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Food Chemistry 85 (2004) 7–12

Food Chemistry

www.elsevier.com/locate/foodchem

Does the winemaking process influence the wine ^{87}Sr/^{86}Sr? A case study

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Received 25 April 2003; accepted 27 May 2003

Abstract

To evaluate the roles of the soil composition and vinification process on the wine strontium isotope ratio ^{87}Sr/^{86}Sr, two different wines (one red table and one fortified wine) from the Douro Portuguese wine-district were selected. The ratio ^{87}Sr/^{86}Sr was determined in the provenance soil, in the grape juices (prepared in the laboratory) and in the wines. Total strontium concentration was determined in the grape juices, skins and seeds and in the samples collected in the different steps of each winemaking process and respective produced wines. All analyses were performed by inductively coupled plasma mass spectrometry. The total strontium concentration increased during the vinification processes. The increase was attributed to the element liberation from grape seeds and skins (richer in strontium than the pulp) during the initial mechanical pressing and fermentation. The ^{87}Sr/^{86}Sr values were statistically identical in the provenance soil, respective grape juice and wine, which indicates that ^{87}Sr/^{86}Sr is a suitable tracer of the origin of the studied wines. The study deserves to be extended to many more wines/provenance soils in order to validate the suitability of that parameter as a tool for the identification of wine provenance region.

Keywords: Wine; Strontium; Strontium isotope ratio; Vinification; ICP-MS

1. Introduction

Normally, isotopic abundances of the elements are constant in nature. However, there are some elements with variable isotopic compositions, among which strontium is included. This alkaline-earth metal has four stable, naturally occurring, isotopes: ^{84}Sr, ^{86}Sr, ^{87}Sr and ^{88}Sr. Only ^{87}Sr is radiogenic, and gradually increases in minerals due to the radioactive decay of ^{87}Rb. Differences in the absolute proportion of ^{87}Sr vary with geological ages and consequently with geographical locations (Bullen & Kendall, 1998).

Measurements of isotope ratios of elements with variable isotopic abundances may be useful for the protection of prestige wines. This variation may be explored in order to determine the origin of a wine. Several articles can be found in the literature reporting the use of stable isotope ratios for that purpose, mostly of carbon, oxygen and hydrogen (Bréas, Reniero, & Serrini, 1994; Day, Zhang, & Martin, 1994; Versini, Monetti, & Reniero, 1999) but also of lead (Augagneur, Medina, & Grousset, 1997; Dean, Elbon, & Massey, 1990; Medina, Augagneur, Barbaste, Grousset, & Buat-Menard, 2000) and strontium (Almeida & Vasconcelos, 2001; Barbaste, Robinson, Guilfoyle, Medina, & Lobinski, 2002).

Trace element concentrations in wines depend, among others factors, on geographical origin. Elements are taken up by the roots of plants, passing to the grapes in the same isotopic proportions as they occur in the soil. Thus, the ^{87}Sr/^{86}Sr isotope ratio (IR) can be used as a tracer of wine origin if a significant correlation between the value of this parameter in the soil and in the wine is observed and if significantly different values are present in wines of different provenance regions. Horn, Hölzl, Todt, and Matthies (1998) and Lancelot, Herrerias, Verdoux, and Lurton (1999) demonstrated the existence of the required correlation for German and French wines, respectively. Nevertheless, studies like those are scarce. As wine processing changes from wine to wine, from winery to winery and from one country to another, more studies are required before one is able to

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0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.05.003

generalise the suitability of the $^{87}Sr/^{86}Sr$ IR as a tracer of wine origin.

The aim of this study was to investigate the relative influence of the provenance soil and of the winemaking process on the $^{87}Sr/^{86}Sr$ IR present in a red table wine and a red fortified wine, similar to Port. These wines were produced in two different wineries with grapes from two vineyards of the Douro wine-district, northeast of Portugal. The strontium IR was determined in the vineyard soil, grape juices prepared in the laboratory and wines. In addition, the total strontium concentration was determined in the grape juices, skins and seeds and samples collected during and at the end of the vinification processes, in order to investigate eventual changes in the strontium concentration during the winemaking process.

2. Materials and methods

2.1. Material and reagents

Suprapure concentrated HNO_3 and a pro analysis 30% H_2O_2 solution, both from Merck, were used without further purification.

A stock $SrCO_3$ (isotopic) standard reference solution (1000 mg l^{-1} Sr) SRM-987, from the National Institute of Standards and Technology (NIST), USA, was prepared by dissolving 1 g of powder in 1% (v/v) HNO_3 solution.

