^{87}Sr/^{86}Sr RATIO IN VINEYARD SOILS FROM PORTUGUESE DENOMINATIONS OF ORIGIN AND ITS POTENTIAL FOR ORIGIN AUTHENTICATION

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Abstract

Aim: The control of geographical origin is one of the most challenging topics regarding wine authenticity. The aim of the present study was to assess the ^{87}Sr/^{86}Sr ratio of vineyard soils from Portuguese Denominations of Origin (DO) and evaluate its suitability as a tool for origin authentication.

Methods and results: An analytical protocol was optimized (chromatographic separation of Sr and Rb, followed by inductively coupled plasma-mass spectrometry (ICP-MS) analysis) for ^{87}Sr/^{86}Sr isotopic ratio determination in soil-wine system. The ^{87}Sr/^{86}Sr ratios of soils from four vineyards located in three Portuguese DO (Dão, Óbidos and Palmela), established on distinct soil types, were determined. Significant differences were found between soils of different DO regions. The soil in the Dão DO, developed on granites, showed a statistically higher ^{87}Sr/^{86}Sr ratio than the other soils, which were developed on sedimentary formations.

Conclusion: The results show clearly that ^{87}Sr/^{86}Sr ratio may represent a suitable fingerprint for these Portuguese DO.

Significance and impact of the study: This study highlights the relevance of setting up an international databank of ^{87}Sr/^{86}Sr values for use for geographical identification and authentication.

Key words: soil, geographical origin, Portuguese DO, ^{87}Sr/^{86}Sr, ICP-MS

Résumé

Objectif : Le contrôle de l'origine géographique est un des sujets les plus difficiles concernant l'authenticité du vin. Le présente étude avait pour but évaluer les ratios ^{87}Sr/^{86}Sr des sols viticoles d'Appellations d'Origine Contrôlée (AOC) portugaises et leur pertinence comme outil pour l'authentification de l'origine.

Méthodes et résultats : Un protocole d'analyse a été optimisé (séparation chromatographique de Sr et Rb, suivie par l'analyse de spectrométrie de masse avec plasma à couplage inductif (ICP-MS) pour la détermination du rapport isotopique ^{87}Sr/^{86}Sr dans le système sol-vin. Les rapports ^{87}Sr/^{86}Sr ont été déterminés sur les sols de quatre vignobles situés dans trois AOC portugaises (Dão, Óbidos et Palmela), avec des types de sols distincts. Nous avons trouvé des différences significatives entre les sols des différents régions. Le sol de l'AOC Dão, développé sur des granites, a montré un ratio ^{87}Sr/^{86}Sr statistiquement plus élevé que les autres sols, développés sur des formations sédimentaires.

Conclusion : Les résultats montrent clairement que le ratio ^{87}Sr/^{86}Sr peut représenter une empreinte digitale appropriée pour ces AOC portugaises.

Signification et impact de l'étude : Cette étude met en évidence la pertinence de la mise en place d'une banque de données internationale des valeurs ^{87}Sr/^{86}Sr, visant son utilisation pour l'identification géographique et l'authentification.

Mots clés : sol, origine géographique, AOC portugaises, ^{87}Sr/^{86}Sr, ICP-MS

INTRODUCTION

The globalization of food markets has raised consumer concerns for product origin and quality. The place of origin of foodstuff is regarded as value-added information and as a guarantee of quality and authenticity. For wine in particular, geographical origin has a direct effect on its quality and commercial value, being one of the most studied products in terms of food authentication (Barbaste *et al.*, 2002 ; Almeida and Vasconcelos, 2003).

The control of the geographical origin of wine based on its chemical composition is one of the most challenging issues in relation to wine authenticity. In the last decade, many efforts have been made to identify potential markers and develop reliable analytical methods to determine the wine's authenticity. Among these "fingerprints", isotopic ratios play an increasingly important role (Almeida and Vasconcelos, 2001 ; Barbaste, 2001 ; Ferreira, 2008 ; Rosner, 2010). The application of methods using stable isotopes of light elements (H, C, N, O, S) started two decades ago, providing information on climate, distance from the sea, altitude, latitude, and technological practices (Ferreira, 2008).

