Ciência Téc. Vitiv. 18 (2), 77-91. 2003

EFFECT OF WINE STYLE AND WINEMAKING TECHNOLOGY ON RESVERATROL LEVELS IN WINES

EFEITO DO TIPO DO VINHO E DA TECNOLOGIA DE VINIFICAÇÃO NO TEOR DE RESVERATROL DOS VINHOS

Baoshan Sun[1], Cristina Ferrão[2] and M. Isabel Spranger[1]

[1]Estação Vitivinícola Nacional, Instituto Nacional de Investigação Agrária e das Pescas, 2565 - 191 DOIS PORTOS, Portugal, E-mail: inia.evn.quim@oninet.pt
[2] Student of "Universidade de Trás-os-Montes e Alto Douro" . 5001 VILA REAL. Portugal

(Manuscrito recebido em 30.12.03 . Aceite para publicação em 28.01.04.)

SUMMARY

Resveratrol (3,5,4'-trihydroxystilbene) is a stilbenic phytoalexin produced by grapevines in response to fungal infection or abiotic stress. Much interest has focused, during the last ten years, on its potent antioxidant activities, which may be associated with health benefit for moderate wine consumers. In this work, a method for analysis of *trans*- and *cis*-resveratrol of wines by HPLC was developed. Using this method, *cis*- and *trans*-resveratrol concentration in different styles of wines (white, rosé and red wines) from several regions of Portugal was determined, and the effects of different winemaking technologies (carbonic maceration, skin fermentation with stem and skin fermentation without stem) on the resveratrol content in wines made from single variety Castelão (*Vitis vinifera* L.) were studied. The developed method appeared specific, practical, sensitive and selective. Red wines present the highest *trans*- and *cis*-resveratrol concentration, followed by rosé wines, while the white wines present the lowest amount of the two isomers, indicating that the resveratrol concentration is dependent on the wine style, which would be associated essentially with maceration of grape solids. The winemaking technology also affects the resveratrol content in wines. Resveratrol concentrations in the wines made by traditional winemaking technologies (fermentation with stems and fermentation without stems) were significantly higher than those in wines made by carbonic maceration. However, there was no significant difference in resveratrol concentrations between stem-contact wines and non stem-contact wine, suggesting that the stems contribute little resveratrol to wines. In addition, prolonged maceration after alcoholic fermentation did not affect the resveratrol concentrations in wines, suggesting that the extraction of resveratrol was complete during or at the end of alcoholic fermentation.

Key words: Resveratrol, wine, HPLC, winemaking technology.
Palavras chave: Resveratrol, vinho, HPLC, tecnologia de vinificação.

INTRODUCTION

Many epidemiological studies have shown that moderate red wine consumption was associated with a decreased risk of cardiovascular diseases. The key components responsible for these beneficial effects have been hypothesized to be some bioactive phenolic compounds present in red wine. Stilbenes, including essentially resveratrol and its glucosides, are one of the most important groups of such phenolic compounds, which have been acclaimed to possess various potent biological activities (Kitanaka *et al.*, 1990; Chung *et al.*, 1992; Frankel *et al.*, 1993; Blond *et al.*, 1995; Bravo Abad, 1996; Fauconneau *et al.*, 1997; Jang *et al.*, 1997; Breuil *et al.*, 1999).

The occurrence of stilbenes in plant tissues is associated to the resistance of plant against fungal diseases such as *Botrytis cinerea*, although they can also occur to abiotic stress, such as UV irradiation. Generally, stilbenes are considered as phytoalexins, and their formation in grape leaves has been correlated with disease resistance (Langcake and McCarthy, 1979; Langcake, 1981). Phytoalexins are a group of plant chemicals of low molecular mass which are inhibitory to microorganisms and their accumulation in plants is initiated by interaction of the plant with microorganisms.

In grape cluster, stilbenes are presented essentially in grape skin and mainly in glucosylated form (Creasy and Coffee, 1988, Roggero and Garcia-Parrilla, 1995). These compounds were also reported to be present in grape seeds (Pezet and Cuenat, 1996) and grape stems (Bavaresco *et al.*, 1997). Resveratrol - the major stilbene of grapes, may present in two isomeric forms, but only its *trans*-isomer has been identified in *Vitis vinifera* grapes (Langcake and Pryce, 1976; Jeandet *et al.*, 1991; Vrhovsek *et al.*, 1997). In wines, both *cis*- and *trans*-resveratrol were detected, with the latter predominant (Lamuela-Raventos *et al.*, 1995; Adrian *et al.*, 2000). The presence of *cis*-resveratrol in wines was generally considered to be due to the photochemical isomerization of partial *trans*- form during winemaking process (Jeandet *et al.*, 1995; Roggero and Garcia-Parrilla, 1995). However, some authors suggested that *cis*-resveratrol might be present in grapes in combined form which could be liberated by hydrolysis during fermentation/maceration process of winemaking (Mattivi *et al.*, 1995; Vrhovsek *et al.*, 1997).

The concentration of stilbenes in wines is generally much lower than other phenolic compounds such as catechins, proanthocyanidins and anthocyanins. Goldberg *et al.* (1996) analysed *cis*- and *trans*-resveratrol and their glucosides in nearly 700 commercial red wines from most of the world's areas of production using direct-injection HPLC method. The concentrations of both resveratrol isomers in majority of red wines analysed were from 5 to 13 μmol/dm^3 (*i.e.* 1,14 to 2,96 mg/dm^3), and the concentrations of both glucosides, from 2 to 10 μmol/ dm^3 (*i.e.* 0,78 to 3,90 mg/dm^3). Furthermore, the rosé wines and white

wines contain much lower amounts of stilbenes than red wines (Romero-Pérez *et al.*, 1996a, 1996b). The *trans*-resveratrol concentrations in most white wines analysed by Siemann and Creasy (1992) were less than 0,03 mg/dm^3. This would indicate that for white and rosé wines, and may be for some red wines, direct injection HPLC method for stibenes is often not valid and thus extraction and pre-concentration of sample prior to HPLC analysis become necessary to ensure its accurate quantification. According to our knowledge, there has been no validated method for this purpose.

