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Trans-resveratrol in wines from the Canary Islands (Spain). Analysis by high performance liquid chromatography

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Abstract

Trans-resveratrol is a phytoalexin from grapes with interesting therapeutic properties. Thus considerable interest has been directed to its study. In this paper the determination of trans-resveratrol in wines from the Canary Islands by high performance liquid chromatography (HPLC), using absorbance and fluorescence detectors in series, is reported. The use of fluorimetic detection improves selectivity and sensitivity for the determination of this compound. The detection limits in real samples were 0.02 and 0.003 mg/l for absorbance and fluorescence detection, respectively. We compared the results obtained by means of both detectors, for 58 red wines from different denominations of origin, and no significant differences were observed, either between methods or among the denominations of origin. The mean level found in bottled red wines was 2.89 mg/l. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: HPLC; *Trans*-resveratrol; Wine

1. Introduction

Trans-resveratrol (3,5,4′-trihydroxystilbene) is one of the major stilbene phytoalexins produced by various families of plants, but grapes and related products are considered the most important dietary sources of these substances (Daniel, Meier, Schlatter, & Frischknecht, 1999). Phytoalexins are a group of low-molecular-mass substances with microbial inhibitory activity (Celottti, Ferrarini, Zironi, & Conte, 1996), and they are produced by the plant as a defence response to some exogenous stimuli, such a ultraviolet radiation, chemical stressors, and particularly, microbial infections (Langcake & Pryce, 1976). *Trans*-resveratrol is synthesized especially in the skin cells and is absent from or its content is low in the fruit flesh. It is present in grapes in both the *cis* and *trans* configurations, in a direct relationship to weather conditions because UV radiation favours the formation of the *cis* isomer (Siemann & Creasy, 1992).

Trans-resveratrol content depends on grape variety. Thus, it has been demonstrated that the content in red wines is much higher than in white wines, regardless of the winemaking technology applied (Mattivi, 1993; Mattivi & Nicolini, 1993; Roggero, 1996). Moreover, *trans*-resveratrol concentrations show marked fluctuations, which seem to be temperature-dependent. Wines from Italy and Spain, that are subject to warmer and drier conditions, tend to have low *trans*-resveratrol concentrations (Goldberg et al., 1995). Another possible cause of difference in *trans*-resveratrol content is the intrinsic resveratrol-synthesizing capacity of the cultivar employed (Goldberg et al., 1995). Higher contents (10 mg/l) are usually present in wines that have had prolonged contact between the must and skins, whereas lower concentrations (0.3 mg/l) are usually present in white and rose wines. Mattivi and Nicolini (1993) studied other winemaking conditions that influence *trans*-resveratrol content such as cap management systems, free-and press-run after dejuicing, carbonic maceration, hyperoxidation of musts and storage conditions.

The *trans*-resveratrol content in wine is very small, and it has no significant influence on sensory characteristics (Mattivi & Nicolini, 1993). However, its ther-

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apeutic properties are totally demonstrated, in as much as a moderate wine consumption is related to a decrease in cardiovascular diseases (St Léger, Cochrane, & Moore, 1979). So, the "French Paradox" refers to the low incidence of coronary artery disease in French people, whose notoriously high-fat diet would predispose to shorter lives and multiple bypass surgeries. Some researchers suggest that this is due to the regular consumption of a moderate amount of red wine (Bolton, 1984; Das et al., 1999; Stacchini, Draisci, & Lucentini, 1994). This protective effect has been associated with an increase in the plasma level of high-density lipoprotein (HDL)-cholesterol and a decrease in platelet activity, because of the effect of ethanol and polyphenolic components (Ruf, 1999). Due to these facts, considerable interest has been focussed on the Mediterranean diet, where grape and wine consumptions play an important role.

Trans-resveratrol has been found in red wines at 0.03–7 mg/l levels (Ribeiro de Lima et al., 1999; Romero-Pérez, Lamuela-Raventós, Waterhouse, & de la Torre-Boronat, 1996). Typical concentrations of this compound are 0.5–3 mg/l (Ribeiro de Lima et al., 1999). At the present time, there are no reports in the literature about *trans*-resveratrol content in wines from the Canary Islands.

The aim of the present study is to optimise the determination of *trans*-resveratrol in wine samples using reversed-phase liquid chromatography with two detectors in series, to apply the method to the determination of *trans*-resveratrol in wines from the Canary Islands and note any differences of content among islands or denominations of origin.