More recently, the study of isotopic ratios of heavy elements such as Pb and Sr came into use in this field of application, providing additional information on the geographical origin, since plants inherit the isotopic signature of these elements from the geological and pedological environment (Horn *et al.*, 1993 ; Barbaste, 2001 ; Rummel *et al.*, 2010).

The use of Sr isotope ratio is a well established tool in earth sciences for dating and tracing the origin of rocks and minerals. Furthermore, some studies indicate that Sr isotope analysis could be very useful for determination and verification of the geographical origin of food.

Sr has four naturally occurring stable isotopes with ranges of natural abundance as follows : ^{84}Sr, 0.55-0.58 % ; ^{86}Sr, 9.75-9.99 % ; ^{87}Sr, 6.94-7.14 % ; and ^{88}Sr, 82.29-82.77 % (Berglund and Wieser, 2011). The ^{84}Sr, ^{86}Sr and ^{88}Sr isotopes occur in constant relative proportions, while ^{87}Sr gradually increases in minerals due to the radioactive β-decay of the ^{87}Rb isotope. The relative abundance of ^{87}Sr varies with geological ages and consequently with geographical locations, providing a fingerprint for different rock types (Capo *et al.*, 1998 ; Vanhaecke *et al.*, 1999 ; Almeida and Vasconcelos, 2001).

The proportion of ^{87}Sr to total Sr increases at a rate dependent on available Rb. Geological environments rich in Rb relative to Sr will have a high $^{87}Sr/^{86}Sr$ ratio, while regions with low Rb relative to Sr will retain low $^{87}Sr/^{86}Sr$ ratios for long periods of geological time. Therefore, the Sr isotopic composition of a geological sample depends on the Rb/Sr concentration ratio and age of the material.

Weathering of the underlying rock and/or sediments is a significant source of strontium for the soil. Pre-Cambrian granitic bedrock and alluvial sands derived from felsic rocks show high $^{87}Sr/^{86}Sr$ ratios (0.710-0.716) reflecting the age of the continental crust and high Rb/Sr ratios from which these materials originated. Limestones have intermediate $^{87}Sr/^{86}Sr$ ratio values (0.706-0.709) and young oceanic basalts and their sediments show the lowest values (0.702-0.705) (Faure, 1986 ; Capo *et al.*, 1998).

Biological processes involved in plant metabolism do not significantly fractionate Sr isotopes (Capo *et al.*, 1998). Elements are absorbed by the plants in the same isotopic proportions in which they occur in soil. Thus, plants reflect the growth environment, such as bedrock, soil and soil water content (Horn *et al.*, 1993 ; Capo *et al.*, 1998 ; Stewart *et al.*, 1998), including all sources of Sr : natural (bedrock weathering, precipitation) and anthropogenic (e.g., fertilizers). To establish correlations between the $^{87}Sr/^{86}Sr$ ratio of wines and soils, a deep knowledge of the region's geological and pedological features is a prerequisite.

Studies by Horn *et al.* (1997) demonstrated that the $^{87}Sr/^{86}Sr$ values of several wines were within the respective ranges for rocks and soils. In the cases in which the isotopic ratio values were inconsistent with their origin, double-blind studies were performed and the wines were found to be falsely labeled. Barbaste (2001) reported values of $^{87}Sr/^{86}Sr$ ratio in wine in agreement with the literature data for the corresponding soils. In addition, Almeida and Vasconcelos (2004) studied the Sr isotopic composition in two wines from the Douro Portuguese Denomination of Origin (DO) by means of quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS) and compared it with the isotopic composition of the respective soil and grape juice. It was shown that the $^{87}Sr/^{86}Sr$ values in soil, grape and wine were statistically identical.

Precise and accurate Sr isotope data is required for origin discrimination. Faraday multi collector-equipped thermal ionization MS (TIMS) or ICP-MS are the techniques of choice to determine the $^{87}Sr/^{86}Sr$ ratio of food, with precision values of 0.002 % (RSD) (Barbaste *et al.*, 2002 ; Rosner, 2010). The lower precision of Q-ICP-MS, typically below 0.1 % (RSD),

can be a limiting factor, especially in studies involving samples with very close $^{87}Sr/^{86}Sr$ values. Nevertheless, Q-ICP-MS is robust and less time consuming, and proved to be suitable to distinguish the Sr isotopic composition of wines (Vanhaecke *et al.*, 1999 ; Almeida and Vasconcelos, 2001).

