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$^{87}Sr/^{86}Sr$ isotopic ratios in vineyard soils and varietal wines from Douro Valley

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Abstract. The assessment of wine authenticity is of utmost importance in the current context of a growing market globalization. The strontium isotopic ratio $^{87}Sr/^{86}Sr$ is a well-established tool for dating and tracing the origin of rocks and minerals with special interest for wine traceability. A study was developed to examine the variation of $^{87}Sr/^{86}Sr$ in wines from Douro Valley taking into account the effects of vineyard location and grape variety. The $^{87}Sr/^{86}Sr$ of soils and wines from six vineyards were determined by using an ICP-MS based analytical procedure. A total of twenty-two monovarietal wines, obtained at micro vinification scale, from relevant white and red grapevine varieties for Douro region, were analysed. The range of $^{87}Sr/^{86}Sr$ values observed in soils and wines was of 0.708–0.725 and 0.711–0.717, respectively. The present study updates the scarce knowledge available on strontium isotopic ratios in soils and wines from Douro Valley, and its results will enlarge global databank on wine composition and support comparison with other world regions.

1. Introduction

Wine traceability and the assessment of its authenticity are of utmost importance in the current context of a growing market globalization [1]. The main issues concern adulterations, geographic origin, grape varieties, and vintage year. Stable isotope analyses, recognized by the International Organisation of Vine and Wine (OIV) for detecting adulterations, are limited in terms of interpreting the data and relating them to the wines provenance. The development of analytical methodologies which can positively identify the geographic origin of a given product is one of the most challenging issues for scientific community.

The strontium isotopic ratio $^{87}Sr/^{86}Sr$ is a well-established tool for dating and tracing the origin of rocks and minerals with special interest for wine traceability. Literature on the progress made since the first application of $^{87}Sr/^{86}Sr$ isotopic ratio for wine traceability purposes in the 1990's is available, with a significant increase number of studies developed in the last years, and involving wine producing regions worldwide [2–13]. Within a research program on strategies for wine fingerprinting carried out by the authors, the transference of $^{87}Sr/^{86}Sr$ signature through soil-wine system was examined. The $^{87}Sr/^{86}Sr$ was identified as a viable tool to distinguish between three Portuguese Protected Designations of Origin (PDO; Dão, Óbidos, and Palmela), where soils are developed on different geological formations [14].

The reliability of $^{87}Sr/^{86}Sr$ for wine fingerprinting is evidenced by the studies. Nevertheless, the heterogeneity of some wine regions and PDO in terms of soils and geological materials is well known, making it difficult to match wines with their substrata data. Furthermore, in some cases, soils from different wine regions and countries have been originated from similar geological formations, which can be a limitation in terms of interpreting the data and relating them to the wines provenance. Thus, aiming to use $^{87}Sr/^{86}Sr$ for traceability and authentication of wine, it is crucial to develop further studies, on other lithological situations and other world regions (where similar geological formations can occur), to confirm the feasibility of $^{87}Sr/^{86}Sr$ fingerprinting and enlarge data.

Sr has been claimed to be absorbed by the vine in the same isotopic proportions in which they occur, under available forms (labile Sr); biological processes involved in vine metabolism do not significantly fractionate Sr isotopes [15,16]. Nevertheless, studies on the

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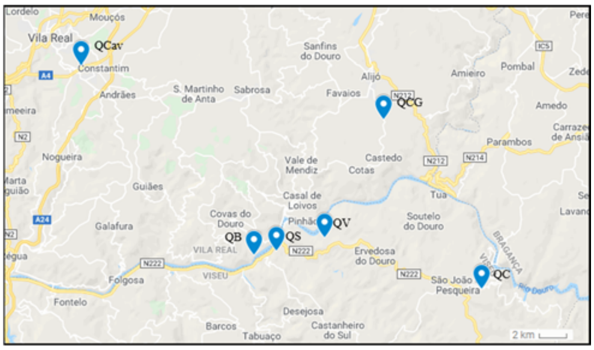

Figure 1. Location of the six experimental vineyards.

hypothetical influence of rootstock and grapevine variety are limited. More, a better understanding of the impacts of anthropogenic factors and technologic processes on this marker is required for its validation. Tailored studies on its robustness to traditional and new technological processes, namely wood aging and nanofiltration for dealcoholisation, were recently published [17,18] and other studies are underway.

