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SHORT COMMUNICATION

Determination of *trans*-Resveratrol Concentrations in Brazilian Red Wines by HPLC

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The levels of *trans*-resveratrol in 36 commercial red wines produced in the southern region of Brazil have been measured using a direct injection isocratic UV-HPLC method. The concentration of *trans*-resveratrol ranged from 0.82 to 5.75 mg/L with a mean value of 2.57 mg/L (R.S.D.% = 1.99).

Key Words: *trans*-resveratrol; red wines; UV-HPLC; phytoalexin; polyphenols.

INTRODUCTION

The polyphenol *trans*-resveratrol (*trans*-3,4′,5-trihydroxystilbene, see Fig. 1 for structure) is a phytoalexin present in a wide variety of plant species, including mulberries, peanuts, and grapes (Langcake and Pryce, 1976; Ingham, 1976; Ector *et al*., 1996; Sobolev and Cole, 1999). This phytoalexin is found in relatively high concentrations in wine, particularly in red wine (Frémont, 2000). The effect of wine consumption toward coronary heart disease has been attributed to the antioxidant and anticoagulation properties of *trans*-resveratrol (German and Walzem, 2000).

Many papers have been published describing some approaches for evaluating the concentrations of *cis*- and *trans*-resveratrol in several selected wines (Goldberg, *et al*., 1995a, b; Soleas *et al*., 1997). Although a number of investigations have focused on the determination of resveratrol in wines of different countries, there is no similar study about the wines produced in Brazil, more precisely in the southern region of the country. This region is responsible for 91% of the Brazilian wine production (Lapolli *et al*., 1995).

In this study, a modified HPLC analytical procedure was employed to determine the *trans*-resveratrol content in samples obtained from 36 commercial red wines produced in the southern region of Brazil. The results indicate that these wines contain significant levels of *trans*-resveratrol.

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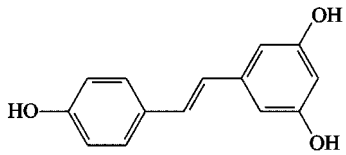

FIGURE 1. Structure of *trans*-resveratrol.

MATERIALS AND METHODS

The HPLC system used was composed by a Perkin-Elmer 785A UV–VIS detector with provision for stopped-flow scanning (190–360 nm), a PE Series 200 pump, a PE 900 Series Interface and a vacuum degasser PE Series 200. An octadecyl column 250 mm long was used, with 5 μm particle diameter, 4.6 mm i.d. (Brownlee, Norwalk, U.S.A.). After filtration in a 0.45 μm membrane, the samples were 6-times diluted with eluent and then directly injected through a 20 μL loop into a C_{18} guard column. Reversed phase HPLC was performed with an isocratic elution (1.5 mL min^{-1} flow rate) using a H_2O : Acetonitrile (75 : 25) solution as eluent. The pH of the solution was adjusted to 3.00 by using concentrated H_3PO_4 (Merck, Darmstadt, Germany). The eluent was prepared by using Milli-Q water and HPLC grade acetonitrile (EM Science, Gibbstown, NJ, U.S.A.). The signal was monitored at 306 nm. The *trans*-resveratrol standards were supplied by PharmaScience Inc. (Montreal, Canada), and the wines were purchased from commercial suppliers. The wines analysed were of the varieties Merlot ($n = 10$), Cabernet Sauvignon ($n = 13$), Cabernet Franc ($n = 4$), Pinot Noir ($n = 3$), Gamay ($n = 3$), Pinotage ($n = 1$), Sangiovese ($n = 1$) and Tannat ($n = 1$). Before being analysed, the wines were kept refrigerated at 4°C. The analyses were performed at 24°C. Each sample was injected four times in the chromatographic system. For quantification, an external standard calibration curve was done ranging from 0.10 to 10.0 mg/L of *trans*-resveratrol. The square regression coefficient of analytical curve was near unit ($r^2 = 0.9986$). Data were processed by TurboChrom™ 4.0 software.

RESULTS AND DISCUSSION

The analytical procedure described by McMurtrey *et al.* (1994) was used as a reference. However, several parameters were modified, in order to obtain a faster and more effective separation of *cis*- and *trans*-resveratrol from the sample matrix. The dilution of the samples was used to avoid column overloading caused by high amounts of sample matrix. This approach requires no gradient elution and is less time consuming. The modifications resulted in an improved resolution of *cis*- and *trans*-resveratrol and a shorter analysis time, as shown in Figure 2. Reproducibility and repeatability of the curves for both the standard and wine were very good, 3.2 and 1.7%, respectively.

