Evaluating Nanofiltration Effect on Wine ^{87}Sr/^{86}Sr Isotopic Ratio and the Robustness of this Geographical Fingerprint

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When aiming to use the strontium isotopic ratio ^{87}Sr/^{86}Sr for the traceability and authentication of wine, it is crucial to understand the impacts of anthropogenic factors and technological processes on this parameter. A study was developed to evaluate the effect of nanofiltration (NF), a membrane process with several applications in oenology, on the isotopic ratio ^{87}Sr/^{86}Sr of wine, testing its robustness as geographical tracer. NF assays, for dealcoholisation, were carried out at laboratory scale with model solutions and white and red wines from Portuguese DO. High rejections to strontium (97%) and tartaric acid (> 70%) and low rejection to ethanol (< 10%) at 15 bar were observed in model solutions. Wines and NF-processed wines (17 bar; 2.1 L/min) were evaluated for physicochemical composition (including phenolic composition, chromatic characteristics and polysaccharides), multi-elemental composition and ^{87}Sr/^{86}Sr ratio by Q-ICP-MS. The preferential membrane permeation of ethanol was associated with a slight increase in the quality parameters of the wine (anthocyanins and polysaccharides). High rejections to mineral elements, including heavy metals, were observed. Rejection to Sr was mostly higher than 95%. No significant differences in the ^{87}Sr/^{86}Sr of wines and corresponding permeate fractions were observed, suggesting that NF does not preclude the use of this parameter for wine traceability purposes.

INTRODUCTION

The control of wine geographic origin and of other agriculture products based on their chemical composition is currently one of the most challenging topics relating to authenticity. Soil-related markers have a primary role to play, considering that there is a relationship between the chemical composition of the wine and the composition of the provenance soil. ^{87}Sr/^{86}Sr is a well-established tool for dating and tracing the origin of rocks and minerals. Isotopes ^{84}Sr, ^{86}Sr and ^{88}Sr occur in constant relative proportions, while ^{87}Sr gradually increases in minerals due to the radioactive β-decay of the ^{87}Rb isotope. Differences in the relative abundance of ^{87}Sr vary with geological age, and consequently with geographical locations, providing a fingerprint for different rock types (Capo *et al*., 1998). Sr is absorbed by the vine in the same isotopic proportions in which they occur as available forms in the soil; the biological processes involved in vine metabolism do not significantly fractionate Sr isotopes. Thus, vine plants reflect the environment of growth: bedrock, soil and soil water (Horn *et al*., 1993; Capo *et al*., 1998).

Recently, within a research programme on strategies for wine fingerprinting, the authors confirmed ^{87}Sr/^{86}Sr as a viable tool for the traceability of Portuguese DO, where soils developed on different geological formations (Martins *et al*., 2014; Catarino *et al*., 2015).

Literature is available on the progress made since the first application of the ^{87}Sr/^{86}Sr isotopic ratio for wine traceability purposes in the 1990s, with an emphasis on the increasing number of studies developed in the last years (Horn *et al*., 1993; 1997; Almeida & Vasconcelos, 2001; Barbaste *et al*., 2002; Almeida & Vasconcelos, 2004; Vorster *et al*., 2010; Marchionni *et al*., 2013; Mercurio *et al*., 2014; Durante *et al*., 2015; Marchionni *et al*., 2016).

The use of this parameter as a geographical origin marker is based on the assumption that there is a relationship between soil, plants and wine. Therefore, the ^{87}Sr/^{86}Sr ratio should not be modified significantly during wine processing. According to Horn *et al*. (1997), fining with bentonites, de-acidification with carbonates and storage in glass showed little effects on wine ^{87}Sr/^{86}Sr ratio. Nevertheless, it is well known that the use of some technological aids, namely

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bentonite, can result in significantly higher concentrations of Sr (Catarino *et al*., 2008a).

Even though it is claimed that Sr isotopes are preserved during winemaking practices (Marchionni *et al*., 2016), the potential effect of technological processes on Sr isotopic composition is still poorly known. Bearing in mind the use of $^{87}Sr/^{86}Sr$ as a tool for unambiguously authenticating the provenance of wines, deep knowledge of the effect of oenological practices is required.

In the wine industry, the high ethanol level in wine can be a serious issue for the quality of the product and it has become an important issue for all the main producing countries (Gonçalves *et al*., 2013). To overcome this problem, the EU has permitted partial dealcoholisation of the wine by means of physical methods. Several techniques are available to the wine industry to reduce the ethanol content; among them, nanofiltration (NF) is certainly an asset not only as far as the dealcoholisation (Gonçalves & De Pinho, 2003) is concerned, but also for the versatility of the control of the mineral composition. NF is a versatile membrane process with several applications in the wine industry, where its use is increasing. In addition to wine dealcoholisation, NF can also be used for reducing the sugar content of musts, being an alternative strategy to reducing alcohol levels in wines; for volatile acidity correction; and to remove excess volatile phenols (De Pinho *et al*., 2007; Santos *et al*., 2008; OIV, 2015).

The abovementioned versatility of NF for the control of mineral composition is associated with the selective transport of ions based on their charge or the ratio between mass and charge (m/z). While NF shows only moderate rejection of monovalent salts, it has been shown to be highly effective in the removal (by rejection) of multivalent ions such as Ca^{2+} (Szöke *et al*., 2005), suggesting a similar effect on Sr^{2+}.

For dealcoholisation purposes, the NF concentrate stream is the most important fraction, since it corresponds to the low alcohol content of wine. NF membranes present low rejection (and therefore preferential permeation) of ethanol and high rejection of aromatic compounds and of macromolecules such as polyphenols and polysaccharides, which allows partial dealcoholisation of wine without significant changes in its physicochemical composition (Gonçalves & De Pinho, 2003; Catarino & Mendes, 2011).

Although NF effects on wine physicochemical characteristics have been studied, namely effects on the main analytical parameters such as alcoholic strength and total acidity, polyphenols and anthocyanins (Massot *et al*., 2008; Gonçalves *et al*., 2013), to the best of our knowledge there is no information concerning its influence on multi-elemental composition and on the $^{87}Sr/^{86}Sr$ isotopic ratio. Considering the use of the Sr isotopic ratio as a marker of geographical origin and its increasing NF application in the wine industry, the study of the influence of NF on $^{87}Sr/^{86}Sr$ is essential.

To this end, white and red wines from three Portuguese DO were treated by NF and the physicochemical composition, including main quality-related parameters (phenolic composition, polysaccharides), multi-elemental composition and $^{87}Sr/^{86}Sr$ isotopic ratio were assessed in the wines and the NF streams (initial feed, final feed and permeate).