Due to the isobaric overlap of ^{87}Sr and ^{87}Rb, an effective Rb/Sr separation is a pre-requisite for the accurate determination of Sr isotope ratios by Q-ICP-MS. In order to perform this separation, ion-exchange chromatography is employed and some elution procedures are described in the literature. The most widely used procedure involves the elution of Rb with a weak HCl solution, followed by the elution of Sr using a stronger HCl solution (Vanhaecke *et al.*, 1999). An alternative approach to the conventional HCl method is the use of a two-step chromatographic separation, described by Almeida and Vasconcelos (2001). Alternatively, Vorster *et al.* (2008) proposed a highly specific separation method using the complexing agent ethylenediaminetetraacetic acid (EDTA), which forms strong chelates with Sr without interacting with Rb.

Data on the isotopic composition of Portuguese vineyards are scarce and no information is available concerning different DO. Therefore, within the "Multi-elemental and isotopic composition as fingerprints of wine geographical origin" research program on wine fingerprinting strategies, a study was developed to investigate whether between-region variation in Sr isotopic composition could be used as a tool for the traceability of Portuguese DO (Dão, Óbidos and Palmela), where soils are developed on different geological formations. For that, we first optimized an analytical protocol suitable for the determination of $^{87}Sr/^{86}Sr$ by Q-ICP-MS in vineyard soil samples, grape must and wine, Sr being separated from Rb by cation-exchange chromatography using EDTA. Then, $^{87}Sr/^{86}Sr$ ratios of soils from selected vineyards in Portuguese DO, established on distinct soil types, were determined and compared.

MATERIALS AND METHODS

1. Vineyards

Four vineyards from three Portuguese DO (Dão, Óbidos and Palmela) were studied (Figure 1). In the Dão DO (Central Portugal), one vineyard was considered : Quinta dos Carvalhais (40º 33´ N, 7º 47´ W), Sogrape Vinhos. It is established on Dystric Cambisols and Dystric Regosols (IUSS Working Group WRB, 2006) soils which were developed on monzonitic granites (Hercynian granites), the most representative geological formation of Dão DO

Figure 1 - Location of the four experimental vineyards in three Portuguese DO (Dão, Óbidos and Palmela).

(Teixeira *et al.*, 1961). In the Óbidos DO (Centre of Portugal), characterized by a larger variety of lithological formations, two vineyards were considered : Quinta do Sanguinhal (39º 15´ N, 9º 09´ W) and Quinta de S. Francisco (39º 11´ N, 9º 10´ W), both of Companhia Agrícola do Sanguinhal. Quinta do Sanguinhal is established on soils (Dystric Regosols) developed on Jurassic sandstones ; Quinta de S. Francisco is established on soils (Eutric Regosols) developed on Cretacic sandstones (Zbyszewski and da Veiga Ferreira, 1966). In the Palmela DO (Southern Portugal), one vineyard was considered : Vinha de Algeruz (38º 34´ N, 8º 49´ W), José Maria da Fonseca Vinhos. This vineyard is established on Eutric Regosols developed on Pliocene sedimentary formations (clays and sands), the most representative geological formation of Palmela DO (Manuppella *et al.*, 1999). The area of each vineyard plot, year of planting, rootstock, vine spacing, row direction and training system are indicated in Table 1. All the vineyards have the same red variety in production (*Vitis vinifera* L., cv Aragonez).

Table 1 - Characteristics of the vineyards

Vineyard / Portuguese DO	Area of the vineyard plot (ha)	Year of planting	Rootstock	Vine spacing (m)	Row direction	Training system
Quinta dos Carvalhais / Dão	2.5	1995	1103P	2.0x1.2	NE-SW	bi-lateral cordon
Quinta do Sanguinhal / Óbidos	2.6	2000	R110	2.7x1.0	N-S	bi-lateral cordon
Quinta de S. Francisco / Óbidos	5.0	2001	R110	2.7x1.0	N-S and E-W	bi-lateral cordon
Vinha de Algeruz / Palmela	3.0	1990	1103P	2.8x1.2	N-S	bi-lateral cordon

The study area has a temperate, Mediterranean-type climate characterized by dry summer and wet autumn/winter/early spring. The Quinta dos Carvalhais, Quinta do Sanguinhal and Quinta de S. Francisco vineyards have a humid temperate climate with dry summer and cold nights (*C*sb, Köppen classification); the Vinha de Algeruz vineyard has a humid temperate climate with dry hot summer and temperate nights (*C*sa, Köppen classification) (Instituto de Meteorologia; IM, 2008).

