Identification and Quantitative Measurement by ^{1}H-NMR Spectroscopy of Several Compounds Present in Romanian Wines

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Wine composition presents a large variety, generated mainly by the type of grapes used, geographical origin and climate. In this paper it was established markers in the high-resolution ^{1}H-NMR spectrum of some Romanian wines. Those markers can be used for identification and quantitative measurement of 14 minor components which can usually be found in wines. Except water and ethanol, main components of wine, identified until now, there are also: carboxylic acids (acetic, citric, lactic, malic, succinic, tartaric), amino acids (alanine, isoleucine, leucine, proline, valine), monosaccharides (fructose, glucose) and glycerol.

Keywords: NMR, wine, carboxylic acids, amino acids, monosaccharides

One of the most important problem of the food chemistry is composition determinations. Although the sensitivity of the NMR method is smaller than for other chemical analyses, the advantage of the NMR method is given by the fact that this method can provide direct information and a total biochemical profile for the sample [1-3]. Also, a single comprehensive profiling procedure could reduce costs by replacing multiple analyses. Classical chemical analyses need predetermined analytical conditions, longer time and can give information regarding only the pre-selected compounds. NMR profiling could be used to detect unknown and unexpected changes and also help selecting the best suited classical methods for further analyses when necessary.

The compositional balance of small molecules (sugars, acids etc.) makes an important contribution to food's sensorial characteristics and can also act as a 'fingerprint that provides evidence of a food's origin [4]. Wine represents a very important subject in the food chemistry, numerous publications dealing with this subject.

One special technique used at the international level for determinations of geographical origin, production year and wine adulteration is SNIF-NMR method. This method distinguishes between ethanol samples from different sources based on the relative amounts of deuterium in the samples and the relative distribution of the deuterium within the ethanol molecule [5-9].

Surprisingly, in spite of the fact that ^{2}H-NMR (SNIF-NMR) became a routine technique in wine analysis almost 20 years ago, ^{1}H-NMR was not extensively used for whole wine sample screening until very recently.

Thus, recent ^{1}H-NMR studies included the composition analysis of wines coming from different geographical areas in Slovenia [10], the determination of minor compounds in wine [11], characterization of the geographical origin of Italian red wines [12] and even a non-destructive method of determining acetic acid spoilage in an unopened bottle of wine [13, 14]. Earlier ^{1}H-NMR studies of wines involved only structural studies of isolated compounds like pigments [15, 16], polyphenols [17] or sugars [18].

Results and discussions

Standard attributions

The first part of this work was to establish the correct attributions in the high-resolution ^{1}H-NMR spectrum, for all compounds used as markers. In order to ascertain exact attributions in the ^{1}H and ^{13}C-NMR spectra it was also recorded for each standard compound the DEPT, COSY, HMQC and HMBC spectra.

Wines attributions

In the usual ^{1}H-NMR spectrum of a wine sample, the water signal overlaps the other signals which are important for this study. In order to observe these smaller signals, it was recorded ^{1}H-NMR spectra with water presaturation.

In order to validate the assignments of signals in the wine sample, it was spiked the whole wine sample with standard compounds. For each standard, only those signals which are not overlapped have been used as markers. Figure 1 exemplifies the area between 3.1-4.3 ppm from in the ^{1}H-NMR spectra of a wine sample (A) without and (B) with added glucose. Some of the peaks (for instance the one from the encircled area) kept their dimensions; meanwhile other peaks are increasing in the spectra for the wine with added glucose (B). It was chosen as marker for glucose, the peak at 3.2 ppm because it is not overlapped and can be easily quantitatively measured.

The same methodology was applied for each standard compound thus obtaining specific markers. Table 1 exemplifies some characteristic peaks, which are identified as markers for the standard substances studied in a Romanian white wine („Blanc Romanesc").

The ^{1}H-NMR spectra of the studied Romanian wines showed the following characteristics:

- dry wines don't include glucose at a distinguishable level;
- red wine in comparison with the white one, contains smaller amounts of carboxylic acids (they don't contain malic and citric acids at a distinguishable level);
- red wines, present a higher content in amino acids in contrast with white wines.