MATERIALS AND METHODS
Wines
In this study, three white wines (WW) and three red wines (RW) from the Portuguese DO Dão, Óbidos, Palmela were used. The grape varieties (*Vitis vinifera* L.) were as follows: Fernão Pires (Óbidos WW); Calladoc, Syrah and Alicante Bouchet (Óbidos RW); Verdelho and Viosinho (Palmela WW); Castelão (Palmela RW); Verdelho (Dão WW); and Touriga Nacional (Dão RW).

White wines from the 2014 vintage were produced by application of the conventional technology and were obtained exclusively by fermentation with grape must extraction preceding alcoholic fermentation.

Red wines from the 2013 vintage 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 method of red winemaking.

Physicochemical characterisation of wines
A summary analysis of the wines, including alcoholic strength, total acidity, volatile acidity, total dry matter, density, pH, free and total sulphur dioxide, reducing substances, colour intensity and tonality parameters was carried out according to the OIV analysis methods (OIV, 2014). The occurrence of malolactic fermentation was verified in the red wines by paper ascending chromatography. Regarding total polysaccharides, the phenol-sulphuric method (colorimetric method) was used after the separation of polysaccharides by ethanol. Total anthocyanins and the total phenol index were determined as described by Somers & Evans (1977).

Multi-elemental analysis
For the multi-elemental analysis, Li, Be, Na, Mg, Al, Sc, V, Zn, Ga, As, Rb, Sr, Y, Zr, Nb, Mo, Cd, Sn, Sb, Cs, Ba, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Tl and Pb were assessed by quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS), a semi-quantitative methodology, according with the protocol described by Catarino *et al*. (2006). A Perkin-Elmer SCIEX Elan 9000 Q-ICP-MS was used, equipped with a Gilson peristaltic sample-delivery pump, a Scott-type spray chamber, a crossflow nebuliser and nickel cones. The system was controlled by software Elan – 6100 Windows NT (Version 2.4).

For the optimisation of Q-ICP-MS operative conditions, mono-element standard solutions of Be, Co and In at 1 000 mg/L (Merck) and a multi-element solution with Mg, Cu, Rh, Cd, In, Ba, Ce, Pb and U at 10 µg/L (Perkin-Elmer) were used. Wash, blank and standard solutions were prepared with ultrapure concentrated HNO_3 (Ultrex II 70% (vol.)) (J.T. Baker). Analytical calibration was established with a standard solution with 30 elements, at 10 mg/L (Perkin-Elmer). For internal standardisation, standard solutions of Rh and Re at 1 000 mg/L (Merck) were used.

$^{87}Sr/^{86}Sr$ isotopic ratio analysis
Sample treatment
Before isotopic analysis by Q-ICP-MS, an effective Rb and Sr separation was required for the accurate determination of the Sr isotope ratio due to isobaric overlap of ^{87}Rb and

^{87}Sr. Wine samples were treated by applying a three-step procedure: digestion by high pressure microwave (HPMW); chromatographic separation of Sr and Rb; and filtration of the Sr chromatographic fraction.

Wine samples were first digested by HPMW following the protocol described by Catarino *et al.* (2010). For the digestion operation, a Milestone ETHOS Plus Microwave Labstation, equipped with a Milestone HPR-1000/6m monoblock high-pressure rotor and TFM Teflon vessels, was used. On each run, a blank solution was prepared for contamination control.

In order to avoid the influence of Rb on the $^{87}Sr/^{86}Sr$ isotopic ratio, Sr and Rb chromatographic separation was carried out according to the protocol described by Martins

et al. (2014). A HIPEX Duran (12 mm internal diameter and 200 mm bed size) column was filled with cationic resin (Dowex 50W-X8/400, Sigma-Aldrich) until around 13 cm. Chromatographic separation included four stages: resin activation/pre-treatment; resin conditioning; sample preparation/dilution; and elution stages. Solutions used were as follows: HCl 32% (v/v) (Merck), EDTA Triplex III p/ analysis (Merck), NH$_3$ 25% (v/v) (Merck), and HNO$_3$ bi-distilled 65% (v/v) (Sigma-Aldrich). A summary scheme of the chromatographic separation procedure of Sr and Rb is shown in Fig. 1.

Finally, in order to avoid/minimise EDTA deposition in Q-ICP-MS equipment, elution fractions with Sr were acidified to 1% with bi-distilled nitric acid and stored at 5ºC
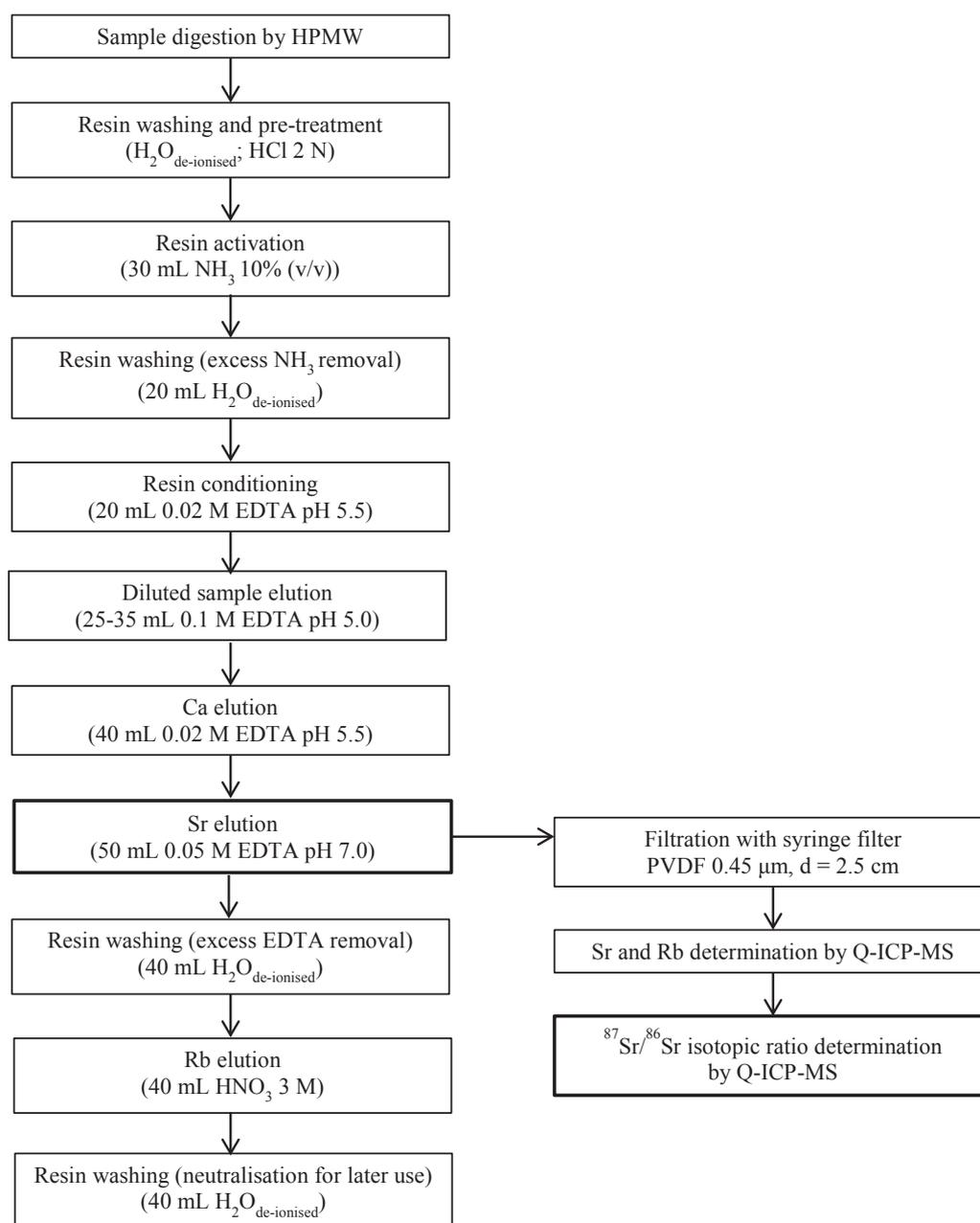