2. Soil sampling

Soil sampling took place in December 2007 (Vinha de Algeruz, Quinta do Sanguinhal and Quinta de S. Francisco) and May 2009 (Quinta dos Carvalhais). In each vineyard, soil samples were collected with a probe, from nine sampling sites distributed along three non-contiguous vine rows (representative of the entire vineyard area), from four depth layers (0-20, 20-40, 40-60 and 60-80 cm), and sealed in plastic bags. The samples used in this study were those collected at the 40-60 cm layer, where root density was higher. Soil samples were dried, ground and then forced to pass through a 2-mm sieve.

3. Analytical procedures

Reagents

Nitric acid (HNO_3) 65 % EMSURE® and hydrofluoric acid (HF) 48 % EMSURE® (Merck) were used for soil digestion. Ultrapure concentrated HNO_3 (J.T. Baker) and Trace Select® 30 % (v/v) hydrogen peroxide (H_2O_2) (Fluka, Sigma-Aldrich) were used for grape must and wine digestion. Ammonia (NH_3) 25 % (v/v) and EDTA Titriplex III of analytical grade (Merck) were used for ion-exchange chromatography.

Monoelement standard solutions of Be, Co, In (1 000 mg/L; Merck) and a multielement solution with Mg, Cu, Rh, Cd, In, Ba, Ce, Pb and U (10 mg/L; Perkin-Elmer) were used for ICP-MS optimization. Ultrapure concentrated HNO_3 (J.T. Baker) was used for washing, blank, and standard solutions. ICP-MS semi-quantitative calibration (for Rb and Sr determinations) was established with a multielement standard solution with 30 elements: Li, Be, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, In, Cs, Ba, Hg, Tl, Pb, Bi and U (10 mg/L; Perkin-Elmer). ICP-MS internal standardization was performed with standard solutions of Rh and Re (1 000 mg/L; Merck).

The standard reference material (SRM) 987 ($SrCO_3$) from the National Institute of Standards and Technology (NIST; Gaithersburg, USA) was used as isotopic standard (external correction of mass bias) and for ICP-MS instrumental parameter optimization. A stock solution of Sr (1 000 mg/L) was prepared by dissolving 1 g of powder in 1 % (v/v) HNO_3. Monoelement standard solutions of Sr (1 000 mg/L; Reagecon) and Rb (1 000 mg/L; Perkin-Elmer) were used for the study of the influence of Rb concentration on Sr isotope ratio measurements.

De-ionized water (conductivity < 0.05 µS/cm) prepared by using a Seralpur Pro 90CN system (Seral, Ransbach-Baumbach, Germany) was used for all solutions. To avoid contamination of the samples, all the material (polypropylene and Teflon PFA) was soaked in 20 % HNO_3 (v/v) for at least 24 hours and then rinsed thoroughly with de-ionized water before use. For decontamination solution preparation, HNO_3 reagent grade was double-distilled using an infra-red sub-boiling distillatory system (model BSB-939-IR, Berghof, Germany).

Soil mineralization

Prior to ion-exchange chromatography, the samples were acid digested using a microwave system (CEM MDS 2000). Four mL of HNO_3 (65 % v/v) and 4 mL of HF (50 % v/v) were added to each subsample of 0.20 g of dried soil (fraction < 270 mesh). The digestion program consisted of one 30-minute step at 630 W (100 psi). The dissolved material was dried, dissolved with HNO_3 (2 M) to 10 mL, and finally diluted to 25 mL with de-ionized water. The efficiency of the digestion procedure was confirmed with the certified reference material Geo PT 25, Basalt HTB-1.

