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Resveratrol content of some wines obtained from dried Valpolicella grapes: Recioto and Amarone

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Abstract

Resveratrol (*trans*-3,5,4′-trihydroxystilbene), a phenolic substance present in both grape skin and wines, is a phytoalexin involved in grey mould resistance. A new interest has surfaced in recent years related to the antioxidative actions of resveratrol, which in vivo could be related to the prevention of cardiovascular diseases linked to lipid metabolism, particularly HDL production, while the antifungal activity may be of interest in wine production technology. These aspects have led to the publication of a number of papers reporting data on the resveratrol content of several kind of wine: for Italian wines, it ranges between 0.5 and 10 ppm, depending on cultivar, area of cultivation, climate and wine-making technology. In this work, resveratrol was quantified in samples of two unusual Italian wines, Recioto (sweet) and Amarone (dry), produced with the same cultivar mixture in the same area (Valpolicella, Verona, Italy) and with the same grape conditioning technique. After resveratrol extraction, reversed-phase HPLC analysis was carried out and several elution conditions were tested. The resveratrol content of Recioto and Amarone wines was lower than the values reported in the literature for other wines, ranging between 0.05 and 0.8 ppm.

Keywords: Wine; Resveratrol; Phenols; Phytoalexins

1. Introduction

Resveratrol (*trans*-3,5,4′-trihydroxystilbene) is a phenolic substance located in several parts of the vine, including the grapes, where it is mainly present in the skin. Resveratrol is considered to be a phytoalexin and its synthesis in grapes and leaves has been related to disease resistance [1–3]. Phytoalexins are a group of low-molecular-mass substances with microbial inhibitory activity, and whose accumulation in plants takes place during plant–microorganism interactions.

Resveratrol can be a constituent found in the woody parts of the plant roots, grapeseeds or stalks, and also an induced substance, as a resistance factor to fungi such as *Botrytis cinerea* and *Plasmopara viticola*.

The formation of resveratrol from tannin degradation does not seem possible, as it is present in both musts and wines; further, its production from tannins as wine ages also seems very improbable [5].

UV radiation, detergents, heavy metals (e.g., copper) or injury are other factors able to induce resveratrol synthesis [5]. Vines cultivated in mountainous areas, where UV radiation is high-

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er, can be induced to synthesize larger amounts of resveratrol.

A number of different hydroxystilbenes are present in several parts of the grape plant; many of them are dimers (ε-viniferin), trimers (α-viniferin, gnetin-H) and tetramers (r-viniferin) of resveratrol [6]. Although studies of grape stilbenes have been carried out in the past with particular attention to their disease-resistance characteristics, in recent years a new interest has emerged amongst enologists and hygienists, as the presence of resveratrol has been related to some "pharmacological" characteristics of red wines [7]. Moderate consumption of red wines has some beneficial effects on cardiac diseases, such as hypoaggregation of blood platelets [7] and increase in HDL cholesterol. Further, resveratrol is an important component of an oriental medicine called Kojo Kon, which has been claimed to have protective action against arteriosclerosis [7].

Red wines usually contain larger amounts of resveratrol than white wines, regardless of the technology applied [8,9]. Higher resveratrol contents (10 ppm) are usually present in wines which have had prolonged contact between the must and skins, whereas lower concentrations (0.3 ppm) are usually present in wines intended to be drunk young, with shorter maceration times [10,11]. The geographical origin, cultivar, agronomic techniques, the state of health of the grapes, wine-making technology and other factors all influence the resveratrol content, whereas the year of production is not of any significant importance. Resveratrol production decreases as ripening proceeds [2]; resveratrol is present in grapes in both the *cis* and *trans* configuration (Fig. 1), in a direct relationship with weather conditions (UV radiation favours the formation of the *cis* isomer [4]). Further, resveratrol is present as a glycoside in a number of cultivars [12].

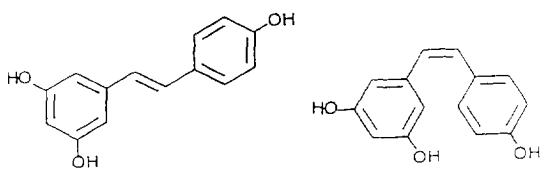

Fig. 1. Structures of (left) *trans*- and (right) *cis*-resveratrol.