FIGURE 1

Procedure scheme of strontium and rubidium ion-exchange separation (HPMW: high power microwave; Q-ICP-MS: quadrupole - inductively coupled plasma - mass spectrometry).

for 24 h. Then, samples were filtrated using a syringe filter (Acrodisc PVDF 0.45 μm) with diameter of 25 mm.

^{87}Sr/^{86}Sr isotopic ratio determination by Q-ICP-MS

The ^{87}Sr/^{86}Sr isotopic ratio was determined by Q-ICP-MS according to the protocol described by Martins *et al.* (2014), using the aforementioned Q-ICP-MS equipment. Instrumental parameters were as follows: sweep mode – peak hopping; 1 MCA channel; dwell time of 30 ms (^{86}Sr), 35 ms (^{87}Sr) and 20 ms (^{88}Sr); 500 sweeps/reading; one reading/replicate; three replicates; time per run of 247 s.

Analytical calibration and stability control in the ^{87}Sr/^{86}Sr isotopic ratio analysis were achieved with SrCO$_3$ (50 μg/L), prepared from the certified reference material NIST SRM 987 (SrCO$_3$). Calibration was established between samples in order to underscore a possible shift over time. Stability control was done periodically. All samples were analysed in duplicate.

Since the ^{87}Rb isotope interferes in the measurement of the ^{87}Sr isotope and residual Rb can still be present in the pre-treated samples, a mathematical correction (using the equipment software) for ^{87}Rb interference in ^{87}Sr (0.385617 × ^{85}Rb) and ^{86}Kr interference in ^{86}Sr (1.505657 × ^{83}Kr) was applied (Perkin-Elmer, 2001).

The purified water (conductivity < 0.1 μS/cm) used in the preparation of the solution and for material washing was produced using a Seralpur Pro 90 CN apparatus. Only plastic material was used for the solution preparation. All the material was immersed in a HNO$_3$ 20% (v/v) bath for at least 24 h and rinsed three times with purified water.

Nanofiltration experiments

NF trials were carried out at laboratory scale in a Celfa P-28 unit for dealcoholisation, initially with model solutions (containing ethanol, tartaric acid and Sr) and later with white and red wines. The Celfa P-28 equipment, containing a feed tank, a heat exchanger, a circulating pump, a flat sheet membrane module, a potentiometer and a valve for circulation flow regulation, a manometer and a valve for pressure regulation, is shown schematically in Fig. 2.

Nanofiltration membrane preparation and characterisation

A laboratory-made membrane was prepared according to the phase inversion method and by using a casting solution of 17% cellulose acetate (Sigma-Aldrich), 30% formamide (≥ 99.5%, Sigma-Aldrich) and 53% acetone (99.7%, Labchem) (Kunst & Sourirajan, 1970). The film-casting conditions were as follows: casting solution at room temperature; solvent evaporation time of 30 s; and a gelation medium consisting of ice-cold water (0 to 3°C). The membrane prepared was annealed by immersion in a deionised water bath at 95°C for 11 min.

The membrane was compacted through the recirculation of deionised water at 18 bar for 3 h in order to avoid pressure effects on the membrane structure during the subsequent experiments. Membrane cleaning was carried out with deionised water until 90% of the initial water permeation flux was recovered, indicating that no important fouling occurred.

The membrane was characterised by hydraulic permeability at transmembrane pressures of between 2 and 6 bar and rejection coefficients for NaCl, Na$_2$SO$_4$, CaSO$_4$ and glucose at a transmembrane pressure of 6 bar and a feed recirculation flow of 1.4 L/min.

The concentration of salts in the feed, retentate and permeate was determined with a Crison GLP 32 conductometer, and glucose concentrations were obtained with a total organic carbon (TOC) Dohrmann 3300 analyser.

In order to assess rejection by the NF membrane, rejection coefficients (f) of solutes were determined using
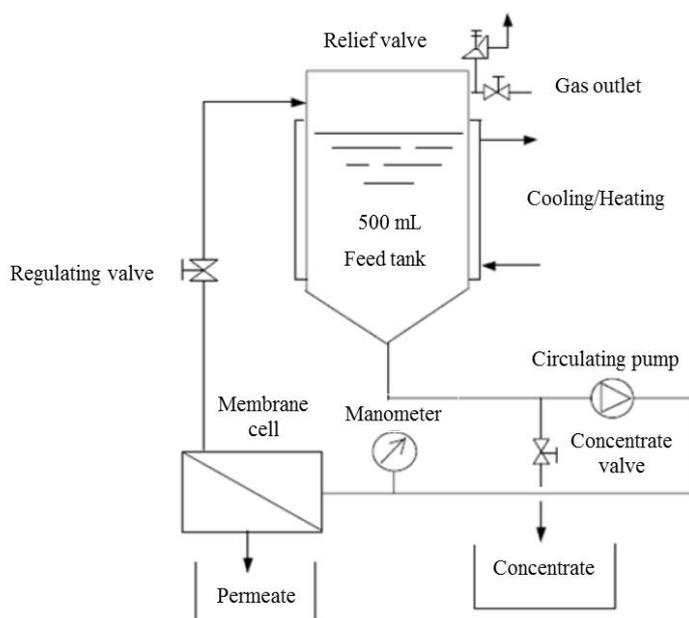

FIGURE 2
Setup of the nanofiltration unit.

equation 1, where C_f and C_p are the concentrations in the feed and permeate respectively.

$$f = (C_f - C_p) / C_f \qquad \text{(Equation 1)}$$

Nanofiltration of model solutions

To better understand the results of the dealcoholisation of the wines by NF, and in particular the Sr permeation, model solutions with 96% ethanol (vol.), tartaric acid (99.5%, Merck) and Sr (1 000 mg/L, 0.5 M HNO$_3$, Reagecon) were processed by NF.

