

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



D-O-C Stable Isotopes, ^{14}C Radiocarbon and Radiogenic Isotope Techniques Applied in Wine Products for Geographical Origin and Authentication

Paraskevi Chantzi, Anastasia-Elektra Poutouki and
Elissavet Dotsika

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/64933>

Abstract

Oxygen, deuterium and carbon isotopes were measured in wine products in two Greek vineyards, Amydaio (north) and Nemea (south). The enriched isotope values in Nemea reflects the higher evapo-transpiration rate and the more arid condition of Southern Greece. White wines were slightly more depleted than red wines of the same year and the same growing region probably due to different harvest periods. Further was pointed out the variety of isotope values with respect to vintage year indicating that the vintage year contributes to the development of isotopes in wine water. In both vineyards the trend lines intersect the oxygen and deuterium isotopes of irrigation water highlighting the source water and the initial isotopic composition of grape berries. $\delta^{13}\text{C}$ values of ethanol confirmed the origin of C3 plants and the authentication of wine products without detecting adulteration with industrial alcohol. The results of ^{14}C measurements in ethanol extracted from Greek wines follow the known pattern of ^{14}C variations in atmospheric CO_2 . The homogeneity of $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{144}\text{Nd}/^{143}\text{Nd}$ isotope values confirms that the territorial and geological signal is transferred through the vineyards in the final product, wine, certifying the exclusively provenance of the wine areas Amydaio and Nemea.

Keywords: oxygen isotope, carbon isotopes, radiocarbon, Greek wines, grape berries

1. Introduction

Wine is a product that has suffered serious damage from adulteration and mislabeling practices. The authenticity of a wine label, which confirms the alignment of the product with the relevant laws of alcohol production, is of great importance in many levels. First of all, the protection of the consumers against the globalization of food market, where the adulteration episodes are strongly existent, should be the dominant objective. On the other hand, the authentication in conjunction with the geographic origin maintains and defends traditional production techniques that influence the particular output features of wine and which are lost through the competition of mass production. In general, modern dietary habits governed by standardized products, as required by the food industry, resulting the lack of the link between products and their geographical origin influenced by the particular environment setting. There is also a great interest by wine producers and traders so that they can guarantee high quality wines offer a reliable label. However, fraud incentives persist and with upgraded methods. Therefore, there is a necessity for the upgrading of the authentication methods of the wine label. Toward this direction the use of stable isotopes ^2H , ^{13}C , and ^{18}O in diet samples started in the early 1970s and later enriched by the use of radiocarbon ^{14}C and radiogenic isotopes Sr and Nd. The recognition of isotopes as food authenticity indicators was a point of reference in the food authentication approach. In fact, stable isotope ratio analysis of wine isotope ratio mass spectrometry is official methods in the European Union for the detection of chaptalization, addition of water, sweetening with sugar, and authentication of geographic origin and year of harvest.

2. Factors that influence carbon and oxygen isotopic values

The nature ratio of stable isotopes of plant tissues is strongly influenced by raw materials of photosynthesis, water and carbon dioxide, as well as the fractionation process during the photosynthetic pathways. Regarding carbon dioxide, it should be noted that the atmospheric reservoir considered as constant where the $\delta^{13}\text{C}$ composition barley varies from -7% [1]. Since then the atmospheric pool supplies fixed carbon stable isotope values, water ("source water") and fractionation processes are responsible for the differentiation in $\delta^{13}\text{C}$ values in plants. According to the photosynthetic pathway that each plant follows three types are distinguished with characteristic range of $\delta^{13}\text{C}$ values: C4 (Hatch-Slack, C4-dicarboxylic acid path-way) plants generally range from -9 to -19% [2, 3] while for C3 (Calvin cycle) plants typically range from -20 (open areas exposed to water stress) to -35% (closed canopy) [2, 3]. The third photosynthetic pathway called crassulacean acid metabolism (CAM) presents $\delta^{13}\text{C}$ values between the end-members of C3 and C4 types. Generally, the CAM cycle corresponds to plants that store quantities of water in their tissues to use under demand conditions; therefore, the biggest distinction is limited to C3 and C4 photosynthetic pathways. The first represents the cycle that the first synthesized sugars are three carbon sugars while in C4 cycle first sugars fixed are four carbon sugars. Consequently, alcohol derived from grapes should reflect $\delta^{13}\text{C}$ values of the corresponding photosynthetic pathway that the grapes belong, which is the C3

type. The addition of different origin alcohol from C4 type (synthetic or beet) could be traced as the $\delta^{13}\text{C}$ values are more enriched than those of the C3 type. This intense discrimination is due to the fact that C3 cycle is “longer” enabling a greater isotopic segregation against the “short” C4 cycle which is closer related to the end of the products [4]. Moreover, since the plant interacts with atmospheric CO_2 any observed fluctuations should be justified by the particular environmental characteristics of the region where the vineyards grow. In more detail, the intake water source for vineyards owns a crucial role in plant growth by determining the sugar content in grapes and therefore the alcohol formation. The “water deficit” causes stress to plant and affects its function process which in an attempt to maintain the needed humidity preventing the loss though evaporation closes its leave stomata [2, 5, 6]. As a direct consequence to this is the limited interaction with environmental CO_2 , which is the main factor in photosynthesis. The disruption of the system “leaf transpiration-carbon fixation per unit leaf area” results in a disruption in sugar formation in grape berries. Stress episodes lead to higher sugar level in fruits in contrast to those where water availability is stable and representative to the actual needs of the plant [7]. Consequently, $^{13}\text{C}/^{12}\text{C}$ isotope analysis was performed in wine samples. Ethanol samples which were extracted by number of wine samples were subjected in $^{18}\text{O}/^{16}\text{O}$, $^2\text{H}/^1\text{H}$, $^{13}\text{C}/^{12}\text{C}$, and radiocarbon (^{14}C) isotope analysis. As oxygen-18 and deuterium isotopes are affected by temperature, precipitation, humidity, altitude, latitude, and the distance from the sea [8, 9], the isotopic signature of vineyard’s “source water” should be related to corresponding geographical region. The emphasis is given to oxygen isotopic values (^{18}O) for the characterization of the plant organic matter as they present a more normal behavior. However, the interpretation of their $\delta^{18}\text{O}$ values is more complicated than that of carbon as it does not present such a linear formation as CO_2 due to the several oxygen sources (precipitation, atmospheric CO_2 , soil water) and the complexity of metabolic processes. The isotopic characteristics of feeding water, environmental conditions, photosynthetic fractionation, and the evapotranspiration effect on plant sap and leave stomata are considered the most dominant factors that gather the fixation of $\delta^{18}\text{O}$ values. However, we should further consider the cellular $\text{CO}_2\text{-H}_2\text{O}$ equilibrium as well as possible contribution of CO_2 and/or H_2O to the organic oxygen in the cell [4, 10, 11].

3. Environmental and hydrogeological setting of study areas vineyards

Tradition of Greece in winemaking is pronounced with the unequivocal uniqueness of Greek wines resulted by the combination of the Greek terroir and the high quality of local wine varieties. Therefore, the authenticity control and origin assignment of Greek wine products have obtained remarkable importance. In the context of this project, two characteristic wine-producing areas of continental Greece: Amydaio in west Macedonia, north Greece, and Nemea in Corinthia, south Greece are selected. The first constitutes a significant Greek vineyard between Vermion and Borra mountains where it is dominated by the cultivation of Xinomauro variety while the second concerns vineyards with the exclusive cultivation of Agiorgitiko variety. So an attempt was made to trace the correspondence of “VQPRD (*Vins de Qualite Produits dans une Region Determinee*) Amydaio and Nemea” labels with their geographical

origin. Many techniques have been performed on geographical origin traceability; however, isotopes have proven to be a valuable tool with robust and reliable results [12–16]. The study model was designed according to the following plan: “source water (precipitation)-plant development (photosynthesis)-wine (final product)” as all the environmental effects on the vine development, reflect their isotope signature in the final product of wine.

