


Effect of Wood Aging on Wine Mineral Composition and $^{87}\text{Sr}/^{86}\text{Sr}$ Isotopic Ratio

Ayse D. Kaya,[†] Raúl Bruno de Sousa,[†] António S. Curvelo-Garcia,[§] Jorge M. Ricardo-da-Silva,[†] and Sofia Catarino^{*,†,§,#} 

[†]LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

[§]Instituto Nacional de Investigação Agrária e Veterinária, 2565-191 Dois Portos, Portugal

[#]CEFEMA, Instituto Superior Técnico, Universidade de Lisboa, Avenida Rovisco Pais 1, 1049-001 Lisboa, Portugal

ABSTRACT: The evolution of mineral composition and wine strontium isotopic ratio $^{87}\text{Sr}/^{86}\text{Sr}$ (Sr IR) during wood aging were investigated. A red wine was aged in stainless steel tanks with French oak staves (*Quercus sessiliflora* Salisb.), with three industrial scale replicates. Sampling was carried out after 30, 60, and 90 days of aging, and the wines were evaluated in terms of general analysis, phenolic composition, total polysaccharides, multielement composition, and Sr IR. Li, Be, Mg, Al, Sc, Ti, V, Mn, Co, Ni, Cu, Zn, Ga, Ge, As, Rb, Sr, Y, Zr, Mo, Sb, Cs, Ba, Pr, Nd, Sm, Eu, Dy, Ho, Er, Yb, Lu, Tl, and Pb elements and $^{87}\text{Sr}/^{86}\text{Sr}$ were determined by quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS) and Na, K, Ca, and Fe by flame atomic absorption spectrometry (FAAS). Two-way ANOVA was applied to assess wood aging and time effect on Sr IR and mineral composition. Wood aging resulted in significantly higher concentrations of Mg, V, Co, Ni, and Sr. At the end of the aging period, wine exhibited statistically identical Sr IR compared to control. Study suggests that wood aging does not affect $^{87}\text{Sr}/^{86}\text{Sr}$, not precluding the use of this parameter for wine traceability purposes.

KEYWORDS: wine, authenticity, wood aging, strontium isotopic ratio $^{87}\text{Sr}/^{86}\text{Sr}$, mineral composition

INTRODUCTION

A large number of studies indicate that mineral composition and concentration of wines can give information about their geological origin and that multielemental analyses can determine the wine fingerprint.^{1–3} By means of statistical techniques, discrimination of elements according to the products' geographical area where they are grown is obtained. However, mineral compositions of rocks and soils change from one region to another and also with different agricultural applications, such as fungicide, pesticide, and fertilization applications, winemaking practices, and environmental conditions.^{4–8}

Heavy elements' isotopic ratios can give information about the plant's origin. To establish a reliable tool for the determination of the geographical origin of wines, the selected abundance ratio of the element must not be altered by the agricultural and winemaking practices through the chain from vineyard to bottle; in other words, the wines must reflect the same ratios as in the soil or in the rocks where the vines are grown.² It is also important to take into consideration anthropogenic human factors such as irrigation water, pollution, and fertilizers that can contribute as mineral sources to the plants.⁹

Analytical techniques such as ICP-MS and thermal ionization mass spectrometry (TIMS) can quantify heavy isotopes by spectroscopic methods. Recently, a high number of studies have been published with especially the ICP-MS technique for characterizing the mineral composition of wines and correlating them with their geographical origin.^{2,4–14}

Moreover, Sr IR values of the substratum and affiliated grapes and wines exhibit correlated results with each other.^{3,15}

Being the element of interest of this study, strontium occurs in nature as four isotopes. Three of them are stable (^{84}Sr , ^{86}Sr , and ^{88}Sr) with constant values, which means they are nonradiogenic, whereas ^{87}Sr is radiogenic and is derived from radioactive β -decay of ^{87}Rb and absolute amounts of the stable ^{86}Sr remain constant.⁸ Hence, Sr IR of a rock, depending on its age and content of Rb, gives particular data in different geological regions. Horn's team stated that soils of respective vineyard regions have different $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. The elements, which are absorbed by plants, maintain the same isotopic ratios as in the soil and rocks. The team used the TIMS method for Sr isotopic ratio measurement. This technique gives both accurate and precise results.⁸ However, being expensive and an inconvenient method with long sample preparation, the technique is less favored and unfeasible. Thanks to its fast and simpler sample preparation and low sample analysis cost, the ICP-MS method has recently been used for isotope ratio determination.

Lately, there have been many research studies that focus on wine authenticity tools, especially by using $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, associating the wines with their vineyards. Many studies suggest Sr IR as one of the most reliable fingerprint tools when it comes to relating the plants to the soil.^{3,15–22}

Almeida and Vasconcelos stated that although winemaking processes and chemical applications in the vineyard change the elemental composition of must and wine, a strong correlation

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in terms of Sr IR between wine and grape juice is still found. A similar study also reports that the analytical results of red and white wines did not show significant differences between enological food chains.¹⁸ Sr isotopic values of red and white wines were not affected by the addition of fining agents (yeast and bentonite) during the winemaking process, proving the close relationship with the vineyard. One recent study by Durante et al.²⁰ that shed light on the use of Sr IR as geographical tracer investigated the cellar practices that used different additives, such as clarification or deacidification agents as well as concentrated musts. The objective in the study was to see whether the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of wine is affected by these practices. Because of the constancy of Sr IR from the soil to the plant and eventually into the wine, samples from grape juice, intermediate products, and wine were taken at each step. In the end, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio was not altered and was suggested to be a powerful tool to discriminate wines on the basis of their region.

A very recent study from Moreira et al. investigated also the effect of nanofiltration (NF) on the Sr IR. It was confirmed that there was no significance on $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of wines.²³

In addition to Sr IR data, the use of combined analytical parameters can provide increasingly robust results for the identification of geographical origin to have complementary information for authentication purpose of wine by means of the relationship between its chemical compositions and the territory where the grapes grow.²⁴ For instance, $^{87}\text{Sr}/^{86}\text{Sr}$ values along with Mg concentrations were the best discriminator minerals of wine origin.²⁵

One study in Australia also justifies the robustness of the strontium isotopic ratio method to differentiate between Australian wines and non-Australian wines. Nevertheless, overlap of the strontium isotopic ratio from different countries was observed. This suggests that the use of only one fingerprinting method may not be sufficient.¹⁴

As commonly described in the literature, aging in wood highly contributes to improved sensory characteristics of wine by means of compounds extracted from the oak into the wine. These extracted compounds are dependent upon wine composition, oak wood composition, and the time that the wine is in contact with the wood.²⁶ Oxidation, copigmentation, polymerization, and condensation phenomena occur during wine and oak contact time that ensure the wine's stability and improve the wine's taste by reducing its astringency.²⁷

It is emphasized by many authors that the technological processes and contact of wine with vineyard equipment can increase metal concentrations during maturation and aging.^{5,6,28–31} Despite several studies indicating that the $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratio remains stable after winemaking processes, the literature lacks information on the influence of wood aging on mineral composition and wine Sr IR. The main aim of this study was to uncover the impact of wood aging by oak staves on strontium composition whether they change the Sr composition or $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratio during aging of red wines and shed some light also on the metal extraction from wood to wine.

