Composition Changes in Wines Produced by Different Growing Techniques Examined Through 1H-NMR Spectroscopy

MARIA-CRISTINA TODASCA1*, LAURA FOTESCU2, NICOleta-AURELIA CHIRA1, CALIN DELEANU3, SORIN ROSCA1
1University Politehnica Bucharest, Faculty of Applied Chemistry and Material Science, „C. Nenitescu” Organic Chemistry Department, 1-7 Polizu St., 011061, Bucharest, Romania
2Researches and Development Institute for Viticulture and Vineyard, Valea Câlugăreasca, Romania
3Romanian Academy, Center of Organic Chemistry “C. D. Nenitzescu”, 202B Spl. Independentei, 060023, Bucharest, Romania

This paper examines the composition of some wines produced from Vitis vinifera in different technological conditions (during cultivation, the vines were subject to different technological sequences). This study becomes necessary in order to improve the management of the current practices, finally obtaining a superior wine and similar crops from one year to the next. In the meanwhile this study offers important compositional information by connecting various technological procedures with wine composition changes.

Keywords: wine, composition, 1H-NMR, integrated wine production system

Rapid and accurate chemical analyses of complex mixtures like wine and other beverages became very important when the demanding for adequate quality in production process increased. Especially for wines, the differentiation in terms of variety, geographical origin and crops year, turned out to be very important in authenticity studies.

An examination of the compositional changes in wines produced in different agro - technical conditions is necessary in order to improve the management of the current practices, finally obtaining a superior wine and similar crops quality from one year to the next.

NMR spectroscopy has proved in various occasions an undisruptive, selective and effective tool for simultaneous detections of numerous compounds with small molecular weight within complex mixtures [1, 2].

C-NMR spectroscopy was used successfully for acids and sugars detection; this information together with amino acids signals were used as “fingerprints” in European wines monitoring [3]. In the mean while wine authentication was investigated using amino acid fractions from different wines and grape juices (must) [4].

Polyphenols play an important role in wine quality. They are the main components responsible for red wine color and have an important role in sensorial characteristics of wine. Polyphenols presents a wide range of biochemical and pharmaceutical effects, like: anti-inflammatory, antimicrobial, antioxidative activity, anticancerous, etc. High resolution NMR spectroscopy was applied for structure elucidation of wine polyphenols and their involvement in solution equilibrium and in the mechanism of wine color stability [5, 6].

1H-NMR spectroscopy method was gainfully used, by a researchers group, for characterization of “minor” components in Slovenian wines. The information obtained from the NMR spectra was analyzed using PCA chemometric method and they obtained a good differentiation among the wines from the costal and the continental areas [1, 7].

1H-NMR spectroscopy method combined with PCA method proved as well to be a valuable tool in monitoring wine fermentation process [8,9]. The same technique confirmed in numerous studies, to be very useful in differentiation and classification of wines according to their geographical origin with essential role in wine authentication [10, 11].

Experimental part

The 1H-NMR spectra have been recorded on a Bruker Avance DRX 400 spectrometer, operating at a field of 9.4 Tesla, corresponding to the resonance frequency of 400.13 MHz for the 1H nucleus, equipped with an inverse detection, two channels multinuclear probe head and field gradients on the z axis.

The NMR samples were prepared by mixing 0.9 mL wine with 0.1 mL solution of 3(trimethylsilyl) propionic acid-d4 sodium salt (TSP) in D2O (Aldrich). This has 10% D2O and an internal quantitative calibration standard. The chemical shifts are reported in ppm as δ values, using as reference the TSP signal at 0 ppm. The samples were analyzed in 5 mm NMR tubes (Norell 507).

Typical parameters for the 1H-NMR spectra where: 35° pulse, 4s acquisition time, 6.4 KHz spectral window, recording 16 scans, with 52 K data points. The FID was not processed prior to the Fourier transformation.

The 1H-NMR water suppression spectra have been recorded with a 90° pulse, 4s acquisition time, 3s relaxation delay, 3s irradiation time, on 6.4 KHz spectral window, with 32 scans, collecting 52 K data points. The FID was processed for 0.3 Hz line broadening prior to the Fourier transformation.

Results and discussions

Five different wine samples, obtained from grapes variety Cabernet Sauvignon, were subject to this study. The wine samples were produced in Valea Calugareasca, in the same year, using different cultivation techniques. The different technological sequences used in each case are presented in table 1.

Wine sample number 5 is obtained using the classical wine production techniques. This sample is used as the “control” sample. The classical wine production techniques have various disadvantages. Among the most important disadvantages we can mention: the variation of crops quality from one year to the next, large production costs, considerable consumptions of phytopharmacological...
products, soil contamination and so on. Wine sample number 4 was obtained in the integrated wine production system that implies the use of 3 main technological sequences mentioned in table 1. Wine samples 1-3 were subject to one specific technological sequence part of the integrated wine production system.

Wine samples were analyzed using $^1$H-NMR spectroscopy method. The method was developed and presented in earlier studies [12].

In order to establish the composition changes in the wine samples produced using different growing techniques we analyzed some of the main components of wine based on their specific marker. The specific markers used for further quantitative measurements of various compounds from the Cabernet Sauvignon wines are shown in figure 1.

Based on those markers several wine compounds where measured for all the samples analyzes. In figure 2 the comparative amount of glycerol measured in the five samples of wine is illustrated.

All five samples contain glycerol in the amount mentioned in literature (between 4 and 9 g/L). Sample 4, the one obtained through the integrated wine production system.

### Table 1

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5 (control)</th>
</tr>
</thead>
</table>

**THE INTEGRATED WINE PRODUCTION SYSTEM**

![Fig. 1. Section of the $^1$H-NMR spectra of a Cabernet Sauvignon wine sample](image1)

![Fig. 2. The average amount of glycerol obtained for each wine sample](image2)
system, contains 4.7 g/L of glycerol. Even the amount of glycerol is not very large in this case there are some advantages: stability from one year to the next and the reduced pollution of the soil during cultivation procedures.