In the first stage, ethanol rejection was evaluated through permeation assays with hydroalcoholic solutions (ethanol concentration of 8, 10, 12, 14 and 16% vol.). These assays were carried out with a feed volume of 500 mL at a transmembrane pressure of 6 bar with a feed recirculation flow rate of 1.4 L/min, and at 15 bar with a feed recirculation flow rate of 2.1 L/min.

In order to determine the rejection coefficient for ethanol, a volume of 10 mL of each NF stream (initial feed, final feed and permeate) was collected. Ethanol concentration was determined by TOC analysis.

In the second stage, in addition to ethanol, tartaric acid and Sr were included in the model solutions at their usual concentrations in wine. Three solutions with different ethanol concentrations (9, 12 and 16% vol.), 0.5 mg/L of Sr and 3 g/L of tartaric acid were permeated, after adjustment of the pH to 3.5 with a NaOH (≥ 98.5%, Sigma-Aldrich) 10 M solution. These assays were carried out at a transmembrane pressure of 15 bar and a feed recirculation flow rate of 2.1 L/min. In order to determine rejection coefficients, ethanol and tartaric acid concentrations were determined by Fourier transform infrared (FTIR), using Foss WineScan FT 120 equipment. The Sr concentration was assessed by Q-ICP-MS, according to the method previously described.

Nanofiltration of wines

The wines were treated by NF at a transmembrane pressure of 17 bar and a feed recirculation flow rate of 2.1 L/min. In order to assess the rejection of wine constituents, NF fractions were evaluated through summary analysis and total polysaccharides, total anthocyanins, total phenolic index, multi-elemental composition and $^{87}Sr/^{86}Sr$. Summary physicochemical characterisation in terms of alcoholic strength, total acidity, volatile acidity, tartaric acid, malic acid, lactic acid, total dry matter, ash, density, pH, total sulphur dioxide, reducing substances, colour intensity and tonality was performed by FTIR. Total polysaccharides, total anthocyanins, total phenol index, multi-elemental composition and $^{87}Sr/^{86}Sr$ were determined as previously described.

Statistical analysis

A one-way analysis of variance (ANOVA) was performed to evaluate the effect of the nanofiltration treatment on the $^{87}Sr/^{86}Sr$ isotopic ratios from wines, along with the respective NF fractions. Fisher's least significant difference (LSD, 95% level) test was applied to compare the different averages. In order to verify the applicability of parametric tests, normal distribution and homogeneity of variance tests were performed. All calculations were carried out using Statistica version 7 (StatSoft Inc., Tulsa, USA).

RESULTS AND DISCUSSION

Physicochemical characteristics and $^{87}Sr/^{86}Sr$ isotopic ratio of wines

The summary analysis of the wines used in the NF essays is shown in Table 1. All the parameters are in accordance with the respective normal variation range, considering the grape varieties, winemaking technology, age and region of origin (Ricardo-da-Silva *et al.*, 1992; Cabrita *et al.*, 2003).

TABLE 1

Physicochemical characteristics of wines used in the nanofiltration experiments (WW – white wine; RW – red wine).

Physicochemical characteristics	Óbidos WW	Palmela WW	Dão WW	Óbidos RW	Palmela RW	Dão RW
Alcoholic strength at 20ºC (% vol.)	13.4	12.6	13.0	12.4	10.8	12.7
Total acidity (g/L tartaric acid)	4.35	5.55	5.70	5.33	5.10	4.80
Volatile acidity (g/L acetic acid)	0.45	0.35	0.38	0.72	0.58	0.60
Total dry matter (g/L)	20.1	19.0	19.8	30.5	28.7	30.0
Density at 20ºC (g/mL)	0.9885	0.9891	0.9889	0.9937	0.9949	0.9931
pH	3.48	3.05	3.27	3.73	3.68	3.68
Free sulphur dioxide (mg/L)	29	20	40	36	27	30
Total sulphur dioxide (mg/L)	103	75	95	58	40	95
Malolactic fermentation	---	---	---	positive	positive	positive
Reducing substances (g/L)	0.7	1.7	1.6	1.7	0.2	2.1
Total polysaccharides (mg/L)	319	243	336	758	914	1125
Absorbance at 420 nm (u.a.)	0.095	0.065	0.069	---	---	---
Colour intensity (u.a)	---	---	---	10.100	8.990	8.590
Tonality	---	---	---	0.662	0.680	0.802
Total anthocyanins (mg/L)	---	---	---	301	252	236
Total phenols index (u.a.)	8	7	7	51	47	57

Regarding multi-elemental composition, the concentrations are in agreement with the literature for all the elements (Catarino *et al*., 2008b), and below the limits established by the OIV (OIV, 2014). Sr concentrations were as follows: 99.8 (0.6) µg/L (Óbidos WW), 194.1 (0.3) µg/L (Palmela WW), 239 (6) µg/L (Dão WW), 431 (10) µg/L (Óbidos RW), 256 (2) µg/L (Palmela RW) and 692 (8) µg/L (Dão RW). The highest values were found in wines from the DO Dão, which is in contrast with previous studies involving wines from the same regions, where the lowest concentrations were found in Dão samples (Catarino *et al*., 2014; 2015). These differences could possibly be explained by the inter-year variability, making evident the drawbacks of multi-elemental-based strategies for the determination of geographic origin. Among Portuguese DO, higher concentrations were recently found in wines from the Douro region (Fernandes *et al*., 2015).

Before ^{87}Sr/^{86}Sr isotopic ratio determination, the ratio of Rb and Sr concentrations was determined in the Sr chromatographic fraction in order to verify the effectiveness of the separation. According to the determined values (always < 1%), Sr and Rb separation was very successful in all the assays.

The ^{87}Sr/^{86}Sr isotopic ratios of the wines were as follows: 0.7075 ± 0.0007 (Óbidos WW), 0.7085 ± 0.0007 (Palmela WW), 0.7128 ± 0.0008 (Dão WW), 0.7093 ± 0.0006 (Óbidos RW), 0.7075 ± 0.0007 (Palmela RW) and 0.7150 ± 0.0008 (Dão RW). Significant differences were not found between wines from the same region ($p < 0.05$).

As expected, the ^{87}Sr/^{86}Sr isotopic ratios were higher for DO Dão, which is characterised by granitic soils (older). It is known that older rocks in general show higher ^{87}Sr/^{86}Sr values than younger ones for the same initial Rb/Sr ratio (Capo *et al*., 1998). Palmela and Óbidos DO, characterised by sandy and clay soils (more recent and with a lower Rb/Sr) respectively, showed lower ^{87}Sr/^{86}Sr ratios.