Therefore, a red wine was aged in stainless steel vats (industrial scale) with oak wood (*Quercus sessiliflora* Salisb.) staves, in triplicate. The wines were sampled once at time zero and after 30, 60, and 90 days of aging and evaluated in terms of general analysis, total polysaccharides, total anthocyanins, total phenols index, multielement composition, and $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratio.

MATERIALS AND METHODS

Wines and Wood Material. A red wine of Castelão grape variety (*Vitis vinifera* L.) from the 2014 vintage from a winery in the Península de Setúbal region of Palmela DO was aged with French oak (*Quercus sessiliflora* Salisb.) staves in stainless steel vats with the volume of 34,000 L during 90 days. The experiment was carried out in industrial scale in the cellars of the winery. The wines did not suffer any stabilization treatment before aging in wood.

The physical–chemical characteristics of wine before starting aging were as follows: alcoholic strength, 13.6% vol; total acidity, 5.40 g/L (expressed in tartaric acid); volatile acidity, 0.7 g/L (expressed in acetic acid); pH 3.62; free sulfur dioxide, 14 mg/L; total sulfur dioxide, 45 mg/L, with completed malolactic fermentation.

Experimental Conditions/Experimental Design. To three stainless steel vats (34,000 L) were added medium-plus toasted (<200 °C) oak staves in dimensions of 91 cm × 6.4 cm × 0.95 cm made from French oak (*Q. sessiliflora* Salisb.) in February 2016. Typical drying time of oak wood was 18–24 months. One oak piece was added per 40 L of wine with the surface to volume ratio of 33.75 cm²/L. Micro-oxygenation was applied with the amount of 3 mg O₂/L per month. Storage temperature of tanks was close to 18 °C. The experiment period of 90 days is representative of that applied in industry for wine aging with oak wood staves and micro-oxygenation. Sampling of the wines was performed each month. It is worth noting that stave dimensions and micro-oxygenation amount were decided by the winery according to their experiences. The experiment was carried out as one control vat, without oak staves, which is designated “control” and three identical vats (triplicates) with oak staves that are designated “wood”.

General Physical and Chemical Analysis. Physical and chemical analysis and determinations were carried out by using International Organization of Vine and Wine (OIV) analytical methods.³² Alcoholic strength was evaluated by distillation and determination by hydrometry; free and total SO₂ by titration with iodine; volatile acidity by distillation followed by acid–base titrimetry; density by aerometry; total dry extract by calculation; pH value by potentiometry; total acidity by acid–base titrimetry; malolactic fermentation verification by paper ascending chromatography, reducing substances, and chromatic characteristics; and total anthocyanins and total phenolics index by spectrophotometry and ash determination. Total polysaccharides content in wine was determined by an adopted method that was established by Segarra et al.³³ In this method, settling the wine samples that are diluted with ethanol (96% vol) at about 12–14 h to eliminate the phenolic and other compounds and then washing the precipitation were important steps for the determination. All of the analyses were carried out in duplicate, with the exception of total polysaccharides performed in triplicate, due to the low precision of the method.

Phenolic Composition and Chromatic Characteristics. The chromatic characteristics of wines (tonality and color intensity) were measured within the framework of OIV standards.³² Total phenol and total anthocyanin concentrations were assessed according to the methods established by Somers and Evans: the first by measuring the absorbance at 280 nm and correcting for absorbing nonphenolics and the second on the basis of their responses to pH change and sulfur dioxide addition.³⁴

Mineral Composition. The wine samples were analyzed in terms of their Na, K, Ca, and Fe contents by flame atomic absorption spectrophotometry methods described by OIV.³² A PerkinElmer (Norwalk, CT, USA) Analyst 100, equipped with an air–acetylene burner and appropriate hollow cathode lamps, was used.

The following elements were determined by quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS) semiquantitative methodology described in the study of Catarino et al.: Li, Be, Mg, Al, Sc, Ti, V, Mn, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Y, Sb, Cs, Ba, Pr, Nd, Eu, Dy, Ho, Er, Yb, Lu, Tl, and Pb.³⁵ A PerkinElmer SCIEX Elan 9000 was utilized with a Gilson pump and a Scott-type spray chamber, a crossflow nebulizer, and nickel cones.

To optimize operational conditions, monoelement standard solutions of Be, Co, and in 1000 mg/L (Merck) and a multielement