The different vinification techniques also affect stilbene contents in wines. Vrhovsek *et al.* (1997) have reported that the yeasts with higher β-glucosidase activity significantly increased the concentrations of *cis*- and *trans*-resveratrol and decreased the concentration of *trans*-resveratrol glucoside in wines; fining treatments with PVPP greatly reduced its resveratrol concentration. More recently, Threlfall *et al.* (1999) studied the effects of grape variety, UV light exposure, enzyme addition, skin contact time, fining agents, carbon and PVPP on resveratrol level of wine. They found that resveratrol levels in wines differed according to variety. UV light exposure of grape clusters and also enzyme addition might increase significantly the resveratrol levels in some wines but did not affect those of other wines. Fining agents, carbon and PVPP, generally reduced the resveratrol level of wine. These authors also found that skin contact time could affect resveratrol extraction but the maximum extraction time was dependent on grape variety. However, since the composition of grapes and winemaking technology are complex, the information of many aspects of winemaking (such as different vinification technologies), which may affect stilbene levels in wine, remained very limited.

Thus, the main objective of this study was to quantify of *cis*- and *trans*-resveratrol in some red wines, rosé wines and white wines originated from Portugal and to verify the effects of different winemaking technologies (skin fermentation with stem-contact, skin fermentation without stem-contact and carbonic maceration) on *cis*- and *trans*-resveratrol contents in a monovarietal red wine, based on one easy, rapid, sensitive and selective HPLC method.

MATERIALS AND METHODS

Materials

Trans-resveratrol was purchased from Sigma Chemical Company (St Louis, MO, USA). *Cis*-resveratrol was obtained by isomerization of *trans*-resveratrol at 254 nm (Goldberg *et al.*, 1995). The C_{18} and tC_{18} Sep-Pak cartridges were purchased from Waters Associates (Bedford, MA, USA). All solvents used were analytical or HPLC grade.

Wines

Several monovarietal (*Vitis vinifera* L.) red wines (Tinta Miúda, Cabernet

Sauvignon, Merlot) and white wines (Fernão Pires, Chardonnay) harvested in different years and/or from different regions of Portugal were produced in the Estação Vitivinícola Nacional - Instituto Nacional de Investigação Agrária e das Pescas (Quinta d'Almoinha, Dois Portos, Portugal) in 1999 and 2000 by traditional winemaking technologies. Rosé wines (Uva do Monte, Robusto, Arieno, Sevares, Casaleiro) were purchased from local supermarket.

Sample preparation

Preliminary assays were done for comparing the two techniques of sample preparation: liquid-liquid extraction and solid-phase extraction.

For liquid-liquid extraction, two solvents were separately tested: diethyl ether and ethyl acetate. Furthermore, one volume of wine (20 cm^3 for red wines, 75 cm^3 for rosé wines and 150 cm^3 for white wines) was sequentially extracted for 3-fold with two volume of diethyl ether (Procedure 1) or ethyl acetate (Procedure 2) at room temperature. The combined organic phases from each procedure were evaporated to dryness at less than 30 °C and the residue was recovered by 2 cm^3 of 50% ethanol in water, filtered by a 0,45 micron PVDF filter (Titan, Scientific Resources Inc, NJ, USA), followed by HPLC analysis.

For solid-phase extraction (SPE), the two Sep-Pak cartridges connected in series (the superior one is tC_{18} Sep-Pak and the inferior is C_{18} Sep-Pak) were used as stationary phase and two elution solvents were chosen: diethyl ether and ethyl acetate. The procedure of pre-condition of cartridges, loading sample and eliminating phenolic acids were identical as already described (Sun *et al.*, 1998). After drying the cartridges, elution was carried out with 25 cm^3 of diethyl ether (Procedure 3) or with 25 cm^3 of ethyl acetate (Procedure 4). The eluent was evaporated to dryness at less than 30 °C and the residue was recovered by 2 cm^3 of 50% ethanol in water, followed by HPLC analysis.

HPLC analysis

The HPLC apparatus was a Waters HPLC system, equipped with a quaternary pump (Waters 626), a dual λ absorbance detector (Waters 2487) coupled to a data processing computer (Millennium 32) and a manual injection valve. The column (250 × 4 mm) was a cartridge of 5-μm Lichrospher 100 RP 18 (Merck, Darmstadt, Germany). The optimised chromatographic conditions were as follows: flow rate was fixed at 1,0 cm^3/min; injection volume was 0,03 cm^3; detection was at 285 nm for *cis*-resveratrol and at 307 nm for *trans*-resveratrol; column temperature was 30 °C; gradient elution from 5% acetonitrile in water (A) to 75% acetonitrile in water (B) during 35 min was used, followed by washing and re-equilibrating column to initial condition.

