

The Isotopic Analysis - a Good Tool for Verifying the Geographical Origin of Wines

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In this paper we intend to set the own characteristics for several varieties of Romanian wines produced in the Valea Călugărească area, characteristics that confer the zone tipicity. This unique fingerprint was established by mass spectrometry, using the isotopic method. The chemical, microbiological and chromatical analyses completed this study.

Keywords: wine, tipicity, geographical origin

Wine authenticity is very important, especially in relation to quality control and consumer information, the determination of geographical origin being one of the primary requirements for certification of wines authenticity, because, the specific characteristics of a wine are induced mainly by geographical location and characteristics of production in the concerned areas. These wines have great quality features, personality, that making them unique [1].

The principal methods used for authentication of geographic origin are: analysis of volatile compounds profile, analysis of amino acidic profile, mineral profile analysis and analysis of stable isotopes. The determination of the $^{18}\text{O}/^{16}\text{O}_{\text{vsSMOW}}$ (‰) isotopic ratio from the water extracted from wine and the appreciation of the $^{13}\text{C}/^{12}\text{C}_{\text{PDB}}$ (‰) isotopic ratio in the ethanol extracted from wine provide relevant information to authenticate the geographic origin of wines.

The composition analysis is required from the wine producers of quality wines by the European Union, being justified due to the fact that the production of wines is diverse and the wine making techniques are more or less correct. To establish the quality of the wine, the physico-chemical analysis can provide information about the natural character and the grapes origin only, without the presence of any fraud or impure. Over time, the Romanian wines were subject to different compositional and authentication studies using, for analysis, various modern physical methods [2-6] combined with chemometrical techniques [7].

Authentication of wines is an important problem to which isotopic analysis by mass spectrometry has made a significant contribution. The use of mass spectrometry to determine the ratios of stable isotopes in the biomolecules now provides the data to prove the botanical and geographical origin of a wide variety of foodstuffs – an application: the authentication and the elimination of the frauds. Starting with 10 September 2007, Ministry of Agriculture and Rural Development from Romania voted the order no. 797 of governing the creation of the analytical data base for Romanian wines for every year production, including isotopic analysis.

The wines components (like the components of any other substance) consist of molecules with atoms which naturally contain the different isotopes with varying atomic

masses. Known as stable isotopes, they occur in particular proportions depending on the origin and the processing history of the substance. The proportion and position of these isotopes create a unique *fingerprint*, measurable by mass spectrometry, which can therefore be used to identify the origin and nature of the substance [8].

In this paper we intend to study the physico-chemical characteristics of two red wines from Valea Călugărească area, features that confer to these samples the zone tipicity. We used the isotopic method in order to prove the authenticity of the geographical origin for these wines.

Experimental part

The studied samples are two red wines from Valea Călugărească area: Merlot (2006) and Fetească Neagră (2007) (produced by ROVIT S.A.), much appreciated, bought from the supermarket.

In the preliminary analysis of the samples studied, we took into account the colour of the wines (objectively determined, using colour space) [5, 9-11] and their microbiological stability [12-14].

The microbiological control was realized by cultivate of wine sample on solid medium YPG (yeast extract peptone glucose) at 28°C for 24 h.

Transmission and absorbance spectra (380–770 nm) were recorded using a Lambda 25 Perkin Elmer UV-Vis spectrophotometer, with double-beam, coupled with a computer IBM-PC, in a 1 mm pathlength quartz cell.

Analysis of colour was made from the UV-Vis spectra data, with the CIE 1964 standard observer (10° visual field), the CIE standard illuminant D65 and bidistilled water as reference solvent. Chromatic analyses were performed using CIE 1976 (L, a, b) space (CIELAB) [15]. The chromatic characteristics of a wine are defined by the colorimetric or chromaticity coordinates: clarity (L^*), red/green colour component (a^*), and blue/yellow colour component (b^*) and by its derived magnitudes: chroma (C^*) and tone (H^*), defined according to the following mathematical function:

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

$$H^* = \text{tg}^{-1}\left(\frac{b^*}{a^*}\right)$$

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After the preliminary examination of samples, a very large set of chemical analysis was performed. For each wine were determined: the relative density and the alcoholmeter titer using the pycnometric method; the total content of the remaining unfermented sugars using the refractometric method; the content of methanol, citric acid and anthocyanins by the spectrophotometric dosage; the content of glycerol, acetaldehyde and SO₂ using the volumetric method; the total acidity and the volatile acidity by titration with a solution of NaOH in the presence of bromothymol blue, respectively phenolphthalein as indicator.