In this work, resveratrol was determined in two unusual Italian wines: Recioto (sweet) and Amarone (dry); these two wines are produced from the same mixture of grapes, in the same geographical area (Valpolicella, Verona) and with the same grape-conditioning technology. The wine production technology involves drying the grapes from vintage time until February, when wine making takes place: in this way, there is a concentration of the sugar content and several modifications linked to endogenous grape enzymes and exogenous enzymes, originating from *Botrytis cinerea*, which can infect grapes during the drying period. The aim of this work was to develop a rapid method to identify and quantify resveratrol in wines obtained with unusual wine-making technology.

A number of methods have been used for resveratrol determination: HPLC techniques are the most commonly used procedures [4,5,8,9,12–15], but gas chromatographic [1–3] and GC–MS techniques [10,16,17] have also been proposed. Extraction techniques are essentially of the liquid–liquid partition type [4], or solid-phase extraction (SPE) [9] when HPLC is used, whereas derivatization [17] is carried out when GC is applied.

2. Experimental

2.1. Chemicals

Methanol (HPLC grade) was supplied by Prolabo, anhydrous sodium sulphate (ACS-RPE), ethyl acetate (ACS-RPE), water (PLUS-RS), glacial acetic acid (ACS) and Acetonitrile (RS-HPLC grade) were supplied by Carlo Erba and *trans*-resveratrol (*trans*-3,5,4′-trihydroxystilbene) (cod R 5010) was supplied by Sigma.

2.2. Apparatus

A Perkin-Elmer Series 3 high-performance liquid equipped with a diode-array UV detector set at 306 nm and a Rheodyne loop injector (5

μl) was used. The column was Partisphere C_{18}, 5 μm (125 × 4.6 mm I.D.) (Whatman) and the mobile phase was water–acetic acid–acetonitrile (75:5:20) at a flow-rate of 0.5 ml/min. A Hewlett-Packard Model 3395 integrator was used.

2.3. Extraction method

The method used is based on Siemann and Creasy's technique [4]. A 5-ml volume of wine was extracted in a test-tube with 3 ml of ethyl acetate with agitation on a vortex mixer for 15 s. The separation of the phases was improved by cooling the tube to 4°C for a few minutes, then to −20°C. The organic phase was recovered with a Pasteur pipette and the aqueous phase was then extracted twice with 2 ml of ethyl acetate. Anhydrous sodium sulphate was added to remove any traces of water. The organic phase was concentrated under reduced pressure (Rotavapor) and the extract was recovered with two 1-ml aliquots of methanol; as the volume of wine was originally 5 ml, the concentration rate obtained was 1:2.5. The methanolic extract was then analysed by HPLC.

3. Results and discussion

3.1. HPLC analysis

The mobile phase used by other workers [15] for the analysis of phenolic fractions of wines was first used in the HPLC determination of resveratrol, i.e. water–acetic acid–acetonitrile 65:5:30. Isocratic elution was carried out at 0.5 ml/min. Under these conditions, a 0.5 ppm resveratrol standard was eluted after 5.1 min. Nevertheless, elution of an authentic extract from Recioto wine did not produce a resolved peak, as is evident from the chromatogram reported in Fig. 2A.

The separation was improved by reducing the acetonitrile content in the mobile phase to 20% and increasing that of water to 75%, i.e., using water–acetic acid–acetonitrile (75:5:20). The retention time of resveratrol under these conditions was 13.5 min, sufficiently separated from any other interfering substances, as shown in Fig. 2B.

3.2. Repeatability and recovery trials

A Recioto wine was extracted eight times with the aim of verifying the repeatability of the method and the instrument response. The average area of the resveratrol peak is reported in Table 1; the variance was expressed as relative standard deviation (R.S.D.). The R.S.D. of 0.11% may be considered adequate for our purposes. Table 1 also gives data for the areas obtained for the same wine containing increasing amounts of added resveratrol (averages of eight replicate analyses) and the R.S.D. values confirm the good repeatability characteristics of the method.

A comparison of the areas of the spiked samples allowed the calculation of a recovery of about 90%. The 0.1 ppm standard solution gave a peak whose dimensions were easily evaluated, so that 0.1 ppm can be considered as the minimum detectable amount; this is usually estimated as the amount of analyte able to give a signal almost three times the instrumental noise: as the concentration factor is 2.5, a concentration of around 0.05 ppm in wine should therefore be detectable. The low R.S.D. values obtained for the spiked samples were similar to those for the standard solution, indicating little influence of the extraction method adopted.