Portugal is the eleventh largest producer of wine and the nine largest exporter, with over six hundred millions of liters produced in 2017 [19]. Nevertheless, there is limited information on $^{87}Sr/^{86}Sr$ of Portuguese wines for their classification according to geographical origin. Located in the northeast of Portugal, the Douro Wine Region was created in 1756 as the first wine appellation in the world, being famous by Porto fortified wines and more recently for high quality Douro dry wines. The region is characterized by terraces that surround the Douro River and its tributaries, in some cases with walls made of schist supporting vineyards, and by the diversity of native grapevine varieties. Most of the vineyards are installed in soils developed on schists. There is little information on strontium isotopic ratio of soils and wines from Douro [3,5,20]. On the basis of these considerations, a study was developed to examine the variation of $^{87}Sr/^{86}Sr$ in wines from Douro region taking into account the effect of vineyard location and grapevine variety.

2. Materials and methods

2.1. Vineyards and soils sampling

Six vineyards, selected based on their geographical location (Douro Region) and on the availability of certain grapevine varieties, were considered: Quinta da Boavista (QB, 41°10′ N, 7°34′ W), Quinta do Casal da Granja (QCG, 41°15′ N, 7°28′ W), Quinta de Cavernelho (QCav, 41°17′ N, 7°43′ W), Quinta de Cidrô (QC, 41°08′ N, 7°23′ W), Quinta do Seixo (QS, 41°10′ N, 7°33′ W), and Quinta de Ventozelo (QV, 41°10′ N, 7°30′ W), property of the enterprises Sogrape Vinhos S.A. and Real Companhia Velha (Fig. 1).

For comparison purposes between $^{87}Sr/^{86}Sr$ values of wines and soils of provenance, soil samples were collected (vineyards QB, QCG, QC and QS) considering two points in the row of vines (for each grape variety), from the depth layer of 50–80 cm. The vineyards located in the Douro valley, are characterized by very hard soil structure as they are mainly originated from schist, a slate-like metamorphic rock, thus imposing the use of a heavy machinery backhoe and drill to access the required soil depth. Soil samples were dried, ground and then forced to pass through a 50 µm sieve.

2.2. Grape varieties and wines

Twenty-two *V. vinifera* varieties were selected from national and international white and red grapevine varieties, and based on their importance to the Portuguese wine sector, more particularly to the Douro region (Table 1). Grape samples from each grapevine variety were harvested in the production year 2012, and were vinified at the National Institute for Agricultural and Veterinary Research (INIAV) in Dois Portos, Portugal.

White wines were produced by applying the conventional technology, being exclusively obtained by the fermentation with grape must extraction preceding alcoholic fermentation. Red wines resulted from the alcoholic fermentation of musts in the presence of the solid parts of the berry (skins and seeds), by application of the conventional red winemaking. More detailed information was described by Pereira et al. [21].

2.3. Strontium isotopic analysis

Prior to $^{87}Sr/^{86}Sr$ determination by quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS), the following three main analytical steps in sequence were performed as described by Martins et al. [14]: sample digestion by high pressure microwave digestion (HPMW) (1), chromatographic separation of ^{87}Sr and ^{87}Rb (2) and determination of Sr and Rb content by Q-ICP-MS (3).

(1) For elimination of organic substances in order to prevent any interference during chromatographic separation, soils and wines were treated by high pressure microwave digestion (HPMW). A subsample of 0.20 g of dried soil was acid digested using a microwave system as described by Martins et al. [14]. Wines were acid digested following a digestion program previously optimized by Catarino et al. [22].

(2) Separation of Sr from Rb is an essential step for correct determination due to isobaric overlapping of ^{87}Rb and ^{87}Sr. An HIPEX Duran column was filled up by using Dowex 50W-X8/400 (Sigma-Aldrich) mesh resin and EDTA as eluent. Separation consists of four phases which are resin activation/pre-treatment; resin conditioning; sample preparation/dilution and elution. Separation was done in duplicates.

(3) Sr and Rb total content and $^{87}Sr/^{86}Sr$ isotopic ratio were measured by the ICP-MS equipment previously described. After first two steps, Sr containing fraction was used for Q-ICP-MS analysis. Determination of Sr and Rb total contents in Sr-containing fractions previously to the isotopic measurement is important in order to keep Rb concentration below 1% of the Sr content in Sr-fraction. The SRM 987 ($SrCO_3$) from National Institute of Standards and Technology (NIST) was used as an isotopic reference material for external correction of mass bias phenomenon. Analytical steps for Sr and Rb and isotopic determination are described in Kaya et al. [18].

After separation of Rb and Sr by ion-exchange chromatography, analytical determinations of Rb and Sr concentration and $^{87}Sr/^{86}Sr$ were carried out by ICP-MS

Table 1. List of 22 grapevine varieties used for wine production followed by the corresponding official code*, abbreviation, berry color, and vineyard of origin.

