



**OIV**  **100**

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## **Testimony by Dr. José Enrique Herbert-Pucheta from Escuela Nacional de Ciencias Biológicas in Mexico**

The OIV method type II No. **OIV-MA-AS2-12**: “Method for  $^{18}\text{O}/^{16}\text{O}$  isotope ratio determination of water in wines and must”, published at the Compendium of International Methods of Analysis of the International Organization of Vine and Wine (OIV), comprises the determination of the  $^{18}\text{O}/^{16}\text{O}$  isotope ratio of water from wine and musts after equilibration with standard carbon dioxide  $\text{CO}_2$ , using the Isotope Ratio Mass Spectrometry technique (IRMS).

The smallest representation of matter in chemistry is defined by atoms, composed by protons and neutrons within their atomic core (this ensemble in turn defined as nucleons) surrounded by electrons. Each element possesses a unique configuration number composed by a specific number of protons and electrons. However, nucleons in elements might change in their number of neutrons. Atoms with the same number of protons (and thus electrons for electrostatic stability) but different number of neutrons are known as isotopes. Different isotopes from the same element might share equivalent chemical properties but differ in mass and therefore in physical properties. The first 80 elements of the periodic table (from hydrogen up to mercury) present almost all of them, at least one stable isotope (meaning that they do not emit any kind of radiation). The following elements (from Thallium-81, Lead-82, up to Ununoctium-118) present at least one radioisotope, that means that they emit radiation. An Isotopic Signature is an established set of isotope ratios between a low abundance isotope with respect its higher natural abundance isotope, of an element within a sample. Isotopic signatures are commonly recognized as tracking & tracing fingerprints of diverse plants', humans', animals', insects' and lands' processes such as anthropological – archaeological ageing; geographical and botanical provenances of foodstuff; food chains and dietary changes in animals; origins, ages and quality of water bodies; atmospheric pollution, amongst others. Natural stable isotope ratios used for the control of the authenticity of food and alcoholic beverages are from hydrogen ( $^2\text{H}/^1\text{H}$ ), carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and oxygen ( $^{18}\text{O}/^{16}\text{O}$ ) stable isotopes. The most common natural sources of oxygen in nature are atmospheric oxygen ( $\text{O}_2$ ), carbon dioxide ( $\text{CO}_2$ ) and water ( $\text{H}_2\text{O}$ ). Oxygen isotopic fractionations naturally occur in diverse evapo-condensation processes. Some important oxygen isotopic relations found in nature are: i)  $^{16}\text{O}$  is the



most abundant source of oxygen with a 99.757% of presence in nature whilst  $^{18}\text{O}$  is the most stable isotope of oxygen with a 0.205% abundance; ii) the  $^{18}\text{O}$  isotope in clouds is in higher amount than the  $^{18}\text{O}$  in ocean water, whilst meteoric water is highly enriched in  $^{18}\text{O}$  isotope, compared to clouds. Wine fermentations do not cause any  $^{18}\text{O}$  water enrichment, but exogenous water does (tap water). In consequence,  $^{18}\text{O}/^{16}\text{O}$  isotopic signatures are measured in oenological samples to detect watering of wines as well as juices' dilutions. Contrary to oxygen,  $^2\text{H}$  enrichment occurs in wine water during fermentation processes. For that, the deuterium ( $^2\text{H}$ , 0.0114% natural abundance) / hydrogen ( $^1\text{H}$ , 0.0114% natural abundance) isotopic signatures are measured to detect  $\text{C}_4$  plant sweetening (sugar canes or corns) in wines (coming from  $\text{C}_3$  vines) and juices, as well as to characterize chaptalization and authenticity of oenological products. Finally  $^{13}\text{C}$  (1.06% natural abundance) /  $^{12}\text{C}$  (98.93% natural abundance) carbon isotopic signatures are mediated by carbon isotopic dynamics of  $\text{CO}_2$  fixation in plants that produces three different isotopic signatures: i)  $\text{C}_3$  plants (Calvin photosynthetic cycle in plants such as vines, potatoes, rices, etc.); ii)  $\text{C}_4$  plants (Hatch and Slack photosynthetic cycle present in corns or sugar canes) and iii) CAM plants (Crassulacean Acid Metabolism) observed in cactus, pineapples, etc. Specific  $^{13}\text{C}/^{12}\text{C}$  fingerprints basically allow to detect sugar sources in wines' and spirits' fermented alcohol.