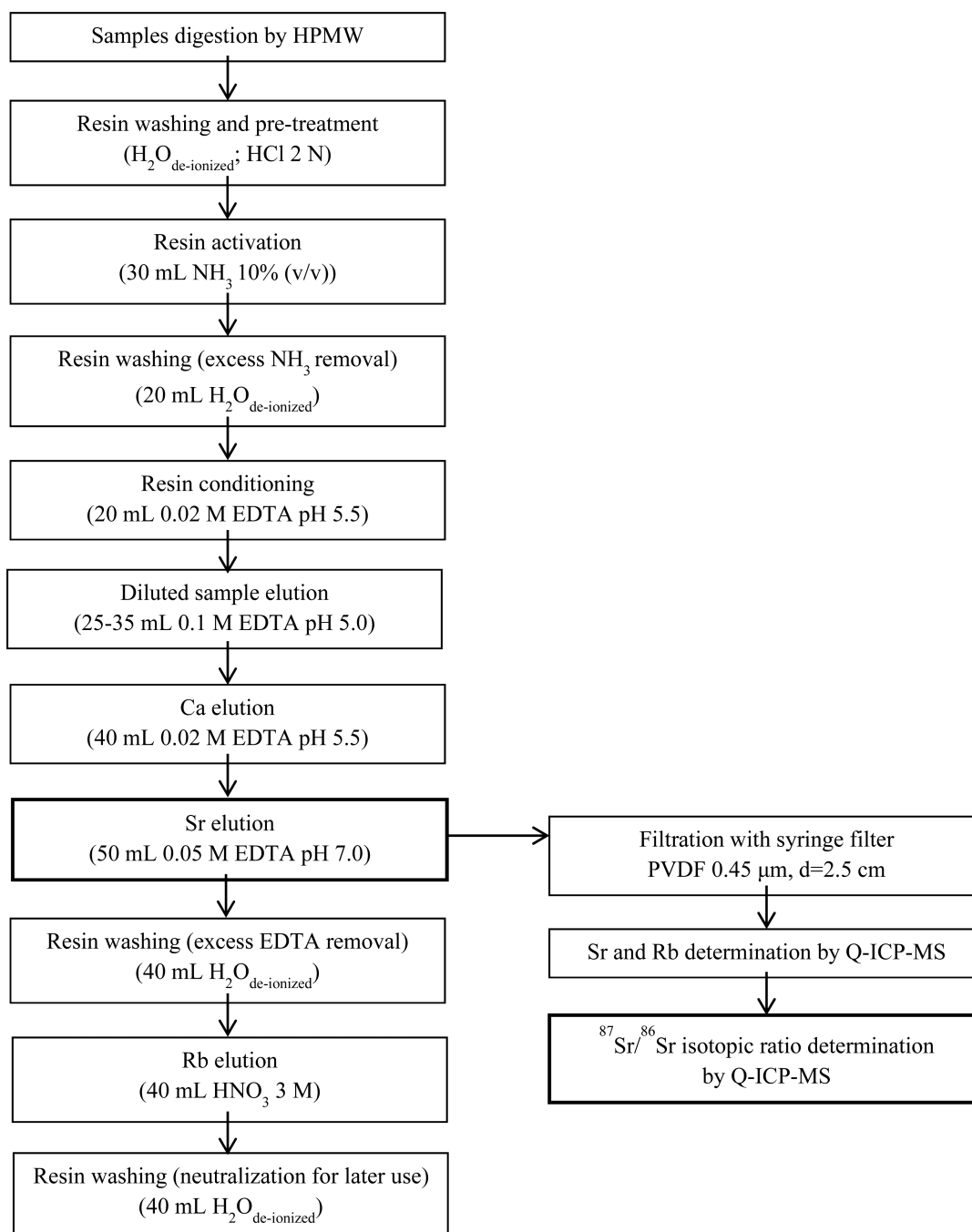


Figure 1. Procedure scheme of strontium and rubidium ion-exchange separation. HPMW, high-power microwave; Q-ICP-MS, quadrupole-inductively coupled plasma–mass spectrometry.

solution with Mg, Cu, Rh, Cd, In, Ba, Ce, Pb, and U 10 $\mu\text{g/L}$ (PerkinElmer) were used. Wash, blank, and standard solutions were prepared with ultrapure concentrated HNO_3 Ultrex II 70% (v/v) (J. T. Baker). Analytical calibration was established with a standard solution with 30 elements, 10 mg/L (PerkinElmer). Standard solutions of Rh and Re 1000 mg/L (Merck) were used for internal standardization.

Strontium Isotopic Ratio Analysis. Sample Preparation. Prior to $^{87}\text{Sr}/^{86}\text{Sr}$ determination by Q-ICP-MS, the following three main analytical steps in sequence were performed as described by Martins et al.: 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).³

(1) For elimination of organic substances to prevent any interference during chromatographic separation, the wine was digested by HPMW (Milestone ETHOS Plus Microwave Labstation).³⁶

(2) Separation of Sr from Rb is an essential step for correct determination due to isobaric overlapping ^{87}Rb and ^{87}Sr . An HIPEX Duran column was filled 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.³ Separation was done in duplicates.

(3) Sr and Rb total content and $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratio were measured by the ICP-MS equipment previously described. After the first two steps, the Sr-containing fraction was used for Q-ICP-MS analysis. Determination of Sr and Rb total contents in Sr-containing fractions prior to the isotopic measurement is important to keep the Rb concentration <1% of the Sr content in the Sr fraction. SRM 987 (SrCO_3) from the National Institute of Standards and Technology (NIST) was used as an isotopic reference material for correction of

Table 1. Effect of Wood Aging and Time on Physical–Chemical Characteristics of Wine^a

physical–chemical characteristic	wood aging			time			time × wood interaction
	wood effect	control	wood	time effect	T-0	T-90	
density (20 °C) (g/mL)	ns	0.9919 (0.0002)	0.9918 (0.0001)	ns	0.9919 (0.0002)	0.9919 (0.0002)	ns
alcoholic strength (% vol)	ns	13.5 (0.1)	13.5 (0.1)	ns	13.5 (0.1)	13.5 (0.0)	ns
pH	ns	3.61 (0.01)	3.61 (0.01)	ns	3.62 (0.00)	3.60 (0.01)	ns
ash (g/L)	ns	3.24 (0.01)	3.11 (0.25)	ns	3.31 (0.13)	2.98 (0.16)	ns
total SO ₂ (mg/L)	ns	56 (13)	56 (7)	***	48 (5) a	63 (3) b	*
free SO ₂ (mg/L)	ns	17 (3)	17 (2)	***	15 (2) a	18 (2) b	ns
volatile acidity (g/L)	ns	0.7 (0.0)	0.7 (0.0)	ns	0.7 (0.0)	0.7 (0.0)	ns
total acidity (g/L)	ns	5.1 (0.2)	5.3 (0.2)	ns	5.3 (0.2)	5.1 (0.2)	ns
total dry matter (g/L)	ns	28.6 (0.2)	28.8 (0.6)	ns	29.1 (0.6)	28.4 (0.0)	ns
reducing substances (g/L)	ns	2.6 (0.3)	2.6 (0.3)	*	2.3 (0.0)	2.9 (0.1)	ns

^aControl, wine without wood staves; wood, wine with wood staves; T-0, time zero; T-90, 90 days. With regard to the wood effect and for each analytical parameter, control and wood results are based on average values of the corresponding levels of time (T-0 and T-90). For the time effect and for each analytical parameter, T-0 and T-90 results are based on average values of control and wood. Relative standard deviations are presented in parentheses. Means followed by the same letter are not significantly different at 0.05* or 0.001*** level of significance; ns, without significant difference.