Using the information obtained from the \textsuperscript{1}H-NMR spectra and the specific markers, the amount of several amino acids from the wine samples was measured. In figure 3 is revealed the amount of various amino acids for the analyses wine samples.

A large amount of amino acids can be damaging for the wine especially when it is drawn too late. It can develop a "yeast" taste and can form precipitates. In the case of sample 4 the amount of amino acids is equilibrated, therefore they can give a good flavor to the wine without causing any damage.

The average amount of carboxylic acids measured in the five wine samples is presented in figure 4.

Lactic acid (and lactate compounds) is formed in wine after malo-lactic fermentation process; a larger amount of lactic acid and mannitol can appear if the wine undergo malo-lactic fermentation with a high level of residual sugars still present. Malic acid (and malate compounds) has a sour grass like taste, but the lactic acid bacteria (LAB) transform the malic acid into lactic acid, reducing in this way the amount of malic acid after malo-lactic fermentation. This fermentation process can occur spontaneously, but most of the times is induced by the producer using LAB cultures. In the case of the five samples analyzed during this research the malo-lactic fermentation was induced after collection of samples. The amount of malic and lactic acid in sample 4, obtained through the integrated wine production system, is lower than the standard limits (0-5 g/L for malic acid, 0.5-1 g/L for lactic acid) \cite{13, 14}. The largest amount of malic and lactic acids was obtained for sample 3 which was subject to phytosanitary protection procedures during cultivation.

Acetic acid (and acetate compounds) is produced as a result of acetic fermentation process which is undesired in wine because, in large amounts, it gives a vinegar taste. All five samples of wine taken into study contain acetic acid within the standard limits (0.3 – 1 g/L) \cite{13, 14}. In comparison with the other four wine samples, sample 4 obtained through the integrated wine production system, contains a small amount of acetic acid similar with sample 1 which was subject only to maintenance and fertilization procedures. The largest amount of acetic acid was obtained in the case of sample 5 using the classical wine production techniques and sample 3 which was subject to phytosanitary protection procedures.

Succinic acid (and succinate compounds) is a secondary product of the alcoholic fermentation process. It has a sour and slightly bitter taste that in small amounts contributes to the general wine savor. All five wine samples contain succinic acid within the standard limits (0.5 - 1.5g/L) \cite{13, 14}. The largest amount of succinic acid was measured in sample 3 which was subject to phytosanitary protection procedures. Sample 4 obtained in the integrated
wine production system contains an equilibrated amount of succinic acid.

Tartaric acid in larger amounts can be damaging for the wine because it produce salts with low solubility which can precipitate and can threaten wine stability. The largest amount of tartaric acid was measured in sample 5, obtained using the classical wine production techniques.

Using the $^1$H-NMR method directly on the wine sample, without any prior separation or concentration of the sample, one can achieve only general information regarding the amount of polyphenols. In figure 5 can be noticed the average amount of polyphenols measured in each wine sample.

Polyphenols are wine important components, but most important of the red wines. Some of the polyphenols are responsible for the color (anthocyanins, flavones) and others contribute to the wine bouquet (tannins). The largest amount of polyphenols was achieved for sample 4, which correspond to the wine obtained in the integrated production system. The lowest amount of polyphenols was measured in sample 5, which corresponds to the wine obtained using the classical wine production techniques.

Another important aspect of this study concerns the possibility to differentiate between the five wine samples, setting the basis for wine differentiation depending on the technological sequence used during cultivation of vine. In order to achieve this objective we analyzed the spectral information with the help of a known statistical method – Principal Component Analysis (PCA).

The $^1$H-NMR spectra of wine samples were processed with MestReNova 5.1.1 software. Each spectrum was integrated from 0.2 to 0.2 ppm, acquiring in this way 49 values. Area between 4.7 - 5.1 ppm, is removed from the spectra due to the vicinity of the suppressed water signal, that may induce errors. The spectral information was analyzed using StatistiXL 8.1 software. Graphical representation of the main component 1 (PCA1) depending on the principal component 2 (PCA2) is shown in figure 6.

From the graphical representation of PCA1/PCA2 one can notice a good differentiation of the wine samples depending on the technological sequence used during cultivation of vine. The distinct separation between sample 1, 2 and 3 emphasizes that each technological procedure used during cultivation of wine imprint specific characteristics to each wine.

There is a similarity between sample 2 produced by following vegetation structures of the vine and sample 5 obtained using the classical wine production techniques. This similarity means a quality equivalence of the two wine samples.

The complete separation of sample 4 in the graphical representation of PCA1/PCA2 certifies that the $^1$H-NMR information combined with chemometrical methods of analyses can provide a powerful tool for wine differentiation depending on the technological sequence used during cultivation of wine.

Conclusions

The technological sequence used for vine cultivation induces significant changes in wine composition that can be quantitatively measured using $^1$H-NMR spectroscopy. Those information are very important for wine producers and can help to improve the management of the current practices, finally obtaining a superior wine and similar crops from one year to the next.

The $^1$H-NMR information combined with chemometrical methods of analyses can provide a powerful tool for wine differentiation depending on the technological sequence used during cultivation of wine.

Acknowledgments: The authors thank for the financial support from CNCSIS grant ID 928 number 240 / 2007-2010 and “Researches and Development Institute for Viticulture and Vineyard, Valea Călugărească” for the wine samples.

Bibliography

1. KOSIR, I. J., KIDRIC, J., Analitica Chimica Acta, 2002, 458, nr. 1, p. 77

Manuscript received: 12.10.2010