These data, referring to resveratrol added to wine, were used to obtain a calibration graph with the following parameters: number of data points from 0.1 to 1.0 ppm = 3 (averages of eight-replicates), standard error = 0.03032, coefficient of determination (r^2) = 0.9965, slope = $5.495 \cdot 10^{-7}$, intercept = −0.024, F value = 441.27 and p level = 0.0303; hence the response linearity and the method reliability were verified.

3.3. Characterization of wines

The method described was able to determine only *trans*-resveratrol; nevertheless, this is the isomer which is usually determined with the aim of carrying out wine characterization [4,5,8–

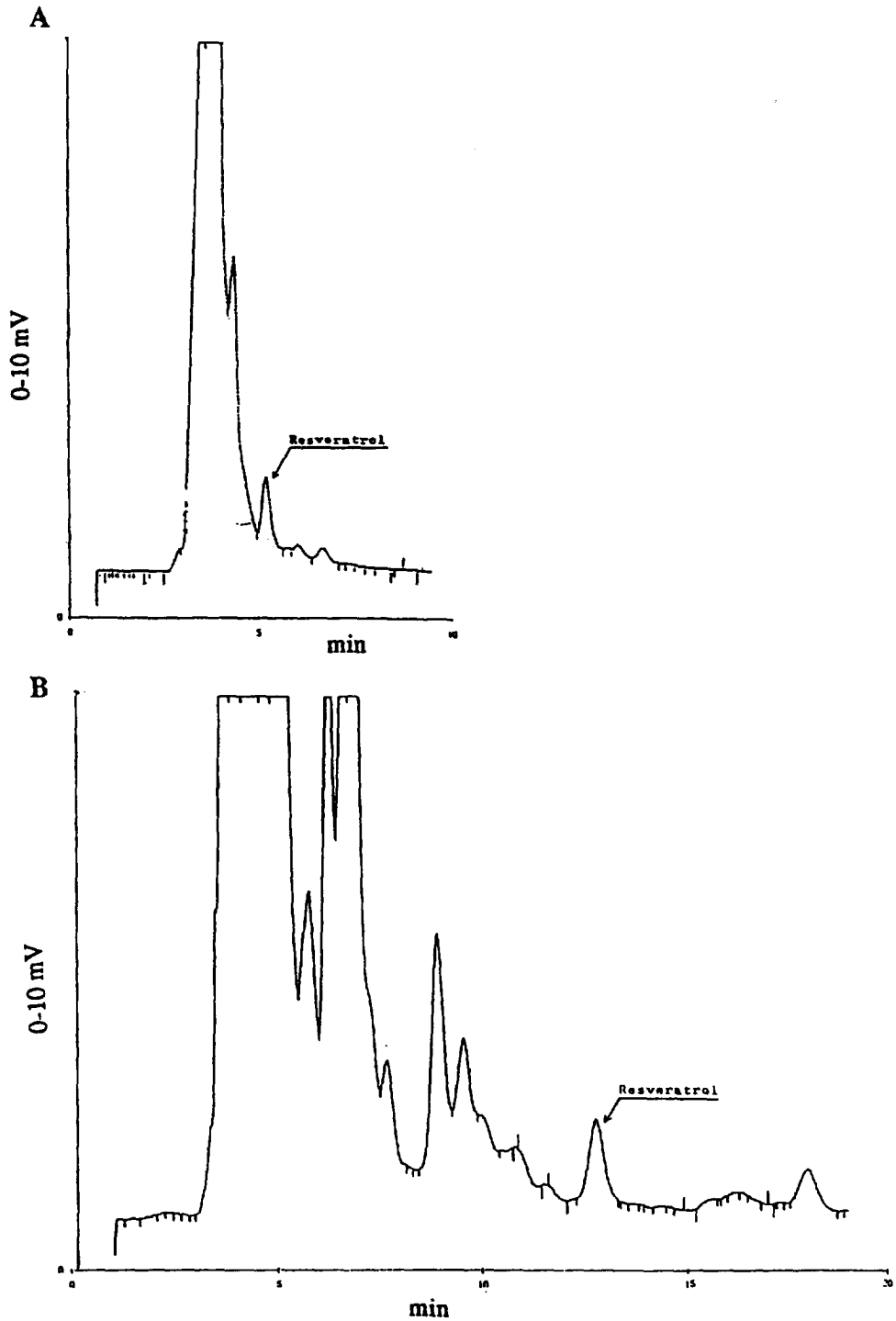

Fig. 2. HPLC traces for resveratrol obtained with different proportions of water–acetic acid–acetonitrile as eluent: (A) 65:5:30; (B) 75:5:20.