The observed values are in accordance with reported ratios for a wine from a Portuguese granitic area (0.71203) (Barbaste *et al*., 2002), and lower than values reported for wines from Douro (0.7130 to 0.7175) (Fernandes *et al*., 2015), where soils are mainly schistous.

Recently, in a study involving soils from Dão, Óbidos and Palmela, the highest ^{87}Sr/^{86}Sr values were found in soils from Dão (Martins *et al*., 2014). The results now find evidence of a relationship between wines and soils of origin.

In this study it was not possible to distinguish between Palmela and Óbidos wines through their ^{87}Sr/^{86}Sr values. The precision of the ^{87}Sr/^{86}Sr measurements ($0.02 \leq RSD \leq 0.27\%$) was the best possible for the Q-ICP-MS technique (RSD $\geq 0.2\%$). Most probably, the use of a more precise technique,
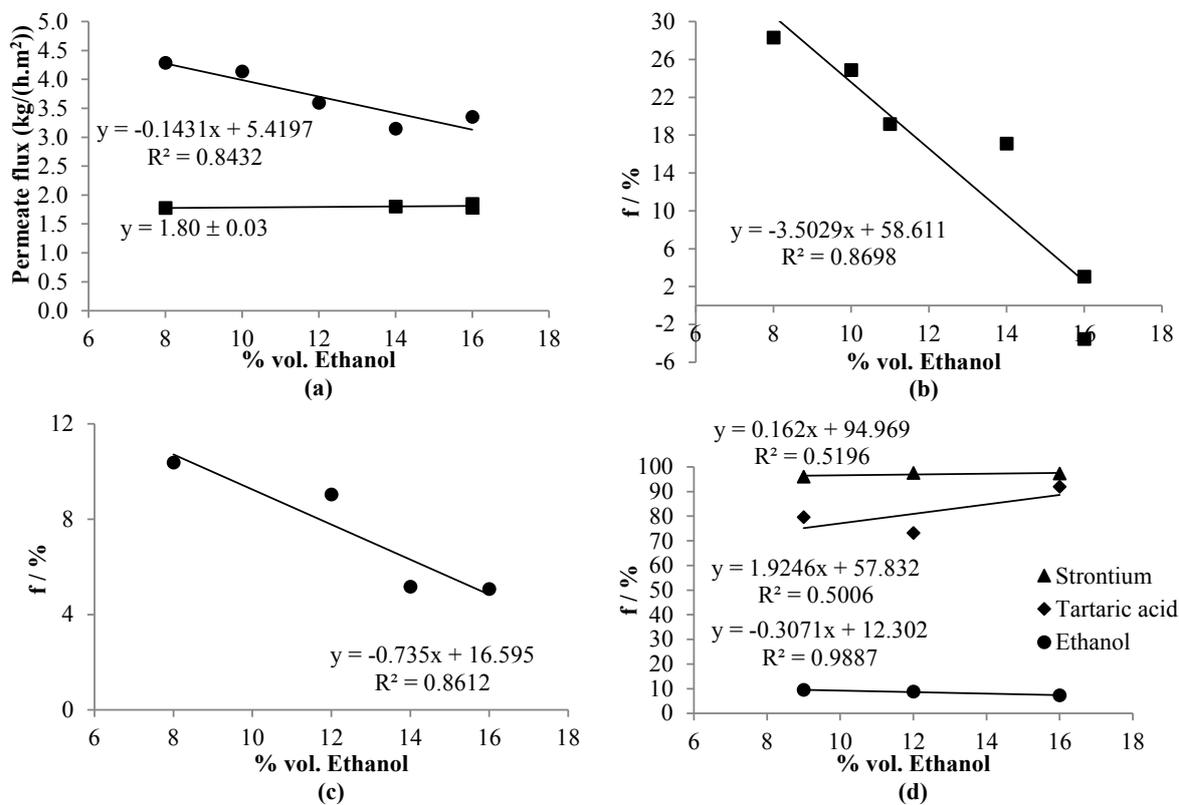
FIGURE 3

Permeation fluxes as function of ethanol concentration in feed, obtained from hydroalcoholic solution permeation at 6 bar (■) and 15 bar (●) **(a)**. Rejection coefficients (f) of ethanol as function of ethanol concentration in feed, obtained from hydroalcoholic solution permeation at 6 bar **(b)** and 15 bar **(c)**. Rejection coefficients (f) of ethanol (●), tartaric acid (♦) and strontium (▲) as function of ethanol concentration in feed, obtained from permeation of model solutions with strontium at 15 bar (quaternary model solutions: aqueous solutions with 9, 12 and 16% (vol.) of ethanol; 0.5 mg/L of strontium; 3 g/L of tartaric acid and pH of 3.5) **(d)**.

such as from a thermal ionisation mass spectrometer (RSD ≤ 0.002%), could facilitate this distinction.

Nanofiltration membrane characteristics
The experimental value of hydraulic permeability was 0.313 kg/(h.m^2.bar). The rejection coefficients observed were 90.3% for NaCl, 95.3% for Na$_2$SO$_4$, 92.8% for CaSO$_4$ and 90.3% for glucose. These permeation characteristics are associated with nanofiltration performance. The selection of this NF membrane was based not only on these features, but was supported mainly by the results obtained by De Pinho *et al*. (1988), demonstrating the preferential permeation of ethanol in the glucose-ethanol-water model system. In fact, this is the key parameter for dealcoholisation by NF.

It should be stressed that membrane interactions with feed solution are dependent on membrane material and structure; therefore, these are characteristics of this specific NF membrane.

Nanofiltration of model solutions
The unique feature of the preferential permeation of ethanol was evaluated by permeation assays with hydroalcoholic solutions (ethanol concentrations of 8, 10, 12, 14 and 16% vol.). The permeation fluxes and rejection coefficients of ethanol are displayed in Fig. 3 (a, b, c).

At 6 bar, the permeation fluxes are practically independent of the ethanol feed concentration and have an average value of 1.8 kg/(h.m^2). At 15 bar they decrease from 4.3 to 3.4 kg/(h.m^2), with an increase of ethanol concentration in the feed (from 8 to 16% vol.). At both operating pressures, of 6 and 15 bar, the rejection coefficients of ethanol decrease with the increase in the ethanol concentration of the feed. Their values are always lower than 28% and, at 15 bar, they are even much lower and decrease from 10% to 5% with the increase in ethanol concentration in the feed from 8 to 16%.

These results suggest that the permeability of the membrane to ethanol increases with the ethanol concentration of the feed. Considering the reduction in the wine alcoholic strength by NF, the efficiency of this operation is dependent on the alcoholic strength of the sample.