The analysis of ratio of the stable isotopes of carbon and, to a lesser extent of oxygen, determined by the mass spectrometry, is an accepted technique for the detection of the illegal markups in the wines and their authentication [16]. The isotopic analyses of carbon 13 in the ethanol extracted from the wine are performed to detect the addition of sugar or synthetic ethanol (not extracted from grapes) [17], the measurements of oxygen 18 are to detect the addition of water [18, 19] and, in particular, to support the geographical component [20]. The method of determination of the isotopic ratio through mass spectrometry with continuous flow (CF-IRMS) involves the on-line preparation of the sample, a smaller amount of sample, the faster analysis and the possibility of coupling with other separation techniques, like gas chromatography. It is produced a fractional distillation of an ethanol-water mixture with an alcoholic strength of 10 % volume, using an Automated Distillation Control System (ADCS), who can distillate simultaneous four wine samples, without contaminating the samples.

The determination of the ¹³C/¹²C_{PDB} (‰) isotopic ratio in the ethanol extracted from wine involves the following steps:

- the ethanol extraction from wines samples through fractional distillation (the minimum concentration of ethanol obtained must be 93% w/w);

- the determination of the ¹³C/¹²C_{PDB} (‰) isotopic ratio from sample with Delta V Plus mass spectrometer coupled with Flash EA/TC: the elemental analyzer, by Dumas combustion.

The δ¹³C determination was made as follows: 0.1 μL of purified ethanol was injected with a syringe of 0.5 μL by the autosampler of liquids, then was taken by the eluent (helium) and introduced into the reactor of the elemental analyzer FlashEA 1112 HT coupled at CF-IRMS, at very high temperature, in the presence of oxygen (the Dumas combustion) (table 1).

Table 1
THE FlashEA 1112 HT SYSTEM SETTINGS

Reactor temperature, °C	1010
GC temperature, °C	45
Carrier flow, ml/min	90
Reference (He) flow, ml/min	60
Oxygen flow, ml/min	250
O ₂ injection time, s	1
Autosampler type	AS 3000
Syringe size, μl	0.5

The determination of the ¹⁸O/¹⁶O_{vsSMOW} (‰) isotopic ratio from the water extracted from wine involves the following steps:

- the extraction of water from the wines samples through fractional distillation;

- the isotopic analysis ¹⁸O/¹⁶O of the sample: the equilibration of the water sample with an equilibration mixture (0.36 % CO₂ in helium) (table 2).

Table 2
THE SYSTEM SETTINGS FOR δ¹⁸O DETERMINATION

Sample size	500 μl of water extracted from wine
CO ₂ /He	0.36 %
Equilibration	20h at 24 °C
Sampling loop	10 μl
Replicates (loop switches)	10
GC column	Pora Plot Q 25 m

Following the small variation of the isotopic abundance, the absolute value of ratios is not measured, but the difference from a standard (etalon) chosen. The expressing of this result is made in units δ calculated by the formula:

$$\delta * X/X(\text{‰}) = \left(\frac{(*X/X)_{\text{sample}}}{(*X/X)_{\text{standard}}} - 1 \right) \times 1000$$

For carbon, the ratio *X/X is ¹³C/¹²C and the standard was the V-PDB carbonate (Viana Pee Dee Belemnite) whereas for oxygen, the ratio *X/X is ¹⁸O/¹⁶O and is reported at the V-SMOW standard (Viana Standard Mean Ocean Water) [21]. The reproducibility of the measurements δ¹³C, δ¹⁸O is ± 0.1 and ± 0.5 ‰, respectively. Positive delta values indicate that there is a greater percentage of the heavier isotope present relative to the standard.