Different winemaking technologies

In order to study the effects of different winemaking technologies on resveratrol content in wines, monovarietal wines were made from Castelão (*Vitis vinifera*

L.) grapes. Microvinifications were performed in duplicate, at the winery of the Estação Vitivinícola Nacional - Instituto Nacional de Investigação Agrária e das Pescas-(Quinta d'Almoinha, Dois Portos, Portugal), by the following vinification techniques:

(1). *Carbonic maceration* Two lots of 33 kg of grape clusters were used for preparation of two carbonic maceration wines with 21 days of maceration time at 25 °C and 35 °C, respectively. In other words, 3 kg of grape clusters of each lot were crushed and put at the bottom of 60 dm^3 stainless steel tank and sulfited with sulfur dioxide (80 mg/dm^3). Then the rest 30 kg of the entire grape cluster was carefully added in the same tank. One tank is stored at 25 °C and another one at 35 °C, both under CO_2 atmosphere. After 21 days of intracellular fermentation/maceration (density = 1013), the mash was pressed. Free-run and press wines were combined, collected in the tank and stored at 25° to undergo extracellular fermentation. After 7 days (density = 1003) when alcoholic fermentation was finished, sulfur dioxide (40 mg/dm^3) was added and the wines stored in 20 dm^3 vessels at room temperature. After one month of conservation, the wines were racked, sulfited with sulfur dioxide (30 mg/dm^3) and stored at room temperature. Second racking was performed after three months and the wines were sulfited with sulfur dioxide (45 mg/dm^3) before bottling. The wines were stored at room temperature for another two months prior to analysis.

(2). *Skin Fermentation with stem.* Two lots of 50 kg of grape clusters were used for preparation of two stem-contact wines at 25 °C, with 7 days and 21 days of maceration time, respectively. The operating procedure was described as follows. The two lots of grape clusters were crushed using a destemmer-crusher (Gandra, Vila Nova de Famalicão, Portugal) and collected respectively in 60 dm^3 stainless steel tanks. Both lots were treated with sulfur dioxide (80 mg/dm^3) prior to undergoing skin fermentation at 25 °C. The cap was punched down two times daily until it remained submerged. After 7 days of maceration when alcoholic fermentation was finished, the mash of one lot was pressed, and its free-run and press wines were combined and stored in 20 dm^3 vessels at room temperature, while another lot continuously underwent maceration until 21 days followed by pressing the mash and collecting its free-run and press wines together in 20 dm^3 vessels. After one month of conservation of each lot, the wines were racked, sulfited with sulfur dioxide (40 mg/dm^3) and stored at room temperature. Second racking was performed after three months and sulfur dioxide (40 mg/dm^3) was added before bottling. The bottled wines were stored at room temperature for another two month prior to analysis.

(3). *Skin Fermentation without stem.* One lot of 50 kg of grape clusters were used for preparation of non stem-contact wines at 25 °C, with 7 days of maceration. Thus, 50 kg grape clusters were crushed and destemmed using a

destemmer-crusher (Gandra, Vila Nova de Famalicão, Portugal) and collected in 60 dm^3 stainless steel tanks and then were sulfited with sulfur dioxide (80 mg/dm^3) prior to undergoing skin fermentation at 25 °C. The cap was punched down two times daily until it remained submerged. After 7 days of maceration when alcoholic fermentation was finished, the mash was pressed. Free-run and press wines were combined and stored in 20 dm^3 vessels at room temperature. After one month of conservation, the wine was racked, sulfited with sulfur dioxide (40 mg/dm^3) and stored at room temperature. Second racking was performed after three months and sulfur dioxide (40 mg/dm^3) was added before bottling. The bottled wines were stored at room temperature for another two month prior to analysis.

Statistical Analysis

All analyses were performed in duplicate or triplicate and the data are presented as mean ± SD. Analysis of variance and comparison of means (LSD, 5% level) were carried out using Statgraphic 5.0 v. (STSC Inc., Rockville, MD).

RESULTS AND DISCUSSION

Sample preparation and HPLC analysis

In many cases, direct-injection HPLC does not permit quantitative analysis of *cis*- and *trans*-resveratrol in wines, particularly white and rosé wines, owing to very low amounts of such compounds. Thus, extraction and pre-concentration of sample prior to HPLC analysis is necessary to ensure its accurate quantification.

In this work, we compared the different procedures for extraction and pre-concentration of the wine resveratrol prior to HPLC analysis: liquid-liquid extraction with diethyl ether (Procedure 1); liquid-liquid extraction with ethyl acetate (Procedure 2); solid phase extraction on C_{18} cartridge using diethyl ether as eluant (Procedure 3); solid phase extraction on C_{18} cartridge using ethyl acetate as eluant (Procedure 4). The efficiencies of these sample preparation methods were verified by HPLC analysis under the optimised elution conditions as described in Materials and Methods. These results were presented in Table 1.

The methods of liquid-liquid extraction have the advantages over those of solid phase extraction as follows: linearity, precision and accuracy. In addition, the liquid-liquid extraction is also easy and rapid and each manipulation could prepare various samples (n = 5 – 10), ready for HPLC analysis. On the other hand, the method of liquid-liquid extraction with diethyl ether has better selectivity than that with ethyl acetate. Furthermore, diethyl ether extracted, in addition to *cis*-and *trans*-resveratrol, only several other monomeric phenols

Table 1

Efficiency of different sample preparation procedures followed by HPLC analysis of *cis*- and *trans*-resveratrol in wine

Eficiência de diferentes processos de preparation da amostra seguido pela análise HPLC do cis- e trans-resveratrol do vinho

	Procedure 1		Procedure 2		Procedure 3		Procedure 4	
	t-resv	*c*-resv	*t*-resv	*c*-resv	*t*-resv	*c*-resv	*t*-resv	*c*-resv
Repeatability (variation coefficient) (%)*	1.7	7.3	2.5	6.1	6.4	11.0	3.7	10.2
Extraction efficiency (%)**	102.0 ± 5.7	116.0 ± 10.5	106.8 ± 5.5	112.6 ± 9.1	63.7 ± 5.0	76.5 ± 9.3	64.6 ± 3.8	69.4 ± 8.0