For total Sr concentration (Sr_{total}) determinations, both a *pro analysis* stock Sr standard solution (1000 mg l^{-1}, from Merck) and a *pro analysis* stock Rh standard solution (1000 mg l^{-1}, from Alfa) were used.

All other reagents used were pro analysis grade or equivalent.

Standard solutions were prepared daily from the stocks, in polyethylene tubes, by weight, with HNO_3 solution.

To prevent contamination, all material used for sampling and sample treatments and all lab ware was soaked in 20% (v/v) HNO_3 solution for at least 24 h, rinsed several times with deionised water (resistivity > 14 MΩ cm, conductivity < 0.1 μS cm^{-1}) and dried in a Class 100 laminar flow hood. To collect the samples, no metallic instruments were used.

The sample manipulation was carried out in a clean room with Class 100 filtered air.

2.2. Sampling

Two vineyards, one 60–70 years of age (referred to as "old vineyard") and other ten years old, raised on a forest-soil ("young vineyard"), from the Douro wine-district, northeast of Portugal, where the soil is schistose, were selected. In both vineyards only treatments with copper sulphate solutions were carried out in the year of the study. Nevertheless, in previous years, several treatment products (pesticides and fertilizers) had been used, particularly in the old vineyard.

The grapes from the polyvarietal vines that have been grown in the old vineyard were used to produce a red fortified wine similar to Port. The vinification process used for that purpose (summarised in Fig. 1A) was performed manually, in an old fashioned way, without automatic controls, and involved a small number of steps, the wine being poured directly from one container to the next. At the end of the vinification process, the fortified wine had been aged in oak barrels for periods between 2 and 20 years, depending on the desirable wine quality. For practical reasons, in the present study, the sample called "final product" was collected after only 1-year of ageing.

In the young vineyard, only "Touriga Nacional" vines were growing and their grapes were used to produce a monovarietal red table wine. Stainless-steel tubes and containers were used in most steps. Plastic containers and tubes (polyethylene, high-density polyethylene and flexible PVC) were also used for the harvest and for transfer of must and wine in some of the steps of the vinification. Ageing of the wine took place in oak barrels. At the end, the wine was stored in glass bottles. In this study, the produced wine was analysed about 1 month after bottling.

Throughout each vinification process, samples were collected in triplicate, in polyethylene tubes, at points where a possible change in the wine's composition could be expected, for instance, as a result of contamination (see Fig. 1A—fortified and 1B—table wine).

In each vineyard, grapes were collected in September, just before the vintage, in three different sampling sites (selected to be representative of the entire vineyard) to prepare grape juice. Soil samples were collected at 20 cm depth in the three selected sampling sites, 4 months before the vintage (when the first grapes appeared). All samples were collected using plastic shovels for soil samples and plastic gloves for grapes and were stored in individual plastic bags, which were immediately closed.

2.3. Sample treatments

2.3.1. Grape juices and samples from the vinification processes

Grape juice was prepared in the laboratory. For this purpose, identical quantities of non-washed grapes collected at the three sampling sites were mixed and smashed (with gloved hands), in plastic cups. The skins and seeds remained intact and were transferred to other vessels for further treatment. The juice obtained was transferred to polyethylene tubes. Aliquots of non-filtered grape juice, as well as non-filtered samples from both vinification processes, were

