2H NMR SPECTROSCOPY OF SOME ROMANIAN COMMERCIAL WINES

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ABSTRACT. The requirements for quality food products have been increased in recent years and the interest in the quality and purity of spirits, wines and fruit juices has grown in this connection as well. In the early 90’s the EU adopted the $^2$H-NMR method for wine analysis as an official method (EEC 2676/90), in order to tackle the problem of over-chaptalization of wines in Europe. A deuterium natural abundance quantitative NMR method (SNIF-NMR) was developed as an efficient and powerful tool capable of characterizing the chemical origins of organic molecules and distinguishing their biological and geographical origin. Usually this type of measurements are performed with dedicated NMR equipments and computerized programs elaborated, commercialized and maintained by Eurofins Scientific (France).

Keywords: $^2$H NMR, wine, isotopic ratio

INTRODUCTION

Making fraudulent profit from misrepresentation of food has been a future of society from historical times. Nowadays frauds in various consumer sectors are commonly practiced. The addition of beet or cane sugar or concentrated rectified must to grape must or wine before or during fermentation is used to increase the natural content of ethanol and therefore the value of wine, which commands higher prices on the market. Consumers are thus deceived since added sugar is not declared on the product. Another type of economic fraud is mixing high quality wines with low quality ones that often originate from other geographical regions or countries. A memorable example is the adulteration of Austrian wine and also some Italian and German wines with poisonous antifreeze ethylene glycol with intention to give the impression of a wine with a greater body. Identifying fraudulence related to fruit juices is also of great economic importance because of the large quantities of juice consumed.

The type of adulteration include diluting with water, the addition of sugar solution, citric and tartaric acid, and colorants to the pure juice, and the addition of cheaper juices originating from other fruits, mainly from grape fruit.

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The consumption of virgin olive oil, which is defined as oil obtained only by mechanical means is increasing due to its nutritional properties arising from the high content of unsaturated acids (oleic and linoleic acids). Natural phenolic compounds present only in virgin olive oil are responsible for its oxidation and for characteristic sensory attributes. The high sensory and nutritional quality and consequently higher commercial value of virgin olive oil has lead to its adulteration with low-grade foreign oils (seed oils), esterified oils or refined olive oils, which due to the refining process and solvent extraction have lost phenolic compounds.

In order to guarantee the quality of marketed products, very drastic requirements tend to be legally imposed. To undertake necessary controls and to detect the adulteration of food products many analytical techniques are used: HPLC, GC-MS, GC-FTIR, IRMS and NMR [1]. Isotopic analyses are now official or standard methods in Europe and North America for routine use in testing the authenticity of several food products. These methods are based on the measurement of stable isotope content ($^2$H, $^{13}$C, $^{18}$O) of the product or of a specific component such as an ingredient or target molecule of the product. The determinations carried out using nuclear magnetic resonance (NMR), and/or Isotopic Ratio Mass Spectrometry (IRMS), provide information on the botanical and geographical origin of the food product.

In the early 1990’s the EU adopted the $^2$H-NMR method for wine analysis as an official method (EEC/2676/90), in order to tackle the problem of over-chapitalization or over-enrichment of wines in Europe [2]. This was followed by the official publication of a method for the determination of $^{18}$O/$^{16}$O isotopic ratios of water from wine by IRMS (EEC/822/97) [3]. The measurement of $^{13}$C/$^{12}$C isotopic ratios of ethanol from wine has been accepted as an OIV Resolution [4].

The main idea behind these authorized methods is that each plant has its own unique pattern of naturally occurring stable isotopes of carbon ($^{12}$C, $^{13}$C), nitrogen ($^{14}$N, $^{15}$N), hydrogen ($^1$H, $^2$H), and oxygen ($^{16}$O, $^{18}$O) whose distribution has been influenced by a number of physical and/or biochemical properties and geoclimatic conditions. The isotope content of natural products depends on their botanic and geographical origin. The most sophisticated and most specific method for detecting adulteration is SNIF - NMR (Site -specific Natural Isotope Fractionation - NMR). This method is based on the measurement of deuterium / hydrogen (D/H) ratios at the specific sites of the ethanol molecule [5]. In the case of European wines, it was decided in 1991 to build an isotopic data bank concerning wines of all Member States producing wine. At this time, the official laboratories of these States supply the data bank that is maintained in the Joint Research Centre of Ispra (Italy) [6].
RESULTS AND DISCUSSION

The purpose of this paper is to implement the SNIF-NMR technique on the BRUKER Avance III 500 UltraShield NMR spectrometer equipped with a special probehead (SEX 500 MHz S2 10 mm) for recording ²H RMN spectra, proton decoupling and lock on ¹⁹F.