Strontium and rubidium separation by ion-exchange chromatography

Ion-exchange chromatography was carried out in a column (internal diameter of 1.1 cm and bed length of 13 cm) filled with Dowex 50W-X8/400 mesh resin (Sigma Aldrich). The procedure described by Vorster *et al.* (2008) employing EDTA as eluent was used as a starting point. After pretreatment and resin conditioning, samples were loaded into the column, and sequential elution of Ca, Sr and Rb was performed with EDTA solutions. Experimental conditions, namely pretreatment and resin conditioning, volume and concentration of EDTA solution at each elution step, and resin washing, were optimized in order to obtain Sr and Rb separation in a single separation step. Experimental tests were conducted using soil, grape must and wine samples after high power microwave (HPMW) digestion and dilution with 0.1 % HNO_3 to a specific volume depending on the required concentration : soil samples were treated as previously described ; grape must and wine samples were mineralized by an adaptation of the HPMW procedure previously optimized by Catarino *et al.* (2010).

To prevent the precipitation of residual EDTA in the ICP-MS instrument, samples were filtered using a Millipore PVDF syringe filter (0.45-µm pore size, 3.0-cm diameter).

ICP-MS analysis

Analytical measurements were carried out with a Perkin-Elmer SCIEX Elan 9000 ICP-MS (Norwalk, CT, USA) equipped with a cross-flow nebulizer, a Ryton Scott-type spray chamber, and nickel cones. A four-channel peristaltic sample delivery pump (Gilson model) and a Perkin-Elmer AS-93 Plus autosampler protected by a laminar-flow-chamber clean room class 100 (Max Petek Reinraumtechnik) were used. The ICP-MS instrument was controlled by Elan 6100 Windows NT software (version 2.4).

The operating conditions of the ICP-MS equipment, optimized daily, were as follows : RF power of 1200 W ; Ar gas flow rates of 15 L/min for cooling, between 0.94 and 0.98 L/min for nebulizer and 1.5 L/min for auxiliary ; and solution uptake rate of 1.0 mL/min.

Rb and Sr concentrations were determined in mineralized soil samples (after 100-fold dilution) by using an ICP-MS semi-quantitative approach, as described by Catarino *et al.* (2006). The contents of Rb and Sr in Sr chromatographic fractions were also determined before isotopic measurements. Between samples or standards, the sampling system was rinsed with 2 % HNO_3 (v/v) for 90 s.

The ICP-MS instrumental parameters were optimized with the Sr isotopic standard (50 µg/L) to obtain the best precision for $^{87}Sr/^{86}Sr$ measurement and, therefore, to allow the detection of small variations in $^{87}Sr/^{86}Sr$ ratios and between samples of different origin. The influence of Sr concentration (between 10 and 50 µg/L) and instrumental parameters on precision was studied : number of replicates (between 3 and 8), dwell time (between 10 and 60 ms) and number of sweeps per reading (between 100 and 1000).

The measurement of the ^{87}Sr isotope suffers from interference by the ^{87}Rb isotope. As residual Rb can still be present in the pretreated samples (HPMW digestion and chromatographic separation), mathematical correction for both ^{87}Rb interference on ^{87}Sr (-0.385617×^{85}Rb) and ^{86}Kr interference on ^{86}Sr (1.505657×^{83}Kr) was systematically carried out using the software described above. Additionally, the influence of Rb was assessed by adding defined amounts of Rb to a 25 µg/L Sr solution and determining $^{87}Sr/^{86}Sr$ ratios.

With the purpose of determining $^{87}Sr/^{86}Sr$ accurately, the SRM 987 ($SrCO_3$) from the NIST (Gaithersburg, USA) was used as isotopic standard for the instrumental mass bias correction (certified value : 0.71034 ± 0.00026). The concentration of the isotopic standard was similar to that estimated for the soil samples (50 µg/L). Between samples or standard, the sampling system was rinsed with 2 % HNO_3 for 120 s.

Under optimized conditions, each soil sample was treated (HPMW digestion followed by chromatographic separation) and analyzed in duplicate (each analysis consisted of three replicates). Periodical calibration between samples was established in order to underscore a possible shift over time.

4. Statistical analysis

The statistical treatment of soil $^{87}Sr/^{86}Sr$ ratios was performed by one-way analysis of variance and comparison of means (Fisher LSD, 95 % level) using Statistica 7.0 software (StatSoft Inc., Tulsa, USA). In an early stage, normal distribution and homogeneity of variance were verified by Normal p-p (distribution of within-cell residuals) and Cochran C tests ($p < 0.05$), respectively.

