

ISOTOPIC CHARACTERISTICS OF FOOD PRODUCTS (WINE) AND THEIR GEOLOGICAL SUBSTRATES: DEFINITION OF THE RELEVANT PARAMETERS TO WINE GEOGRAPHICAL TRACEABILITY

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In recent years, the international scientific community has paid great attention to the development of innovative and suitable methodologies for solving the problem of traceability in the food industry (*e.g.*, Kelly *et al.*, 2005). Wine is one of the foods most subjects to adulteration. His production spans many countries and its commercialization has spread around the world. For these reasons, specific regulations will protect the authenticity either through the document checks and the determination of chemical parameters. The main adulteration concern sweetening and dilution, on the one hand, and the variety and geographical origin on the other hand. The analytical protocols based on the determination of the isotopes of light elements (*e.g.*, H, O, and C), made by mass spectrometry, are based on the acquisition of a particular isotope ratio (*i.e.*, δD ‰, $\delta^{18}O$ ‰).

Fractionation of the isotopes of light elements is favored by biotic and environmental factors, as well as by the presence of certain enzymes or by the characteristics of evapotranspiration in the region of production. In addition, the isotopic ratios can also vary on an annual basis, where rainfall and average temperatures influence the isotopic composition (*e.g.*, Cristoph *et al.*, 2004; Zoppi *et al.*, 2004). It is therefore necessary to make regional authentication rules based on the comparison of the isotopic characteristics of grapes and wines collected on an annual basis. In contrast, the authentication methods that are based on the geographic determination of the chemical composition (trace elements) and isotopic characteristics (*e.g.*, Sr, Nd, Pb) of the inorganic component is not affected by the problem related to climatic and seasonal variations of the production area.

The first oenological study that took into account the Sr isotope ratio values as a geography tracer were published in the early 90's by Horn *et al.* (1993). The authors have shown that there is a relationship between the values of the isotopic ratio $^{87}Sr/^{86}Sr$ of wines and the different region of wine provenance providing encouraging results for the use of $^{87}Sr/^{86}Sr$ isotopic ratio for geographical traceability of wines. In the following years, scientific research has focused primarily on the methodological and, in particular, on the experimental verification of the assumption that the values of the $^{87}Sr/^{86}Sr$ isotope ratio of the wines were not changed during the winemaking process (Almeida & Vasconcelos, 2001). Therefore, any portion of the territory where it lodges a vineyard has a specific isotopic ratio $^{87}Sr/^{86}Sr$ which may represent a fingerprint of wine compared to its geographical origin. However, pioneeristic studies were of foremost importance for indicating the way to follow, but they fail to provide an analytical method with uncertainties as low as those typical of geological studies. The isotopic ratios of Sr, Nd and Pd in geological materials constituting the substrate (rocks and soils) vary in a systematic way depending on the age of the rocks and their genesis, nature, and composition. Wine takes its nutrients, and therefore also the inorganic elements from the soil, and transfers them through the water to the biological tissue. The concentration of trace elements in the final product (*i.e.*, the wine) is in the range of 10-100 ppb (ng/g).

In this study we present a new analytical procedure for the determination of $^{87}Sr/^{86}Sr$ in wines with reproducibility consistent with that generally reported for geological materials (~ 20 ppm), which finalise the preliminary studies by Boari *et al.* (2008). In addition, we accurately analysed $^{87}Sr/^{86}Sr$ in wines produced with different grape vintage years from the same vineyard. Eventually, we applied the method to a large set of Italian bottled wines with certification of origin (*e.g.*, IGT, DOC, and DOCG) from four different wine regions of the Italian peninsula characterized by clear lithologic, and hence Sr isotope differences, to evaluate the presented

analytical method as a possible tool for tracing wine provenance using combined wine and geochemical isotopic data (Marchionni *et al.*, 2013). The actual experimental work has developed through three main phases of action.

The determination of any isotopic ratio of multi-elemental material has its critical point in the chemical treatment, measurement techniques, and correction for isotope fractionation and isobaric interference. Cation exchange chromatographic methods performed in a clean laboratory are of the foremost importance in obtaining highly precise $^{87}\text{Sr}/^{86}\text{Sr}$ analyses of wine by TIMS due to the comparable contents of Sr and Rb in wines, with the latter interfering on the measure of mass 87 in Sr (*e.g.*, Sr = 480-1600 μgL^{-1} and Rb = 370-1000 μgL^{-1}).