2. Materials and methods

2.1. Apparatus and materials

A Waters HPLC system was used, consisting of two pumps (Models 510), a Waters automated gradient controller (Model 680), an injector (Rheodyne Model 7125 with a 20-μl loop), a UV detector (Waters Model 486 tuneable absorbance detector) and a scanning fluorescence detector (Waters Model 470 programmable fluorescence detector). A diode-array detector from Beckman was used to check peak purity. A Baseline Workstation 810 software (Waters) and a personal computer were employed for data storage and evaluation. The analytical column was a Waters (Milford, MA, USA) Nova-Pak C18 150×3.9 mm i.d., 4 μm particle diameter. A Nova-Pak C-18 precolumn was employed as a guard column to protect the analytical column. Sep-Pak Plus C-18 cartridges (Waters) were used to prepare wine samples. Membranes (Millipore) of 0.45 μm were used to filter solutions.

2.2. Reagents and solutions

All chemicals were of analytical grade. *Trans*-resveratrol was obtained from Sigma (Sigma Chemical Co., St. Louis, MO, USA). A stock standard solution of 40 mg/l was prepared in a matrix solution (15% v/v ethanol and 3 g/l tartaric acid in water) and stored at −4 °C in the darkness. Working standard solutions were prepared by diluting the stock solution with the matrix solution. Oxygen in all solutions was eliminated with a nitrogen stream to avoid decomposition.

Methanol, acetic acid and ethanol (all HPLC grade) were obtained from Merck (Darmstadt, Germany). Ultrapure water from Milli-Q system (Millipore, Bedford, USA) with a conductivity of 18 MΩ was used throughout. Chromatographic mobile phase: methanol–acetic acid–water (10:2:88) as solvent A, and methanol–acetic acid–water (90:2:8) as solvent B.

2.3. Samples

Fifty-eight bottled red wines from the 1999 harvest, belonging to seven Denominations of Origin of the Canary Islands (Spain) were analysed: Abona (5), Valle de Güímar (9), Valle de la Orotava (11), Tacoronte-Acentejo (13), Ycoden-Daute-Isora (11), Lanzarote (6) and Gran Canaria (3). Likewise, six white wines were analysed. The grape varieties used in the elaboration were mainly "listán negro", with small amounts of "negramoll" in some cases, for red wines and "malvasía" for white wines.

2.4. Sample preparation

Cartridges were previously conditioned with 4 ml of methanol, followed by 4 ml of water. Then 5 ml of wine were introduced, next the cartridge was dried with a nitrogen gas stream and finally the compounds were eluted with 3 ml of methanol. This solution was injected into the HPLC system after filtering through a 0.45 μm membrane.

2.5. Chromatographic conditions

The chromatographic separation was carried out using a three stage linear gradient: solvent A from 100 to 85% in 15 min, from 85 to 50% in 10 min and from 50 to 30% in 9 min, with a total flow rate of 1.0 ml/min. A wavelength of $\lambda = 280$ nm was used for absorbance detector and a $\lambda_{ex} = 360$ nm and $\lambda_{em} = 374$ nm for fluorescence detector. Chromatographic peak was identified by comparing retention times of samples with that of the standard compound. Injected samples were interspersed with standards to ensure accurate quantitative analysis. Duplicate injections were performed and average peak areas were used for the quantitative

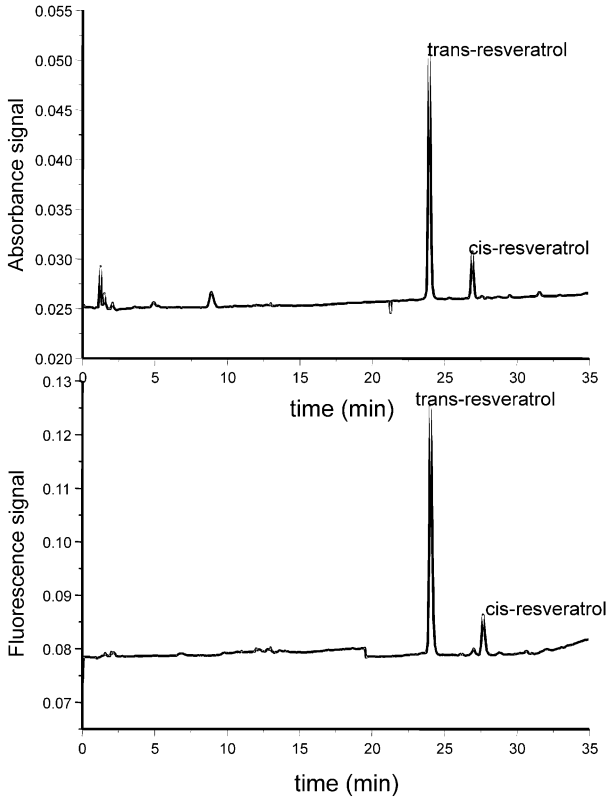

Fig. 1. Chromatograms of a standard solution of *trans*-resveratrol using absorbance and fluorescence detectors.