RESULTS AND DISCUSSION

1. Strontium and rubidium separation by ion-exchange chromatography

Figure 2 presents the optimized procedure scheme for Sr and Rb ion-exchange separation. In order to overcome the lack of specificity of the resin for Sr, a pretreatment step with 30 mL of 10 % NH_3 (v/v) for resin particles conversion to NH_4^+ form was performed, followed by excess NH_3 removal with 20 mL of de-ionized water. A volume of 20 mL of 0.02 M EDTA (pH 5.5) was used to adjust the column pH (conditioning step). By applying the correct EDTA concentration, at a suitable pH for complex formation,

cations are eluted from the column (Vorster *et al.*, 2008). Soil samples, grape must and wine contain high concentrations of cations, which saturate the resin causing a loss of Sr. To avoid this problem, samples were diluted with 0.1 M EDTA (pH 7.0) to 15 mL. As cations preferentially bind to EDTA rather than to the resin, they are selectively removed from the column. The sample solution (pH 5.0) was loaded into the

Table 2 - Optimized ICP-MS instrumental parameters for the measurement of $^{87}Sr/^{86}Sr$ in wine, grape must and soil samples

Parameter	Settings
Scanning mode	peak hopping
MCA channels	1
Dwell time	30 ms (^{86}Sr) 35 ms (^{87}Sr) 20 ms (^{88}Sr)
Sweeps per reading	500
Readings per replicate	1
Replicates	3
Time per run	247 s
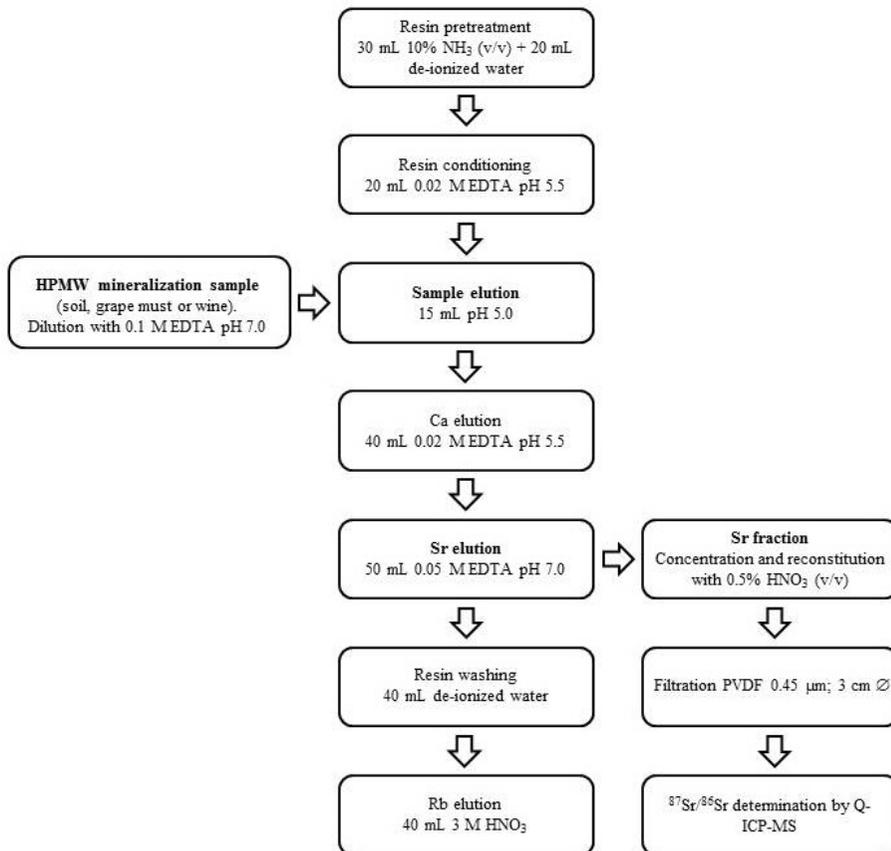
Figure 2 - Procedure scheme of strontium and rubidium ion-exchange separation. HPMW, high power microwave; Q-ICP-MS, quadrupole - inductively coupled plasma - mass spectrometry.

column, with the cations being retained by the resin. During the first elution step with 40 mL of 0.02 M pH 5.5 EDTA, Ca was removed. During the second elution step with 50 mL of 0.05 M pH 7.0 EDTA, Sr was removed. At this stage, the column was washed with 40 mL of de-ionized water to remove excess EDTA. Since EDTA does not form chelates with alkali metals, Rb remains in the column. The elution of this element was carried out with 40 mL of 3 M HNO_3, following the removal of EDTA by de-ionized water. The Sr-containing fraction was evaporated to dryness and dissolved with HNO_3 (0.5 % v/v) to 20 mL.

