet

Grapes were sqashed, then marc was pressed by the help of hydro-pneumatic vertical press. *Table 2* presents some physico-chemical characteristics of musts (***, 2005). Fermentation was done by *Sacharomyces cerevisiae* - type EC 1118 yeasts, at a temperature of 19°C, until the consumption of sugars. After fermentation, samples were extracted from yeast deposits, and the correction of sulphur dioxide was done by adding potassium metabisulphite, in order to avoid the introduction of exogenous water. For maturation, wines were bottled into large bottles of 15 l. After 4 months, wines were extracted again from yeast deposit, which was formed mainly of tartrates. After a few days, wines were filtered through a mean porosity filtering plate and bottled in bottles of 750 ml. Closing the bottles was done by synthetic material stoppers, for limiting the influence of other natural materials. Having in view the stratification of the liquid in storing vessels, the great bottles were encoded. The wine from bottles was noted with **A** at the upper half and with **B**, the wine from the other half.

Table 2
Physico-chemical characteristics of musts from 2002 harvest

Vineyard	Grapes weight (Kg)	Must volume (L)	Refraction index	Acidity g/L tartaric acid
Cotești	36.5	19	1.3641	6.37
Tohani	43	24	1.3697	5.52
Ștefănești	40	22	1.370	4.7
Aiud	34	19.5	1.3658	6.41
Cotnari	34	17.5	1.3679	7.21
Bucium	31	16	1.3626	8.04

The isotopic analysis of samples is done as follows:

- ^2H (D) was measured from the residue, which remained after wine distillation, by a special mass spectrometer used for deuterium isotopic analyses from water;
- ^{18}O was measured both in wine and distillation residue. For the isotopic analysis of oxygen, the sample balance with CO_2 , in a special balancing line, was necessary. Then, the analysis with mass spectrometer with double collection of isotopic composition of ^{18}O from CO_2 , balanced with sample, was also necessary;
- ^{13}C was measured from the ethanol obtained by wine distillation, then ethanol was submitted to the method of excess oxygen combustion, in order to obtain CO_2 , on which ^{13}C determination was done with double collection mass spectrometer, and by reporting to a certain standard (Ough, Amerine, 1988).

Measurement of the isotopic ratio of carbon

In nature, there are two stable isotopes of carbon: ^{12}C and ^{13}C . The most frequent isotope is ^{12}C , with an atomic concentration of 98.9%, while the heaviest one, ^{13}C , has a concentration of 1.1%. These two isotopes are not equally distributed in natural compounds, because of isotopic fractioning, which takes place within physical, chemical, and biological processes involved in the cycle of carbon. During the fixation of carbon in plant through photosynthesis, the discrimination of carbon isotopes from CO_2 source (carbon dioxide from air) is produced. This source is used by plant in the photosynthesis process. The discrimination results in plant containing less ^{13}C than CO_2 , consumed from air.

The isotopic composition of carbon is, generally, measured on CO_2 , by the technique of mass spectrometry. The matter from plants is converted into CO_2 , by combustion. The isotopic ratio **R** is obtained by mass spectrometer analysis. The ratio is defined as follows:

$$R = \frac{^{13}\text{CO}_2}{^{12}\text{CO}_2}$$

Preparation of samples

^{13}C analysis of wine is carried out from ethanol extracted by distillation from wine sample.

Any method of ethanol extraction may be used as long as 98-98.5% of the total alcohol from wine is recovered by distillate (distillate must contain 92-93% alcohol mass percentage, that is 95% in volumes).

The liquid is collected at temperatures between 78 and 78.2 $^{\circ}\text{C}$; if the temperature exceeds 78.5 $^{\circ}\text{C}$, collecting is stopped for about 5 minutes. This proceeding allows that 98-98.5% of the wine alcohol be recovered in distillate, which reaches a power of 95% in volume. After the collecting is over, the obtained distillate is weighed.

A homogeneous sample of 60 mL from the residue obtained at distillation is kept in a balloon of 60 mL and represents water from wine. If necessary, the D/H isotopic ratio for water from wine will be determined on this sample.