The predominately climate type in Greece is the Mediterranean with warm to hot, dry summers and mild to cool, wet winters; however, the unique topography of Greece constitutes to a large variation of microclimates which enhance the diversity and variety of traditional products. Amydaio located in the northwestern part of Greece, 33 km from Florina town (West Macedonia) at an altitude of 650 m above sea level without any influence of the Aegean Sea. Its climate is characterized as purely continental but it becomes milder because of the presence of Vegoritida Lake. Specifically, the climate presents as temperate continental with heavy winters accompanied by snow and low temperatures. The plateaus exhibit a southeastern direction without interfering mountains along them and the prevailing northerly winds to keep the temperature low. Summers are mild with higher temperatures presented at lower altitudes. The minimum average temperature in the winter months and the average maximum temperature during the summer are about 2.5°C and 29.6°C, respectively. Although the region of Western Macedonia belongs in the eastern mainland characterized by less rainfall compared to the corresponding west, the climate displays wet character (up to 75% humidity in the winter months of December–February) due to the combination of mountain landscape and lakes (Vegoritida, Zazari, Petron, and Cheimaditida lakes). The main volume of precipitation is observed in the autumn months. The wider area of Amydaio belongs to a tectonic basin with NE-SW trend formed during the Tertiary, as a consequence of strong tensile stress in the region. The Amydaion basin is divided into two elongated sub(tectonic wells), characterized by different geological-stratigraphic evolution and surface morphology [17]. The basin was gradually sinking during the Medium-Upper Miocene, which led to the stratification of the basin and the creation of lignite deposit. Tectonic events that occurred during the Pleistocene and Holocene caused further subduction and sediment deposition, which reached 350 m. The repeatability deposition in lake-marshy environment allowed the creation of lignite in the region. The vineyards of VQPRD Amydaion developed in alluvial area of the basin, dominated by lacustrine sediments (clay, sand, and gravel).

Nemea is a town of Corinth Prefecture, located 42 km southwest of Corinth, near Mount Prophet Elias, at an altitude of 320 m and is presented as the greater wine-growing region of Greece which produced the renowned wines VQPRD (Vins de Qualite Produits dans une Region Determinee) Nemea. The climate is characterized as warm and temperate with little rainfall throughout the year. The rainfall distribution is uneven with respect to seasons (85% of precipitation falls on a wet season from October to April). In the western part (Feneou areas and Stymfalias) where the higher altitudes are observed, the precipitation episodes are often in contrast to the Eastern part which is characterized by lower altitudes. Temperature and sunlight follow the same trend related to altitude presenting the lower values in January and the highest in July. The inverse distribution is observed for moisture regime where the winter

presents a peak (December) while July is the driest month. The prevailing winds that occur are those of NE and east direction without any strong episodes. The dominant geological formations that are encountered include both pre- and postalpine formations of terrestrial, lacustrine, and marine origin. Three large plane sections constitute the relief portrait of the wider study area: the coastal area between Corinth and Kiato known as the Vochas plane, the plane between Agios Basilios and Spathovouni villages, and the plane area around the historical city of Nemea. In contrast, at the SW and SE edge steeper scene is observed with carbonate formations of the pre-alpine basement to set up a mountainous terrain with steep slopes, deep ravines, and sharp mountain peaks. Intermediate areas are developed in a hilly or semimountainous setting with moderate slopes and rounded mountain peaks. The drainage network is developed due to the creation and activity of WNW-ESE faults and vertical to these transform faults (**Figure 1**) [18, 19].

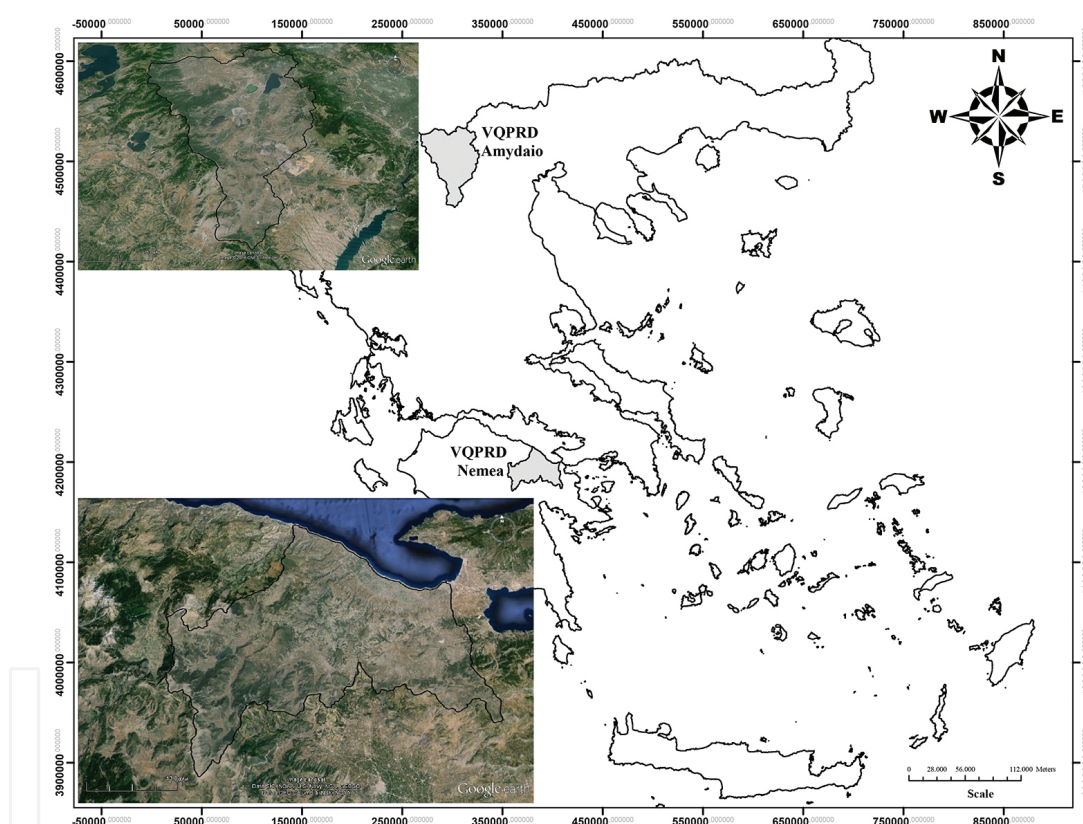


Figure 1. Study area of Amydaio, north Greece, and Nemea, south Greece, Greek vineyards (Google earth modified picture).