In order to verify the chromatographic separation efficiency, Rb and Sr concentrations in Sr-containing fractions were determined. The results indicated that the optimized ion-exchange separation method using the complexing properties of EDTA could be used reliably to separate Sr and Rb.

2. Accuracy and precision of $^{87}Sr/^{86}Sr$ measurement by ICP-MS

The ICP-MS acquisition parameters for $^{87}Sr/^{86}Sr$ measurement, under optimized conditions, are given in Table 2. As mentioned above, mass bias correction was carried out with a Sr isotopic standard solution. The correction factor (automatically calculated) generated through several studies ranged between 1.020197 and 1.025118. The instrumental stability during measurements was investigated by repeating

the analysis of a 50 µg/L Sr solution and no drift effect was observed.

It was noted that 1 µg/L of Rb in a 25 µg/L Sr solution could influence the $^{87}Sr/^{86}Sr$ ratio, resulting in values higher than in reality.

Since the application of this method to sample discrimination is based on relative differences between samples and not on absolute ratios, its success is more dependent on precision than on accuracy. The precision of $^{87}Sr/^{86}Sr$ measurement ranged between 0.1 and 0.2 % (RSD) and proved not to be related to Sr concentration (10-50 µg/L). As expected, this precision is poor as compared to that provided by TIMS, but similar or slightly better than that obtained by several authors (Almeida and Vasconcelos, 2001 ; Vorster *et al.*, 2008) using a similar mass separator device (Q-ICP-MS).

3. $^{87}Sr/^{86}Sr$ in soils from Portuguese Denominations of Origin

$^{87}Sr/^{86}Sr$ determination in soil samples from different Portuguese DO was performed using the optimized analytical protocol. Table 3 presents the total Rb and Sr concentration, Rb and Sr concentration ratio, Rb and Sr levels in Sr chromatographic fraction, and $^{87}Sr/^{86}Sr$ ratio for each vineyard. In addition, Table 4 shows the LSD test *P*-values for the $^{87}Sr/^{86}Sr$ ratio of the different vineyards and DO.

Table 3 - Soils from Portuguese DO vineyards: identification, classification, total Rb and Sr concentration, Rb/Sr ratio, Rb and Sr levels in Sr chromatographic fraction, and $^{87}Sr/^{86}Sr$ ratio

Vineyard / Portuguese DO	Soil classification	Soil			Sr fraction after ion-exchange separation		$^{87}Sr/^{86}Sr$
		Sr (ug/g)	Rb (ug/g)	Rb/Sr	Sr (ug/L)	Rb (ug/L)	
Quinta dos Carvalhais / Dão	Dystric Cambisols / Regosols from monzonitic granites	34±3	323±28	9.5	29	0.4	0.737±0.0017 c
Quinta do Sanguinhal / Óbidos	Dystric Regosols from Jurassic sandstones	50±7	176±34	3.5	50	0.5	0.714±0.0011 b
Quinta de S. Francisco / Óbidos	Eutric Regosols from Cretacic sandstones	54±3	179±9	3.3	41	0.2	0.714±0.0010 b
Vinha de Algeruz / Palmela	Eutric Regosols from Pliocene clayey sands	52±7	141±27	2.7	55	0.4	0.711±0.0003 a
	NIST SMR 987				Measured value:		0.710±0.00054
					Certified value:		0.71034±0.00026

Means followed by the same letter are not significantly different at the 0.05 level of significance

Table 4 - LSD test *P*-values for the $^{87}Sr/^{86}Sr$ ratio of the different vineyards and DO regions