Abbreviation: *t*-resv = *trans*-resveratrol; *c*-resv = *cis*-resveratrol
*Values obtained by 10 replications of the same wine.
**Mean value ± SD (n = 5).

such as catechins and gallic acid of wines, whereas ethyl acetate also extracted many other phenolic compounds such as oligomeric procyanidins, piceid, astringin and low amount of anthocyanins. In consequence, using diethyl ether liquid-liquid extraction method, the extracts of wine samples can be much concentrated prior to HPLC analysis, and thus permit quantifying the wines with trace amount of *cis*- and *trans*-resveratrol. However, in many cases, liquid-liquid extraction with ethyl acetate can also be used as sample preparation method. Ethyl acetate also presented high extraction efficiency for both resveratrol isomers (106.8±5.5 for *trans*-resveratrol and 112.6±9.1 for *cis*-resveratrol), If not only *cis*- and *trans*- resveratrol, but also other low-molecular phenolic compounds are interested, such as resveratrol glucosides and oligomer procyanidins, the liquid-liquid extraction with ethyl acetate should be used, instead of liquid-liquid extraction with diethyl ether. Figure 1 presented typical HPLC chromagrams of red wine extracts recorded at 307nm (λ_{max} of *trans*-resveratrol) and 285nm (λ_{max} of *cis*-resveratrol), following sample preparation by liquid-liquid extraction with diethyl ether.

Thus, according to these preliminary assays, only the method of liquid-liquid extraction with diethyl ether followed by HPLC analysis was selected and was further validated according to Bouvier (1994) and Monteiro and Bertrand (1994). The detail procedure of the method validation was presented previously (Ferrão, 1992). Here, only several characteristics of this method are summarised as follows:

(1) Practicability – the method is practical. Only routine-laboratory reagents and equipment are needed. The sample preparation is easy and rapid, and each manipulation permits preparing 5 to 6 samples. Each HPLC analysis requires only about 55 minutes.

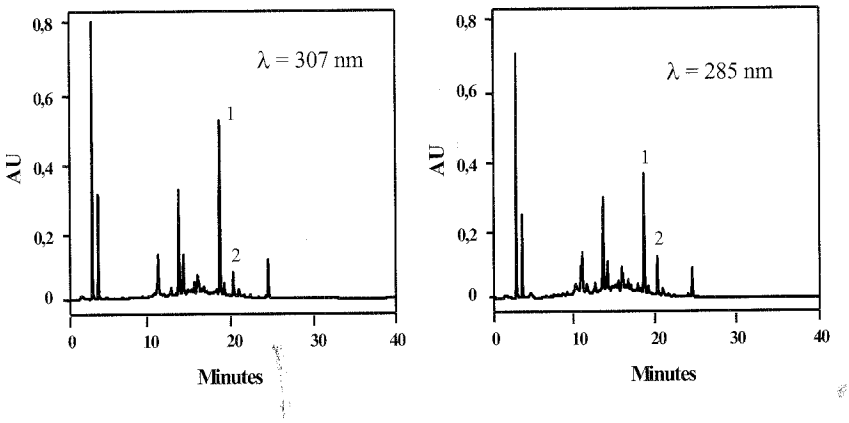

Figure 1 – Typical HPLC chromatogram of red wine extract recorded at 307nm (λ_{max} of *trans*-resveratrol) and 285nm (λ_{max} of *cis*-resveratrol), following sample preparation by liquid-liquid extraction with diethyl ether. 1 – *trans*-resveratrol; 2- *cis*-resveratrol.

Cromatograma tipo em HPLC de extracto de vinho tinto a 307nm (λ_{max} de trans-resveratrol) e 285nm (λ_{max} de cis-resveratrol) após a preparação da amostra por extracção Líquido-líquido com dietileter. 1 – trans-resveratrol; 2- cis-resveratrol.

(2) Quantification – the linearity is excellent in the range of 0 – 40 mg/dm^3 for *trans*-resveratrol (calibration curve: y = 256,61x + 9,8887; r = 0,9993) and in the range of 0 – 20 mg/dm^3 for *cis*-resveratrol (calibration curve: y = 160,73x – 27,446; r = 0,9996), which satisfy the following statistical criteria: the slope is significantly different from zero, the intercept is not significantly different from zero, the correlation coefficient r > 0,999 and the residual variance due to the adjustment error is not significant (P = 99,5%). The limit of detection is 0,006 mg/L for *trans*-resveratrol and 0,021 mg/dm^3 for *cis*-resveratrol, and the limit of quantification is 0,02 mg/dm^3 for *trans*-resveratrol and 0,07 mg/dm^3 for *cis*-resveratrol;

(3) Specificity and selectivity – This was evaluated by spiking the same wine with each of five increasing concentrations of standards solutions within the concentration range, in duplicate. The percentage of recovery varies from 96% to 107% for *trans*-resveratrol and from 106% to 126% for *cis*-resveratrol.

(4) Repeatability – the variation coefficient of method is 1,7% for *trans*-resveratrol and 7,3% for *cis*-resveratrol.

These results indicate that this method is valid for analysis of *cis*- and *trans*-resveratrol in wines, although the value of the percentage of recovery for *cis*-resveratrol was a little high. Furthermore, this high percentage of recovery may probably due to high instability of *trans*-resveratrol, easily isomerised to

its *cis*- form which may occur during analysis process. Due to high selectivity of diethyl ether for resveratrol, the method could be used especially for wines poor in resveratrol (rosé wines, white wines, and some red wines).