Regarding the assays with quaternary model solutions at 15 bar, a decrease was observed in the permeate fluxes with an increase in ethanol concentration in the feed, ranging from 4.5 to 3.1 kg/(h.m^2) (results not shown). These fluxes are very similar to the permeation fluxes of hydroalcoholic solutions at 15 bar, suggesting that neither the Sr nor tartaric acid content have some effect on them.

Rejection coefficients of ethanol, tartaric acid and Sr are shown in Fig. 3 (d). Regarding tartaric acid, an increase in the rejection coefficient (> 70%) was observed with an increase in the ethanol concentration in feed. Sr and ethanol rejections were constants with ethanol concentration in feed, corresponding to an average value for the three assays of 97.0% and 8.5% respectively. The high rejection coefficient of Sr indicates that this element is maintained in the nanofiltered wine and only a minimal amount is permeated.

Nanofiltration of wines
The NF of each wine yielded permeate fluxes very similar for the same types of wine. For white wine, these fluxes, with an average value of 2.7 kg/(h.m^2), were higher than the red wine fluxes, with an average value of 1.7 kg/(h.m^2). This was expected, as the red wines have a more complex matrix and a higher concentration of macromolecules. These high molecular compounds increase the fouling effect on the membrane due to concentration polarisation and adsorption on the membrane material, leading to lower permeate fluxes. In the published data, lower permeate fluxes were also observed during the NF of red wines due to high molecular weight compounds such as polyphenols and polysaccharides (Salgado *et al*., 2013).

TABLE 2
Physicochemical characteristics of white wine nanofiltration streams (IF – initial feed; FF – final feed; P – permeate)

NF streams	Óbidos			Palmela			Dão		
	IF	FF	P	IF	FF	P	IF	FF	P
Alcoholic strength at 20ºC (% vol.)	13.0	13.0	12.2	12.1	12.1	11.3	12.6	12.5	11.7
Total acidity (g/L tartaric acid)	3.37	3.50	0.92	4.92	5.77	0.65	5.60	4.86	0.74
Volatile acidity (g/L acetic acid)	0.35	0.36	0.20	0.26	0.29	0.18	0.29	0.28	0.18
Tartaric acid (g/L)	0.96	0.82	nd	1.20	1.49	nd	1.51	1.17	nd
Malic acid (g/L)	nd	nd	nd	1.48	1.70	nd	1.90	1.56	nd
Lactic acid (g/L)	1.01	1.08	0.46	0.09	0.09	0.16	0.08	0.16	0.16
Total dry matter (g/L)	17.2	19.0	1.8	17.1	20.5	1.2	20.7	17.9	1.5
Ash (g/L)	2.25	2.39	0.43	1.51	1.81	0.35	2.17	1.92	0.43
Density at 20ºC (g/mL)	0.9878	0.9884	0.9827	0.9889	0.9902	0.9836	0.9897	0.9887	0.9832
pH	3.23	3.25	3.12	3.06	3.06	3.09	3.22	3.22	3.13
Total sulphur dioxide (mg/L)	102	115	47	74	79	59	95	89	56
Reducing substances (g/L)	3.06	3.39	0.25	2.78	3.35	0.78	3.24	2.57	0.62
Total polysaccharides (mg/L)	343	593	nq	372	529	Nd	416	583	nq
Total phenols index (u.a.)	6	8	1	8	16	1	6	7	1

nd – not detected; nq – not quantified

The basic analytical parameters of the NF streams are shown in Tables 2 and 3. Ethanol was preferentially permeated by the membrane, as expected, corroborating the observations on model solutions and previous studies (Gonçalves *et al.*, 2013). The rejection coefficients of ethanol were the following: 6.4% (Óbidos WW); 6.2% (Palmela WW); 7.0% (Dão WW); 4.9% (Óbidos RW); 7.2% (Palmela RW); and 6.5% (Dão RW). Alcoholic strength in the initial feed was not significantly different from the final feed due to the volumes processed and the operation being in total recirculation mode. Considering NF use for wine dealcoholisation, the alcoholic strength in the final feed can be reduced further by the reintroduction of the permeated water, after separation from ethanol by reverse osmosis or distillation, for example. Furthermore, the concentration effect in nanofiltered wine could be corrected totally by the reposition to the initial volume through the addition of supplementary water, although this practice is not currently allowed, since it would imply the incorporation of exogenous water.

The rejection of tartaric, lactic, malic and acetic acids were in general high, which indicates that the wine acidic composition of nanofiltered wine is similar to that of the original wine. Density and pH values were maintained during NF. For some parameters, such as total dry matter, total acidity and reducing substances, a concentration effect was observed due to water removal. In relation to polysaccharides, an almost complete retention was verified (always ≥ 95%). Regarding colour intensity and tonality, an increase was observed in both parameters. The results relative to the total phenol index and total anthocyanins suggest a high rejection of phenolic compounds. These results confirm the high selectivity of NF and its suitability for wine dealcoholisation purposes, without important changes in the main parameters related to wine characteristics and sensory quality, namely structure, body and colour.

Multi-elemental composition

Table 4 shows the multi-elemental composition of NF streams from Dão white wine and Palmela red wine. For the other wines in this study, the effect of NF on the multi-elemental composition was similar in accordance with the type of wine (white and red), and with each element.

In general, high rejection was more evident for the elements Li (98.7%), Na (f > 93.5%), Mg (f > 99.7%), Rb (f > 96.7%) and Cs (f > 94.4%). Concentrations in the final feed were slightly higher than concentrations in the initial feed, due to the removal of water and ethanol.

The rejection coefficients obtained for Sr were 98.0% (Óbidos WW), 98.7% (Palmela WW), 99.4% (Dão WW), 98.8% (Óbidos RW), 88.7% (Palmela RW) and 99.5% (Dão RW). Since Sr is in wines in both salt forms (e.g. strontium tartrate) and combined with polysaccharides (Pellerin & O'Neill, 1998; Doco *et al.*, 2000), which are high molecular weight compounds, the high rejections obtained by the NF membrane are expected.

In the permeate the Sr concentrations were very low, with the highest value of 6.61(0.09) µg/L corresponding to red wine from Palmela. The very low transference of Sr to permeate suggests that NF does not modify the $^{87}Sr/^{86}Sr$ ratio of wine.

It should be highlighted that information was obtained on the retention of 36 elements, including several heavy metals. Bearing in mind the potential toxicity of some heavy metals (e.g. As, Cd and Pb at high concentrations), their involvement in wine oxidation-reduction phenomena (e.g. Cu and Fe) and physicochemical instability phenomena (e.g.

TABLE 3

Physicochemical characteristics of red wine nanofiltration streams (IF – initial feed; FF – final feed; P – permeate).