Table 1
Analysis of standard solutions and recovery tests

Sample[1]	Recovery (%)[b]	R.S.D. (%)	Peak area ($\mu V\ s^{-1}$)[c]
Recioto only	–	0.11	707 237
Extract of Recioto + 0.1 ppm resveratrol	108	0.10	952 560
Extract of Recioto + 0.5 ppm resveratrol	102	0.13	1 721 043
Extract of Recioto + 1.0 ppm resveratrol	86	0.063	2 534 729
Standard, 0.1 ppm	–	0.10	91 000
Standard, 0.5 ppm	–	0.10	399 380
Standard, 1.0 ppm	–	0.07	850 000

[a] Standard solutions of resveratrol were in methanol.
[b] The reported value was calculated considering the dilution factor of 2.5 that originates from the concentration effect of the injected sample.
[c] Averages of eight replicates.

10,12]. Under the experimental conditions used, the *cis*-isomer did not interfere, as its maximum absorbance was well separated from that of the *trans*-isomer [4].

The method was then applied to a series of 24 Recioto and 53 Amarone wines from Valpolicella. The amounts of resveratrol detected in these samples are presented in Table 2 as classes of frequency distribution. As can be seen, the amounts detected were generally very small and the data were highly dispersed (the median was very far from mean); these data were in the lower part of the range reported in the literature, which covers values between 0 and 10 ppm [1,2,4,5,8–10,13,15]; on the other hand, the technology applied is very different to that for musts produced immediately after harvest, and is similar to that for fruit supermaturation. If the presence of resveratrol is a grape reaction to *Botrytis cinerea* infection, as reported in the literature, it could be stated that, in the case of Recioto and Amarone, it is of very low magnitude, even considering the long period of contact between skin and must. Furthermore, the conditions under which drying was carried out could induce resveratrol degradation processes or antagonistic biosynthesis mechanisms. No relationship was noted between resveratrol content and other chemical composition characteristics, such as glycerol and gluconic acid; as is well known, these parameters are related to *Botrytis* infection. This should also confirm that the *Botrytis* infection, if present, was of a very small magnitude and almost certainly not an important condition for Recioto and Amarone production technology. These data suggest an interest in future research to clarify why resveratrol is not significantly represented in these wines: usually, Italian red wines have higher resveratrol contents, as shown by the data in Table 3, obtained in our laboratory with the analytical method

Table 2
Resveratrol contents of Recioto and Amarone wines as a frequency distribution

Recioto (ppm)	Frequency (%)	Amarone (ppm)	Frequency (%)
n.d.[a]	75.0	n.d.[a]	45.3
0.05–0.1	12.5	0.05–0.1	11.3
0.1–0.15	0.0	0.1–0.15	11.3
0.15–0.2	4.17	0.15–0.2	5.7
0.2–0.25	4.17	0.2–0.25	3.8
0.25–0.4	4.17	0.25–0.8	22.6
Average	0.044	Average	0.154
S.D.	0.094	S.D.	0.203
Median	0.00	Median	0.093

[a] Not detectable.

Table 3
Resveratrol contents of some red wines produced without grape drying

Wine	Resveratrol (ppm)
Il Poggiolo Carmignano '89	0.577
Arione Barolo '88	0.479
Cantucci Nobile di Montep. '90	1.395
Quintarelli Valpolicella '88	0.118
Vino Novello '94	0.184
Salice Salentino Rosso Ris. '90	0.219
Colli Euganei Cabernet '90	1.895
Chianti Grigio S. Felice Ris. '88	0.895
Average	0.72
Standard deviation	0.596
Median	0.528

described. Data for these red wines were much more homogeneous, as the median value was much closer to the average. These data could demonstrate that drying may have an important influence in drastically reducing the resveratrol content.

4. Conclusions

The proposed HPLC procedure for resveratrol determination was suitable for the purpose of determining this compound in wine at levels of $\geq$0.05 ppm. The technique was sensitive and rapid enough to allow a large number of analyses to be carried out. The data indicate that drying grapes is not a technique which induces resveratrol synthesis; further, a wide range of values were recorded without any relationship to other wine compounds.

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