Sample preparation and Sr purification

All samples have been prepared and measured at the Dipartimento di Scienze della Terra of the University of Florence, Italy. Sample preparation and purification prior to isotopic measurement have been performed in a clean laboratory (class 1000 environment). Sample digestion has been performed within a horizontal HEPA-filtered laminar flow hood sited inside a fume cupboard, which ensures a low-blank working area. Subsequent purification has been performed using cation exchange chromatography within a vertical HEPA-filtered laminar flow hood (class 100 environment). High-purity chemical reagents and Milli-Q water (18.2 $\text{M}\Omega\text{ cm}^{-1}$) have been used throughout the entire chemical procedure. Some 5 mL of wine sample was evaporated to dryness at 90 °C in cleaned PFA beakers. The residue was dissolved twice in 3 mL of ultrapure quality H_2O_2 (30 wt.%) at 40 °C for 1 day and subsequently evaporated to dryness at 90 °C. The sample was then dissolved twice in 2 mL of suprapure quality HNO_3 (67 wt.%) at 150 °C for 1 day, evaporated to dryness, and dissolved again in 1 mL of 3N HNO_3 for Sr chromatography. This procedure has been set up to oxidize organic matter to CO_2 . Sr was finally purified by extraction chromatography using precleaned disposable Sr-Spec resins (100-150 μm , Eichrom) placed in 140 μL pure quartz microcolumns. After sample loading onto the column, some 14 column volumes (CV) of 3N HNO_3 was added in three steps (including rinsing two times with 2 CV) to elute all major and trace element except Sr, which is retained in the resin bed. Sr was eventually collected in clean PFA beakers using some 13 CV of Milli-Q water in three steps. The procedure was designed to collect > 500 ng of Sr given a concentration range between 0.1 and 0.5 ppm determined by atomic absorption spectrometry in representative wine samples for each wine production area. Total procedural Sr blank was tested using an ^{84}Sr -enriched (> 80%) spike at a concentration of 0.92 ppb, yielding values between 100 and 300 pg, which are negligible with respect to the sample size. Sr isotope compositions were measured using the last-generation Thermo Fisher (Bremen, Germany) Triton-Ti magnetic sector field thermal ionization mass spectrometer (TIMS) designed to separate charged atoms or molecules on the basis of their momentum along their way through the magnetic sector field.

Multidynamic mass collection procedure (*i.e.*, peak jumping) has been used to avoid further uncertainties due to bias among different signal collecting channels. In addition, the multidynamic mass collection procedure gives the possibility to obtain two simultaneous but independent measurements of the $^{87}\text{Sr}/^{86}\text{Sr}_{\text{double}}$ ratio during the run. The two $^{87}\text{Sr}/^{86}\text{Sr}$ double exponential law corrected ratios are geometrically averaged to obtain a single $^{87}\text{Sr}/^{86}\text{Sr}$ triple ratio, which is then a more accurate and precise value. The accuracy and precision of the procedure were tested through replicate analyses of the NIST SRM987 reference standard, yielding a long-term mean value $^{87}\text{Sr}/^{86}\text{Sr} = 0.710248 \pm 16$ (2σ , $n = 173$, equivalent to an error of 23 ppm), identical to the widely accepted recommended value of Thirlwall (1991) $^{87}\text{Sr}/^{86}\text{Sr} = 0.710248 \pm 11$. The within-run precision (*i.e.*, 2σ , internal precision) of $^{87}\text{Sr}/^{86}\text{Sr}$ measurements has been typically ≤ 10 ppm.

The excellent external reproducibility shown by the NIST SRM987 reference standard during the measuring period might be considered not representative of that of the wine samples due to the different matrix between the wine samples and the reference standard. To tackle this issue we have processed and measured the $^{87}\text{Sr}/^{86}\text{Sr}$ values of 31 different aliquots of wine from a single bottle of the Guardiolo wine. The internal precision (either 2σ or 2 SE, *i.e.*, 2 standard errors of the mean at 95% confidence level) of the replicate measurements has been < 10 ppm, and the statistics performed on all of the 31 replicates provide a log-normal distribution with excellent parameters: the average, median, and mode values are identical; the external precision at 95%

confidence level (2σ) is ± 0.000017 (i.e., ± 23 ppm). This give us confidence in the reliability of the Sr isotope compositions measured in wine samples and permits us to *i*) propose to the scientific community our analytical procedure, and *ii*) properly investigate the relationship between wine and geology for obtaining reliable information about wine traceability.