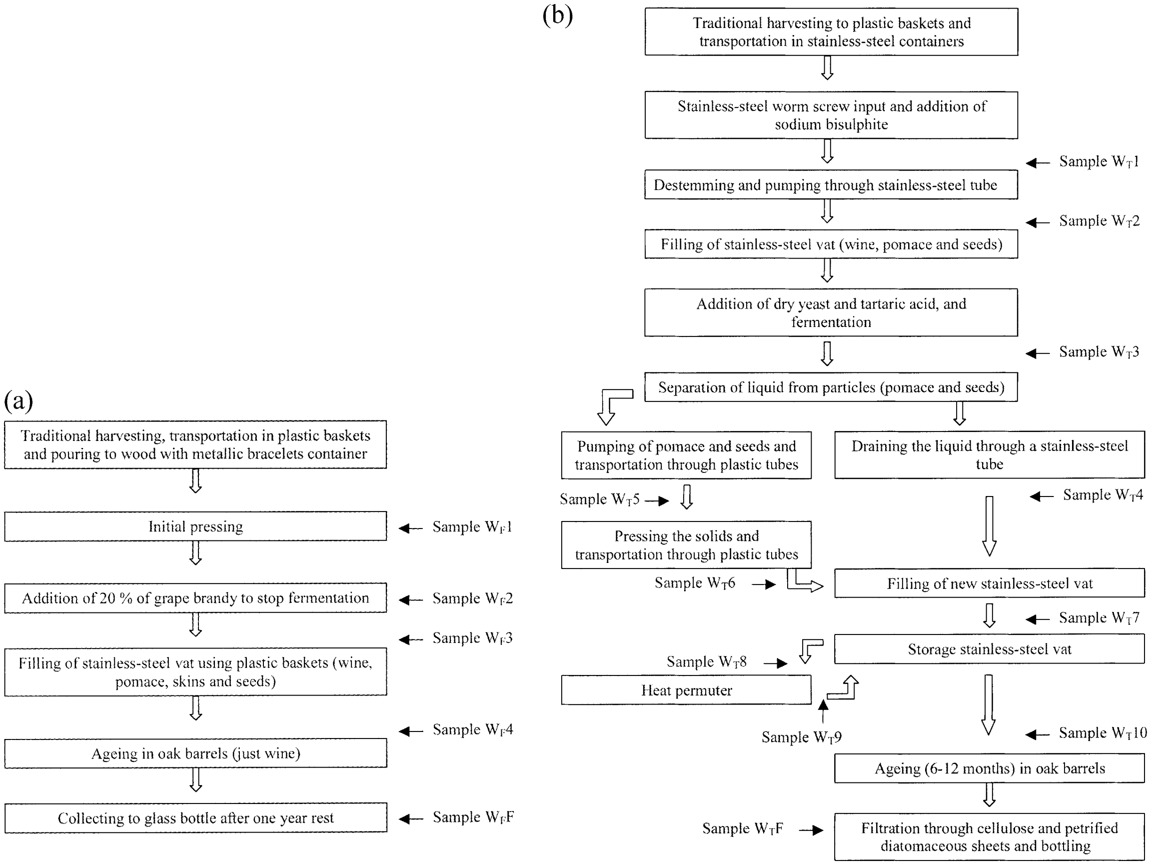

Fig. 1. Schematics of the vinification processes with indication of the points where wine samples were collected. (a) red fortified wine produced from grapes of the old vineyard (from W_F1 to W_F4 and W_FF); (b) red table wine produced from grapes of the young vineyard (from W_T1 to W_T10 and W_TF).

pre-treated by UV-irradiation (Almeida & Vasconcelos, 1999). For Sr_{total}, the solutions were diluted afterwards with a solution containing HNO_3 and Rh. For Sr IR determinations, solutions were diluted with deionised water, afterwards being submitted to a cation-exchange chromatographic procedure (Almeida & Vasconcelos, 2001) for the removal of most of the Rb, which is also present in the samples and is an interferent in ICP-MS analysis of $^{87}Sr/^{86}Sr$.

2.3.2. Vineyard soil, grape skins and seeds

The samples of vineyard soil, grape skins and seeds were first dried in an oven to constant weight. Soil was fractioned and homogenized by sieving through nylon nets of 2 mm and 200-mesh (Reeve, 1994). A composite sample was prepared with identical aliquots of the dried 200-mesh fractions. Grape skins and seeds were crushed and homogenized.

The soil samples were digested with concentrated HNO_3 (Smith & Arsenault, 1996) by high-pressure, microwave assisted (HPMW), which was carried out in closed PTFE vessels using a MLS-1200 Mega system, from Millestone. The digestion programme consisted of three steps of 5 min each, at 250, 400 and 500 W, respectively, and was run twice. The efficiency of the digestion procedure was tested with standard soil reference material: San Joaquin Soil SRM-2709, from NIST. Subsequently to the HPMW digestion, the solutions, after appropriate dilution, were submitted to a cation-exchange chromatographic procedure, similar to that used for wine, for elimination of most of the Rb.

Grape skins and seeds were digested with concentrated HNO_3 and 30% H_2O_2, using, as starting point, literature data (Smith & Arsenault, 1996). The HPMW was also used, with a programme similar to that used for soil samples but run only once. Before analysis of Sr_{total}, suitable dilutions with a solution containing Rh were carried out.

2.4. ICP-MS analysis

The analytical measurements were carried out in a Perkin-Elmer SCIEX Elan 5000 ICP-MS (Perkin-Elmer, Norwalk, CT, USA) apparatus equipped with a crossflow nebulizer, nickel cones and a peristaltic sample delivery pump. The operating conditions were described elsewhere (Almeida & Vasconcelos, 2001).