The water content from distillate is determined by Karl Fischer Method, by using 0.5 ml distillate with known alcoholic power.

^{13}C analysis, by mass spectrometry, is done on CO_2 resulted from excess oxygen combustion of ethanol, and obtained by distillation and cryogenic purification.

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For measuring isotopic ratios of carbon ($^{13}\text{C}/^{12}\text{C}$), the technique of mass spectrometry with double collector is used. The great accuracy with which these isotopic ratios are measured, is obtained by simultaneous collecting of the three interest ion beams ($^{12}\text{C}^{16}\text{O}^{16}\text{O}^+$, $^{13}\text{C}^{16}\text{O}^{16}\text{O}^+$, $^{13}\text{C}^{16}\text{O}^{18}\text{O}^+$), and by comparing sample to a certain standard, during the isotopic ratio determination. The result of measuring with this technique is not the absolute isotopic ratio of sample, but the relative difference between the isotopic ratio of sample and reference gas. For expressing the relative differences between isotopic ratios of sample and reference gas (standard), $\delta^{13}\text{C}$ is used, which is defined as:

$$\delta^{13}\text{C}(\text{‰}) = \left[\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right] \times 10^3,$$

where $R_{\text{standard}} = R_{\text{PDB}}$ is the absolute isotopic ratio ($^{13}\text{C}/^{12}\text{C}$) of international standard for carbon, and it represents: $R_{\text{PDB}} = 0.0112372$.

By international convention, $\delta^{13}\text{C}$ is always expressed in relation to the value $\delta^{13}\text{C}$, for the standard of calcium carbonate, known as PDB. This standard is a carbonate obtained from *Belemnitella americana* fossil. The base of PDB scale is the value $\delta^{13}\text{C} = 0\text{‰}$ for this standard. $\delta^{13}\text{C}$ value indicates if sample has a greater (+) or lower (-) $^{13}\text{C}/^{12}\text{C}$ ratio than PDB.

PDB standard quantity being limited, other primary standards were used. Measurements were done with an ATLAS 86-type commercial spectrometer, produced by Varian AMT Firm, Bremen.

Method for determining ^{18}O from wine

Preparation of wine samples for ^{18}O measurements. Method of $\text{H}_2\text{O} - \text{CO}_2$ balance and proceedings. $\delta^{18}\text{O}$ measurements from wine and the residue remaining after distillation, can not be done on liquid samples, because they have a corrosive action inside the metallic sides of the spectrometer. That's why we have tried to process wine/distillation residue samples, so that gas be introduced in the spectrometer. Measurements are done easily on this gas, and the presence of errors is diminished. This gas is carbon dioxide. The simplest method, used for preparing wine samples for ^{18}O isotopic analyses, is $\text{CO}_2\text{-H}_2\text{O}$ isotopic balance, applied in wine samples and wine distillation residues. Balancing is carried out between wine/residue and carbon dioxide. The method is based on the reaction between water from wine and residue, and carbon dioxide, with formation of carbonic acid. The carbonic acid, being instable, is decomposed again in carbon dioxide and water. In the $\text{CO}_2\text{-H}_2\text{O}$ system, C-O bonds being formed and neutralized, it appeared the opportunity of changing oxygen isotopic composition from interest water (water from wine or residue) and carbon dioxide.

If water mass exceeds carbon dioxide mass, to which is found in connection, the modification of the isotopic composition of oxygen from water is insignificant, while from CO_2 , it will show the composition of oxygen from water.

As we have already mentioned, the isotopic concentration for natural variations of isotopes is not expressed by absolute ratios, but by relative variations $\delta\text{‰}$.

The positive values indicate an enriching in heavy isotope from sample, compared to reference standard, while the negative values show a diminution in heavy isotope from sample. In the above-mentioned relation, the multiplication by 1000 was chosen, because the relative deviation of the sample is very low in comparison with the standard.