RESULTS

The first phase tested the eventual consistency of the initial hypothesis, that there was a relationship between wine and geographical area of origin by analyzing bottled wines with certification of origin (IGT, DOC, DOCG) acquired by the dealer focusing on four regions Italian: Tuscany, Lazio, Campania, and Basilicata. For these four regions experimentation has focused on single cultivar wines from wine-sector consortia of which the geological literature presents a thorough knowledge of the isotopic distribution of Sr in the rocks of their substrate. Experimental work has shown good correlation between Sr isotopes of wines and those of the substrate sets the vineyard. We also observed in a preliminary way as for white wines have values that differ from those of the substrate very likely due to the addition of additives, such as bentonite. The experiment showed that $^{87}\text{Sr}/^{86}\text{Sr}$ ratio can be used as a tracer geographical wine (Marchionni *et al.*, 2013) since *i*) the isotope ratio of Sr is preserved in time (Fig. 1), *ii*) there isn't Sr change during the process of winemaking, and

iii) organic wines from vineyards set on the same substrate have the same $^{87}\text{Sr}/^{86}\text{Sr}$ ratio. The wine regions chosen for the first part of this study are distributed along the Italian peninsula, from Tuscany to Basilicata: the area of Chianti Classico in Tuscany, one of the Cesanese in southern Lazio, those of Piediroso Campania Aglianico in Campania, and the Aglianico del Vulture in Basilicata. In addition to these large red wines, the Ansonico wine from the island of Giglio (Tuscany) was also analyzed; it is a limited production white wine with set screws on a geological substratum of intrusive igneous nature.

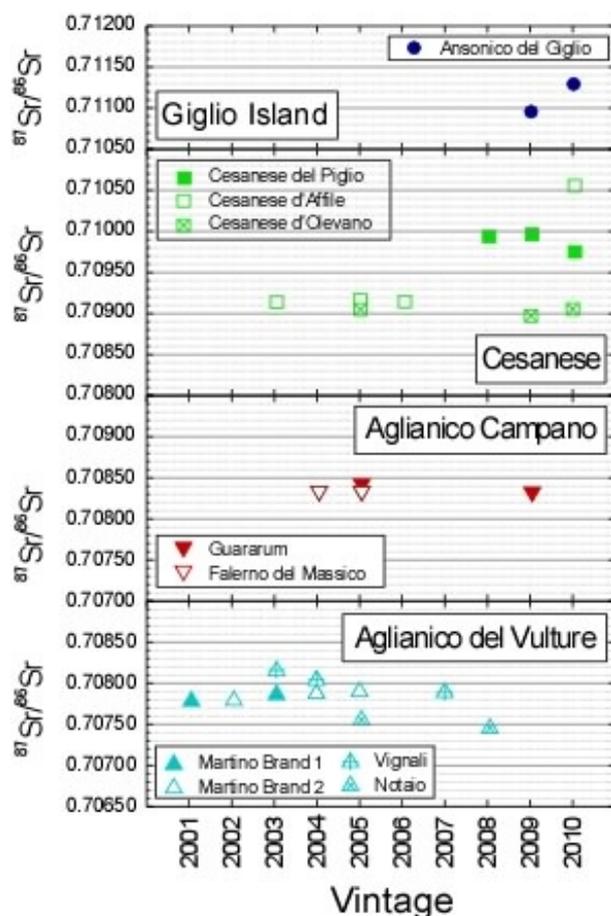


Fig. 1 - Bottled wine samples from the same winery but different vintage years to check the constancy of the Sr isotope geological signature through time. Note that the analytical error (2 SE) is smaller than the symbol size when error bars are not shown.