Vineyard/ DO	*P*-value
Quinta dos Carvalhais / Dão *vs* Quinta do Sanguinhal / Óbidos	0.000000
Quinta dos Carvalhais / Dão *vs* Quinta de S. Francisco / Óbidos	0.000000
Quinta dos Carvalhais / Dão *vs* Vinha de Algeruz / Palmela	0.000000
Quinta do Sanguinhal / Óbidos *vs* Quinta de S. Francisco / Óbidos	0.579584
Quinta do Sanguinhal / Óbidos *vs* Vinha de Algeruz / Palmela	0.003171
Quinta de S. Francisco / Óbidos *vs* Vinha de Algeruz / Palmela	0.013087

Rb was successfully removed from Sr-containing fraction, and its residual content in the samples (less than 1.5 % of the Sr content) had a minimal contribution to the $^{87}Sr/^{86}Sr$ ratio.

The precision of Sr isotope ratio measurements, expressed as RSD (%), ranged between 0.04 and 0.23, which allowed to distinguish variations in isotopic abundance between soil samples.

Regarding the $^{87}Sr/^{86}Sr$ ratio, significant differences were found between soils from different regions. The two soils from the Óbidos region displayed the same $^{87}Sr/^{86}Sr$ ratio (0.714) and were significantly different from the soils of the Dão and Palmela regions (0.737 and 0.711, respectively). Despite being developed on different geological materials, the soils from the Óbidos DO showed identical ratios, demonstrating the suitability of $^{87}Sr/^{86}Sr$ as fingerprint for this DO.

The lowest values of $^{87}Sr/^{86}Sr$ ratio and Rb content were observed in the soil of the Palmela region, developed on the youngest parent material considered in this study. The Dão region $^{87}Sr/^{86}Sr$ ratio was distinctively higher than in the other regions and is in agreement with values reported for granitic rocks of the same region (Costa, 2006). This value is also in accordance with the results of Almeida and Vasconcelos (2004) for one soil (0.732) and wines (0.729) from the Douro DO, a mainly schistous region located in the northeastern Portugal. According to the literature, high $^{87}Sr/^{86}Sr$ values are characteristic of granitic and, therefore, older rocks (Costa, 2006).

The results show that Rb/Sr ratio is a good indicator of the $^{87}Sr/^{86}Sr$ values and level of similarity between soils of these vineyards. Considering the evolution of $^{87}Sr/^{86}Sr$ in geological systems, in general older rocks, such as granites in continental crust, with higher Rb/Sr ratios will develop higher $^{87}Sr/^{86}Sr$ ratios than younger ones with lower Rb/Sr ratios (Capo *et al.*, 1998). Dão has the highest Rb/Sr, the two vineyards from Óbidos have similar intermediate Rb/Sr, and Palmela shows the lowest Rb/Sr values. These values are in agreement with the ones obtained for $^{87}Sr/^{86}Sr$.

With the exception of the Douro region (Almeida and Vasconcelos, 2004), no reference to the Sr isotopic composition of vineyard soils from Portugal was found in the literature. In this study, $^{87}Sr/^{86}Sr$ values for three major DO regions (Dão, Óbidos and Palmela) were assigned, with the specific purpose of discriminating between vineyard soils from different regions. The $^{87}Sr/^{86}Sr$ ratio may represent a suitable fingerprint for the studied DO and may be used in conjunction with other tracers. In a previous study within the referred research program, we showed the potentialities of rare earth element (REE) patterns as fingerprint for origin authentication (Catarino *et al.*, 2011). Regarding the vineyards/DO of the present study, it was shown that REE patterns can represent a suitable fingerprint for wine origin authentication.

The developed analytical protocol for $^{87}Sr/^{86}Sr$ determination by Q-ICP-MS showed sufficient precision and accuracy to allow the detection of variations in isotopic abundance between soil samples.

The most important result of this study was the confirmation that $^{87}Sr/^{86}Sr$ ratio could be a viable tool for origin identification for these three Portuguese DO regions. In addition, the scarce knowledge of $^{87}Sr/^{86}Sr$ ratios in Portuguese vineyard soils was extended.

In this work, only the most representative lithological formations of each DO were considered. Studies using $^{87}Sr/^{86}Sr$ should be performed on the other lithological formations of these DO, in order to characterize each region, and on other Portuguese DO. Besides providing geological and pedological background, this information may be integrated in an international wine databank, together with other parameters, for use in geographical identification and authentication.

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