In isotopic analyses of waters, carried out by mass spectrometry, the following reference international standards are used:

- SMOW (Standard Mean Ocean Water) for $^{18}\text{O}/^{16}\text{O} = (1993.4 \pm 2.5) \times 10^{-6}$ and $\text{D}/\text{H} = (158 \pm 2) \times 10^{-6}$;
- V-SMOW (Vienna SMOW) for $\text{D}/\text{H} = (155.76 \pm 0.05) \times 10^{-6}$

Measurement of D/H isotopic ratio

Deuterium content from sugars and water from must will be re-distributed after fermentation in wine molecules: $\text{CH}_2\text{D}-\text{CH}_2-\text{OH}$, $\text{CH}_3-\text{CHD}-\text{OH}$, $\text{CH}_3-\text{CH}_2-\text{OD}$, HOD .

The isotopic content of sample is usually expressed under the shape of relative concentration to the concentration of an international standard V-SMOW (Vienna Standard Mean Ocean Water) in a thousand :

$$\delta (\text{‰}) = [(R-R_s)/R_s] \times 1000 = (R/R_s - 1) \times 1000,$$

where: R- D/H ratio of sample; R_s - D/H ratio of international standard V-SMOW (Vienna Standard Mean Ocean Water); $R_s = 155.76 \pm 0.005$ ppm or $\delta_s = 0$ (‰).

The above parameters are determined by nuclear magnetic resonance (^1H -RMN) of deuterium for the ethanol extracted from wine (Guillou, Martin, 1993). In case of European Community, for the creation of database for wines, authentic samples with the same origin (variety, vintage place, and year) must be also taken over, besides the samples to be verified.

RESULTS AND DISCUSSION

Table 3 presented data on isotopic composition: ^{18}O deviation compared to ^{16}O ($\delta^{18}\text{O}$) from wine and residue, ^{13}C deviation compared to ^{12}C ($\delta^{13}\text{C}$) from wine ethanol, deuterium deviation compared to hydrogen (δD) from residue, and deuterium/hydrogen ratio (R_D).

Table 3

Isotopic data on wines from 2002 harvest

No	Sample	$\delta^{18}\text{O}$ (‰)		$\delta^{13}\text{C}$ (‰)	δD (‰)	R_D (ppm)
		wine	residue			
1.	1A=Fetească Albă, Tohani	-0.57	+6.27	-25.75	23.8	159.36
2.	2A=Fetească Albă,Bucium	-0.34	+3.12	-25.75	9.13	157.18
3.	3A=Fetească Albă,Cotnari	+0.70	+1.89	-25.86	10.02	157.3
4.	4A= Fetească Albă,Cotești	-	+2.94	-25.16	16.56	158.31
5.	5A=FeteascăAlbă,Stefă-nești	+1.26	+2.51	-25.13	12.58	157.74
6.	6A = Fetească Albă, Aiud	-2.60	+3.03	-25.61	26.24	159.64
7.	1B= Fetească Albă,Tohani	+0.52	+7.34	-25.15	31.3	160.42
8.	2B=Fetească Albă,Bucium	-3.58	+7.07	-26.84	26.76	159.93
9.	3B= Fetească Albă,Cotnari	-0.61	-0.94	-26.05	7.64	156.95
10.	4B= Fetească Albă,Cotești	+3.28	+10.23	-26.22	48.02	163.24
11.	5B=FeteascăAlbă,Stefănești	-0.68	+5.32	-25.61	41.13	162.17

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These isotopic analyses were related to present standards from Romanian laboratories for carbon, oxygen, and deuterium. Without a special standard for wine, these results had only an informative character.

Data from *Table 3* confirmed the observations from other similar studies on the interdependence between the ratio of stable isotopes with geographical origin and climatic conditions of the year.

The calculation of $\delta^{13}\text{C}$ is not important in differentiating the areas of Romania. It has only an informative role. If we compare the values for $\delta^{18}\text{O}$ and δD to the type of climate from the vineyard, a logical distribution of values appears from plus to minus for ^{18}O , and an increasing one at deuterium, as we go inside the continent. We also mention that $\delta^{18}\text{O}$ may be different for the same geographical region, for instance Iași region

CONCLUSIONS

The results confirm the variability of the ratio between ^{18}O and D stable isotopes, according to geographical origin and annual climatic conditions.

The isotopic variability of ^{13}C is not sufficiently relevant for delimiting-identifying wines from different regions.

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