

Methodological issues of grape and wine water evaluation by quantitative nuclear magnetic resonance deuterium spectroscopy ($^2\text{H(D)}$ -qNMR)

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Abstract. The new methodological approach of rapid evaluation of intracellular grape and wine water without sample preparation, based on the quantitative nuclear magnetic deuterium spectroscopy ($^2\text{H(D)}$ -qNMR) is suggested and developed under the research program carried out under the scientific grant of the International Organization of Vine and Wine (O.I.V.). The approach provides a quantitative high precision measurement of the deuterium content in water based on the direct dependence of the areas of NMR signals on the number of nuclei responsible for these signals. The developed approach uses similar to the well-known SNIF-NMR method with an internal reference substance (IRS) - dimethyl sulfoxide (DMSO). The IRS signals in the NMR spectrum do not overlap with the signals of main components of the analyte (e.g., grape must/juice, wine). In addition, it is possible to change the deuterium content using an available NMR solvent - DMSO- d_6 . At the same time, the amount of deuterium in the IRS-DMSO is increased by adding DMSO- d_6 so that the proportion of the standard, equal to 10-15% by volume, gives a signal commensurate in its intensity with the signal of water contained in the analyte. The exact amount of deuterium in the IRS is determined by comparing the integral intensities of signals in the $^2\text{H(D)}$ -NMR spectrum in a standard water sample (VSMOW) with a known deuterium content. After calibration of the deuterium content in IRS, the analyte and DMSO in quantities of 0.5 and 0.075 ml, respectively, are placed in a standard tube, then the $^2\text{H(D)}$ -NMR spectrum is measured in the following conditions: 90° pulse, 2.5 sec acquisition time, 3 sec delay relaxation, 1000 scans. Additionally, to assess the amount of water in the analyte, the ^1H -NMR spectrum is recorded, from which the contents of main sample components, for example, ethanol, glycerol, sugars, etc., are determined. The calculation of the deuterium content is carried out by quantifying the integral intensities of the IRS signals and the water contained in the sample. The developed methodological approach has been validated in experimental tests to study the natural content of deuterium in intracellular water of grapes of different seasons, as well as to study changes in the quantitative balance of deuterium in the case of adding extension water to musts before and after fermentation.

1 Introduction

A number of analytical methodologies are used, for example, the method of measuring water potential using a pressure chamber according to Scholander et al. [1] to study the characteristics and state of water as a participant in physiological and biochemical processes. The mass spectrometric IRMS/SIRA method for analysis of the ratios of stable isotopes of light elements is widely used to study the thermodynamics of the water exchange of plants with the environment, in particular in terms of

the formation of specific properties of water, which characterize in the case of grapes - the level of natural and climatic influence, in the case of wine products - the origin and use of third-party components (e.g., water). There are various approaches in the IRMS/SIRA method for solving evaluation problems by analyzing the isotope composition of water in grapes and wine products, for example, direct measurement of the $^{18}\text{O}/^{16}\text{O}$ oxygen isotope ratio in water [2] or an indirect approach based on

analyzing the distribution of $^{13}\text{C}/^{12}\text{C}$ carbon isotopes in characteristic components (e.g., sugars, organic acids) of plants and products of their processing, followed by an assessment of the relationship between the results of studies with the water balance of plants and the external conditions for growing grapes [3-7]. This paper presents the new qNMR spectroscopy method of deuterium nuclei ($^2\text{H(D)}$ -qNMR) for their direct quantitative determination in the structural elements of water without its preliminary isolation from grape musts and wines.

2 Materials and methods

2.1 Grape and wine samples

The samples of the 6 white and red grape varieties were used for the method approbation: white grapes – Aligote and Rkatsiteli, red grapes - Cabernet Sauvignon, Merlot, Pinot Noir and Bastardo.

The main areas of cultivation of grapes are concentrated in 12 major zones of the Crimean Peninsula, which include, for example, Western Seaside-steppe, Submontane, South Coast, Eastern Steppe and other areas. The sampling of the authentic fresh grapes was carried out in 3 viticulture areas with different grape growing conditions: Western zone of the South Coast area (Livadia), Western Foothills-Maritime area (Uglovoye) and Western Maritime-Steppe area (Evpatoria) – see Fig. 1. The sampling was conducted according to the requirements for collection of an average sample of grapes [8].