4. Material and methods

In order to identify the isotopic link between the environmental factors of vineyards and wine products precipitation, ground water, wine water, as well as grape must samples during the wine-making process were collected for $\delta^{18}\text{O}$, δD , $\delta^{13}\text{C}$, and ^{14}C isotopic analysis. Sr and Nd

radiogenic analysis was also performed in selected wine samples. Rain samples were collected (11 samples for Nemea and 12 samples for Amydaio) by using 125 mL glass bottles. Ground water samples (10 samples for Nemea and 17 samples for Amydaio) were taken from irrigation water boreholes related to vineyards by using 125 mL glass bottles. In total 70 (54 samples for Amydaio and 16 samples for Nemea) different bottled vintage wines were chosen and prepared for stable isotopic analysis by extraction (approximately 40 mL from each bottle) through the cork using a 10 mL Hamilton Gastight Syringe with Gauge 26 Point Style 5 (side hole) needles. Finally, unfermented grape must samples (16 samples for Nemea and 33 samples for Amydaio) were collected immediately after harvest. Then they were centrifuged for approximately 1 hour to extract grape solids and larger yeasts, syringe-filtered with a 0.45 mm and 0.22 mm Cameo IV filter and stored into 20 mL glass bottles (without the addition of sulfur dioxide). Moreover, 10 samples of wine ethanol were collected by using a Cadiot column distillation system to quantitatively separate the ethanol, according to the procedure described in OIV MA-AS-311-05.

All isotopic analyses were performed in Laboratory of Stable Isotope and Radiocarbon of Nanoscience & Nanotechnology, Institute in N.C.S.R. Demokritos (Athens, Greece). Stable isotope analysis of $^{18}\text{O}/^{16}\text{O}$, D/H, and $^{13}\text{C}/^{12}\text{C}$ ratio in Greek wines, wine water, meteoric, and ground waters were carried out on a continuous flow Finnigan DELTA V plus (Thermo Electron Corporation, Bremen, Germany) stable isotope mass spectrometer [20, 21].

^{13}C , ^{18}O , and ^2H isotope analysis in ethanol with Thermo Scientific DELTA V Isotope Ratio Mass Spectrometers was performed according to Ref. [22]. Specifically, for $\delta^{13}\text{C}$ determination 1 μL of purified ethanol was injected into a small tin container for liquids and closed securely to prevent evaporation. The Flash Elemental Analyzer (FlashEA) with a single reactor system combining combustion and reduction in one reaction was used. The Thermo Scientific ConFlo interface diluted the CO_2 sample peak with 1 bar helium resulting in a split of about 1:12. For $\delta^2\text{H}$ and $\delta^{18}\text{O}$ determination 0.1 μL of pure ethanol was injected with a 0.5 μL syringe into a glassy carbon reactor in a high temperature carbon reduction system of a FlashEA. The autosampler was used and samples were stored in 2 mL vials with standard caps and septa.

The results are expressed in standard delta notation (δ) as per mil (‰) deviation from the standard V-SMOW as: $\delta = ((R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}) \times 1000$, where R_{sample} and $R_{\text{standard}} = ^2\text{H}/^1\text{H}$ or $^{18}\text{O}/^{16}\text{O}$ or $^{13}\text{C}/^{12}\text{C}$ ratios of sample and standard, respectively.

Measurement precision, based on the repeated analysis of internal standard waters, was 1.5, 0.5, and 0.2‰ for $\delta^2\text{H}$, $\delta^{18}\text{O}$, and $\delta^{13}\text{C}$, respectively.

All measurements were carried out according to laboratory standards that were periodically calibrated based on the international standards recommended by the IAEA.

^{14}C activity was determined by using liquid scintillation counting (LSC) on a Packard Tri-Carb TR/SL. Radiogenic isotope of Sr and Nd were measured with thermal ionization mass spectrometry (TIMS, Nd) and inductively coupled plasma mass spectrometry (ICP-MS, Sr).

5. Results and discussion

5.1. Isotopic composition of meteoric water

Twenty-nine water samples (ASt1 to ASt17 irrigation water samples and ASt18 to ASt29 precipitation water samples) were collected at the region of Amydaio in West Macedonia in North Greece, corresponding to the growing season of 2013. From Nemea, northeastern Peloponnese in southern Greece, a total of 21 water samples (NSt1 to NSt10 groundwater samples and NSt11 to NSt21 precipitation water samples) were collected, corresponding to the same growing season. The $\delta^{18}\text{O}$ and δD values of Amydaio and Nemea are shown in **Figure 2**. In the same figure are also pictured the Global Meteoric Water Line with correlation equation: [23]

$$\delta\text{D} = 8 \times \delta^{18}\text{O} + 10 \quad (1)$$

and the Local Meteoric Line that correspond to Greece (LMWL) with correlation equation: [24]

$$\delta\text{D} = 8.7\delta^{18}\text{O} + 19.5 \quad (2)$$

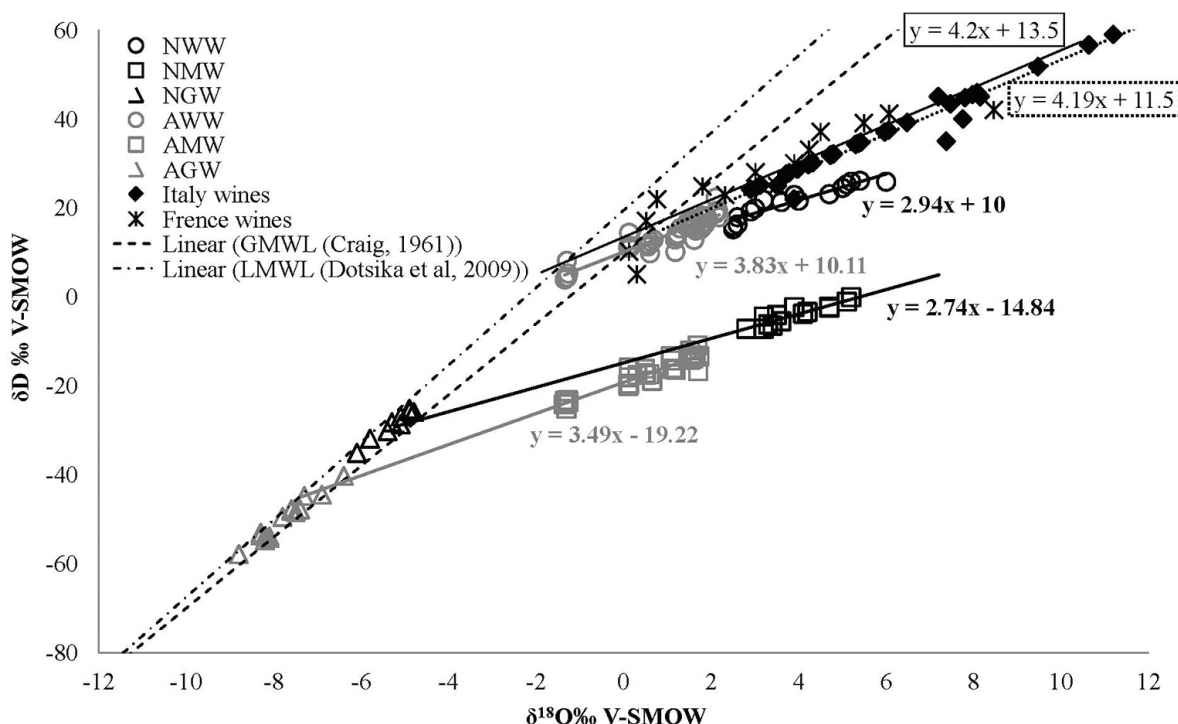


Figure 2. Equation correlation $\delta^{18}\text{O}$ – $\delta^2\text{H}$ both in wine water and grape berries of Greece and Europe generally.

Generally, an isotopic correlation between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ with a slope close to 8 indicates fresh precipitation of all types [23], as well as for surface water not subjected to excessive evaporation relative to input [24]. In contrast, slopes ranging from 6 to 4 reflect isotopic relations between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ for waters which are subjected to excessive evaporation relative to input [23].