Resveratrol content in some white, rosé and red wines

Using the validated method, *i.e.,* liquid-liquid extraction with diethyl ether followed by HPLC analysis, *cis*-and *trans*-resveratrol contents in some white, rosé and red wines were quantified. These results are presented in Table 2.

Table 2

Cis- and *trans*-resveratrol contents in some white, rosé and red wines (mean value ± SD)

Teores em cis- e trans-resveratrol nos alguns vinhos brancos, rosés e tintos (valor media ± DP)

	Name	Region	Vintage	Resveratrol concentration (μg/dm^3)			
				Trans-resveratrol	*Cis*-resveratrol	Total	Trans / cis
White wine	Fernão Pires	Estremadura	1997	21.6 ± 1.9	26.9 ± 4.0	48.5 ± 4.4	0.8 ± 0.2
	Fernão Pires	Estremadura	1998	24.3 ± 2.5	20.7 ± 1.9	45.0 ± 3.1	1.2 ± 0.1
	Fernão Pires	Estremadura	1999	34.7 ± 0.2	18.8 ± 0.1	53.5 ± 0.2	1.8 ± 0.0
	Chardonnay	Estremadura	1999	21.9 ± 2.9	11.4 ± 4.7	33.3 ± 5.5	1.9 ± 0.4
Rosé wine	Mateus	Bairrada	2000	103.7 ± 0.7	30.4 ± 0.1	134.1 ± 0.7	3.4 ± 0.0
	Uva da Monte	Estremadura	2000	47.6 ± 7.6	38.6 ± 0.6	86.2 ± 7.6	1.2 ± 0.2
	Robusto	Estremadura	2000	151.4 ± 9.4	96.9 ± 8.8	248.3 ± 12.9	1.6 ± 0.1
	Arieno	Estremadura	2000	78.2 ± 0.2	46.4 ± 7.3	124.6 ± 7.3	1.7 ± 0.2
	Sevares	Ribatejo	2000	46.9 ± 2.3	75.0 ± 6.3	121.9 ± 6.7	0.6 ± 0.1
	Casaleiro	Ribatejo	2000	74.5 ± 1.0	52.2 ± 1.3	126.7 ± 1.6	1.4 ± 0.0
Red wine	Tinta Miúda	Península de Setúbal	1999	18825.1 ± 342.2	6105.3 ± 447.3	24930.4 ± 563.2	3.1 ± 0.1
	Tinta Miúda	Península de Setúbal	2000	5291.3 ± 158.7	2267.3 ± 113.4	7558.6 ± 195.1	2.3 ± 0.1
	Cabernet Sauvignon	Ribatejo	2000	895.2 ± 24.2	402.3 ± 16.1	1297.5 ± 29.1	2.2 ± 0.0
	Cabernet Sauvignon	Península de Setúbal	2000	526.7 ± 26.3	441.5 ± 13.3	968.2 ± 29.5	1.2 ± 0.1
	Merlot	Península de Setúbal	1999	2633.8 ± 52.8	1089.1 ± 65.5	3722.9 ± 84.1	2.4 ± 0.1
	Merlot	Península de Setúbal	2000	7323.6 ± 75.8	2842.3 ± 198.9	10165.9 ± 212.9	2.6 ± 0.1

As expected, the concentrations of resveratrol isomers in red wines are much higher than those in rosé wines, while only trace amounts of such compounds were found in the white wines (Table 2). In all wines analysed except for a Fernão Pires (1997 harvest) white wine and a Sevares (2000 harvest) rosé wine, *trans*-resveratrol presented higher contents than its isomer. It should be noted that the *cis*-resveratrol concentrations in majority of white and rosé wines analysed were inferior to that of limit of quantification (70 μg/dm^3), indicating that direct injection of these wines to HPLC for analysing their *cis*-resveratrol content is impossible. In other words, the analysis of these white and rosé wines could be realised (Table 2) due to pre-extraction (liquid-liquid

extraction with diethyl ether) and concentration of the samples (75 times for white wines and nearly 40 times for rosé wines) before their injection to HPLC.

It should especially be mentioned that the wine made with Tinta Miúda grapes harvested in 1999 present the highest amount of resveratrol (24,9 ± 0.6 mg/ dm^3). This value is even higher than the highest values reported until now by various authors (Lamuela-Raventos *et al.*, 1995; Goldberg *et al.*, 1996; Adrian *et al.*, 2000). Although the resveratrol concentration in wine made with Tinta Miúda grapes harvested in 2000 is much lower (7,6 ± 0.2 mg/dm^3) than that harvested in 1999, this value is also higher than that of nearly all other wines analysed (except for Merlot red wine, 2000 harvest) or even higher than other reported wines of various origin (Goldberg *et al.*, 1996).

Tinta Miúda is a traditional Portuguese grapevine variety. Although this variety is generally difficult to ripen, it is often used, together with other varieties, to make high-quality red wines. It has been found that the Tinta Miúda red wines made both by traditional winemaking technologies or by carbonic maceration contained high concentrations of bioactve phenolic compounds - catechins and proanthocyanidins (Sun *et al.*, 2001). The fact that this wine is also rich in resveratrol, a potent antioxidant, indicate that the Tinta Miúda red wine would be a potential bioactive phenolic-rich wine or healthy wine.

Effect of different winemaking technologies on resveratrol content in wine

Figure 2 shows the effect of various winemaking technologies on resveratrol content in Castelão red wines.