2.4.1. Total strontium concentration

The Sr_{total}, in the grape juices, skins and seeds and samples from the vinification processes was measured using a semi-quantitative mode of ICP-MS multi-element analysis optimised previously for wines (Almeida & Vasconcelos, 2002), being carried out in a single day's work. Two red wines (one table and one Port), with previously determined multi-element concentrations (Almeida & Vasconcelos, 2002), were analysed together with the samples to check possible instrument bias. Daily variations (short-term precision) of around 2% were observed for Sr in both "reference" wines.

2.4.2. Strontium isotope ratio

The $^{87}Sr/^{56}Sr$ IR was measured using a data acquisition procedure optimised previously (Almeida & Vasconcelos, 2001), all the values being internally corrected for mass discrimination phenomena using the constant $^{88}Sr/^{86}Sr$ IR.

The $SrCO_3$ (isotopic) standard NIST SRM-987 was run regularly for quality control.

For each sample, three independent replicates were prepared for analysis. Blank solutions, treated as samples, were regularly analysed in order to subtract the background signal.

3. Results and discussion

3.1. Total strontium concentration

The levels of Sr_{total} in the grape juices (GJ_F and GJ_T for the fortified and table wines, respectively) and in the samples collected throughout and at the end of the vinification processes (see Fig. 1A—fortified and 1B—table wine) are presented in Fig. 2. For the fortified wine, Sr_{total} increased about 215% during the vinification, from 0.49±0.04 mg l^{-1} in GJ_F to 1.56±0.01 mg l^{-1} in the wine (W_FF). For the table wine, Sr_{total} increased about 70%, from 0.85±0.01 mg l^{-1} in GJ_T to 1.45±0.02 mg l^{-1} in the wine (W_TF).

A more detailed analysis of the data (Fig. 2) showed that the increase of Sr_{total} throughout both vinification processes was not regular. For the fortified wine, the Sr_{total} only increased significant and markedly (about 190%) at the beginning of the vinification (concentration higher in WF_1 than in GJ_F). A fraction of Sr may come from natural sources, namely seeds and skins of the grapes. This hypothesis is supported by the relatively high Sr_{total} content in the grape seeds, 42±3 μg $g_{dry\ grape\ seed}{}^{-1}$, and skins, 27±3 μg $g_{dry\ grape\ skin}{}^{-1}$, when compared with that in the grape juice, 0.49±0.04 mg l^{-1} (assuming a grape juice density of 1). It is noteworthy, that W_F1 was obtained by mechanical pressing of the entire grapes (pulp, seeds and skin) in the winery, whereas GJ_F was prepared in the laboratory by manually pressing only the grape pomace.

In the table wine, also, a significant increase in Sr_{total} (about 110%) was observed in the first steps of the vinification process, between W_T1 to W_T4, when compared with the Sr_{total} level in GJ_T. In the following steps, in which only a liquid phase was present (no further contact with grape seeds and skins) a slight but significant decrease in the Sr_{total} level was observed, which could be related to the precipitation or co-precipitation of Sr with suspended colloidal particles during ageing. Decreasing of the concentration of other elements during ageing of wines has been observed, as discussed in detail by Almeida and Vasconcelos (2003a, 2003b). As before, grape skins and seeds displayed much higher Sr_{total}, 41±1 μg $g_{dry\ grape\ skin}{}^{-1}$ and 111±6 μg $g_{dry\ grape\ seed}{}^{-1}$, respectively, than the grape juice, 0.85±0.01 mg l^{-1}.

Heterogeneous distribution of Sr in the grapes is compatible with previous reports. It is known, for instance, that the distribution of Ca, Fe, K, Mg and Na throughout the berry is not homogeneous (Curvelo-Garcia, 1988): the berry pulp is richer in K and Na than the seeds or skin, while seeds and skin are richer than pulp in Ca and Mg and berry skin is particularly rich in Fe. Teissedre, Cabanis, Champagnol, and Cabanis (1994) also found significant differences in the levels of