Oxygen and deuterium isotope values for Amydaio precipitation water samples range from -9.9‰ to -9.6‰ and from -66.7‰ to -63.8‰ , respectively, while for groundwater range from -8.8 to -6.4‰ and from -56.6‰ to -40.9‰ , respectively. The correlation equation for Amydaio groundwater is $\delta\text{D} = 6.86 \cdot \delta^{18}\text{O} + 3.24$ where the slope 7.59 suggests that the samples have not undergone evapotranspiration process. For Nemea precipitation water samples of $\delta^{18}\text{O}$ and δD values range from -7.9‰ to -6.0‰ and from -48.1‰ to -35.2‰ , respectively, while for groundwater samples range -6.1‰ to -4.8‰ and from -35.1‰ to -25.2‰ , respectively. Evapotranspiration process seems not to have effected remarkably the irrigation water samples as their slope is 7.27 ($\delta\text{D} = 7.27 \cdot \delta^{18}\text{O} + 9.70$). Groundwater from both study areas do not exhibit remarkable variations in $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values indicating the lack of seasonal and altitude impacts affecting the meteoric waters [25].

Similar equations with slightly greater slopes of 7 and 7.5 were proposed for the northern part of Eastern Macedonia and for the Eastern Nemea, respectively [26]. A slope lower than 7 was reported for Central Macedonia [27], the southern part of Eastern Macedonia and Thrace [28], as well as the northern part of Epirus [29].

5.2. Isotopic composition of wine water

Amydaio vineyards are represented by 54 bottles of wine (AW1–AW54) consisting of 6 (AW1–AW6) Roditis (white wine), 24 (AW7–AW30) Xinomauro (red wine), and 24 ((AW31–AW54) Tannat (red wine). Nemea vineyards are represented by 16 bottles of wine (NW1–NW16) consisting of 11 (NW1–NW11) Agiorgitiko (red wine), 1 (NW12) Merlot-Agiorgitiko (red wine), 1 (NW13) Moschofilero-Roditis (white wine), and 3 (NW14–NW16) Roditis-Chardonnay (white wine).

The stable isotope values of Greek wine waters are presented in **Figure 2**. As Western and Southern Europe constitutes the most significant region, both in terms of vineyard area and quantity of production, French and Italian wines are also plotted in the same figure. Amydaio presents isotopic values ranging from -1.3‰ to 2.3‰ (mean value 1.1‰) for $\delta^{18}\text{O}$ and from 3.9 to 22.3‰ (mean value 14.2‰) for δD , while Nemea samples range from 2.5‰ to 6.1‰ (mean value 3.8‰) and from 15.2‰ to 26.1‰ (mean value 21.4‰) for $\delta^{18}\text{O}$ and δD , respectively. A first observation is the fact of isotope enrichment in the north-south direction. This trend has been also suggested by some authors for olive oils [30, 31]. $\delta^{13}\text{C}$ values also related to the north-south enrichment observation with wine water values to range from -29.4‰ to -26.1‰ (mean value -27.7‰) for Amydaio wines and from -26.2‰ to -24.7‰ (mean value -25.6‰) for Nemea wines. Moreover, Nemea wines present slightly more positive values with larger variations verifying the warmer and drier conditions than those of Amydaio where the conditions are cooler and the precipitation episodes more

frequent and constant. Indeed, ambient conditions affect intermediate steps in plant functions [32, 33] and proceeds for sugar formation, therefore carbon isotope could trace deferent environmental conditions. The enzymes favor the ^{12}C isotope for the photosynthesis than ^{13}C as it is found in greater concentration in atmospheric CO_2 (98.9‰ for ^{12}C and 1.1‰ for ^{13}C). Moreover, this difference mass which cause a higher diffusion of $^{12}\text{CO}_2$ than that of $^{13}\text{CO}_2$ combined to the fact that ^{13}C forms stronger chemical compounds as it is heavier than ^{12}C induce in lower concentrations of ^{13}C in plants than atmosphere [3]. Especially in water deficit conditions leaves stomata close resulting in a reduced atmospheric CO_2 interchange, which leads to changes in $\delta^{13}\text{C}$ values among C3-photosynthesis plants such as grapes [2, 5].

Wine water and groundwater samples correlated well enough in both vineyards implying that the “source water” represented by groundwater could be linked to wine products. The isotopic composition of groundwater and precipitation are mainly related to latitude, distance from the sea, and altitude [27]. Groundwater samples from Amydaio and Nemea reflect their meteoric origin without remarkable variations (**Figure 2**) so it is assumed that environmental setting of the vineyards control the isotopic composition of wine products. Schmidt et al. have already reported the $\delta^{18}\text{O}$ correlation between carbohydrates and $\delta^{18}\text{O}$ of leaf water which are linked to the isotopic composition of groundwater with the evapotranspiration ratio to be influenced by humidity and temperature [9].

Amydaio wine samples are represented by two major vintage labels: 2009 and 2010. The correlation equation for all samples corresponding to both vintages is $\delta\text{D} = 3.86 \cdot \delta^{18}\text{O} + 10.01$ with characteristic slope of 3.86. Correlation equations for 2009 and 2010 are $\delta\text{D} = 3.75 \cdot \delta^{18}\text{O} + 10.38$ and $\delta\text{D} = 3.78 \cdot \delta^{18}\text{O} + 9.82$, respectively. Both vintage years retain their characteristic slope (3.75 for 2009 and 3.78 for 2010) with the vintage year of 2010 to present a better correlation against 2009 ($r^2 = 0.69$ for 2009 and $r^2 = 0.93$ for 2010). Nemea's wine samples present a correlation equation $\delta\text{D} = 2.79 \cdot \delta^{18}\text{O} + 10.71$ with characteristic slope of 2.79. Moreover, French and Italian wines exhibit slope of 4.19 and 4.20, respectively. The fact that Italian wines concern the north-west part of central Italy explains their similarity with French wines. Amydaio is also the most northerly of Greek vineyards, with vines growing at an altitude of 620–710 m and a significant network of lakes (Zazari, Cheimaditida, Petron, and Vegoritida lakes) to be presented; therefore, its slope is closer to those of French and Italy reflecting the strong moisture regime. The slope of Nemea wines is lower reflecting the more arid condition of Southern Greece. The slopes that both vineyards present reflect the different ambient conditions indicating the evapotranspiration process as the critical factor for the observed oxygen and deuterium isotope enrichment [34].

5.3. Isotopic effect of fermentation

Raco et al. demonstrated that for detection of geographical origin of wine $\delta^{18}\text{O}$ and $\delta^2\text{H}$ analysis should be carried out not only in wines but in grape berries and grape must as it clarifies the impact of fermentation process more precisely. The isotopic values of water from grape must from Amydaio and Nemea vineyards are presented in **Figure 2**. Oxygen and deuterium isotopic values of Amydaio grape must samples range from -1.4‰ to 1.7‰ and from -25.2‰ to -10.9‰ , respectively, while Nemea grape must range from 2.5‰ to 5.2‰

and from -7.2‰ to -0.1‰ , respectively. Moreover, in **Figure 2** are also reported the stable isotopic values of groundwater samples corresponding to vinification areas. Grape must samples are marked further away from groundwater-source water as during their maturation the transpiration process results in a water loss which leads to their isotopic enrichment. However, their transpiration lines with correlation equation

$$\delta D = 3.49 \times \delta^{18}O - 19.20 \quad (r^2 = 0.89) \quad \text{Amydaio area} \quad (3)$$

$$\delta D = 2.75 \times \delta^{18}O - 14.84 \quad (r^2 = 0.84) \quad \text{Nemea area} \quad (4)$$

intersect the Meteoric Water Lines in the area of groundwater samples highlighting the source water of vineyards and the initial isotopic composition of grape berries.