According to various published works, winemaking technology affected considerably the contents of phenolic compounds in wines. Sun et al. (2001) studied the effect of different winemaking technologies on phenolic composition in Tinta Miúda red wines. According to this work, the wine made by carbonic maceration contained highest amounts of both catechins, oligomeric and polymeric proanthocyanidins, followed by the wine made by fermentation with stem contact, whereas the wine made by fermentation without stem contact contained the lowest of these compounds; on the contrary, the concentrations of total anthocyanins and nearly all individual anthocyanins in carbonic maceration wines were lower than in skin fermentation wines.

It has been reported that catechins and proanthocyanidins in grape stem were easily transferred into wine during fermentation/maceration (Sun *et al.*, 1999) and thus stem-contact wine contained much higher contents of these compounds that non stem-contact wine (Sun *et al.*, 1999, 2001). Bavaresco *et al.* (1997) reported that grape stem contained considerable amount of resveratrol. Thus, it is reasonable to suggest that for a given grape variety, wine made by

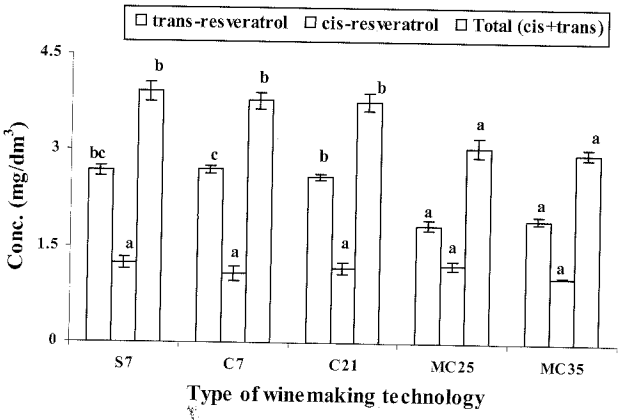

Figure 2 – Effect of winemaking technology on resveratrol concentration in Castelão red wine. C7 = stem-contact wine with 7 day's maceration; S7 = non stem-contact wine with 7 day's maceration; C21 = stem-contact wine with 21 day's maceration; MC25 and MC35 = wines made by carbonic maceration at 25 °C and 35 °C, respectively.

Efeito da tecnologia de fermentação da casta Castelão na concentração em resveratrol dos vinhos. C7 = sem desengace com 7 dias de maceração; S7 = com desengace e 7 dias de maceração; C21 = sem desengace com 21 dias de maceração; MC25 e MC35 = vinhos de maceração carbónica a 25 °C e 35 °C.

fermentation with stem would contain higher concentration of resveratrol than that made by fermentation without stem. However, in this work, there was no significant difference between total resveratrol concentration in stem-contact wine and that in non stem-contact wine (Figure 2). These results would indicate that, although grape stem contain high amount of resveratrol, it contribute little resveratrol to wine. This may be explained by the low transfer rate of this compound from grape stem to wine during vinification. It can further suggest that resveratrol might be present in stem tissue in strongly combined form. This also support that grape skin is the major source of resveratrol in wine (Creasy and Coffee, 1988).

It can be seen from Figure 2 that increasing maceration time of the wine made by skin fermentation with stem does not affect the concentration of total resveratrol, suggesting that the extraction of this compound during alcoholic fermentation was complete. This may be explained by the fact that the major source of resveratrol is the soft tissue - grape skins, which is easily extracted.

As compared with the wines made by traditional winemaking technologies (skin fermentation with stem and skin fermentation without stem), carbonic maceration wines presented lower concentration of total resveratrol (Figure 2), indicating that this technology did not favor extracting this compound. In addition, variation of carbonic maceration temperature did not affect significantly *cis*-, *trans*- and total resveratrol contents.

Finally, it is interesting to note that although there is significant difference in *trans*-resveratrol concentration between the traditional and carbonic maceration wines, there is no significant difference in *cis*-resveratrol concentration among all these wines. This would indicate that *cis*-resveratrol concentration in wine might be independent on the winemaking technology used.

CONCLUSIONS

The proposed method for quantification of *cis*- and *trans*-reveratrol in wines, *i.e.*, liquid-liquid extraction with diethyl ether followed by HPLC analysis, is simple, rapid, sensitive and selective, which is particularly suitable for wines with trace amount of *cis*- and *trans*-resveratrol, such as white wines and rosé wines. However, if other phenolic compounds, such as oligomer procyanidins, piceid and astringin, are also interested, sample preparation by liquid-liquid extraction with ethyl acetate might be selected.

For all wines analyzed, red wines contained much higher concentration of both *trans*- and *cis*-resveratrol than rosé wines, while only trace amounts of such compounds were found in the white wines. It is surprisingly found that Tinta Miúda red wine (1999 harvest) contained very high concentration of resveratrol, i.e., 24,9 $\pm$ 0.6 mg/dm^3. This value is even higher than the highest values in wines reported before.

The wines made by traditional winemaking technologies (skin fermentation with stem and skin fermentation without stem) contained higher levels of total resveratrol than those made by carbonic maceration technique. Prolonged maceration after alcoholic fermentation did not affect the total resveratrol concentration in finished wines, suggesting that the extraction of resveratrol from grape skins should be complete before or at the end of alcoholic fermentation. Unexpectedly, grape stem did not contribute significantly the resveratrol to wines.