NF streams	Óbidos			Palmela			Dão		
	IF	FF	P	IF	FF	P	IF	FF	P
Alcoholic strength at 20ºC (% vol.)	11.9	11.9	11.3	10.6	10.6	9.9	12.4	12.4	11.6
Total acidity (g/L tartaric acid)	4.71	4.98	1.31	6.06	5.70	1.63	4.47	5.07	1.16
Volatile acidity (g/L acetic acid)	0.56	0.56	0.44	0.76	0.76	0.64	0.52	0.56	0.41
Tartaric acid (g/L)	0.87	0.83	nd	1.10	1.09	0.19	0.37	0.58	nd
Lactic acid (g/L)	1.72	1.89	0.44	2.46	2.25	0.52	1.24	1.29	0.45
Total dry matter (g/L)	27.5	30.7	2.6	28.6	25.8	2.2	28.6	33.5	2.5
Ash (g/L)	3.32	3.60	0.43	3.03	2.83	0.49	2.83	3.34	0.40
Density at 20ºC (g/mL)	0.9931	0.9943	0.9841	0.9951	0.9941	0.9857	0.9930	0.9949	0.9837
pH	3.59	3.63	3.14	3.52	3.49	3.14	3.54	3.64	3.13
Total sulphur dioxide (mg/L)	53	57	67	6	19	67	66	62	72
Reducing substances (g/L)	3.12	3.60	0.26	1.65	1.53	0.42	3.24	3.70	0.27
Total polysaccharides (mg/L)	717	977	nq	820	1161	Nd	1139	1363	nd
Colour intensity (u.a)	13.810	14.945	0.012	13.375	14.775	0.017	10.800	13.040	0.012
Tonality	0.697	0.760	21.000	0.768	0.738	27.273	0.759	0.789	20.000
Total anthocyanins (mg/L)	229	296	13	140	170	2	187	217	17
Total phenols index (u.a.)	53	62	1	46	54	2	55	66	1

nd – not detected; nq – not quantified

K, Ca, Cu and Fe), this information is crucial to guarantee that NF does not adversely affect wine quality.

Regarding the elements As, Cd and Pb, slightly higher concentrations were found in the final feed due to the concentration effect described previously. Nevertheless, it should be stressed that the final concentrations were always very low (< 1 μg/L for As and Cd; < 30 μg/L for Pb), and quite distant from the OIV maximum acceptable limits in wines of 0.2 mg/L, 0.01 mg/L and 0.15 mg/L respectively.

$^{87}Sr/^{86}Sr$ isotopic ratio

In order to verify the chromatographic separation efficiency, Rb and Sr concentrations in Sr-containing fractions were determined. The Rb and Sr concentration ratios obtained were lower than 1% for all the NF fractions, meaning that Rb and Sr chromatographic separation was very successful. Fig. 4 shows the $^{87}Sr/^{86}Sr$ ratios of the wine NF fractions.

The scale of $^{87}Sr/^{86}Sr$ ratios represents the range of values observed in natural materials referred to by Rosner (2010). During wine NF, $^{87}Sr/^{86}Sr$ ranged between 0.7080 ± 0.0008 and 0.7095 ± 0.0007 for DO Óbidos, between 0.7075 ± 0.0007 and 0.709 ± 0.000 for DO Palmela, and between 0.713 ± 0.000 and 0.715 ± 0.001 for DO Dão. The $^{87}Sr/^{86}Sr$ ratio in the final feed was not significantly different from the $^{87}Sr/^{86}Sr$ ratio in initial feed, indicating that no preferential permeation of any strontium isotopes occurred. The $^{87}Sr/^{86}Sr$ ratios in the permeate tend to be lower than in the initial feed, even though no significant differences were observed.

To the best of our knowledge, no information regarding the effect of NF on the $^{87}Sr/^{86}Sr$ isotopic ratio is available. Nevertheless, relative to the influence of NF on other isotopes, high rejections were reported. Regarding the removal of $^{226}Ra^{2+}$, $^{228}Ra^{2+}$, UO^{2+}_2, $UO_2(CO_3)_2^{2-}$, $UO_2(CO_3)_3^{4-}$ and ^{222}Rn from contaminated wastewater by NF, rejections of 99% of all isotopes were verified (Mohammad *et al.*, 2014). According to published results, the rejection of heavy isotopes is high, both in ionic and complex forms, being those forms of Sr occurring in wine, namely as strontium tartrate, and in organic complexes, namely as rhamnogalacturonans II, as referred to previously.

Previously, the potential influence of some technological processes, including cold-soak, on-skin fermentation, malolactic fermentation and filtration, on the $^{87}Sr/^{86}Sr$ isotopic ratio of wine was studied and no significant effects were verified (Almeida & Vasconcelos, 2004; Vinciguerra *et al.*, 2015; Marchionni *et al.*, 2016). To the best of our knowledge, this work is the first to study the possible modification of the Sr isotopic composition of wine induced by NF treatment, with a significantly increasing use in oenology. Our results demonstrate the robustness of the $^{87}Sr/^{86}Sr$ ratio in the NF process, confirming $^{87}Sr/^{86}Sr$ as a technologically advanced tool for assessing the authenticity and geographic origin of wine.

CONCLUSIONS

The results show preferential NF permeation of ethanol and high rejection of the main quality-related compounds of wine (e.g. phenolic compounds and polysaccharides), confirming that NF is an asset for wine dealcoholisation without adversely affecting wine quality.

During wine processing by NF, the high rejection of mineral elements, including heavy metals, was verified. Bearing in mind the potential toxicity of some heavy metals, and their involvement in oxidation-reduction phenomena and in physicochemical instability phenomena, this information is of major relevance and also new.

In the experimental conditions of this study, no significant differences were observed in $^{87}Sr/^{86}Sr$ isotopic ratio during the NF process (for dealcoholisation), indicating that the application of NF in the wine industry is not a limiting factor for the use of $^{87}Sr/^{86}Sr$ as marker of geographical origin and confirming the robustness of this marker. However, it is recommended that more information should be collected on the effects of NF in the $^{87}Sr/^{86}Sr$ isotopic ratio under different experimental conditions, namely with other NF membranes
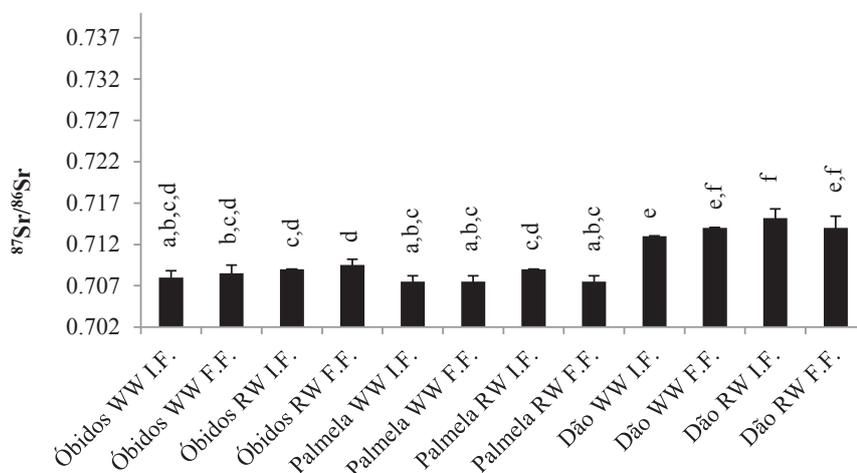

FIGURE 4

$^{87}Sr/^{86}Sr$ isotopic ratio of wine nanofiltration streams (WW – white wine; RW – red wine; IF – initial feed; FF – final feed). Values correspond to the average of two independent replicates and the respective standard deviation. Values associated with the same letter are not significantly different at the 0.05 level of significance.