The wine line and transpiration line of grape must samples corresponding to Amydaio are differentiated about 0.37‰ for $\delta^{18}O$ and 29.21‰ for δD while the difference for Nemea's wines and grape must samples is about 0.03‰ for $\delta^{18}O$ and 25.55‰ for δD . The controlling factor of this isotopic setting is clearly the difference in deuterium isotopic values. The δD isotope values of grape must samples is referred to the member of the water molecule; however, the δD isotope values of wine water reflect hydrogen members of sugar and ethanol [35]. Refs. [44–48] demonstrated that during fermentation, deuterium (D) transfer from exchangeable hydroxyls or from nonexchangeable sites of sugars leading to significant differences in the “site-specific” hydrogen isotopic values of wine.

5.4. Influence of wine variety and vintage on isotopes

Isotopic values of $\delta^{18}O$ and δD for wine water in red wines produced in Amydaio range from 0.1‰ to 2.2‰ and from 9.8‰ to 22.3‰ , respectively, while in white wines they range from -1.3 to -1.2‰ and from 3.9‰ to 8.1‰ , respectively. For Nemea red wines the $\delta^{18}O$ and δD values of wine water range from 2.5‰ to 6.1‰ and from 15.2‰ to 26.1‰ , respectively, while in white wines they range from 3.0‰ to 5.2‰ and from 19.9‰ to 25.9‰ , respectively. In both vineyards the white wines are slightly more depleted than red wines of the same year and the same growing region. White and red wine grapes are harvested in different periods; red wine grapes usually harvested 1–3 weeks after white wine grapes. This interval leads to longer period of transpiration for red wine grapes resulting isotopically enriched red wine than white wine. However, it is revealed that the control factor that distinguishes the wine water values is their origin. Amydaio and Nemea wines clustered individually into two groups where the first concerns cooler-wetter climate and the second warmer-drier climate, respectively.

The distinct variation of wine water values based on their origin is also highlighted to the diagrams of $\delta^{18}O$ and δD versus vintage year (**Figures 3 and 4**). In the same figures, it is further pointed out the variety of isotope values with respect to vintage year indicating that the vintage year contributes to the development of isotopes in wine water. The special yearly

weather variations play a critical role in establishing the basic isotope characteristics of wine on larger scale than geographical origin.

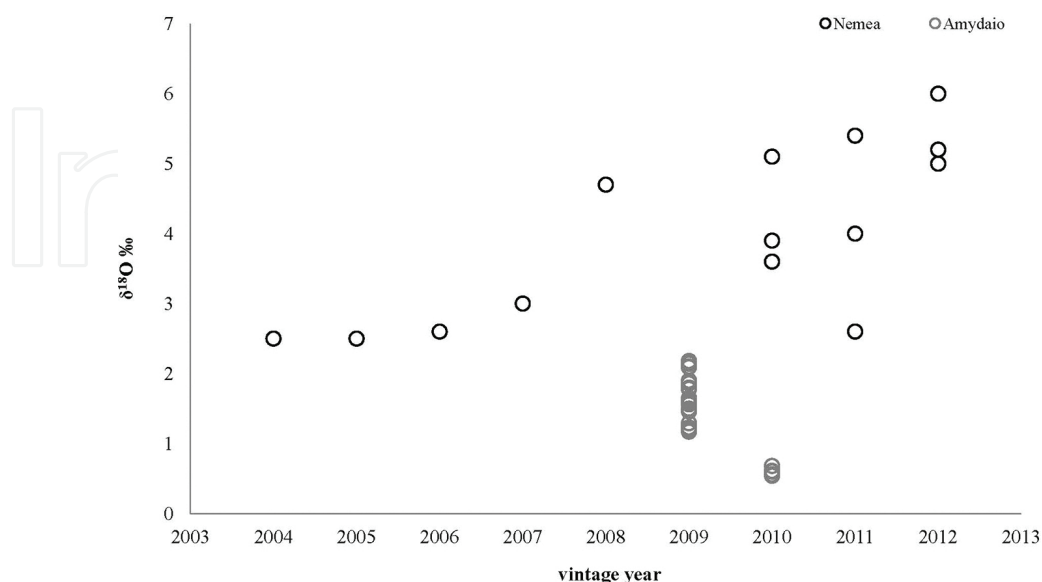


Figure 3. $\delta^{18}\text{O}$ ‰ values of the analyzed wine samples versus the vintage year.

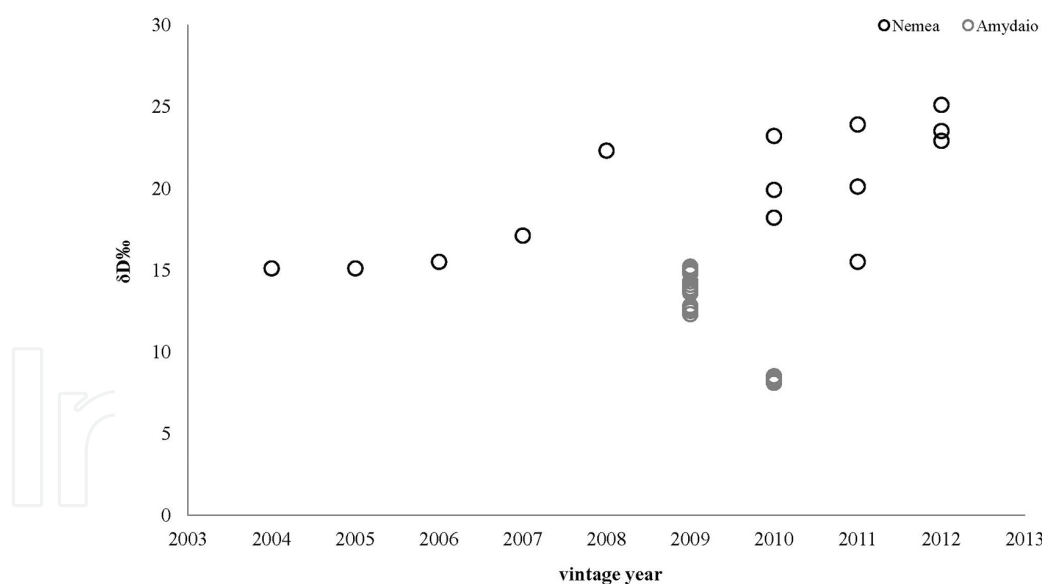


Figure 4. δD ‰ values of the analyzed wine samples versus the vintage year.

5.5. Authentication: adulteration control

The formation of carbon isotope values in plants depends on both biotic and environmental factors. Photosynthetic pathways that referred to terrestrial plants are C3 (Calvin plants: first product of photosynthesis is a 3-carbon molecule), C4 (Hatch-Slack: first product of photo-

synthesis is a 4-carbon molecule, and crassulacean acid metabolism, which respond in different way to the atmospheric carbon dioxide (CO_2) concentration, to temperature oscillations, and water availability.