The position of the marker signals can suffer some small adjustments with the *p*H of the wine sample. This aspect should be taken in consideration and the errors

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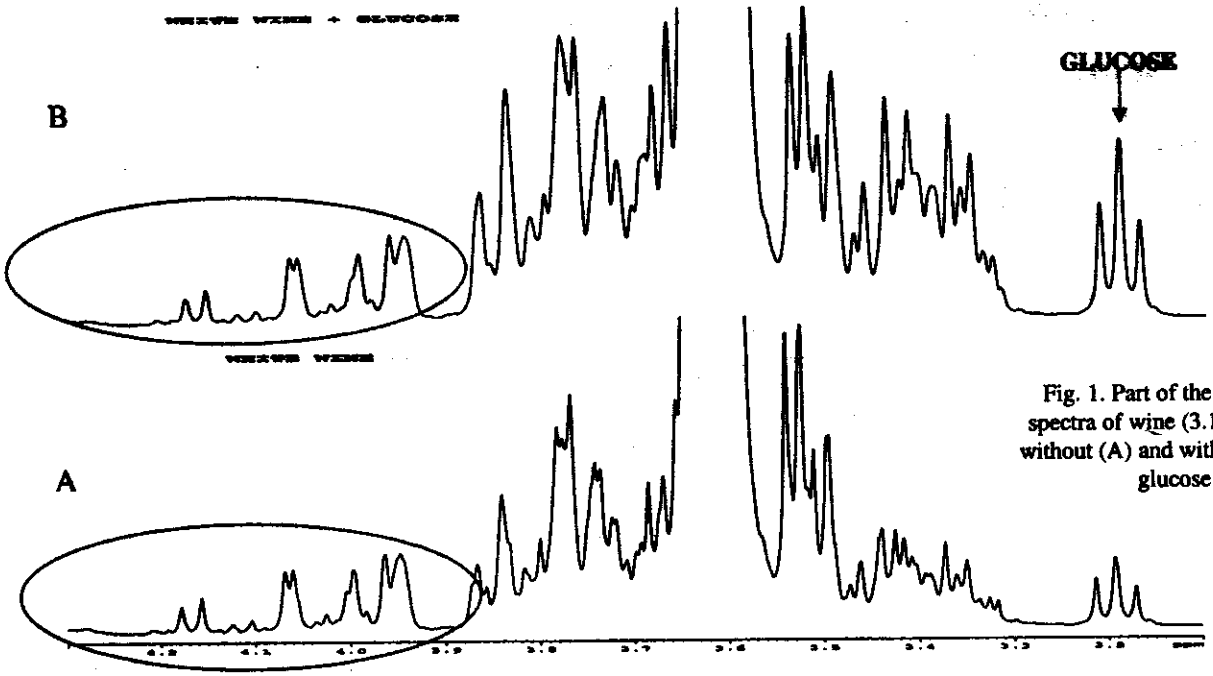

Fig. 1. Part of the ^{1}H-NMR spectra of wine (3.1-4.3 ppm), without (A) and with (B) added glucose

Table 1

No.	Standard Compound	δ (ppm) in water	δ (ppm) in wine	No.	Standard Compound	δ (ppm) in water	δ (ppm) in wine
1.	Acetic acid	1.90	1.84, s	8.	Proline	1.96	1.94, s
2.	Lactic acid	1.16	1.18, d	9.	Alanine	1.46	1.42, d
3.	Malic acid	2.82	2.70, d	10.	Valine	2.26	2.22, m
4.	Tartaric acid	4.68	4.57, s	11.	Leucine	1.73	1.65, m
5.	Succinic acid	2.64	2.59, s	12.	Fructose	4.10	4.05, d
6.	Citric acid	2.97	2.93, d	13.	Glucose	3.25	3.17, t
7.	Isoleucine	1.99	1.93, m	14.	Glycerine	3.50	3.45, q

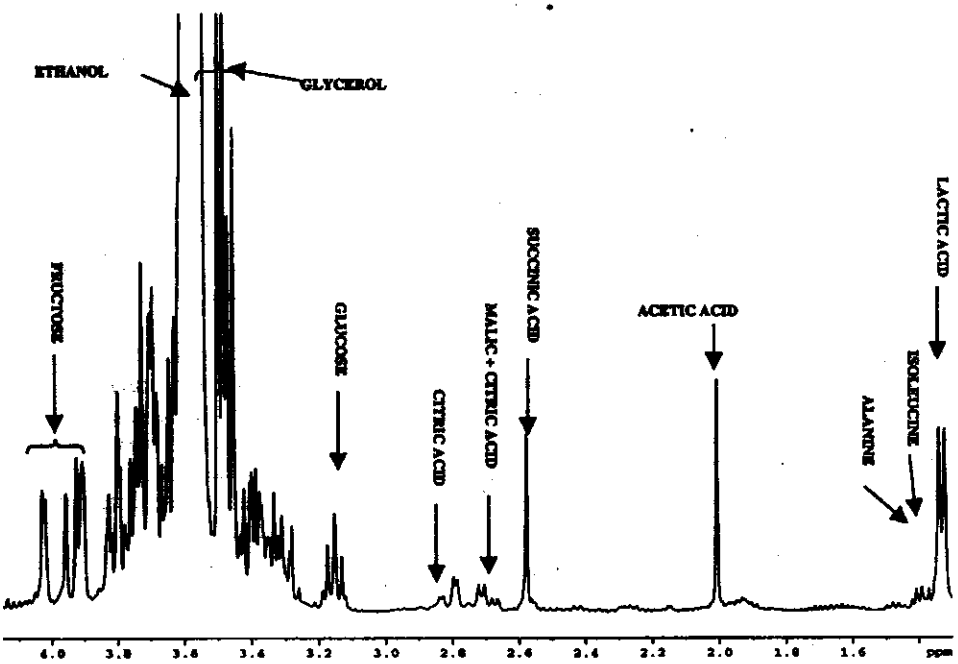

Fig. 2. Part of the ^{1}H-NMR spectra of a white wine sample - "Fetească Regală"