Figure 1. Grape sampling areas on the Crimean Peninsula.

Dry white and red wines (residual sugar <math><4.0\text{ g/l}</math>) have been made from the fresh musts under laboratory conditions. The fermentation of fresh musts was carried out using a pure 47K yeast culture (collection of the Magarach Research Institute), which was added in an amount of 2% of the must volume. The SO_2 in an amount of 75-100 mg/l was added to musts before fermentation. Fermentation of musts was carried out at a temperature of 20-22 °C. Prior to the qNMR measurements the musts and wines were stored at a temperature from 2 to 4 °C in sealed sterile glass containers without access of air. In addition of grape and wine samples, model systems were

used to validate the correctness of qNMR measurements. A 30% sucrose solution and a 12% solution of ethanol of grape origin, prepared with tap water from the Moscow city supply network, were chosen as model systems.

2.2 qNMR-Measurement

A BRUKER Avance™ NEO 700 NMR spectrometer (Germany) with an operating frequency for Protium ^1H 700 MHz and Deuterium $^2\text{H(D)}$ 107 MHz was used in the study. To provide increased resolution and accuracy of measurements, this model of NMR spectrometer is equipped with a cryoprobe and an autosampler. The NMR spectrometer is shown in Fig. 2. The device has the ability to work with 5 and 10 mm probes without stabilizing the resonance conditions at the ^{19}F fluorine frequency, since the field drift during spectrum registration is insignificant. Calibrated tubes with a diameter of 4.97 ± 0.013 mm and a length of 178 mm were used for measurements in this study. A mixture consisting of dimethyl sulfoxide (DMSO) and deuterated dimethyl sulfoxide (DMSO- d_6 , 99.9% D, Sigma Aldrich, Cat. # 2206-27-1) was chosen as an internal standard to be used in the method.



Figure 2. Magnet unit of the BRUKER NMR spectrometer Avance™ NEO 700.

The deuterium content of the internal DMSO standard is calibrated before starting the study. After calibration, the amount of deuterium according to the $^2\text{H(D)}$ NMR spectrum of the sample should provide an integral signal intensity comparable to the water signal of the must or wine material when 75 μl of DMSO is added to 500 μl of sample. The ratio (D/H) in the prepared solution is calculated after providing the necessary amount of

deuterium in the standard by comparing in the NMR ²H spectrum the integral signal intensities of DMSO and a IAEA standard water sample – “Vienna Standard Mean Ocean Water” (VSMOW) with known deuterium content. For this purpose, a solution consisting of 500 μl of the VSMOW standard water sample and 75 μl of DMSO is prepared. Then a ²H NMR spectrum is recorded under standard measurement conditions: 90° pulse, 3 s delay, 01 – 5 ppm, sweep – 25 ppm, 8K points per spectrum, acquisition time – 2.5 s, 1000 scans. The spectrum was processed using Bruker TopSpin 4.1.3 software. Automatic baseline correction, manual phase correction, exponential multiplication for 2.0 Hz were used to process the spectrum. An acceptable measurement accuracy is provided by the technical characteristics of the used high-resolution NMR spectrometer equipped with a cryoprobe, and by the applied measurement conditions.

2.3 Calculation of measurement results

The ratio $(D/H)_{st}$ in DMSO was calculated according to Eq. (1), by the same way as [9]:

$$(D/H)_{st} = \frac{N_{H2O} \cdot M_{st} \cdot m_{H2O} \cdot I_{st}}{N_{st} \cdot M_{H2O} \cdot m_{st} \cdot I_{H2O}} \cdot (D/H)_{H2O} \quad (1)$$

where N_{H2O} is the stoichiometric number of hydrogen atoms in VSMOW water,

N_{st} - stoichiometric number of hydrogen atoms in DMSO,

M_{H2O} - molecular mass of water, g/mol,

M_{st} - molecular mass of DMSO, g/mol,

m_{H2O} - weight of a sample of VSMOW, g,

m_{st} - weight of DMSO, g,

I_{H2O} - integrated signal intensity of VSMOW water,

I_{st} - integrated signal intensity of DMSO,

$(D/H)_{H2O}$ – isotope ratio of the VSMOW water, ppm.