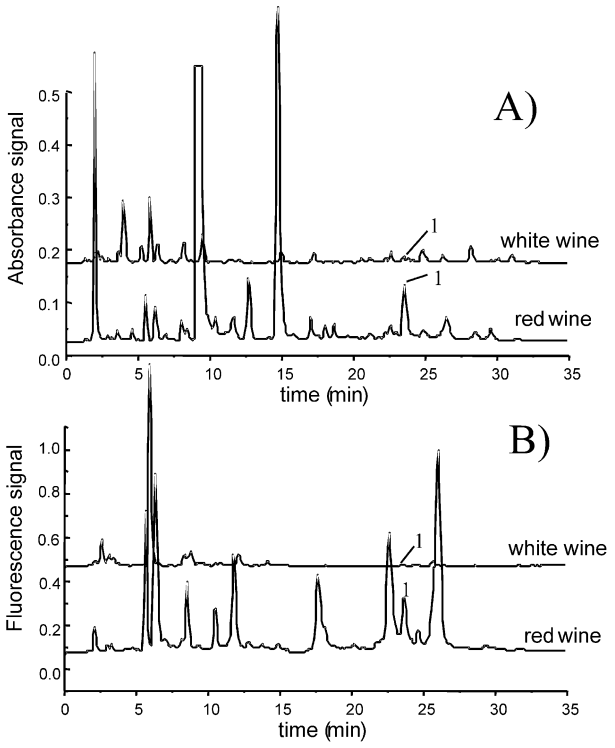

Fig. 2. Chromatograms of a red and a white wine using (A) absorbance and (B) fluorescence detectors.

analysis. Quantitation was accomplished by comparison with a standard response curve prepared from dilutions of the standard solution.

2.6. Statistics

Statistical parameters and analysis of variance (ANOVA) were performed by computer, using Statgraphics V.4 Plus for Windows from Statistical Graphics Corporation.

3. Results and discussion

The method described can be used to determine both *trans*- and *cis*-resveratrol, but we focused attention on determining the more interesting *trans*- form (Mattivi, 1993; Roggero & Archier, 1994; Siemann & Creasy, 1992). Under the experimental conditions used, the *trans*- and *cis*- isomers are well separated and no compound present in the wine interferes in the determination.

A peak in the chromatogram of wine was identified as *trans*-resveratrol, by comparison of its spectra and retention time with the standard prepared in a synthetic matrix solution of wine. Fig. 1 shows the chromatograms of the standard solution using both detectors. As can be seen, the fluorescence detector showed a much higher sensitivity than the absorbance detector. In accordance with that, the detection limit (DL) found for fluorimetric detection analysis was 0.003 mg/l, while that with UV-Vis detection was 0.02 mg/l, considering the detection limit as the analyte concentration giving a signal three times higher than the blank value (Rodríguez-Delgado, Malovanà, Pérez, Borges, & García Montelongo, 2001). These results are similar to those found by Viñas, López-Erroz, Marín-Hernández, and Hernández-Córdoba (2000).

The results of the calibration are shown in Table 1. It was performed by plotting the peak area of the standard against concentration, in the range 0.4–4.0 mg/l. Each point of calibration is the mean value from two independent area measurements. The precision of the method and the detection limits were calculated using the regression lines for the standards according to Bolton (1984). Furthermore, the values of the retention time and the areas are the same after 200 injections, showing the usefulness of the developed method for routine analysis.

The procedure was applied to a series of 58 red wines and six white wines from the Canary Islands. In Fig. 2 we present the chromatograms belonging to one red and one white wine using absorbance (A) and fluorescence (B) detectors. A considerable difference exists in *trans*-resveratrol content between red and white wines. These findings are consistent with Goldberg et al. (1995).

Table 1
Calibration results for determining *trans*-resveratrol in wines[a]

Detector	Equation	S.D.	*r*	LOD (mg/l)
Absorbance		909	0.999	0.02
	$Y = 0.8270 \times 10^5\ x - 0.0190 \times 10^5$			
Fluorescence		829	1.000	0.003
	$Y = 1.8775 \times 10^5\ x + 0.0456 \times 10^5$			

[a] S.D., standard deviation; *r*, correlation coefficient; LOD, limit of detection.

Table 2
Statistic comparison of fluorescence and UV-Vis detector systems for bottled red wines from the Canary Islands (mg/l)

	Fluorescence detector	UV-Visible detector
No. of samples	58	58
Mean value	2.89	3.03
Standard deviation	1.22	1.10
Minimum value	0.18	0.25
Maximum value	5.66	5.46

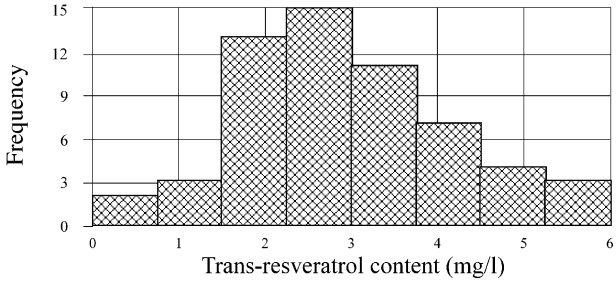

Fig. 3. Frequencies histogram of *trans*-resveratrol contents (mg/l) in bottled red wines from the Canary Islands using a flurescence detector.

