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Article

Ultraviolet Irradiation of Trans-Resveratrol and HPLC Determination of Trans-Resveratrol and Cis-Resveratrol in Romanian Red Wines

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A reversed-phase high-performance liquid chromatography method with diode array detection was developed for the determination of *trans*- and *cis*-resveratrol in red wines. Separation was achieved after direct injection by the use of a BDS Hypersil C18 column (250 × 4.6 mm) with gradient elution (solvent A: acetic acid 2%, solvent B: acetonitrile). Detection of *trans*- and *cis*-resveratrol was performed at 306 and 286 nm, respectively. The retention times of *trans*- and *cis*-resveratrol were 22.2 and 26.1 min, respectively. Good linearity and precision were obtained for the two isomers. Detection limits of 0.004 mg/L for *trans*-resveratrol and 0.02 mg/L for *cis*-reveratrol were obtained. The developed method was applied to determine *cis*- and *trans*-resveratrol in 30 red wines produced in Oltenia (south-western Romania). The wines came from different vineyards harvested in various vintages. The concentration of *trans*-resveratrol ranged from 0.287 to 7.188 mg/L, while the content of *cis*-resveratrol ranged from 0.718 to 6.587 mg/L. The highest amount of *trans*-resveratrol was found in Merlot from Vanju Mare, Mehedinti (7.188 mg/L), followed by Sirah from Corcova, Mehedinti (4.738 mg/L), both from the 2010 harvest. The paper also approaches the study of the transformation of *trans*-resveratrol into the *cis* form after ultraviolet irradiation through glass and quartz. At the irradiation of a *trans*-resveratrol solution through quartz, the formation of another two compounds apart from *cis*-resveratrol was observed.

Introduction

Phenolic compounds are partly responsible for the color, astringency and bitterness of wine, and for numerous physiological properties associated with wine consumption (1).

Although phenolic substances are routinely synthesised during the processes of plant growth, the synthesis of some substances is also induced by stress factors caused, for example, by fungal diseases or ultraviolet (UV) radiation (2).

The group of phenolic substances involves a number of compounds ranging from simple phenols, phenolic acids and their derivatives to coumarins, flavonoids and stilbenes, tannins and lignins (3). The roles of these compounds within plants are very different: they protect plants against pests and UV radiation, attract pollinators, function as antioxidants, and endow sensory properties and color to their fruit (4–6).

One of the important phenolic bioactive constituents in wine is resveratrol (3,4′,5-trihydroxystilbene), a naturally occurring phytoalexin produced by some spermatophytes, such as grapevines, in response to injury (7). Resveratrol is the parent compound of a family of molecules, including glycosides (piceid) and polymers (viniferins), existing in *cis* and *trans* configurations classified as stilbenes. The essential structural skeleton comprises two aromatic rings linked by a methylene bridge (Figure 1) (8).

Resveratrol and piceid are primarily present in grape and wine derivatives, and their concentrations can vary depending on factors such as grape cultivar, mechanical injury, fungal infection, primarily by *Botrytis cinerea* and *Plasmospora viticola* (9–13), vinification procedures (14, 15), environmental conditions (temperature, humidity, latitude, height above sea level and geochemical characteristics) (16, 17) and abiotic stresses such as UV (11, 18).

In addition to grapes, a large variety of fruits, including mulberry, bilberry, lingonberry, sparkle-berry, deerberry, partridgeberry, cranberry, blueberry, and jackfruits, peanuts, pistachios and a wide variety of flowers and leaves also contain resveratrol (19–24). The importance of resveratrol and piceid food sources depends on food composition and the amount of consumption of these foods.

Resveratrol is raising a lot of interest in nutrition and medicine due to its potential health benefits. Several studies have demonstrated that trans-resveratrol is an effective antioxidant and that it inhibits lipid peroxidation of low-density lipoprotein (LDL), prevents the cytotoxicity of oxidized LDL, protects cells against lipid peroxidation, decreases tumour promotion activity by inhibiting cyclooxygenase-1 (COX-1) and platelet aggregation (25–28). Resveratrol was found to inhibit tumorigenesis in rodent cancer models (29), to inhibit proliferation and induce apoptosis in several human cancer cells (30, 31), including B-cell malignancies (32), and to affect a series of critical events associated with tumor initiation and progression (33, 34), including up-regulation of p53 and p21 levels, induction of nitric oxide, inhibition of cyclooxygenase, protection against reactive oxygen intermediates, down-regulation of survival factors and down-regulation of proteinases (35, 36).