The results of the chromatographic analyses of the 36 different Brazilian wines are summarized in Table 1. The concentration of *trans*-resveratrol ranged from 0.82 to 5.75 mg/L with a mean value of 2.57 mg/L (R.S.D.% = 1.99). Mean values of red wines *trans*-resveratrol concentrations reported in literature include 0.132 mg/L in California (Lamuela-Raventos *et al.*, 1993), 0.157 mg/L in Japan (Okuda and Yokotsuka, 1996), 0.77 mg/L in Canada (Soleas *et al.*, 1995), 0.873 mg/L in Greece (Dourtoglou *et al.*, 1999), 0.998 mg/L in California (Goldberg *et al.*, 1996), 1.00 mg/L

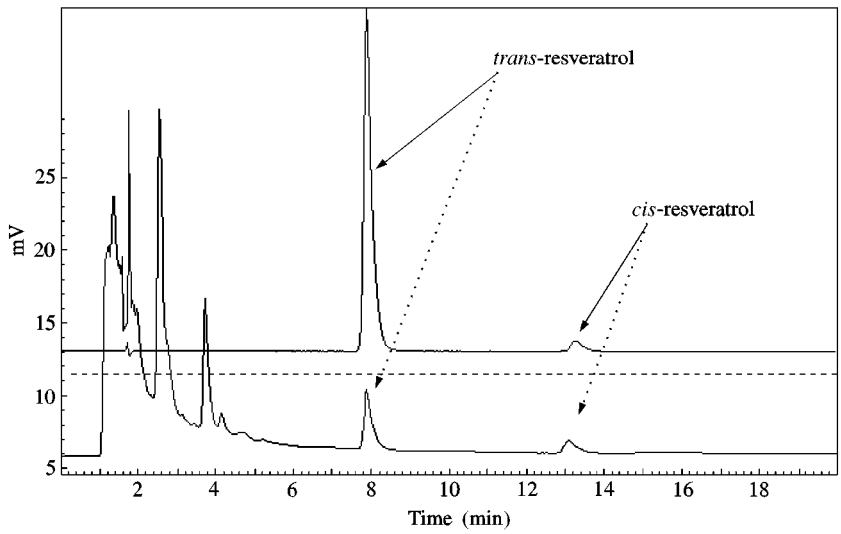

FIGURE 2. HPLC chromatograms of a *trans*-resveratrol standard solution (top), containing 4.23 mg/L and a 1997 vintage Merlot wine sample diluted six times (bottom).

TABLE 1

Trans-resveratrol concentration in Brazilian red wines

		Concentration (mg/L)		
Wine	Year[1]	Min	Max	Average (R.S.D.%)[2]
Merlot	1999 (2)	4.97	5.23	5.10 (1.65)
	1998 (1)	—	—	3.90 (1.80)
	1997 (7)	0.91	5.43	3.12 (1.77)
Cabernet Sauvignon	1998 (6)	1.17	3.57	2.01 (1.93)
	1997 (5)	0.82	2.33	1.53 (1.95)
	1994 (1)	—	—	2.33 (1.91)
	1991 (1)	—	—	1.25 (2.03)
Cabernet Franc	1999 (1)	—	—	1.60 (1.80)
	1997 (2)	1.83	2.07	2.10 (1.90)
	1990 (1)	—	—	1.07 (2.08)
Pinot Noir	1991 (1)	—	—	1.07 (1.99)
	1998 (1)	—	—	3.36 (1.11)
	1996 (1)	—	—	4.21 (1.01)
Gamay	1999 (2)	0.91	2.37	1.64 (1.76)
	1998 (1)	—	—	1.27 (2.05)
Pinotage	1997 (1)	—	—	3.43 (1.55)
Sangiovese	1993 (1)	—	—	5.75 (0.87)
Tannat	1997 (1)	—	—	4.17 (1.03)

[1] Number of samples in brackets.
[2] Relative standard deviation (R.S.D.%) of four determinations of each sample.

in Portugal (Lima *et al*., 1999), 1.21 mg/L in Chile/Argentina (Goldberg *et al*., 1995b), and 2.46 mg/L in California (McMurtrey *et al*., 1994). The number of samples analysed by the authors varied from $n = 9$ (Okuda *et al*., 1996) to $n = 34$ (Lima *et al*., 1999). These differences could be attributed to environmental conditions, such as humidity and fungal disease, which are factors influencing the production of *trans*-resveratrol by grapevines (Goldberg *et al*., 1995b). Additionally, Soleas *et al*. (1997) state that comparisons between concentration values of *trans*-resveratrol have to take into account not only the statistical parameters, but also the sample preparation (direct injection or not), and the chromatographic method (GC or HPLC). As seen above, wines from California presented mean values varying from 0.132 to 2.46 mg/L.

CONCLUSIONS

In this study, we focused for the first time on the determination of *trans*-resveratrol levels in red wines produced in southern Brazil. Thirty-six commercially available red wines were analyzed and significant concentrations of this phytoalexin were found. T*rans*-resveratrol levels vary according to several factors, including ambient conditions and the analytical methodology employed. Additional and more detailed examination is required. The analytical procedure proved to be fast and effective, and no sample pre-treatment was needed.

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