Results and discussions

The preliminary analysis shows that the studied wines are stabilized and did not contain viable yeast cells. Thus the alcoholic fermentation will not continue in the studied wines.

Using the dates from the transmittance spectra, we calculated: the clarity (L*), the a* parameter (red/green colour component) and b* parameter (blue/yellow colour component). Chroma (C*) had completed the chromatic parameters, by CIELAB space (table 3).

Table 3
CIELAB (D65/10°) COORDINATES FOR WINE SAMPLES

Wine	L*	a*	b*	Chroma
Fetească Neagră	87.42	46.42	0.029	46.42
Merlot	88.66	42.15	-0.009	42.15

Due to the fact that wines display colours in the red range, in the CIELAB scale, a* is higher than b* (in module). The a* parameter is a measure of the total contribution of anthocyanins to the red colour of a wine. Because the a* parameter is positive for all samples, (higher for Merlot), the wines are located on red part of axis red to green. The b* parameter is negative for Merlot samples, (this wine is

Sample	$\delta^{18}\text{O}/^{16}\text{O}(\text{‰})$ vs. VSMOW	Standard deviation	$\delta^{13}\text{C}/^{12}\text{C}(\text{‰})$ vs. PDB	Standard deviation
Feteasca Neagra - 2007	2.09	0.08	- 25.45	0.06
Merlot - 2006	2.08	0.09	- 25.40	0.08
Reference material IAEA: SMOW	0.00	0.09	-	-
Reference materials BCR-659 (synthetic wines)	- 7.18	0.10	-	-
Internal reference material	- 8.6	0.11	-	-
Reference materials BCR-656 (96% ethanol)	-	-	- 26.91	0.07
Internal Reference materials: ethanol absolute	-	-	- 24.01	0.05

Table 4
THE VALUES OF $\delta^{13}\text{C}$ AND
 $\delta^{18}\text{O}$ IN THE ETHANOL
AND WATER EXTRACTED
FROM WINE

located on blue part on an axis that ranges from blue to yellow) and the Fetească Neagră is on yellow part on axis (because the b^* parameter is positive).

We have done some analysis of key components from the wine, in order to establish the parameters of composition that defining the quality of wines and to detect any falsification. Thus, for each wine were determined: the relative density, the alcoholmeter titer, the total acidity and the volatile acidity, the content of methanol, glycerol, citric acid, acetaldehyde, SO_2 and anthocyanins.

The values obtained for the content of methanol (25 mg/L- Merlot and 360 mg/L - Fetească Neagră) and the content of acetaldehyde (120 mg/L - Merlot and 155 mg/L- Fetească Neagră) show a medium degree of degradation of these wines, as indicated by their ionic/real acidity (pH): 3.76 - Merlot and 3.75 - Fetească Neagră [22]. The values of other parameters (alcoholmeter titer, total acidity, volatile acidity, total SO_2 , total sugars, anthocyanins, glycerol) [22] are within normal limits for the red semi-dry wines, established by the Oeno resolutions of the OIV and the EU norms, the Law of Vine and Wine 2004/2002 and other regulations governing the quality of wine. Thus, despite the weak ionic acidity, two red wines from the Valea Călugărească region can be classified as superior wines.

The values from table 4 represent the results (the $^{18}\text{O}/^{16}\text{O}$ and $^{13}\text{C}/^{12}\text{C}$ ratios) from the original wines in the same area, but different varieties: Feteasca Neagră (2007) and Merlot (2006). The proportion and position of the isotopes carbon 13 and oxygen 18 creates a unique fingerprint for every variety and year of production respective, measurable by mass spectrometry CF-IRMS.

Conclusions

The chromatically parameters, obtained by CIELAB method, allow an objective appreciation of wines colour. They are different for studied wines, with yellow (for Fetească Neagră) and blue (for Merlot) contribution on the predominant colour - red. The chromatic and chemical characteristics are normal for the wines from this area and those years.

The isotopic analysis completes the characterization of these wines, coming up with useful information about the botanical origin of the raw material in the wines, the geographical origin, the year of production. This analytical method can be a powerful tool for verifying and confirming the nature of drinks on the market in our country, in particular wines.

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Manuscript received: 26.08.2011