Table 2. Effect of Wood Aging and Time on Total Polysaccharides, Phenolic Composition, and Chromatic Characteristics^a

	wood aging			time			time × wood interaction
	wood effect	control	wood	time effect	T-0	T-90	
total polysaccharides	ns	437 (83)	416 (77)	***	350 (26) a	493 (28) b	ns
total phenolics index	*	53 (2) a	55 (2) b	ns	54 (1)	55 (2)	***
total anthocyanins	ns	320 (32)	303 (36)	***	278 (23) a	337 (12) b	ns
color intensity	ns	10.5 (0.6)	10.7 (0.5)	***	11.1 (0.1) b	10.2 (0.3) a	ns
tonality	ns	0.771 (0.012)	0.779 (0.022)	***	0.760 (0.002) a	0.795 (0.013) b	*

^aControl, wine without wood staves; wood, wine with wood staves; T-0, time zero; T-90, 90 days. With regard to the wood effect and for each analytical parameter, control and wood results are based on average values of the corresponding levels of time (T-0 and T-90). For the time effect and for each analytical parameter, T-0 and T-90 results are based on average values of control and wood. Relative standard deviations are presented in parentheses. Means followed by the same letter are not significantly different at 0.05* or 0.001*** level of significance; ns, without significant difference.

mass bias phenomenon.³ Analytical steps for Sr and Rb separation and isotopic ratio determination are summarized in Figure 1.

⁸⁷Sr/⁸⁶Sr Isotopic Ratio Determination. After separation of Rb and Sr by ion-exchange chromatography, analytical determinations of Rb and Sr concentration and ⁸⁷Sr/⁸⁶Sr were carried out by ICP-MS using the PerkinElmer SCIEX 9000 ICP-MS equipment mentioned before. This is illustrated in Figure 1. The detailed analytical protocol is in the study of Martins et al.

Statistical Analysis. The two-way analysis of variance (ANOVA) was performed to evaluate the effects of wood aging and aging time on the studied wines. Significant differences were compared with post hoc LSD (Tukey's test) by using Statistica 7 software (StatSoft, Inc. 2004). *p* values <0.05 were considered to be significant (*p* < 0.05).

RESULTS AND DISCUSSION

General Physical and Chemical Analysis. Results of the physical–chemical analyses of the wines are listed in Table 1. For each analytical parameter control results are based on average values of the analytical duplicates, whereas wood results are based on average values of three experimental replicates and respective analytical duplicates. The values from each month's sampling are presented, and they are in the usual range of variation for each parameter in wines.³⁷ Castelão wine exhibits the following physical and chemical characteristics of the wine at time zero: 13.6% vol alcoholic strength, 0.9921 g/mL density, 3.62 pH, 3.2 g/L ash, 45 mg/L total SO₂, 14 mg/L free SO₂, 0.7 g/L volatile acidity, 5.4 g/L total acidity, 30 g/L total dry matter, and 2.4 g/L reducing substances. As seen in Table 1, there is no significant effect of wood aging and time on the

majority of parameters. Exceptionally, total SO₂ and free SO₂ concentrations significantly changed, which might be explained by SO₂ addition to the wines. Statistical significance was found also on reducing substances. However, it is considered to be a slight increase in terms of wine parameters.

Total Polysaccharides. Total polysaccharide concentrations at the initial state and after 90 days are given in Table 2. On the basis of the results obtained by application of this analytical method, wood aging did not have a significant impact on total polysaccharides. The wood is known to have a high amount of cellulose, its main polysaccharide, and hemicellulose, besides lignin. Pectins, which also represent a large proportion of polysaccharides in wood and pulp fibers, and some minor polysaccharides are also present.³⁸ Hence, the potential release of polysaccharides from wood to wine should be considered. However, as seen in Table 2, wood did not have a significant impact, which might be explained by the short period of time of aging and by the low sensitivity of the analytical method.

On the other hand, there is a significant increase of total polysaccharides over time. This increase might be also due to the addition of any enological product that has polysaccharide content, even though to our knowledge there was no addition of any additives.

Phenolic Composition and Chromatic Characteristics. *Total Phenolics Index (TPI).* The phenolic composition of wines widely varies with grape variety, geographical origin, vintage (in straight relationship with climatic year), and anthropogenic factor.³⁷ In terms of the TPI, the wines show

Table 3. Effect of Wood Aging and Time on Major Element Composition (mg/L)^a

element	wood aging		time				
	wood effect	control	wood	time effect	T-0	T-90	time × wood interaction
Na	ns	30 (1)	30.4 (0.2)	***	30.4 (0.3) b	29.9 (0.9) a	***
Mg	*	56 (1) a	58 (2) b	ns	57 (1)	59 (3)	ns
K	ns	1064 (97)	1102 (37)	***	1135 (11) b	1050 (50) a	*
Ca	ns	56 (1)	54.4 (0.1)	***	56 (2) b	53 (1) a	ns

^aControl, wine without wood staves; wood, wine with wood staves; T-0, time zero, T-90, 90 days. With regard to the wood effect and for each analytical parameter, control and wood results are based on average values of the corresponding levels of time (T-0 and T-90). For the time effect and for each analytical parameter, T-0 and T-90 results are based on average values of control and wood. Relative standard deviations are presented in parentheses. Means followed by the same letter are not significantly different at 0.05* or 0.001*** level of significance; ns, without significant difference.

relatively higher values (54.3 at initial time) than wines studied by Dallas, produced with Castelão grapes from the Ribatejo region with different added amounts of sulfur dioxide (TPI from 29.0 to 35.5).³⁹ On the other hand, Spranger et al. reported a study about the effect of our different winemaking techniques on the same grape variety.⁴⁰ TPI values of the samples of our experiment are in accordance with this study; the slight difference is most probably due to different vintage year and winemaking techniques. They still fall within the medium range as usual TPI values for red wines.³⁷ As is seen in Table 2, there is a slight but significant increase by the wood effect, from 53 to 55. This most probably might be explained by the phenolic compounds that are released from wood to wine that are ellagic tannins, as the main extractable compounds from oak wood.⁴¹ Our results also justify the findings of Cruz et al. as they had found out that the TPI significantly increased after wood aging of wine brandies.⁴²

Total Anthocyanins. The average anthocyanin concentration values are given in Table 2. The values are similar to those observed by Spranger et al. in wines from the same grape variety.⁴⁰ When compared to other grape varieties, according to the literature, Castelão wines seem to have relatively lower anthocyanin levels, as they range from 100 to 1500 mg/L.³⁷ As is seen, there is a significant increase in the concentrations at the end of 90 days. However, the use of wood did not have a significant impact on anthocyanin levels.