The effects of resveratrol have been documented in a wide variety of cell types, including macrophages, polymorphonuclear cells, platelets, osteoblasts, neurons and adrenal cells, and it was suggested that resveratrol has therapeutic potential for allergy and neurologic disorders (36–39). The biological effects have primarily been studied *in vitro*, although there is also growing *in vivo* evidence (40). Some effects required a high concentration of resveratrol in tissues, although chemopreventive and chemotherapeutic anticancer effects are an exception. In this case, resveratrol, at micromolar concentrations, affects the activity of transcriptional factors involved in proliferation and stress responses and leads to the modulation of

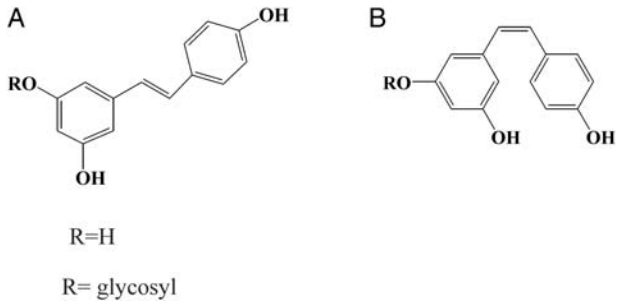

Figure 1. Structures of resveratrol and derivatives: *trans*-resveratrol (A, R = H); *cis*-resveratrol (B, R = H); *trans*-piceid (A, R = glycosyl); *cis*-piceid (B, R = glycosyl).

survival and apoptotic factors in carcinogenesis. In atherosclerotic and neurodegenerative diseases, the effects of resveratrol are not only due to its antioxidant and scavenging activities, but also to its participation in the modulation of signal transduction pathways and in the activation of several enzymes at micromolar concentrations (8).

Due to these health-promoting properties, resveratrol is used in food supplements and is a promising substance to obtain functional foods (41).

In natural foods, plants or wine, resveratrol exists naturally as both *cis*- and *trans*-isomers, the *trans* isomer being the major and more stable natural form. *Cis*-isomerization can also occur when the *trans* isoform is exposed to sunlight or to artificial or natural UV radiation (42, 43). According to Merillon *et al.* (44), the *in vitro* antioxidant activity of *cis*-resveratrol is seven times weaker than that of *trans*-resveratrol, while according to Belleri *et al.* (45), *trans*-resveratrol is more effective than the *cis* isomer in inhibiting angiogenesis and tumor growth *in vivo*. Kim *et al.* (46) found that *cis*-resveratrol has a less potent anti-platelet activity than the trans-isomer, while Rius *et al.* (47) demonstrated that the *in vivo* anti-inflammatory activity of resveratrol is produced by its *trans* isomer, whereas *cis*-resveratrol produced no significant effect. Nevertheless, other studies about the biological activity of *cis*-resveratrol showed that it exerts antioxidant and anti-inflammatory activities similar to those exerted by the *trans* form (48).

Both isomeric forms were detected in white, rosé and red wine. The isomer *trans* is primarily located in the grape berry skins (50–100 mg/g), and it is absent in the pulp (49). Wines produced with skin contact contain higher amounts of resveratrol and piceid than wines made without skin contact. The *cis*-isomer is probably transformed during the vinification process but usually does not reach the concentration of the *trans*-isomer in the wine. In red wine, the concentrations of the *trans*-isomer, which is the major form, generally range between 0.10 and 15 mg/L. Wines of the rosé type exhibit intermediate values between red and white wines (7).

The different vinification techniques also affect *trans*- and *cis*-resveratrol contents in wines (17). 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, while fining treatments with polyvinylpolypyrolidone greatly reduced its resveratrol concentration. UV light exposure of grape clusters and enzyme addition might significantly increase the resveratrol levels in some wines, but does not affect those of other wines. Skin contact time can affect resveratrol extraction, but the maximum extraction time is dependent on grape variety (50).

Many investigations on the resveratrol concentrations of commercial wines have been conducted. High-performance liquid chromatography (HPLC) techniques are the most commonly used procedures, but gas chromatography (GC), GC–mass spectrometry (MS) (51) and capillary zone electrophoresis (CZE) (52, 53) techniques have also been proposed. HPLC and GC can be coupled to various types of detectors, including UV (14, 54, 55), photodiode array (DAD) (11, 13, 17, 56-58), chemiluminescent detection (CL) (59), fluorescence detection (FD) (60) and MS (41, 61, 62).

Many papers have been published describing some approaches for evaluating the concentrations of *cis*- and *trans*-resveratrol in wines of different countries (16, 41, 51–58, 63–69). In this study, a modified RP-HPLC analytical procedure was employed to determine the *trans*- and *cis*-resveratrol content in samples obtained from 30 commercial red wines produced in Oltenia, a southern region of Romania with a tradition of producing quality wines.

Another purpose of this article is to describe the results of our experiments in which *trans*-resveratrol was UV irradiated through glass and quartz, transformations that have been followed using HPLC coupled with DAD.

Materials and Methods

Reagents

Pure resveratrol (*trans*-3,4',5-trihydroxystilbene) was purchased from Sigma Chemical Co. and stock solution (1,000 mg/L) was prepared by dissolving 25.00 mg of the commercial product, without previous purification, in 25 mL of methanol. Acetonitrile, methanol and acetic acid were of liquid chromatographic grade (Merck). The water used was ultrapure, Basic TWF.