The value of $(D/H)_{st}$ after calibration of the internal standard is used to determine the deuterium content of the studied samples. Before NMR changes in the studied samples, the determination of the water content was carried out. In grape musts, soluble solids content was determined on a RM40 laser refractometer (Mettler Toledo, Switzerland). Water content of wine was determined by ¹H NMR spectroscopy and subtraction of the main components - ethanol and glycerol - in the obtained NMR spectrum. Typical ¹H and ²H NMR spectra of the wine materials are shown in Figs. 3 & 4.

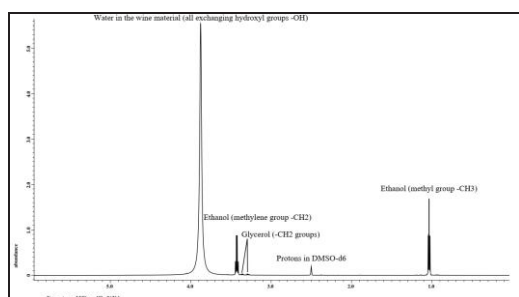


Figure 3. ¹H NMR spectrum of wine.

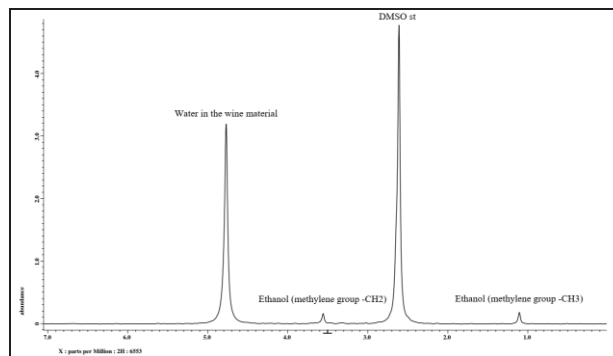


Figure 4. ²H NMR spectrum of wine.

Isotope ratio $(D/H)_a$ of water of the studied grape musts and wine materials was calculated by Eq. (2), similar to the official SNIF-NMR OIV-method [10]:

$$(D/H)_a = \frac{N_{st} \cdot M_{H2O} \cdot m_{st} \cdot I_{H2O}}{N_{H2O} \cdot M_{st} \cdot m_{st} \cdot I_{st}} \cdot (D/H)_{st} \quad (2)$$

where N_{st} is stoichiometric number of hydrogen atoms in DMSO,

N_{H2O} – stoichiometric number of hydrogen atoms in water,

M_{H2O} – molecular mass of water, g/mol,

M_{st} – molecular mass of DMSO, g/mol,

m_a – weight of a sample, g,

m_{st} – weight of DMSO, g,

I_{H2O} – integrated signal intensity of samples water,

I_{st} – integrated signal intensity of DMSO,

ω_{H2O} – the water content in sample,

$(D/H)_{st}$ – isotope ratio of DMSO, ppm.

3 Results and Discussion

The results of approbation of the method are shown in Tables 1 and 2. During the approbation, to verify the accuracy of the determination of the deuterium content, a series of experiments with model systems was additionally carried out. As part of this series, the content of deuterium in a 30% solution of sucrose and a 12% solution of ethanol of grape origin was investigated. Both solutions were prepared using Moscow city tap water. Isotope ratio of water samples was 142.65 and 142.77 ppm for the sucrose and ethanol solutions, respectively. Isotope ratio of city tap water in Moscow is 142.15 ppm. The estimated measurement error in the developed method is less than 2%.

The results of the study show that during the production of wines in controlled laboratory conditions the statistically significant reduction of deuterium content in wine water was observed. The difference between the initial and obtained $(D/H)_a$ values ranges from 1.19 to 9.66 ppm (for the crop 2020) and from 5.40 to 12.09 (for the crop 2022). The effect of reduction of deuterium content in the wine water compared to the original grape must is considered proven, but it requires a further research.