Generally, total anthocyanin concentration is expected to decrease by aging due to thermal and oxidative breakdown reactions and transformation, namely, polymerization with condensed tannins involving or not acetaldehyde.⁴³ However, in our study total anthocyanins seem to increase in concentration from 278 to 337 mg/L in the wine without oak staves after 3 months. There is a slight decrease at 30 days, and then they reach a higher concentration after 2 months. Therefore, this slight rise might be due to the low specificity of the method despite taking into account the complex composition of wine. Jarauta et al. stated that there are several phenomena regarding the concentration of compound changes during wine aging.⁴⁴ For example, compounds such as aliphatic lactones are wood-extractable lactones that are released into the wine and also compounds highly extracted from the wood surface (such as fatty acids) at the end of 3 months of aging. In our study, most probably, we have extracted compounds only from the wood surface due to the short aging time, but keeping in mind the extraction process with oak staves is faster than that with oak barrels will help to interpret these results. Nevertheless, a study in which six different winemaking techniques were evaluated, including wood chips, revealed that anthocya-

nin concentration was not significantly altered with wood contact.⁴⁵

Color Intensity. Mean values of color intensity with corresponding standard deviation values are also given in Table 2. It is known that phenolic content has the highest contribution to color intensity. Interactions that occur during wine aging such as condensation and polymerization of tannins, promoted by aeration, play an important role in the color. In the course of wood aging, despite the decrease in total anthocyanin concentration, due to breakdown phenomena and precipitation of free anthocyanins, polymeric pigments are formed, resulting in the increase in color intensity and stabilizing it. Due to the presence of oxygen, oxidation of ethanol forms traces of acetaldehyde during aging and stimulates an increase in color.⁴⁶

In our experiment, there was a significant decrease in color intensity. This might be due to precipitation of colloidal colorant matter. Therefore, loss of those molecules leads to a decrease in color intensity. Also, it might be due to the short aging time, which was not sufficient to create condensation reactions with tannins that eventually induce an increase of color intensity. The statistical data show that oak stave addition did not affect the color.

It is worth noting that condensation reactions are slow and the experiment ended after 90 days. It is possible that also anthocyanin breakdown reactions occurred and led to color loss.

Color intensity values of the Castelão variety in the Évora region from another study by Cabrita et al. present relatively lower values than ours in the years 1997, 1998, and 1999 that are 5.54, 5.01, and 6.05, respectively.⁴⁷ This difference might be due to *terroir* and vintage year difference and also different winemaking practices.

Tonality. The absorbances at different wavelengths represent different colors. Absorbance at 520 nm represents the red color, which is mainly related to free anthocyanins, whereas that at 420 nm is responsible for yellow.

As seen in Table 2, time had a significant impact on tonality, causing modification in red color as expected. This increase in tonality might be due to the anthocyanin and tannin breakdown reactions and the wine's tendency to yellowish color. Our wine may be considered not very rich in tannins and also relatively low in anthocyanins (varying from 273 to 347 mg/L); the red color did not develop as expected. These reactions depend on the phenolic content and structure of the wine.

Mineral Composition. The mineral composition of wines is of relevant interest due to its potential effect on physical–chemical stability, sensory characteristics, wine safety, and legal

Table 4. Effect of Wood Aging and Time on Minor and Trace Elements^a

element	wood aging			time			time × wood interaction
	wood effect	control	wood	time effect	T-0	T-90	
Li	ns	11300 (162)	11756 (640)	ns	11451 (318)	11790 (720)	ns
Be	ns	102 (47)	110 (36)	ns	91 (26)	126 (39)	ns
Al	ns	180 (12)	184 (7)	***	188 (4) b	178 (8) a	ns
Sc	ns	671 (169)	915 (121)	ns	858 (212)	860 (53)	ns
Ti	ns	1253 (84)	1346 (104)	ns	1313 (122)	1333 (93)	ns
V	***	672 (277) a	961 (56) b	***	938 (90) b	839 (245) a	***
Mn	ns	770 (22)	806 (47)	ns	777 (24)	812 (52)	ns
Fe	ns	2.7 (0.4)	2.9 (0.3)	***	3.1 (0.1) b	2.6 (0.2) a	ns
Co	***	2392 (534) a	2833 (110) b	***	2866 (92) b	2580 (412) a	***
Ni	***	33691 (24380) a	53440 (2440) b	***	55094 (708) b	41911 (18205) a	***
Cu	ns	21673 (639)	24365 (6241)	ns	22018 (7083)	25367 (2814)	ns
Zn	ns	323 (37)	330 (21)	***	344 (20) b	313 (19) a	ns
Ga	ns	2770 (171)	2865 (137)	ns	2903 (88)	2779 (172)	ns
As	ns	976 (98)	1058 (71)	ns	1060 (48)	1016 (107)	ns
Rb	ns	1174 (41)	1220 (47)	ns	1187 (32)	1231 (54)	ns
Sr	*	240 (7) a	258 (12) b	ns	249 (6)	258 (17)	ns
Y	ns	401 (48)	418 (41)	ns	406 (33)	423 (51)	ns
Sb	ns	76 (7)	67 (6)	ns	67 (9)	72 (13)	ns
Cs	ns	4941 (443)	5202 (208)	***	5338 (117) b	4935 (273) a	*
Ba	ns	976 (98)	1058 (71)	ns	1060 (48)	1016 (107)	ns
Pr	ns	50 (12)	45 (8)	ns	43 (11)	49 (6)	ns
Nd	ns	123 (11)	181 (78)	ns	189 (89)	135 (18)	ns
Eu	ns	109 (20)	111 (18)	ns	106 (19)	115 (16)	ns
Dy	ns	32 (17)	43 (8)	ns	36 (11)	47 (9)	ns
Ho	ns	13 (5)	13 (3)	ns	15 (3)	11 (4)	ns
Er	ns	30 (13)	44 (10)	ns	42 (6)	38 (16)	ns
Yb	ns	37 (13)	44 (12)	ns	47 (12)	38 (12)	ns
Lu	ns	15 (5)	13 (3)	ns	13 (3)	13 (3)	ns
Tl	ns	310 (21)	324 (15)	ns	322 (13)	320 (21)	ns
Pb	ns	4912 (1292)	6567 (2678)	***	7956 (2353) b	4351 (449) a	ns

^aResults are expressed in ng/L except for Al, Mn, Zn, Rb, Sr, and Ba ($\mu\text{g/L}$) and Fe (mg/L). Control, wine without wood staves; wood, wine with wood staves; T-0, time zero; T-90, 90 days. With regard to the wood effect, control and wood results correspond to mean values of the corresponding levels of time (T-0 and T-90). For time effect, for each level (T-0 and T-90), the results are based on average values of control and wood. Relative standard deviations are presented in parentheses. Mean values followed by the same letter are not significantly different at 0.05* or 0.001*** level of significance; ns, without significant difference. Two-way ANOVA is applied only to T-0 and T-90 values.

limits as well as a discriminating tool of the wines as their composition changes from rock to soil to vines. There are several factors that can alter the multielemental composition of grapes and wines such as winemaking practices, agricultural practices, and climatic conditions.⁶ The concentrations with two-way ANOVA results are given below in Tables 3 and 4.