Grapevine variety	Official code	Abbreviation	Berry color	Vineyard
Alicante Bouschet	PRT53808	AB	Red	QB
Aragonez	PRT52603	Ar	Red	QS
Cabernet Sauvignon	PRT53606	CS	Red	QC
Chardonnay	PRT53511	Ch	White	QC
Côdega do Larinho	PRT51317	CL	White	QB
Donzelinho Tinto	PRT52306	DT	Red	QB
Fernão Pires	PRT52810	FP	White	QCG
Gouveio	PRT52112	Gou	White	QCav
Malvasia Fina	PRT52512	MF	White	Qcav
Merlot	PRT50518	Mer	Red	QV
Moscatel Galego	PRT52915	MG	White	QCG
Pinot Noir	PRT53706	PN	Red	QC
Rufete	PRT52106	Ruf	Red	QS
Tinta Barroca	PRT52905	TB	Red	QS
Tinta Francisca	PRT52502	Tfi	Red	QS
Tinto Cão	PRT53307	TC	Red	QS
Touriga Fêmea	PRT50705	Tfe	Red	QS
Touriga Franca	PRT52205	TF	Red	QS
Touriga Nacional	PRT52206	TN	Red	QS
Trincadeira	PRT53006	Tr	Red	QS
Vinhão	PRT51902	Vin	Red	QS
Viosinho	PRT52715	Vio	White	QB

* Portaria n° 380/2012.

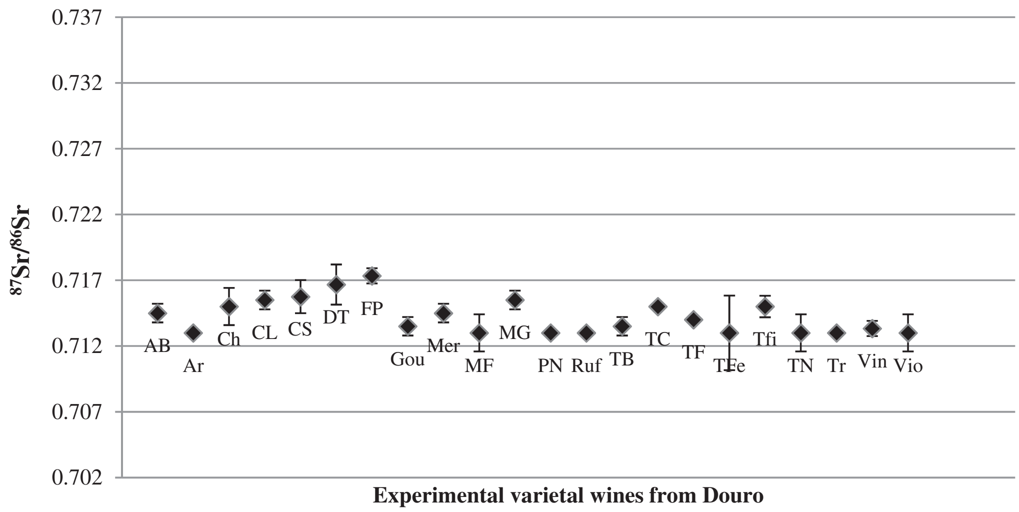

Figure 2. $^{87}Sr/^{86}Sr$ values of white and red experimental varietal wines from Douro Valley. Results correspond to mean value (and corresponding standard deviation with error bars) of analytical duplicates.

using a Perkin-Elmer SCIEX 9000 ICP-MS equipment. The detailed analytical protocol can be found in the study of Martins et al. [14].

In addition, total strontium concentration was analysed in wines by ICP-MS semiquantitative method as described by Catarino et al. [23].

2.4. Statistical analysis

The statistical treatment of soil and wines $^{87}Sr/^{86}Sr$ ratios was performed by one-way analysis of variance and comparison of means (Fisher LSD, significance level of 0.05) using Statistica version 7 software (StatSoft Inc., Tulsa, USA).

3. Results and discussion

3.1. $^{87}Sr/^{86}Sr$ of the wines from Douro Valley

The values of $^{87}Sr/^{86}Sr$ found in the experimental monovarietal wines from the six vineyards from Douro region are shown in Fig. 2. The $^{87}Sr/^{86}Sr$ range of variation, from 0.713 (MF, QCav) to 0.717 (FP, QCG), is very narrow reflecting the geographical proximity of the wines. These values are in line with those reported by Almeida and Vasconcelos [3] for two fortified wines from Douro, of 0.716 and 0.721, using the same analytical strategy applied in this study (ICP-MS and external calibration for mass bias correction).

In comparison to Dão region, mainly granitic, these values are lower than those reported by the same authors,

Table 2. $^{87}Sr/^{86}Sr$ isotopic ratio of the wines by vineyard of provenance.