As has already been mentioned, the carbon isotopic signature of sugar in grape berries is totally correlated to photosynthetic pathway. As the isotopic discrimination that carbon offers depends on plant type (C3, C4, or CAM), the $\delta^{13}\text{C}$ values are a reliable tool for the detection of C3 or C4 sugar origin [3, 32]. The $\delta^{13}\text{C}$ values of C3 plants, where grape berries belong, range from -30‰ to -22‰ and for C4 plants from -14‰ to -10‰ . So the carbon isotope values that referred to both Amydaio and Nemea vineyards confirm the C3 origin of their sugar and consequently the authentication of wine products. Although the addition of C4 plant sugars could be detected through carbon isotope values of wine water an adulteration through C3 plants would be harder to be traced. Such a challenge is addressed by the combined use of deuterium content of sugars and carbon isotopes of wines [34, 36, 37]. The equation $\text{D}/\text{H}_{\text{eth}} = \{[(\delta^2\text{H}/103) + 1] * 155.7$, where -54‰ is the fractionation of $\delta^2\text{H}$ of water with that of ethanol [38] was used in order to calculate the $(\text{D}/\text{H})_i$ of wine ethanol. $(\text{D}/\text{H})_i$ for Greek wines ranged between 102.4 and 105.8 ppm and they are consistent with the corresponding values given for wine (98–108 ppm). The extreme values correspond to only to the 3–5% of Greek wines. In fact, the upper limit of 98 ppm reflects wines originate from northern Greece for the years with strong precipitation episodes while the limit of 108 ppm reflects wines originate from the southern part of Greece (e.g., Crete) for the years that present increased temperature and almost absence of precipitation. In **Figure 5**, carbon isotope values versus deuterium contents of sugars that referred to Amydaio and Nemea wines are presented where reference alcohol from beet, cane [3], are given. Based on the adulteration triangle it is concluded that at the analyzed wine samples no sugar addition was detected. However, based on the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values with respect to the authentic wine of EU wine isotopic database (**Figure 6**) some suspicious samples are detected for Roditis 2010, Xinomavro 2010, and Syrah 2009 from Amydaio. These samples present low $\delta^{18}\text{O}$ values (-1.3‰ to 0.7‰) where this fact could reflect watering of wines and/or mixing with other varieties, respectively. However, it should be taken into account that Amydaio is characterized by purely continental climate with important annual variation in temperature due to the lack of significant bodies of water nearby. Negative oxygen values referred to white wines (Roditis) and as it has already been documented above their harvest were performed earlier than red wines with the evapotranspiration process to be shorter.

The concentration of Sr minerals in wines depends on many factors that are related to their geographic origin. Minerals found in the soil pass through the roots and transported to the plant with isotopic composition similar to that of soil. This way, the isotopic ratio $^{87}\text{Sr}/^{86}\text{Sr}$ can be used as a tracer of wine origin, where there is a strong correlation between the isotopic composition of the soil in the production area and the final wine that is produced [41–43]. The same approach underlies the use of neodymium isotopes $^{143}\text{Nd}/^{144}\text{Nd}$. Combined with $^{87}\text{Sr}/^{86}\text{Sr}$ provides useful information to geochemical analysis. Isotope ratios $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ reflect the isotopic composition of the soil that are affected by silicate formations in Amydaio area, while in Nemea carbonate minerals also have intense participation.

Finally, stable carbon isotope values of ethanol samples range between -29.2‰ and -25.9‰ reflecting the C_3 origin of ethanol in Greek wines. The same conclusions, regarding the authenticity of Greek wines from Amydaio and Nemea, were reached as well by ^{14}C analysis in wine ethanol which compared with the atmospheric ^{14}C data. The results of ^{14}C measurements in ethanol extracted from Greek wines follow the known pattern of ^{14}C variations in atmospheric CO_2 . A possible fraud episode with synthetic oil ethanol would result in lower values in the ^{14}C activity concentration and could therefore be detected.

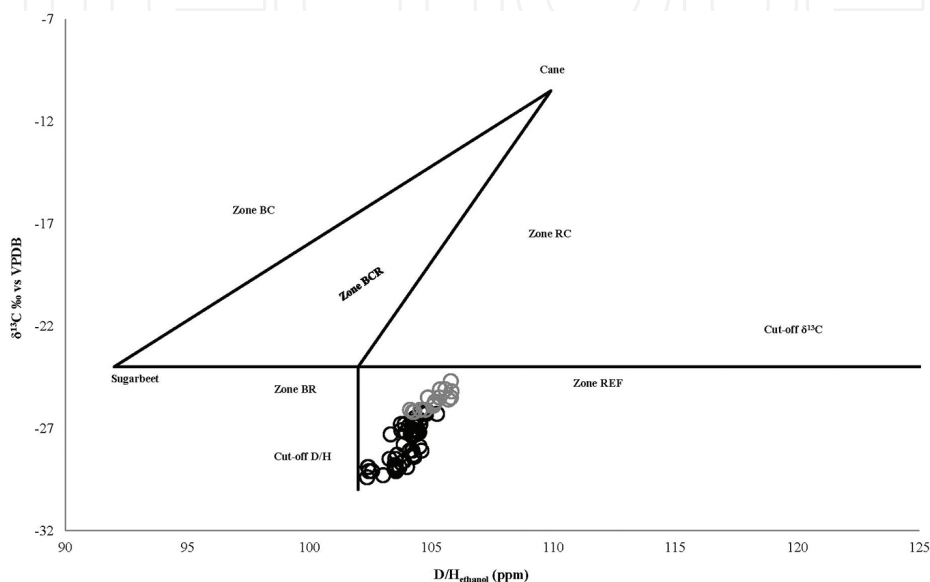


Figure 5. Correlation between $\delta^{13}\text{C}$ value of wine and alcoholic fraction of the isotope ratio of H ([39], as modified).

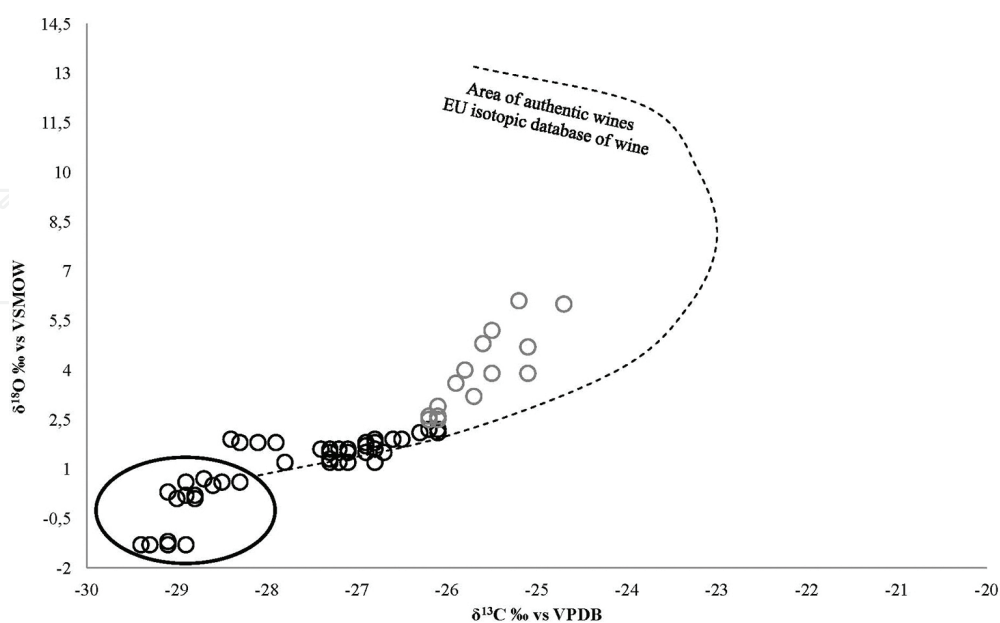


Figure 6. Correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of wine [40].