RESUMO

Efeito do tipo do vinho e da tecnologia de vinificação no teor de resveratrol dos vinhos

O resveratrol (3,5,4' – tri-hidroxiestilbeno) é uma fitoalexina, produzida pela videira como resposta à infecção fúngica ou ao *stress* abiótico. Durante os últimos dez anos, acentuou-se o interesse pelas suas actividades antioxidantes, provavelmente associado ao benefício que o consumo moderado de vinho pode trazer à saúde humana. No presente trabalho, foi desenvolvido um método para a análise do *cis*- e *trans*-resveratrol do vinho por HPLC foi desenvolvido. Com base nesse método, efectuou-se a quantificação do resveratrol nos três tipos de vinhos (vinhos brancos, vinhos rosés e vinhos tintos) oriundo de diversos regiões de Portugal, e estudou-se os efeitos de diferentes tecnologias de vinificação (maceração carbónica, curtimenta com engaço e curtimenta sem engaço) sobre o teor de resveratrol em vinhos da casta Castelão (*Vitis vinifera* L.). O método desenvolvido demonstrou ser prático, sensível, selectivo e específico. Os vinhos tintos apresentaram

teores de resveratrol mais elevados, seguidos pelos vinhos rosés, enquanto os vinhos brancos apresentavam os menores teores destes compostos. Este resultado indica que os teores de resveratrol dependem do tipo de vinho, o que pode ser associado principalmente ao tempo de maceração. O tipo de tecnologias de vinificação também afectam a concentração deste composto no vinho. As concentrações do resveratrol nos vinhos obtidos por vinificação clássica (curtimenta com ou sem engaço) são mais elevadas do que nos vinhos de maceração carbónica. Contudo, não é observada diferença significativa entre os teores de resveratrol de vinhos de curtimenta com engaço e os vinhos de curtimenta sem engaço, o que parece indicar que os engaços pouco contribuem para o teor de resveratrol dos vinhos. Por outro lado, o aumento do tempo de maceração após a fermentação alcoólica não influencía a concentração deste composto no vinho, sugerindo que a extração do resveratrol se completou durante fermentação alcoólica.

RÉSUMÉ

Influence du type de vin et de la technologie de vinification sur la concentration du resveratrol dans les vins

Le resvératrol (3,5,4'-trihydroxystilbène) est une phytoalexine stilbènique produisée par la vigne à la réponse à l'infection fongique ou *stress* abiotique. Beaucoup d'intérêt a été concentré, pendant la dernière dix années, sur ses activités antioxydants, qui pourrait être associées au fait que la consumation modérée du vin é favorable à la santé humane. Dans le présent travail, une méthode modifiée pour l'analyse de *cis*- et *trans*-resvératrol du vin par HPLC a été développée. Par cette méthode, *cis*- et *trans*-resvératrol dans les différents types du vin (Vins blancs, vins rosés et vins rouges) de divers régions du Portugal a été dosée, et les effets de différentes technologies de vinification (macération carbonique, fermentation avec rafles et fermentation sans rafles) sur la concentration de resvératrol dans les vins du cépage monovariétal – Castelão (*Vitis vinifera* L.) ont été étudiés. La méthode développée paraissait spécifique, pratique, sensitive et selective. Les vins rouges présentent la teneur en *cis*- et *trans*-resvératrol le plus élevée que les vins rosés, tandis que les vins blancs présentent une concentration de *cis*- et *trans*-resvératrol le plus basse. Ce résultat indique que la teneur en resvératrol dépend du type de vin, qui pourrait être associés essentiellement avec le temps de la macération. Le type de technologies de vinification influence aussi la teneur en resvératrol dans les vins. Les concentrations du resvératrol dans les vins faite par vinification classique (fermentation avec ou sans rafles) étaient significativement plus élevées que celles dans les vins de macération carbonique. Cependant, la différence significative en concentration du resvératrol entre les vins faites par fermentation avec rafles et les vins faites par fermentation sans rafles n'été pas observée, suggérant que les rafles contribuent peu avec resvératrol pour les vins. De plus, prolongation du temps de macération après la fermentation alcoolique n'influence pas la teneur en resvératrol dans les vins faites par fermentation avec rafles, suggérant que l'extraction du resvératrol avait été complète pendant la fermentation alcoolique.

REFERENCES

Adrian M., Jeandet P., Breuil A.C., Levite D., Debord S., Bessis R., 2000. Assay of resveratrol and derivative stilbenes in wines by direct injection High Performance Liquid Chromatography. *Am. J. Enol. Vitic.*, **51:** 37-41.

Bavaresco, L., Cantù E., Fregoni M., Trevisan, M., 1997. Constitutive stilbene contents of grapevine cluster stems as potencial source of resveratrol in wine. *Vitis*, **36**: 115-118.

Blond J.P., Denis M.P., Bezard J., 1995. Antioxidant action of resveratrol in lipid peroxidation. *Sci. Aliments*, **15**: 347-358.

Bouvier M., 1994. Guide d'évaluation et de la validation d'une méthode d'analyse chimique. INRA CEE, 1990. *Official Journal of the European Communities*, L272, volume 33, 3 October, 84-87.

Bravo Abad, E., 1996. Resveratrol in wine: contribution to potential cardiovascular protective activity. *Alimentaria*, **269**: 71-72.

Breuil A.C., Loreau N., 1999. Comparative efficacy of resveratrol and its derivatives with respect to antioxidant activity as assessed using human low density lipoproteins and blood hemolysis. Presented at the "Polyphenols, Wine, Health" Congress, Bordeaux, France.

Chung M.I., Teng C.M., Cheng K.L., Ko F.N., Lin C.N., 1992. An antiplatelet principle of *Veratrum formosanum*. *Planta Med.*, **58**: 274-276.

Creasy L.L., Coffee M., 1988. Phytoalexin production potencial of grape berries. *J. Am. Soc. Hortic. Sci.*, **113**: 230-234.

Fauconneau B., Waffo Teguo P., Huguet F., Barrier L., Decendit A., Merillon J.M., 1997. Scavenger and antioxidant properties of astringin and phenolic compounds extracted from *Vitis vinifera* cell cultures. *Life Sci.*, **61**: 2103-2110.