The experimental data obtained using the developed method correlate with the results of preliminary studies of the period 2015-2016 [11], according to which the isotope ratio of water in wines from the Crimea and Krasnodar region was from 157.0 to 166.0 ppm, and isotope ratio of surface and ground water in Krasnodar region did not exceed 148.0 ppm.

The cumulative results of studies obtained during the preliminary experiments, as well as in the modern measurements based on the developed method ²H(D)-qNMR allow to propose the so-called a limit value, characterizing the natural level of deuterium in authentic wines, $(D/H)_{LV} \geq 157.0$ ppm. This limit value is a provisional value. The specified limit value is subject to adjustment in case of receipt of new experimental data received in the process of annual monitoring researches of grapes grown in different natural-climatic zones, and also researches of products of its processing (for example, mash, wines), made in industrial conditions and/or delivered on the Russian market on import from the countries of near and far abroad.

Considering the practical importance of the developed method ²H(D)-qNMR and the proposed limit value for the isotope ratio of water of authentic wines, it seems acceptable to calculate the proportion of external water (EW) in the wine products according to the Eq. (3):

$$EW (\%) = \frac{D/H_{sample} - D/H_{lv}}{D/H_{water} - D/H_{lv}} \times 100 \quad (3)$$

where $(D/H)_{sample}$ is the measured deuterium content in the sample (e.g., must, wine), ppm,

$(D/H)_{water}$ – measured deuterium content in water of surface and underground sources (e.g., 148.0 ppm), ppm,

$(D/H)_{lv}$ – limit value (e.g., provisional limit of 157.0), ppm.

Table 1. Deuterium level in musts and wines from grapes of the selected areas of the Crimean Peninsula (crop of 2020).

Grape variety	Climate zone	(D/H) _a , ppm	
		must	wine
Bastardo	Maritime-Steppe	163.74	156.53
Rkatsiteli	Maritime-Steppe	160.17	158.67
Pinot Noir	Foothills-Maritime	160.85	159.66
Rkatsiteli	Foothills-Maritime	160.61	157.22
Merlot	Foothills-Maritime	160.46	157.65
Aligote	South Coast	164.80	155.14
Aligote	Maritime-Steppe	158.87	156.54

Table 2. Deuterium level in musts and wines from grapes of the selected areas of the Crimean Peninsula (crop of 2022).

Grape variety	Climate zone	(D/H) _a , ppm	
		must	wine
Merlot	Maritime-Steppe	171.10	159.01
Rkatsiteli	Maritime-Steppe	164.44	159.04
Cabernet Sauvignon	South Coast	166.28	158.96
Rkatsiteli	Foothills-Maritime	163.75	158.23
Merlot	Foothills-Maritime	168.88	157.97
Aligote	Foothills-Maritime	165.89	158.65

4 Conclusions

On the basis of the developed method of high-resolution nuclear magnetic resonance spectroscopy ²H(D)-qNMR a new methodical approach for the study of isotope ratio in water of grapes and products of their processing - musts and wines is proposed. In contrast to existing NMR methods [12-14] the new methodological approach allows the use of spectrometers not equipped with fluorine lock and self-made standards enriched with deuterium.

The developed Deuterium-²H(D)-qNMR method, in contrast, for example, to the IRMS/SIRA method of mass spectrometry of stable isotope ratios of light elements, does not require preliminary extraction of searched compounds from sample matrix before measurement that not only provides selectivity of determination, but also essentially increases reliability and accuracy of its results. The high resolution Deuterium-²H(D)-qNMR nuclear magnetic resonance spectroscopy method and the new methodological approach based on it are designed for simultaneous research of the component composition of wine products by means of ¹H NMR spectroscopy of the sample (e.g., for analysis of ethanol, secondary fermentation products, carbohydrates, organic acids etc.) and measurement of isotope ratio to study characteristics of water contained in the products. Research will be continued.

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Conflict of Interest

The authors declare no conflict of interest.

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