6. Conclusions

Stable isotopes (^{13}C , ^{18}O , ^2H), radiocarbon (^{14}C), and radiogenic isotopes ($^{87}\text{Sr}/^{86}\text{Sr}$, $^{143}\text{Nd}/^{144}\text{Nd}$) have been applied to determine the origin assignment and verify the geographical provenance of VQPRD Amydaio and Nemea wine products, which is considered as important characteristics both for consumers and the international regulations of wines. Stable isotope analyses of $^{18}\text{O}/^{16}\text{O}$, D/H, and $^{13}\text{C}/^{12}\text{C}$ ratio were performed in irrigation water, wine water, and grape must samples in order to detect the origin and the adulteration of wine. The $\delta^{13}\text{C}$ analysis of ethanol and wine water $\delta^{18}\text{O}$ underlines the importance of the photosynthetic pathway and the environmental conditions of wine (mean $\delta^{18}\text{O}_{\text{wine}}$ 1.5‰ with $\delta^{18}\text{O}_{\text{rain}}$ -8‰ for Amydaio and mean $\delta^{18}\text{O}_{\text{wine}}$ 4.5‰ with $\delta^{18}\text{O}_{\text{rain}}$ -6‰ for Nemea). The main factors that are responsible for the differentiation of the oxygen isotope ratios of wine water were discussed enhancing the relation of Amydaio and Nemea wine products with the corresponding vineyards. Data interpretation demonstrated the efficacy of $\delta^{18}\text{O}$ analysis in both wine and grape berry samples. The determination of $\delta^{18}\text{O}$ with the $\delta^2\text{H}$ isotope content of wine is proposed for achieving greater results on the detection of the geographical origin of wine. The range of $\delta^{13}\text{C}$ values and radiocarbon (^{14}C) analysis of ethanol corresponded to all wine labels of VQPRD Amydaio and Nemea vineyards confirming the C3 photosynthetic pathway origin which implies the authentication of wine product and not adulteration with industrial alcohol and/or sweetening agents (sugar beet or cane). The efficiency of direct and precise analysis of ethanol in liquid scintillation counter should be noted. $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of all wine labels of VQPRD Amydaio and Nemea vineyards are compared with an isotopic database of authentic European wines. The wine products of the zone VQPRD Amydaio and Nemea grouped with all the original European wines demonstrating the absence of adulteration episodes. A slight deviation of Amydaio wines from the EU database, due to more negative oxygen isotope values, attributed to the continental climate in area with important annual variation in temperature due to the lack of significant water bodies nearby. Moreover, $\delta^{13}\text{C}$ values of wine water and D/H_{ethanol} values of ethanol extracted from wines of VQPRD Amydaio and Nemea define them as authentic products certifying nonadulteration process and an authenticity label. The homogeneity of $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{144}\text{Nd}/^{143}\text{Nd}$ isotope values confirms that the territorial and geological signal is transferred through the vineyards in the final product, wine, certifying their exclusive provenance.

Author details

Paraskevi Chantzi^{1*}, Anastasia-Elektra Poutouki¹ and Elissavet Dotsika^{1,2}

*Address all correspondence to: p.chantzi@inn.demokritos.gr

¹ Laboratory of Stable Isotope and Radiocarbon, Institute of Nanoscience and Nanotechnology, NCSR Demokritos, Agia Paraskevi, Attiki, Greece

² Institute of Geosciences and Earth Resources, Pisa, Italy

References

- [1] Keeling, C.D., 1961: The concentration and isotopic abundances of carbon dioxide in rural and marine air. *Cheochimica et Cosmochimica Acta*, 24Q, 277–298.
- [2] Ehleringer, J.R., Hall, A.E., & Farquhar, G.D., Eds., 1993: *Stable Isotopes and Plant Carbon-Water Relations*. San Diego, CA: Academic Press.
- [3] O'Leary, M.H., 1988: Carbon isotopes in photosynthesis. *BioScience*, 38, 328–336.
- [4] Hillaire-Marcel, G., 1986: Chapter 12—Isotopes and Food, In *Handbook of Environmental Isotope Geochemistry*, edited by P. Fritzj and C. Fontes. Amsterdam: Elsevier. Part B, pp. 507–548, *The Terrestrial Environment*, B, ISBN 9780444422255.
- [5] Farquhar, G.D., Ehleringer, J.R., & Hubick, K.T., 1989: Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Molecular Biology*, 40, 503–537.
- [6] Freeman, B.M., Kliewer, W.M., & Stern, P., 1982: Influence of windbreaks and climatic region on diurnal fluctuation of leaf water potential, stomatal conductance and leaf temperature of grapevines. *American Journal of Enology and Viticulture*, 31, 233–236.
- [7] Roby, G., & Matthews, M.A., 2004: Relative proportions of seed, skin and flesh, in ripe berries from Cabernet-Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. *Australian Journal of Grape and Wine Research*, 10, 74–82.
- [8] Clark, I., & Fritz, P., 1997: *Environmental Isotopes in Hydrogeology*. New York: Lewis Publishers. pp. 35–78.
- [9] Rossmann, A., Reniero, F., Moussa, I., Schmidt, H.L., Versini, G., & Merle, M.H., 1999: Stable oxygen isotope content of water of EU data-bank wines from Italy, France and Germany. *Zeitschrift für Lebensmittel-Untersuchung und Forschung A*, 208 (5), 400–407.
- [10] Epstein, S., Thompson, P., & Yapp, C.J., 1977: Oxygen and hydrogen isotopic ratios in plant cellulose. *Science*, 198 (4323), 1209–1215, doi: 10.1126/science
- [11] Fehri, A., & Letolle, R., 1977: Transpiration and evaporation as the principal factors in oxygen isotope variations of organic matter in land plants. *Physiologie Vegetale*, 15, 363–370.
- [12] Asche, S., Michaud, A.L., & Brenna, J.T., 2003: Sourcing organic compounds based on natural isotopic variations measured by high precision isotope ratio mass spectrometry. *Current Organic Chemistry*, 7 (15), 1527–1543.
- [13] Ingraham, N.L., & Caldwell, E.A., 1999: Influence of weather on the stable isotopic ratios of wines: Tools for weather/climate reconstruction? *Journal of Geophysical Research [Atmos.]*, 104 (D2), 2185–2194.