Major Elements. The concentrations of major elements in wines during wood aging, namely, Na, K, and Ca, measured by flame atomic spectrometry, and Mg, measured by ICP-MS, are reported in Table 3. Figure 2 displays the evolution of Na (a), Mg (b), K (c), and Ca (d) concentrations over time in both control and wood.

For Na, K, and Ca slight but significant decreases were observed in concentration by time.

Most probably, K and Ca depletions are explained by their precipitation as potassium bitartrate and calcium tartrate, respectively, during the aging period, as natural phenomena of wine physical–chemical stabilization. It should be highlighted that the concentration of Ca in wine at the beginning of the experiment was under the empiric limit of 80 mg/L with regard to the susceptibility to calcium tartrate precipitation.

Because of the low precision (9% RSD) of K measurement in control wine (T-90), the results for this element should be interpreted with prudence.

Having in mind that the main components of wood ash are K, Ca, and Mg, but also Na and Fe, the potential transference of these metals to wine during aging is expected.⁴⁸ With regard to wood aging effect, only for Mg was a slight but significant higher concentration observed, however, without relevant technological interest. In general, wines are in the range of 60–150 mg/L in terms of Mg content.³⁷ The mean concentration of Mg in wine samples is 58 mg/L, which can be considered a medium concentration. Without other special enological interest, Mg plays an essential role in the buffer capacity and metallic structure of wine, along with K and Ca elements.

A reasonable explanation for these results can be the short time of contact between wine and wood. Probably, the amounts of K and Ca extracted from wood to wine have been not high enough to counterbalance their loss as tartrate salts.

As shown in Table 3 and Figure 2, for Na, a very highly significant interaction between wood and time was observed. Not all of the wines showed the highest concentration at the same time level. Although for the control the highest

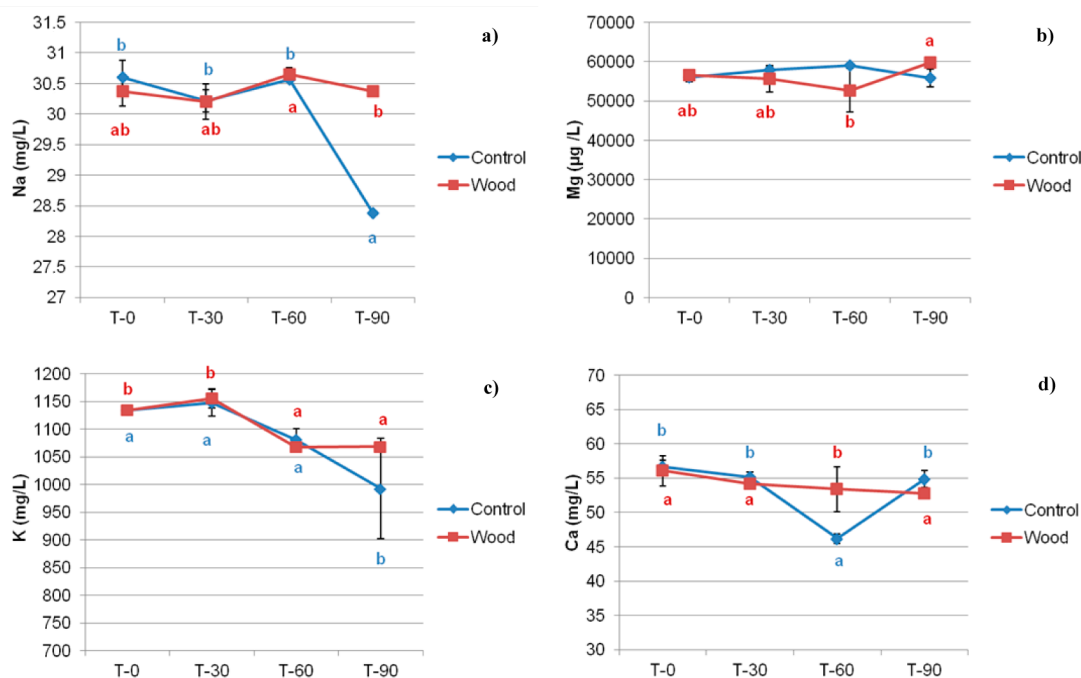


Figure 2. Evolution of Na (a), Mg (b), K (c), and Ca (d) concentrations over time. Control, wine without wood staves; wood, wine with wood staves; T-0, time zero; T-30, 30 days; T-60m 60 days; T-90, 90 days. With regard to control wines, results correspond to mean values of the corresponding analytical duplicates ($n = 2$); for wood wines, results are mean values (and corresponding standard deviation in parentheses) of three experimental replicates and corresponding analytical duplicates ($n = 6$).

concentration was observed at T-0, for wood the highest concentration was observed at T-90.

Minor and Trace Elements. The concentrations of minor and trace elements in wines during wood aging, determined by ICP-MS, are reported in Table 4. Neither wood aging nor time had a significant effect on the concentrations of Li, Be, Sc, Ti, Mn, Cu, Ga, As, Rb, Y, Sb, Ba, Pr, Nd, Eu, Dy, Ho, Er, Yb, Lu, and Tl elements.

Time had a significant impact on the concentrations of Al, V, Fe, Co, Ni, Zn, Cs, and Pb. Figure 3 displays the evolution of these element concentrations, and also of Sr, over time in both control and wood wines. The decrease of concentrations is in accordance with the precipitation phenomena of heavy metals as insoluble salts, namely, as sulfides, over time, favored by sulfur dioxide addition. The depletion of these metals in wine is positive from the wine quality point of view, due to their potential involvement in physical–chemical instability phenomena and their potential toxicity.

Fe forms soluble compounds with organic acids in wine. However, excessive amounts of this metal can cause turbidity known as ferric casse. Concentrations >10 mg/L (empiric technical limit) can cause undesired sensory characteristics in wine.³⁷ The concentration of Fe in the control wine decreased 16% after 3 months of aging, being of 2.42 mg/L, representing no risk of iron casse or toxic effect.