Nowadays there is a need to establish worldwide  $^{18}\text{O}/^{16}\text{O}$ ,  $^2\text{H}/^1\text{H}$  and  $^{13}\text{C}/^{12}\text{C}$  isotopic signatures of diverse oenological samples by creating robust data basis, in agreement to the 2020-2024 / 2025-2029 OIV Strategic Plan, in order to have global harmonic regulations for controlling authenticity and counterfeiting. The use and regulations of diverse Artificial Intelligence (AI) algorithms, in combination with high-resolution analytical techniques such as IRMS or Site-specific Natural Isotopic Fractionation Nuclear Magnetic Resonance Spectroscopy (SNIF-NMR) is undoubtedly the prelude of a Novel Generation in the OIV Sub-commission of Methods of Analysis, whereas the accurate validation of both analytical methods and AI algorithms, shall fulfil some of the United Nations-Sustainable Development Goals (UN-SDGs), defined at the recently created OIV Digital Transition Plan.



## **Testimony by Dr. Alexander Kolesnov from Peoples' Friendship University of Russia named after Patrice Lumumba in Russia**

The IRMS/SIRA-Mass-spectrometry for analyzing the isotopic composition of light elements, mainly carbon and oxygen, takes one of the central places in the overall analytical potential of modern viticulture and winemaking. Despite the fact that this methodology has now become classical and has reached the maximum of its capabilities, its contribution to the development of analytics in winemaking can hardly be overestimated. Since the first half of the 20th century, when the world theorists of the practical mass-spectrometry - Arthur Dempster, Kenneth Brainbridge and Alfred Nier, who created the first models of instruments capable of making measurements with the required accuracy and reliability, the analysis of ratios of stable isotopes of light elements has gone a long way from an exclusive high-sophisticated methodology of fundamental scientific research to a familiar routine analytical method, available for use in a wide range of laboratories, including industrial ones.

With the resolution OIV-OENO 2/96, the database of international analytical methods was first supplemented in 1996 with the mass-spectrometric method for determining the ratios of oxygen isotopes  $^{18}\text{O}/^{16}\text{O}$  in wine and must water, which was subsequently amended by the Resolution OIV-OENO 353/2009 and is currently available for use by all interested parties as the official OIV method (OIV-MA-AS2-12, type II method). Along with other mass-spectrometric methods for determination of isotopic ratios, mainly carbon, in a number of components (carbohydrates, ethanol, glycerin and carbon dioxide), this analysis implements a methodological approach that allows to obtain objective information about the isotopic composition of oxygen in water, which is not only one of the main components of wine, but also to a greater extent, the environment in which the biochemical fermentation processes take place, as well as the processes of maturation and eventually the formation of authentic and, last but not least, organoleptic properties of products. The results of the analysis with a sufficiently degree of reliability allow us to answer the question about the nature of wine and must water, which in turn is of great importance for the objective assessment of quality, especially when solving problems of identifying adulterated products made by water extension. Since its approval as an official OIV document, this method, together with other methods of analysis, has been a reliable analytical resource for preserving the authenticity of wine and preventing manipulation of its composition.



The prerequisite for the successful application of the method was the base of scientific knowledge laid down in the 20th century from 1953 to 1997 in the works of a number of researchers, which provided the necessary level for the interpretation of analysis results. The experience of using the mass-spectrometric method for determination of ratios of oxygen isotopes  $^{18}\text{O}/^{16}\text{O}$  and expert interpretation of its results, as well as modern scientific achievements and knowledge about the isotopy of light elements in metabolic processes during the ripening of grapes and its technological processing have created the necessary foundation for the development and introduction of new approaches for evaluation of wine water in the current century. In this regard, the classical efficiency and reliability of the mass-spectrometric method for determination of isotopic ratios of oxygen  $^{18}\text{O}/^{16}\text{O}$  in wine water will be appropriately developed and supplemented by use of new advanced methods, which undoubtedly include high-resolution nuclear magnetic resonance spectroscopy of deuterium and protium nuclei ( $^2\text{H}/^1\text{H}$ -NMR).

This is the only way to ensure both scientific progress and continuity in applied analytics in winemaking, which in turn consist of three components - fundamental knowledge about the basic principles of measurement, professionalism in their implementation and, last but not least, the effectiveness of the practical application of the developed methods of analysis. Without the combination of all these components, it is impossible to develop sustainably the full analytical potential for the evaluation of quality and safety of wines, as well as increase the confidence in results of the application of analysis methods on the part of both producers and consumers of wine.