can be eliminated by recording the spectra at the same *p*H level.

The signals can be quantitatively measured in two ways: in relative ratio with ethanol (which is known) or in absolute concentration, in ratio with an intern standard (e.g. TSP added in a concentration of the same order of magnitude as the studied compounds).

As an example, figures 2 and 3 present the area between 1.3-4.1 ppm from a white wine sample (2) and a red wine sample (3).

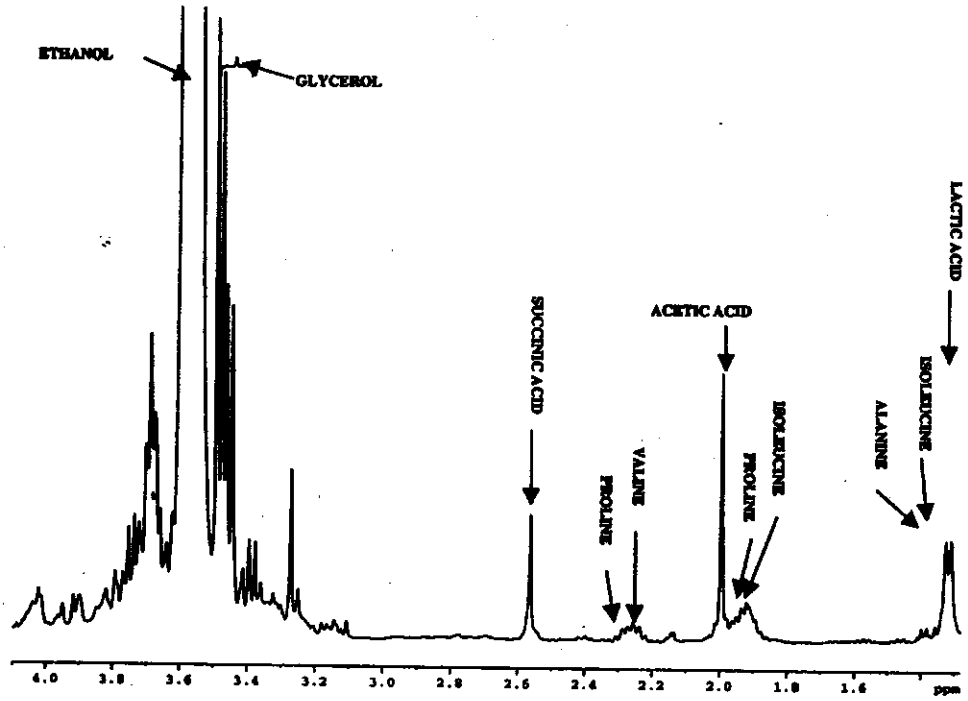

Fig. 3. Part of the ^{1}H-NMR spectra of a red wine sample - "Murfatlar"

There are significant differences in the composition of the minor compounds for different types of Romanian wines. Also it was noticed major differences in comparison with the profile of some Slovenian wines analyzed by Kisir and Kidric [6].

Eperimental part

The NMR spectra have been recorded on a Bruker Avance DRX 400 spectrometer, operating at a field of 9.4 Tesla, corresponding to the resonance frequencies of 400.13 MHz for the ^{1}H nucleus and 100.61 MHz for the ^{13}C nucleus, equipped with an inverse detection two channels multinuclear probehead and field gradients on the z axis.

The samples were analyzed in 5 mm NMR tubes (Norell 507).

The NMR samples where prepared by adding 10% v/v D2O (Aldrich) to the wine to ensure the lock of the spectrometers' magnetic field.

The chemical shifts are reported in ppm as δ values, using as reference the water signal at 4.78 ppm.

Typical parameters for the ^{1}H-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 ^{1}H-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.

Conclusions

Through identification and quantitative measurement of 14 minor components, there were obtained "fingerprints" for several Romanian wines. These fingerprints could be used to establish wine identity.

Thus, the NMR method is a powerful tool for quantitative measurements and identification of the organic compounds without any previous separation of the wine samples. The method has a high potential for application in some other food branches.

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