TABLE 4
Multi-elemental composition of nanofiltration streams from DO Dão white wine and DO Palmela red wine. Results are expressed in µg/L, corresponding to the average of duplicate analysis and respective standard deviation.

Element	Dão WW			Palmela RW		
	initial feed	final feed	permeate	initial feed	final feed	permeate
Li	1.88 (0.05)	2.25 (0.06)	0.005 (0.001)	0.76 (0.02)	0.878 (0.007)	nd
Be	0.13 (0.05)	0.15 (0.02)	nd	0.027 (0.001)	0.05 (0.02)	0.020 (0.008)
Na	4 067 (154)	4 829 (146)	71 (3)	26 680 (484)	30 121 (328)	1 730 (70)
Mg	67 454 (2 492)	79 862 (3350)	105 (1)	89 276 (3387)	101 454 (1482)	300 (17)
Al	45.6 (0.3)	55 (2)	26.6 (0.3)	192 (7)	217 (2)	41 (2)
Sc	nd	1.4 (0.4)	0.02 (0.02)	1.33 (0.07)	0.9 (0.4)	nd
V	0.083 (0.003)	0.511 (0.008)	0.077 (0.006)	0.164 (0.004)	1.405 (0.005)	0.23 (0.03)
Zn	-	-	-	1153 (60)	1352 (1)	62237
Ga	0.55 (0.05)	0.67 (0.01)	0.026 (0.001)	0.55 (0.02)	0.74 (0.02)	0.037 (0.003)
As	0.25 (0.02)	0.34 (0.01)	0.919 (0.003)	0.35 (0.03)	0.63 (0.02)	1.49 (0.04)
Rb	891 (32)	1042 (30)	16.89 (0.02)	473 (17)	506 (4)	11.6 (0.3)
Sr	49 (1)	63.2 (0.6)	0.29 (0.01)	58 (2)	89.9 (0.6)	6.61 (0.09)
Y	0.0670 (0.0008)	0.080 (0.006)	0.024 (0.002)	0.063 (0.005)	0.077 (0.002)	0.10 (0.01)
Zr	0.00015 (0.00001)	0.000294 (0.000001)	0.00032 (0.00003)	0.00020 (0.00004)	0.00062 (0.00007)	0.000108 (0.000003)
Nb	0.000035 (0.000005)	0.00041 (0.00001)	0.000012 (0.000001)	0.000057 (0.000008)	0.00099 (0.00003)	0.000023 (0.000007)
Mo	0.00150 (0.00006)	0.0165 (0.0004)	0.00095 (0.00006)	0.00177 (0.00004)	0.0343 (0.0002)	0.0052 (0.0003)
Cd	0.0358 (0.0009)	0.07 (0.01)	0.67 (0.04)	0.055 (0.008)	0.01 (0.02)	2.7 (0.1)
Sn	0.33 (0.07)	3.17 (0.05)	20.2 (0.3)	1.55 (0.07)	17.5 (0.5)	6.8 (0.2)
Sb	0.060 (0.002)	0.069 (0.008)	2.046 (0.002)	0.07 (0.01)	0.108 (0.005)	3.0 (0.2)
Cs	5.74 (0.08)	6.76 (0.07)	0.114 (0.003)	1.33 (0.06)	1.50 (0.02)	0.036 (0.005)
Ba	29 (5)	31.1 (0.6)	7.76 (0.05)	21 (1)	27.51 (0.04)	50.0 (0.3)
Ce	0.075 (0.002)	0.088 (0.005)	0.060 (0.003)	0.069 (0.002)	0.17 (0.01)	0.13 (0.02)
Pr	0.007 (0.003)	0.010 (0.004)	0.0062 (0.0008)	0.0081 (0.0004)	0.016 (0.006)	0.013 (0.003)
Nd	0.026 (0.002)	0.026 (0.001)	0.022 (0.003)	0.02 (0.01)	0.0574 (0.0006)	0.050 (0.002)
Sm	0.01 (0.01)	nq	nq	nq	0.005 (0.007)	0.007 (0.002)
Eu	0.012 (0.003)	0.013 (0.002)	0.006 (0.002)	0.0118 (0.0001)	0.013 (0.003)	0.023 (0.009)
Gd	nq	0.006 (0.002)	nq	0.006 (0.001)	0.0083 (0.0006)	0.009 (0.002)
Tb	nq	0.008 (0.005)	nq	nq	nq	nq
Dy	0.009 (0.001)	0.007 (0.003)	nq	0.0085 (0.0002)	0.0077 (0.0009)	0.0100 (0.0002)
Ho	0.00299 (0.00003)	nq	nq	nq	0.0029 (0.0001)	0.003 (0.001)
Er	0.008 (0.002)	0.007 (0.005)	nq	nq	0.005 (0.003)	0.004 (0.005)

and on an industrial scale. With a view to the authentication of wine in terms of geographical origin, this study should be expanded to other technologies and oenological practices.

In more general terms, the present study constitutes a significant contribution to the evaluation of the influence of oenological practices on the $^{87}Sr/^{86}Sr$ isotopic ratio and its use for the evaluation of geographic provenance and authenticity.

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TABLE 4 (CONTINUED)

Element	Dão WW			Palmela RW		
	initial feed	final feed	permeate	initial feed	final feed	permeate
Tm	nq	0.003 (0.001)	nq	nq	0.0028 (0.0003)	nq
Yb	0.011 (0.004)	0.011 (0.003)	nq	nq	0.005 (0.005)	nq
Lu	0.004 (0.001)	0.00352 (0.00006)	nq	nq	0.0032 (0.0006)	nq
Tl	0.189 (0.002)	0.215 (0.003)	0.019 (0.002)	0.13 (0.01)	0.133 (0.002)	0.031 (0.009)
Pb	2.1 (0.2)	3.5 (0.3)	nq	8.5 (0.4)	21.7 (0.5)	nq

nd – not detected; nq – not quantified

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