- [14] Martin, G.J., & Martin, M.L., 2003: Climatic significance of isotope ratios. *Phytochemistry Reviews*, 2 (1–2), 179.
- [15] Martin, M.L., & Martin, G.J., 1999: Site-specific isotope effects and origin inference. *Analusis*, 27 (3), 209–213.
- [16] Zhang, B.L., Buddrus, S., Trierweiler, M., & Martin, G.J., 1998: Characterization of glycerol from different origins by H-2- and C-13-NMR studies of site-specific natural isotope fractionation. *Journal of Agricultural and Food Chemistry*, 46 (4), 1374–1380.
- [17] Steenbrink, J., Hilgen, F.J., Krijgsman, W., Wijbrans, J.R., & Meulenkamp, J.E., 2006: Late Miocene to early Pliocene depositional history of the intramontane Florina–Ptolemais–Servia Basin, NW Greece: Interplay between orbital forcing and tectonics. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 238 (1–4), 151–178, ISSN 0031-0182.
- [18] Seger, M., & Alexander, J., 1993: Distribution of Plio-Pleistocene and modern coarse grained deltas south of the Gulf of Corinth, Greece. *Special Publications International Association of Sedimentologists*, 20, 37–48.
- [19] Zelilidis, A., 2000: Drainage evolution in a rifted basin, Corinth graben, Greece. *Geomorphology*, 43, 253–262.
- [20] Duhr, A., & Hilkert, A.W., 2004: Application note 30049: Automated H₂/H₂O Equilibration for δD Determination on Aqueous Samples Using Thermo Scientific GasBench II. Bremen, Germany: Thermo Fisher Scientific.
- [21] Hilkert, A.W., & Avak, H., 2004: Application Note 30048: ¹⁸O-Equilibration on Water, Fruit Juice and Wine Using Thermo Scientific GasBench II. Bremen, Germany: Thermo Fisher Scientific.
- [22] Kracht, O., Hilkert, A.W., Racz-Fazakas, T., 2008: Application Note 30147: ¹³C and Simultaneous ¹⁸O and ²H Isotope Analysis in Ethanol with Thermo Scientific DELTA V Isotope Ratio Mass Spectrometers. Bremen, Germany: Thermo Fisher Scientific
- [23] Craig, H., 1961: Isotopic variation in meteoric waters. *Science*, 133, 1702–1703.
- [24] Dotsika, E., Lykoudis, S., & Poutoukis, D., 2010: Spatial distribution of the isotopic composition of precipitation and spring water in Greece. *Global and Planetary Change* 71 (3e4), 141–149.
- [25] Dotsika, E., Poutoukis, D., Michelot, J.L., & Raco, B., 2009: Natural tracers for identifying the origin of the thermal fluids emerging along the Aegean Volcanic arc (Greece): Evidence of Arc-Type Magmatic Water (ATMW) participation. *Journal of Volcanology and Geothermal Research*, 179 (1–2), 19–32, ISSN 0377-0273
- [26] Leontiadis, I., Payne, B.R., Letsios, A., Papagianni, N., Kakarelis, D., & Chadjiagonakis, D., 1984: Isotope hydrology study of Kato Nevrokopi of drama. *Proceedings of the Symposium on Isotope Hydrology 1983*, International Atomic Energy Agency, Vienna. pp. 193–206.

- [27] Christodoulou, T., Leontiadis, I.L., Morfis, A., Payne, B.R., & Tzimourtas, S., 1993: Isotope hydrology study of Axios River plain in northern Greece. *Journal of Hydrology*, 146, 391–404.
- [28] Leontiadis, I.L., Vergis, S., & Christodoulou, T., 1996: Isotope hydrology study of areas in Eastern Macedonia and Trace, Northern Greece. *Journal of Hydrology*, 182, 1–17.
- [29] Leontiadis, I.L., & Nikolaou, E., 1999: Environmental isotopes in determining ground-water flow systems, northern part of Epirus, Greece. *Hydrogeology Journal*, 7, 219–226.
- [30] Angerosa, F., Bréas, O., Contento, S., Guillou, C., Reniero, F., & Sada, E., 1999: Application of stable isotope ratio analysis to the characterization of the geographical origin of olive oils. *Journal of Agriculture and Food Chemistry*, 47, 1013–1017. doi:10.1021/jf9809129.
- [31] Bréas, O., Guillou, C., Reniero, F., Sada, E., & Angerosa, F., 1998: Oxygen-18 measurement by continuous flow pyrolysis/isotope ratio mass spectrometry of vegetable oils. *Rapid Communications in Mass Spectrometry*, 12(4), 188–192.
- [32] O'Leary, M. H., 1995: Environmental effects on carbon isotope fractionation in terrestrial plants. In (E. Wada, T. Yoneyama, M. Minigawa, T. Ando & B. D. Fry, Eds) *Stable Isotopes in the Biosphere*. Kyoto, Japan: Kyoto University Press, pp. 78–91.
- [33] Schmidt, H.-L., Werner, R.A., & Roßmann, A., 2001: ^{18}O pattern and biosynthesis of natural plant products. *Phytochemistry*, 58 (1), 9–32, ISSN 0031-9422.
- [34] Dunbar, J., & Wilson, T., 1983: Oxygen and hydrogen isotopes in fruit and vegetable juices. *Plant Physiology*, 72, 725–727.
- [35] Raco, B., Dotsika, E., Poutoukis, D., Battaglini, R., & Chantzi, P., 2015: O–H–C isotope ratio determination in wine in order to be used as a fingerprint of its regional origin. *Food Chemistry*, 168, 588–594, ISSN 0308-8146.
- [36] Bréas, O., Guillou, C., Reniero, F., Sada, E., & Tanet, G., 1996: Deuterium/Hydrogen Measurements in Wines and Fruit Juices by Platinum Catalysed Equilibration Method. *Rapid Commun. Mass Spectrometry*, 10: 246–249.
- [37] Dunbar, J., & Schmidt, H.-L., 1984: *Fresenius Z. Analytical Chemistry*, 317, 853.
- [38] Bréas, O., Thomas, F., Zeleny, R., Calderone, G., Jamin, E., & Guillou, C., 2007: Performance evaluation of elemental analysis/isotope ratio mass spectrometry methods for the determination of the D/H ratio in tetramethylurea and other compounds-results of a laboratory inter-comparison. *Rapid Communications in Mass Spectrometry*, 21, 1555–1560.
- [39] Ogrinc, N., Košir, I.J., Spangenberg, J.E., & Kidrič, J., 2003: The application of NMR and MS methods for detection of adulteration of wine, fruit juices, and olive oil. A review. *Analytical and Bioanalytical Chemistry*, 376, 424–430.

- [40] Calderone, G., & Guillou, C., 2008: Analysis of isotopic ratios for the detection of illegal watering of beverages. *Food Chemistry*, 106, 1399–1405.
- [41] Almeida, C.M., & Vasconcelos, M.T., 2004: Does the winemaking process influence the wine $^{87}\text{Sr}/^{86}\text{Sr}$? A case study. *Food Chemistry*, 85, 7–12.
- [42] Horn, P., Hoelzl, S., Todt, W., & Matthies, D., 1998: Isotope abundance ratios of Sr in wine provenance determinations in a tree-root activity study and of Pb in a pollution study on tree-rings. *Isotopes in Environmental and Health Studies*, 34, 31–42.
- [43] Lancelot, J., Herrerias, J., Verdoux, P., Lurton, L., 1999: The use of strontium isotopes geochemistry for a high resolution identification of wines from the Rhone Valley. In: *Fifth European Symposium of Food Authenticity*, La Baule, 9–11 June, 1999.
- [44] Martin, G.J., Zhang, B.L., & Martin, M.L., 1983: Application of quantitative deuterium NMR to the study of isotope fractionation in the conversion of saccharides to ethanols. *Biochemical and Biophysical Research Communications*, 111, 890–896.
- [45] Martin, G.J., Zhang, B.L., Naulet, N., & Martin, M.L., 1986: Deuterium transfer in the bioconversion of glucose to ethanol studied by the specific isotope labeling at the natural abundance level. *Journal of the American Chemical Society*, 108, 5116–5122.
- [46] Martin, G.J., Guillou, C., Martin, M.L., Cabanis, M.T., Tep, Y., & Aemy, J., 1988: Natural factors of isotope fractionation and the characterization of wines. *Journal of Agricultural and Food Chemistry*, 36, 316–322.
- [47] Martin, G.J., Martin, M.L., & Zhang, B.L., 1992: Site-specific natural isotope fractionation of hydrogen in plant products studied by nuclear magnetic resonance. *Plant, Cell & Environment*, 15, 1037–1050.
- [48] Zhang, B.L., Yunianta, V.C., & Martin, M.L., 1995: Site-specific isotope fractionation in the characterization of biochemical mechanisms. *Journal of Biological Chemistry*, 270, 16023–16029.

IntechOpen