The SNIF method is based on the measurement of deuterium / hydrogen (D/H) ratios at the specific sites of the ethanol. In ethanol, deuterium may be located in the sites:

\[
\begin{align*}
\text{CH}_2\text{DCH}_2\text{OH} & \quad \text{CH}_3\text{CHDOH} & \quad \text{CH}_3\text{CH}_2\text{OD} \\
(\text{I}) & \quad (\text{II}) & \quad (\text{III})
\end{align*}
\]

Scheme 1

The (D/H) ratios are determined at the methyl (D/H)\text{I} and methylene (D/H)\text{II} sites of the ethanol molecule.

(D/H)\text{I} mainly characterizes the vegetable species which synthesized the sugar and to a lesser extent the geographical location of the place of harvest (type of water used during photosynthesis).

(D/H)\text{II} represents the climatology of the place of production of the grapes (type of rain water and weather conditions) and to a lesser extent the sugar concentration of the original must.

\[ R = \frac{2^{(D/H)\text{II}}}{(D/H)\text{I}} \]

expresses the relative enrichment or depletion of the methylene site, the methyl site being arbitrary given the statistical weight 3. A random distribution of deuterium within the ethyl fragment would therefore be characterized by a value of R = 2. Since the area of an NMR signal is proportional to the number of nuclei resonating at the considered frequency, the isotope parameters are directly accessible from peak area measurements. Thus, if \( S_1 \) and \( S_2 \) denote the areas of the methyl and methylene signals in the deuterium spectrum of a distillate, \( R = \frac{S_2}{S_1} \). The value of R varies according to the fermentation process and the sugar used.

Using this method we present the obtained results for a series of Romanian wines:
- sample 1, semi-sweet white wine;
- sample 2, semi-dry red wine;
- sample 3, semi-dry white wine;
- sample 4, semi-dry white wine.
Figure 1 represents a $^2$H NMR spectrum, recorded on a sample of ethanol obtained from wine by distillation. In this spectrum, we can see the signals for the three sites of ethanol.

Table 1. The $R$ values and their standard deviations for each studied samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R$</th>
<th>$\bar{R} \pm SD$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.37548</td>
<td>2.403±0.026</td>
</tr>
<tr>
<td></td>
<td>2.41916</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.38682</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.42974</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.31089</td>
<td>2.357±0.044</td>
</tr>
<tr>
<td></td>
<td>2.35831</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.41488</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.34192</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.47975</td>
<td>2.408±0.057</td>
</tr>
<tr>
<td></td>
<td>2.33918</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.43982</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.42170</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.36202</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.46609</td>
<td>2.404±0.057</td>
</tr>
<tr>
<td></td>
<td>2.47893</td>
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<td></td>
<td>2.34082</td>
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</tr>
<tr>
<td></td>
<td>2.40770</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.36967</td>
<td></td>
</tr>
</tbody>
</table>
The obtained values of R and their standard deviations are given in Table 1. The number of experiments / sample was 4. In order to obtain more quantitative data, mainly (D/H)$_{II}$, (D/H)$_{I}$, and R, we need a reference substance with very well defined D/H ratio. SNIF NMR recommend that tetramethylurea (TMU) supplied by IRMM, Geel Belgium to be used. In order to decide if a certain wine was modified or not, the producer must supply an original wine sample which will be considered as reference one. Detailed results will be published in a forthcoming paper.

CONCLUSIONS

In this paper, were performed $^2$H NMR measurements on ethanol distilled from wine by distillation. For each sample four measurements were made and the R average value and standard deviation for each sample were obtained separately.

Our results prove that R value can be obtained with a standard deviation of maximum ± 0.06.

EXPERIMENTAL SECTION

NMR measurements were performed the BRUKER Avance III 500 UltraShield NMR spectrometer equipped with a special probehead (SEX 500 MHz S2 10 mm) for recording $^2$H RMN spectra, proton decupling and lock on $^{19}$F.

All measurements were performed using the following acquisition parameters:

Spectral width 10 ppm
Data points 8 K
Pulse width 23.5 μs
Frequency 76.77 MHz
Number of scans 256
FID resolution 0.093 Hz
Acquisition time 5.33 s
Relaxation delay 5 s
Temperature 302 K

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REFERENCES