In addition, Fe plays a primary role in nonenzymatic oxidation mechanisms occurring in wines, namely, in oxidation of polyphenols containing a catechol or a galloyl group to semiquinone radicals and benzoquinones. The participation of reduced transition metal ions, essentially Fe^{2+} and Cu^+ , in these reactions has been described.⁴⁹

With regard to aging impact on the mineral content, it can be seen from Table 4 that V, Co, Ni, and Sr are the only elements significantly affected by wood aging. Because V, Co, and Ni are

heavy metals, particular attention must be paid to the differences in their concentrations between control and wood wines. For the rest of the elements, although no significant effect of wood treatment was observed, metal concentrations tend to be higher in the wine treated with oak staves. Maximum acceptable limits by OIV of critical elements are defined as 1, 5, 0.2, and 0.15 mg/L for Cu, Zn, As, and Pb, respectively. Our wines show low amounts of these elements, which are 0.02, 0.0004, 0.001, and 0.01 mg/L, respectively.³²

Higher concentrations of V, Co, and Ni in wood in comparison with control wine are most probably explained by its more intense precipitation through time in the wine without wood staves. This hypothesis is supported by the concentrations of V, Co, and Ni in control wine at the beginning of the experiment.

Depending on the oxidation–reduction potential of wine, metal ions can be present under different oxidation forms in different proportions, decisively influencing insolubilization and precipitation phenomena. It is well-known that wine is more susceptible to ferric haze and precipitation after aeration, as this increases the proportion of Fe^{3+} form responsible for the phenomenon. Unlike ferric casse, Cu insolubilization develops when the oxidation–reduction potential reaches an appropriately low level for Cu^{2+} reduction to Cu^+ . In this experiment, micro-oxygenation was applied with the amount of 3 mg of $\text{O}_2/\text{L}/\text{month}$ to wine aged with oak staves. Having in mind the slight but significant increase of total phenolics index in wood wine and that phenolic compounds are the main oxygen consumers in wines, most probably part of the added oxygen was consumed in oxidation reactions. In future studies, the oxidation–reduction potential of control and wood-aged wines should be assessed and the results related with their metal composition.

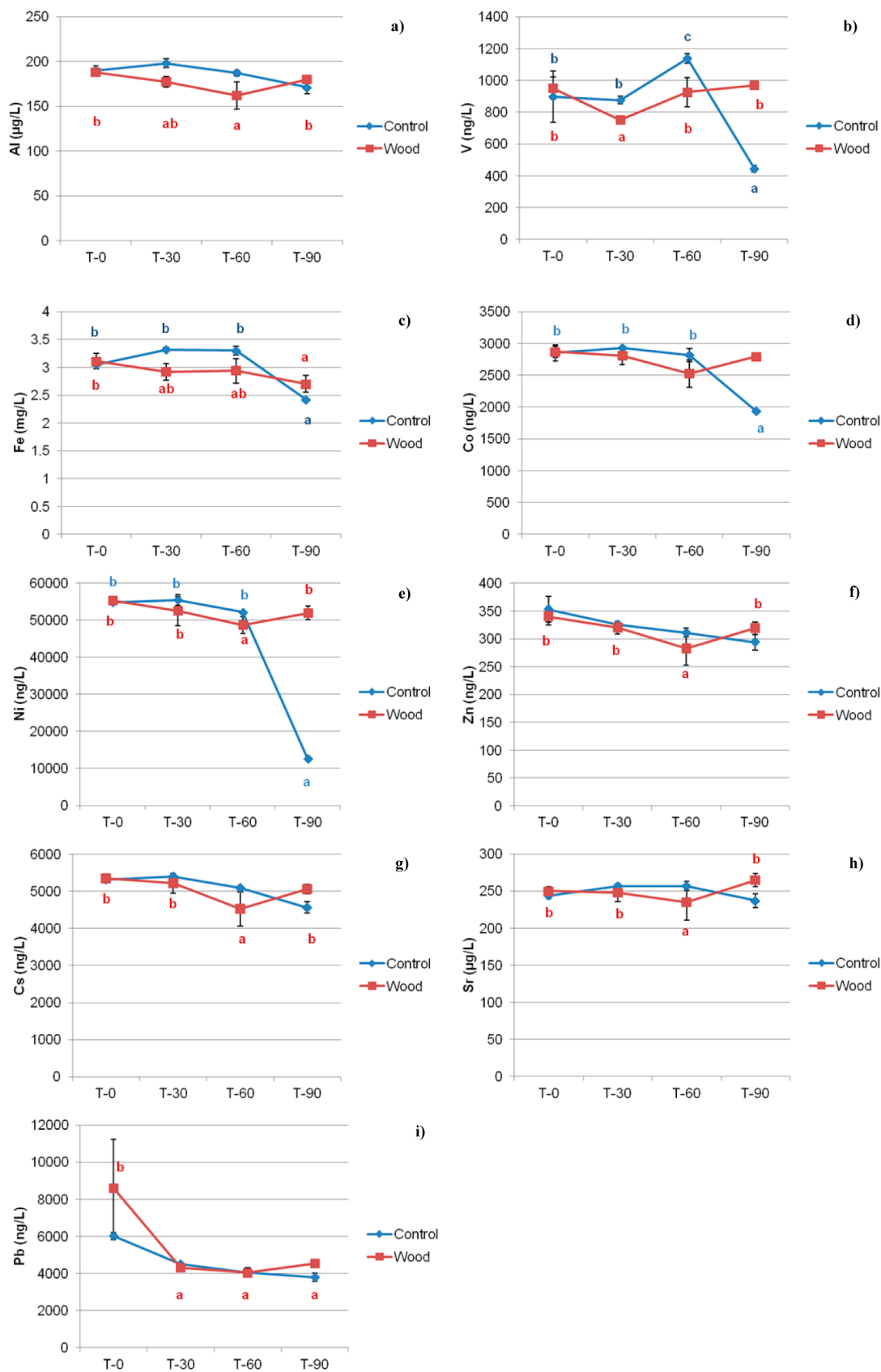


Figure 3. Evolution of Al (a), V (b), Fe (c), Co (d), Ni (e), Zn (f), Sr (g), Cs (h), and Pb (i) concentrations over time. Control, wine without wood staves; wood, wine with wood staves; T-0, time zero; T-30, 30 days; T-60, 60 days; T-90, 90 days. With regard to control wines, results correspond to mean values of the corresponding analytical duplicates ($n = 2$); for wood wines, results are mean values (and corresponding standard deviation) of three experimental replicates and corresponding analytical duplicates ($n = 6$).