The second phase focused on a specific experiment on the area of the Cesanese including the consortia of Cesanese Affile, Cesanese Olevano, and Cesanese Piglio. As far as this part of the study area (Lazio) had the chance to analyze also grapes, grape must, soils and substrates in addition to the wines of different vintages. In this phase have been selected 6 wineries which have supplied grapes, musts, and wines of different vintages and allowed the sampling of both the rocks and the substrate soil. These companies provide control over the origin of wines from well-defined areas, culture, and the ways single variety of wine making. For each company have been identified one or two portions of land of limited size (from one to four acres) planted with red grape varieties and characterized by a lithologically homogeneous substrate. The experiment has shown

that the isotope ratio of Sr remains unchanged during the winemaking process (Fig. 2), preserving the isotopic ratios typical of the substrate. Contrary to what was observed by Marchionni *et al.* (2013), also because of the greater detail of their experimental study, it appears that the isotopic ratio to keep in mind as comparison value for the validation of the geographical origin of wines that of the leached soil solution, rather than those of the soil

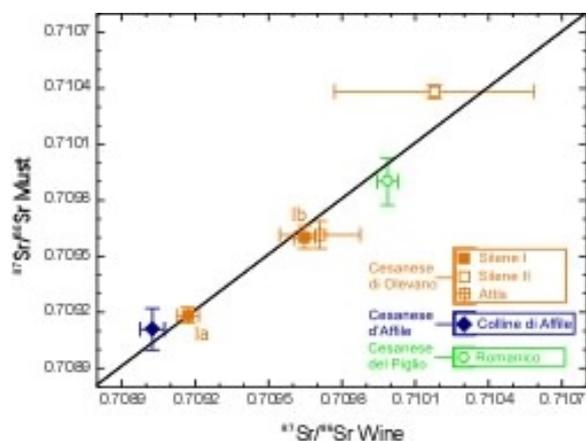


Fig. 2 - Average Sr isotope composition of wine vs. average Sr isotope composition of must in the different vineyards of the Cesanese Consortium. Wines and musts are from table 2. Error bars are standard deviation.

For each vintage micro-vinification for each row of vines were made. The Sr isotope data showed a constancy in time of micro-vinification and an unbreakable bond between Sr isotopes of the finished product and those of the extractable fraction of soil, even at a local scale.

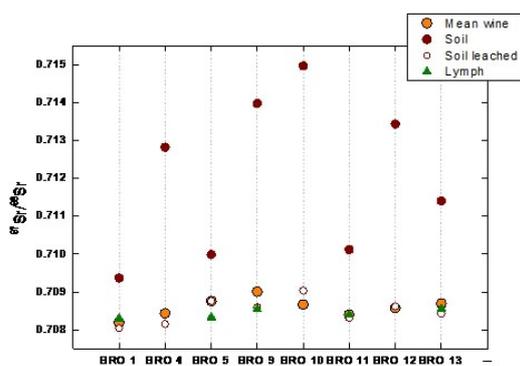


Fig. 3 - Average Sr isotopic values for the wine, the total soil, the soil leached and lymph in the Barone Ricasoli vineyard of the Chianti Area.

variability is due to the heterogeneity of the soil, although the presence of bioavailable Sr in soils can be also influenced by fertilizers and pesticides.

analyzed in its total component. To get the soil leached we used UNIBEST® resin capsules that simulate the behavior of the plant roots.

The third phase of the experiment was developed in collaboration with researchers at the institution CRA and developed with the support of the winery “Barone Ricasoli SpA”.

The trial took place in company where the urban noises were sampled on rows of vines defined soil substrate and three different vintages and grape sap. In relation to the proposed objectives is to be noted that the strengths with which the project was conceived are substantially related to the use of a technique of micro-vinification in the laboratory of the individual samples of grapes picked, which allows a tight control on the progress in preparation and to the knowledge of the detail of the sampling point which allows to have a good completeness of the information boundary.

The wines obtained from the individual sampling points show a specific and distinctive isotopic value and demonstrated that (Fig. 3): 1) the wines made by individual sampling points show a specific and distinctive isotopic ratio, thus showing that the variability of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio in wine should be also investigated at a local scale; 2) the ratio $^{87}\text{Sr}/^{86}\text{Sr}$ is well preserved over time, as observed in this case study for the four vintages of harvest, and therefore the reproducibility of ratio $^{87}\text{Sr}/^{86}\text{Sr}$ for wines is good also in time.

From this study we can conclude that it is possible to discriminate wines, sampled regularly and set on different soils, by assigning them a distinctive $^{87}\text{Sr}/^{86}\text{Sr}$ ratio; in fact, the process of transfer of Sr from the roots to the wine, *i.e.*, the final product, is not dependent of time; the observed

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