	Vineyard effect	QB	QCG	QCav	QC	QS	QV
$^{87}Sr/^{86}Sr$	*	0.7149 (0.0005) a,b	0.7164 (0.0008) b	0.7133 (0.0008) a	0.7146 (0.0006) a,b	0.7136 (0.0003) a	0.715 (0.001) a,b

Results are based on average values (QB: $n = 4$ wines; QCG: $n = 2$ wines; QCav: $n = 2$ wines; QC: $n = 3$ wines; QS: $n = 10$ wines; QV: $n = 1$ wine). Means followed by the same letter are not significantly different at 0.05* level of significance. Relative standard deviations are presented in parenthesis.

of 0.728, and similar to those reported by Moreira et al. [17], between 0.713 and 0.715. Comparing with other Portuguese regions, these ratios are higher than those found in wines from Bairrada (0.710), Borba (0.710), Madeira (0.708), Óbidos (0.708–0.710), and Palmela (0.708–0.710) [3,17,18].

Information about $^{87}Sr/^{86}Sr$ values in wines from worldwide producing regions is described in the literature: Argentinean wines from different regions (Córdoba, Mendoza, San Juan) and different grape varieties ($^{87}Sr/^{86}Sr$ lower than 0.910) [24]; Australian wines (mean values close to 0.7115, $n = 231$) [25], Bordeaux wines (0.7086–0.71005) [4]; wines from Canada (Quebec, 0.70988–0.71546) [12]; a wine from Chile (0.70471) [4]; relevant data is available for Italian wines from different regions (0.7086–0.7126) [7–11,13]; Romanian wines (0.71015–0.72311) [26]; South African wines, from 0.7070 (Stellenbosch) to 0.7154 (Robertson) [6].

Table 2 displays for each vineyard the $^{87}Sr/^{86}Sr$ mean value of the corresponding wines. Significant differences were observed, with Quinta do Cavernelho and Quinta do Seixo showing the lowest values, and Quinta do Casal da Granja exhibiting the highest value.

In respect to wine type (white and red), no significant difference was observed between white and red wines in terms of $^{87}Sr/^{86}Sr$ isotopic ratio. The $^{87}Sr/^{86}Sr$ mean value corresponding to the white wines ($n = 7$) was of 0.7147 (0.0005), while for red wines ($n = 15$) the value was of 0.7140 (0.0003).

3.2. $^{87}Sr/^{86}Sr$ of vineyard soils from Douro Valley

The values of $^{87}Sr/^{86}Sr$ found in soils from the four vineyards (selected for evaluation of soil-wine correlation) ranged from 0.715 (QS) to 0.7183 (QCG and QC), without significant differences between vineyards.

3.3. Comparison of the $^{87}Sr/^{86}Sr$ obtained for vineyards soils and wines from Douro Valley

No significant difference was observed between vineyards soils and wines in what concerns strontium isotopic ratios. The $^{87}Sr/^{86}Sr$ mean value corresponding to the soils ($n = 19$) was of 0.7157 (0.0007), while for wines ($n = 22$) the value was of 0.7142 (0.0007). These results are in line with the trend observed in several studies, of lower $^{87}Sr/^{86}Sr$ values in wines than in the soils of provenance [5,6,12]. In fact, recent research indicates that $^{87}Sr/^{86}Sr$ of wines is mainly determined by the bioavailable fraction of the soils on which the vineyards are settled instead of by the bulk soil [7]. For each vineyard, $^{87}Sr/^{86}Sr$ values of soils and wines were compared (Table 3). With exception of Quinta do Cidrô no significant differences were observed between soils and wines.

Table 3. $^{87}Sr/^{86}Sr$ of soils and wines, for each vineyard, from Douro Valley.

Vineyard	Sample effect	Soils	Wines
QB	*n.s.*	0.716 (0.001)	0.715 (0.001)
QCG	*n.s.*	0.718 (0.003)	0.716 (0.003)
QC	*	0.7183 (0.0006) b	0.7146 (0.0006) a
QS	*n.s.*	0.715 (0.001)	0.714 (0.001)

Results are based on average values (QB: 4 soils and 4 wines; QCG: 2 soils and 2 wines; QC: 3 soils and 3 wines; QS: 10 soils and 10 wines). Means followed by the same letter are not significantly different at 0.05* level of significance; *n.s.* – without significant difference at 0.05* level of significance. Relative standard deviations are presented in parenthesis.

4. Conclusions

The present study represents a development background for building an authentic wine reference database (e.g. official or wine organisation, PDO consortium) to evaluate the provenance of wine labelled as Douro, or to be integrated in a global wine database (e.g. EU wine databank) of great usefulness for industry. Nevertheless, despite the potential of $^{87}Sr/^{86}Sr$ for determining the provenance of wines, it seems it can be difficult to differentiate them both at the country and regional level only through $^{87}Sr/^{86}Sr$, indicating that it should be used together with other discriminating parameters. Finally, the results of this study indicate that it is crucial to know the soil geochemistry background so a reliable wine origin relationship can be established.

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