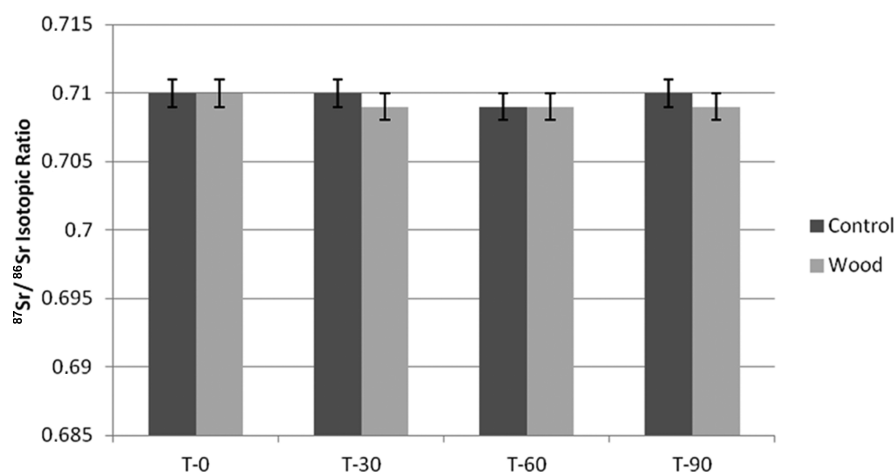


Figure 4. $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratios in wines over time. Control, wine without wood staves; wood, wine with wood staves; T-0, time zero; T-30, 30 days; T-60, 60 days; T-90, 90 days. With regard to control wine, results correspond to mean values of the corresponding analytical duplicates ($n = 2$); for wood wines, results correspond to mean values (and corresponding standard deviation with uncertainty bars) of three experimental replicates and corresponding analytical duplicates ($n = 6$).

Cu and Fe play roles in the oxidation of ellagic tannins released from wood to wine that makes them important for aging phenomena.⁵⁰ However, there was no impact of wood aging on Cu and Fe.

As shown in Table 4 and Figure 3, for V, Co, and Ni elements, a very highly significant interaction between wood and time was observed, evidencing different trends in control and wood wines.

The Sr concentration was altered by wood from 249 to 258 $\mu\text{g}/\text{L}$. As Sr is an important element regarding wine fingerprinting concerning its $^{87}\text{Sr}/^{86}\text{Sr}$ isotopic ratio, an increase in its concentrations might cause also a change in this ratio. It is important to observe if alterations in the concentration cause changes in Sr abundance ratios to contribute to authenticity verification applications.

One of the most explored fingerprinting techniques combines multielement analysis and multivariate statistical analysis of the chemical data to obtain classification of wines according to geographical origin.^{2,11,51} Its successful application strongly depends on the selection of suitable elements that would reflect the link with soil geochemistry and thus with discriminating potential. Otherwise, the wine's classification will reflect not only the geographical provenance but also anthropogenic factors. In this study, slightly higher concentrations of Mg, Sr, and some heavy metals were found in wood in comparison with control wine, evidencing an impact of wood aging on wine mineral composition. Therefore, wood aging may limit the usefulness of wine multielement composition as a fingerprint of the wine region of origin.

In future studies, information on the evolution of mineral composition of wine during aging should be crossed with data of wood elemental composition before and after extraction, for mass balance purposes.

Strontium Isotopic Ratio. *Separation of Rubidium and Strontium.* As previously stated, the separation of Sr and Rb is a mandatory step before isotopic analysis by ICP-MS. It can be confidently said that in the samples that the Sr isotopic ratios measured were not interfered with by the ^{87}Rb isotope, because in all of the chromatographic fractions containing Sr, the ratio Rb/Sr was always $<0.93\%$. Relative standard deviation of measurements ranged between 0.1 and 0.2%.

$^{87}\text{Sr}/^{86}\text{Sr}$ Isotopic Ratio. $^{87}\text{Sr}/^{86}\text{Sr}$ isotope abundance ratio values were determined. In total, 32 wine samples were analyzed from initial time until the end of 90 days. Yet, as previously indicated, it is important to consider anthropogenic sources of strontium while using it as a geographical indicator.

The strontium isotopic ratio that is taken by the roots of the vines shows identical values as in the soil as well as in the wine after wood aging. As before stated, with regard to Sr concentration, the wine samples at the end of 3 months exhibit significantly higher concentration values. However, as is illustrated in Figure 4, strontium isotopic ratios were not modified after wood aging, and they are significantly identical before and after, which are independent from the Sr concentrations even though the concentration has slightly increased by wood aging factor. These results justify our hypothesis about using the Sr isotopic ratio as a wine provenance tool. Our findings are also in accordance with results obtained in previous studies.³ The team analyzed the isotopic ratio of the soil from the Palmela region, which was 0.711 ± 0.0003 . In this study the ratios vary from 0.709 to 0.710. This suggests a strong relationship of $^{87}\text{Sr}/^{86}\text{Sr}$ between soil and wines. Nevertheless, more studies are necessary to prove the reliability of this method, and a database must be developed for this purpose.

After 3 months of aging with oak staves in stainless steel tanks with three experimental replicates, it was found that wood aging and time did not alter $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. However, some significant changes in elements' compositions occurred that might be explained by enological treatments, precipitations, and interactions between several compounds in wine such as anthocyanins, proteins, and tannins. The effect of wood aging on the chromatic characteristics and total polysaccharide content of wine was also studied.

This study allowed us to reveal the impacts of wood aging on wine mineral composition and on strontium isotopic ratio as a novelty, to the best of our knowledge. Findings of this work can shed some light on the authenticity methods and contribute to future similar studies and can be developed in different regions, especially the ones that have appellations, by analyzing the soil and the wines. In this way, establishing a database will help new studies that can relate soils' Sr isotopic ratios with the wines. It is also worth mentioning that this method can be very useful in

adding value to high-quality fine wines and preventing false declarations.

This study was carried out with one type of wine, treated with oak staves from a single wood species, during a short period of time, but it can be developed by assaying aging wines with different oak species, oaks with different cooperage processes, with oak barrels, and with diverse winemaking techniques to reveal impacts of such practices and alterations on the strontium isotopic ratios of wine.

AUTHOR INFORMATION

Corresponding Author

*(S.C.) E-mail: sofiacatarino@isa.ulisboa.pt. Phone: +351 21 3653246.

ORCID

Sofia Catarino: [0000-0002-6223-4377](https://orcid.org/0000-0002-6223-4377)

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Notes

The authors declare no competing financial interest.

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