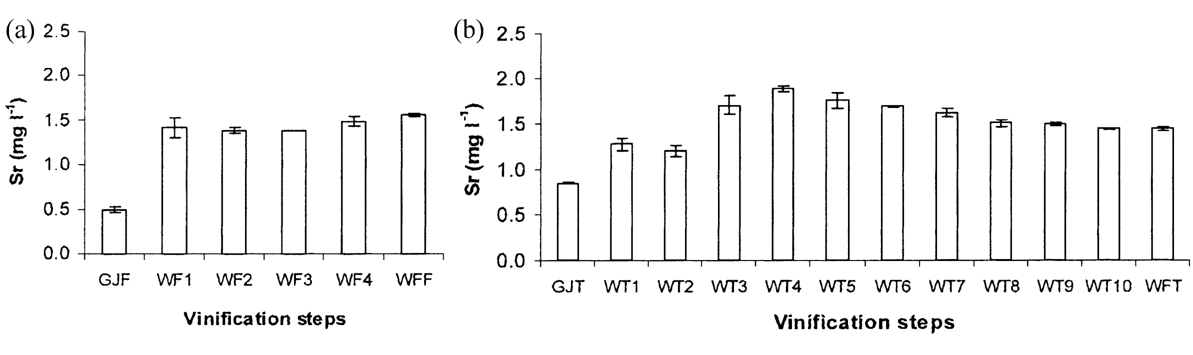

Fig. 2. Values of strontium total concentration obtained in the grape juices (GJ), in the samples collected throughout the vinification processes (from W_F1 to W_F4 for fortified and from W_T1 to W_T10 for table wine) and in the final products (WF). (a) red fortified wine produced with grapes from the old vineyard; (b) red table wine produced with grapes from the young vineyard.

Pb in the different parts of the grape berries, the seeds having the highest content of Pb and the pulp the lowest.

3.2. Strontium isotope ratio

As previously mentioned, $^{87}Sr/^{56}Sr$ IR may vary with the origin of the element. If anthropogenic contamination does not occur during the vinification, that Sr IR will remain constant through the winemaking process. To confirm this hypothesis, the Sr IR was determined in the vineyard soil, GJ and WF. The measurements took place in a single working day and the short-term precision of the Sr IR was measured in the $SrCO_3$ (isotopic) standard NIST SRM-987, being 0.4%. The relative standard deviation (RSD) associated with the Sr IR in the standard solutions varied between 0.2 and 0.7%, indicating some instability of the instrument, which was not possible to compensate.

The mean and the standard deviation of the Sr IR determined in soil, GJ and WF are presented in Table 1. The RSDs were around 0.7%, identical to those obtained for standard solutions. Table 1 shows that the values of $^{87}Sr/^{86}Sr$ IR were similar in GJ and respective produced wines. Therefore, the vinification processes did not significantly influence the Sr IR, which is compatible with no significant Sr anthropogenic contamination. This result corroborates the hypothesis formulated above, that the increase of Sr_{total} in the first steps of the vinification processes basically resulted from release into the must of Sr from skins and seeds of the grapes. A statistical comparison through paired *t*-test (Miller & Miller, 1984) between the values of Sr IR in wines and soil indicated that they also did not differ significantly, which makes $^{87}Sr/^{86}Sr$ suitable for use as a tracer of the origin of the studied wines.

In addition, the $^{87}Sr/^{86}Sr$ IR obtained for both studied wines was identical to that observed previously (Almeida & Vasconcelos, 2001) in other wines from the same region—Douro wine-district, northeast of Portugal, and different, for instance, from those observed in wines from Bairrada and Borba wine-districts, from the centre and southeast of Portugal, respectively (Almeida & Vasconcelos, 2001).

Table 1
Sr IR[a] values obtained in grape juices, wines and soil

	Old vineyard			Young vineyard	
	Grape juice	Wine	Soil	Grape juice	Wine
$^{87}Sr/^{86}Sr$	0.727	0.729	0.732	0.731	0.729
SD	0.006	0.005	0.006	0.004	0.006

[a] Mean and standard deviation (S.D.) calculated, attending to errors of propagation ($n = 3$). Results corrected for mass bias discrimination with $^{88}Sr/^{86}Sr$.

4. Conclusions

The total strontium concentration was significantly higher in the produced wines than in the respective grape juices, the increase being higher in the fortified wine than in the table wine. The enrichment in strontium was attributed to release of the element into the must from the seeds and skins of the grapes instead of anthropogenic contamination. This hypothesis was compatible with the statistically identical $^{87}Sr/^{86}Sr$ values observed in grape juices and in the respective wines. The $^{87}Sr/^{86}Sr$ values were also statistically identical in the wines and soil. These results indicate that $^{87}Sr/^{86}Sr$ is suitable for use as a tracer of these wines' origins.

In more general terms, the present and some previous data indicate that $^{87}Sr/^{86}Sr$ can be a valuable fingerprint of wines, particularly when combined with other tracers, such as multi-element composition.

Acknowledgements

Mr. António Sousa is thanked for his help in the sampling and in some experimental work. The winegrowing Company "Domingos Alves de Sousa" is thanked for logistic support for sampling and valuable information on the winemaking processes and the "Fundação para a Ciência & Tecnologia", Lisbon, Portugal, for the C.M.R.A. PhD. scholarship (PRAXIS XXI/BD/16028/98) and equipment (Proj. 27/M/90).

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