Ferrão C.C.A., 2002. Pesquisa de resveratrol e outros derivados estilbénicos em vinhos. Optimização de um méthodo de doseamento por HPLC. *Relatório Final de Estágio de Licenciatura em Enologia.* Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal.

Frankel E.N., Waterhouse A.L., Kinsella J.E., 1993. Inhibition of human LDL oxidation by resveratrol. *The Lancet*, **341**: 1103-1104.

Goldberg D.M., Karumanchiri A., Ng E., Yan J., Diamandis E.P., Soleas G., 1995. Direct gas chromatographic-mass spectrometric method to assay *cis*-resveratrol in wines: preliminary survey of its concentration in commercial wines. *J. Agric. Food Chem.*, **43**: 1245-1250.

Goldberg D. M., Ng E., Karumanchiri A., Diamandis E.P., Soleas G.J., 1996. Resveratrol glucosides are important components of commercial wines. *Am. J. Enol. Vitic.* **47**: 415 – 420.

Jang, M., Cai L., Udeani G.O., Slowing K.V., Thomas C.F., Beecher C.W.W., Fong H.H.S., Farnsworth N.R., Kinghorn A.D., Mehta R.G., Moon R.C., Pezzuto J.M., 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, **275**: 218-220.

Jeandet P., Bessis R., Gautheron B., 1991. The production of resveratrol (3,5,4'-trihydroxystilbene) by grape berries in different developmental stages. *Am. J. Enol. Vitic.*, **42**: 41-46.

Jeandet P., Bessis R., Maume B.F., Meunier P., Peyron D., Trollat P., 1995. Effect of enological practices on the resveratrol isomer content of wine. *J. Agric. Food Chem.*, **43**: 316-319.

Kitanaka S., Ikezawa T., Yasukana K., Yamanouchi S., Takido M., Sung H.K., Kim I.H., 1990. (+)-α-viniferin, an anti-inflammatory compound from *Caragana chamlagu* root. *Chem. Pharm. Bull.*, **38**: 432-435.

Lamuela-Raventós R. M., Romero-Pérez A. I., Waterhouse A.L., De la Torre-Boronat, 1995. Direct HPLC analysis of *cis*- and *trans*-resveratrol and piceid isomers in Spanish red *Vitis vinifera* wines. *J. Agric. Food Chem.* **43**: 281 – 283.

Langcake P., 1981. Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ε-viniferin, α-viniferin and pterostilbene. *Physiol. Plant Pathol.*, **18**: 213-226.

Langcake P., McCarthy W.V., 1979. The relationship of resveratrol production to infection of grapevine leaves by Botryis cinerea. *Vitis*, **18**: 244 – 253.

Langcake, P., Pryce R.J., 1976. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. *Physiol. Plant Pathol.*, **9**: 77-86.

Mattivi, F., Reniero F., Korhammer S., 1995. Isolation, characterisation, and evolution in red wine vinification of resveratrol monomers. *J. Agric. Food Chem.*, **43**: 1820-1823.

Monteiro M.J.P., Bertrand A., 1994. Validation d'une méthode de dosage. Application a l'analyse des amines biogénes du vin. *Feuillet Vert* n° 970.

Pezet R., Cuenat Ph., 1996. Resveratrol in wine: extraction from skin during fermentation and post-fermentation standing of must from Gamay grapes. *Am. J. Enol. Vitic.*, **47**: 287-290.

Roggero J.P., Garcia-Parrilla C. 1995. Effects of ultraviolet irradiation on resveratrol and changes in resveratrol and various of its derivatives in the skins of ripening grapes. *Sci. Aliments*, **15**: 411-422.

Romero-Pérez A., Lamuela-Raventos, R.M. *et al.*, 1996a. Resveratrol and piceid as varietal markers of white wines. *J. Agric. Food Chem.*, **44**: 1975-1978.

Romero-Pérez A.I., Lamuela-Raventos R.M., Waterhouse A.L., de la Torre –Boronat M.C., 1996b. levels of *cis* and *trans*-resveratrol and piceid isomers in rose and white Vitis vinifera wines. *J. Agric. Food Chem.*, **44**: 2124-2128.

Siemann E. H., Creasy L.L., 1992. Concentration of the phytoalexin resveratrol in wine. *Am. J. Enol. Vitic.* **43**:49-52.

Sun B., Spranger I., Roque-do-Vale, F., Leandro C., Belchior P., 2001. Effect of different winemaking technologies on phenolic compositon in Tinta Miúda red wines. *J. Agric. Food Chem.*, **49**: 5809-5816.

Sun B.S., Leandro M.C., Ricardo-da-Silva J.M., Spranger M.I., 1998. Separation of grape and wine proanthocyanidins according to their degree of polymerization. *J. Agric. Food Chem.*, **46**: 1390-1396.

Sun B.S., Pinto T., Leandro M.C., Ricardo-da-Silva J.M., Spranger M.I., 1999. Transfer of catechins and proanthocyanidins from grape solids into wine, *Am. J. Enol. Vitic.*, **50**: 179-184.

Threlfall R. T., Morris J.R., Mauromoustakos A., 1999. Effect of variety, ultraviolet light exposure, and enological methods on the *trans*-resveratrol level of wine. *Am. J. Enol. Vitic.*, **50**: 57-64.

Vrhovsek U., Wendelin S., Eder R., 1997. Effects of various vinification techniques on the concentration of *cis*- and *trans*-resveratrol and resveratrol glucoside isomers in wine. *Am. J. Enol. Vitic.*, **48**: 214-219.