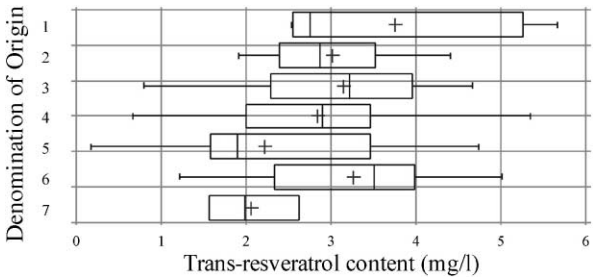

Fig. 4. Box-and-Whiskers plot of *trans*-resveratrol content (mg/l) in bottled red wines from the Canary Islands according to the Denomination of Origin using fluorescence detector (1. Abona; 2. Valle de Güímar; 3. Valle de La Orotava; 4. Tacoronte-Acentejo; 5. Ycoden-Daute-Isora; 6. Lanzarote and 7. Gran Canaria).

Table 3
Statistical summary of *trans*-resveratrol content in red wines from the Canary Islands according to the Denomination of Origin and white wines (mg/l)[a]

DOs/Type of wine	Sample No.	Mean value	S.D.	Min. value	Max. value
Abona	5	3.75	1.57	2.54	5.66
Valle de Güímar	9	3.01	0.83	1.91	4.41
Valle de La Orotava	11	3.15	1.60	0.80	4.66
Tacoronte-Acentejo	13	2.84	1.18	0.66	5.35
Ycoden-Daute-Isora	11	2.22	1.33	0.18	4.74
Lanzarote	6	3.26	1.32	1.22	5.02
Gran Canaria	3	2.06	0.54	1.56	2.63
Total red wines	58	2.89	1.22	0.18	5.66
White wines	6	0.095	0.02	0.07	0.13

[a] Results of fluorescence detector.

Table 2 shows the global results obtained for red wines with both detectors. The *t*-test, to compare the means of the two groups, indicates that there is no statistically significant difference between the fluorescence and UV-Vis detection systems (*P*-value = 0.507) at the 95% confidence level.

Fig. 3 shows the amounts of *trans*-resveratrol as classes of frequency distribution for red wines, and it can be seen that no homogeneous Gaussian distribution exists. All analyzed wines contain *trans*-resveratrol and 82% of them present a content that ranges from 1.5 to 4.5 mg/l. A summary of the results for the red wines, by denomination of origin, and white wines is presented in Table 3. Red wines have a greater content than white wines, and wines from Abona present a higher mean concentration than wines from other Denominations of Origin. The Canary Islands are characterized by a wide variety of orographical features with different microclimates (López Arias, Armas Benítez, & Criado Ortega, 1993). There are microclimate differences, not only among islands, but within the same island. Thus, in the case of Tenerife, the north of the island (Valle de La Orotava, Tacoronte-Acentejo and Ycoden-Daute-Isora) is under the influence of the Atlantic trade winds, that modify temperatures throughout the year and make that area wetter. By contrast, the south of the island (Abona) is

subject to warmer and drier weather (Rodríguez Rodríguez, 1973). But these differences in microclimate are not clear enough to differentiate the mean content in *trans*-resveratrol among the different DOs and thus, an ANOVA study shows that there are no significant differences at the 95% confidence interval in accord with the Denomination of Origin in the case of red wines. This fact is visualized when the results obtained for each Denomination of Origin are presented in the form of a Box–Whisker plot, Fig. 4. As is known, the box contains 50% of the data, those that are between the first and third quartiles, and the whisker extends up to a maximum of 1.5 times the interquartile range (Bolton, 1984). As can be seen, there is a weak different tendency in the data of DO Abona, but there is important overlapping among the boxes of the different Denominations of Origin, which explains the absence of significant differences.

The concentration of *trans*-resveratrol in our red wines is similar to that obtained by Goldberg et al. (1995) in wines produced in France (3.66 mg/l) and Central Europe (3.26 mg/l), and higher than those of wines from California (1.47 mg/l), Australia (1.47 mg/l), Italy (1.76 mg/l), Spain and Portugal (1.64 mg/l), and South America (1.21 mg/l).

4. Conclusions

The *trans*-resveratrol content in wines from the Canary Islands was determined using a highly selective and sensitive method. The DO Abona, in south Tenerife, presented the highest mean concentration of trans-resveratrol, but no significant differences were observed among the different DOs. The level of this important compound is similar to those of wines from France and Central Europe.

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