HPLC analysis

Stock solution of *cis*-resveratrol was prepared by UV irradiation of a standard solution (1,000 mg/L) of *trans*-resveratrol for 10 h, through glass and quartz. Samples were taken after every 2 h. The sample obtained by irradiation through glass was used for calibration curve.

The calibration curves of *trans*-resveratrol and *cis*-resveratrol were obtained by plotting the peak area of each standard against concentration, in the ranges 5–100 and 2–50 mg/L, respectively. The number of calibration points was eight for *trans*-resveratrol and five for *cis*-resveratrol. Each calibration point was the mean of three independent measurements.

Reversed-phase HPLC was performed with a Surveyor Thermo Electron system comprising vacuum degasser, Surveyor Plus LCPMPP pump, Surveyor Plus ASP autosampler and diode array detector with 5-cm flow cell. Integration, data storage and processing were performed by Chrom Quest 4.2 software.

The analytical column was a BDS Hypersil C18 (25 cm × 4.6 mm i.d., 5 μm particle diameter). The mobile phase was filtered through a polyamide membrane (0.2 μm) and degassed with a DK 102p Bandelin ultrasonic bath.

The chromatographic separation was performed using elution gradient, and mobile phases were acetic acid 2% (A) and acetonitrile (B). The chromatographic separation was performed using a five-stage linear gradient: 90% solvent A from 0 to 5 min, from 90% to 60% A in 22 min, 60% A for 3 min, from 60 to 90% A in 2 min and 3 min 90% A to re-establish the initial conditions, before the injection of another sample. The total gradient time was 35 min, with a flow rate of 1.0 mL/min. An injection volume of 5 μL and thermostatic control of the system to maintain a temperature of 20°C were used.

The eluent was monitored at 306 and 286 nm, optimum absorbancies of *trans*- and *cis*-resveratrol, respectively, and the UV spectra of the eluents were obtained. Identification was based on retention time and spectrum data. All analyses were performed in triplicate and the data are presented as mean ± error (95% confidence level, F = 4, $n = 5$).

Performance of the method

Accuracy of the method was studied as two components: accuracy and precision. In the absence of a certified reference material, accuracy was investigated by recovery (70). Accurate amounts of standards were added to the wine sample, and the recovery values were obtained by comparing the increase of the peak areas before and after the addition of standard analytes.

The precision of the method was confirmed by repetitive analyses, calculating the average relative standard deviation (RSD) for six replicate determinations. The limit of detection [(LOD), signal-to-noise (S/N) = 3] of the individual compounds was calculated at their absorbance maxima.

Samples

The levels of *trans*- and *cis*-resveratrol were determined in 30 samples of red wines from Oltenia (southwestern Romania). Samples were purchased from local markets or obtained from Romanian wineries and kept at 4°C in darkness until analysis. Samples included wines made from Cabernet Sauvignon, Merlot, Pinot Noir, Syrah, Feteasca neagra and Negru de Dragasani grapes from 2007–2010 vintages. The wines were analyzed by direct HPLC injection, after filtration through a 0.45-μm membrane.

Results and Discussion

Performance of the method

Linearity

The calibration graphs for *trans*-resveratrol were produced by injecting standard solutions in the range 0.01–50 mg/L. Because no other compound was formed at the irradiation through glass, values for *cis*-resveratrol standards were assigned on the basis of the decrease in *trans*-resveratrol following irradiation.

Each point of the calibration graph corresponded to the mean value obtained from three independent area measurements. The corresponding regression equations and other characteristic parameters for the determination of both isomers are shown in Table I.

To test peak area and retention time reproducibility, Chrom Quest software was used for calculation of the RSDs for the retention times of the analytes for all levels of the calibration graph and for peak area at each calibration level.

Table I
Calibration Results for Determining *Cis*- and *Trans*-Resveratrol in Wines

Analyte	λ (nm)	Retention time (min)	Equation	r^2
Trans-resveratrol	306	22.223	$y = 5.46026e - 006x$	0.99967
Cis-resveratrol	286	26.125	$y = 1.02624e - 005x$	0.99959

The RSDs for the retention time were 0.074% for *trans*-resveratrol and 0.099% for *cis*-resveratrol; therefore, in standard solutions, the developed HPLC method provides stable retention times. RSDs for peak areas were between 0.057% and 0.307% for *cis*-resveratrol and between 0.090% and 0.440% for *trans*-resveratrol. Moreover, the calculated RSDs also prove stability in terms of peak height and asymmetry.

Precision

To test the precision of the HPLC method, standards of 20 and 5.5 mg/L of *trans*- and *cis*-resveratrol, respectively, were analyzed by 10 repeated injections. RSD values were 0.063 and 0.088% for retention time and 0.096 and 0.123% for peak areas for *trans*- and *cis*-resveratrol, respectively.

To evaluate the method, a wine sample was independently analyzed 10 times. This analysis was repeated over three days. For retention time, RSD values were 0.065 and 0.233%, while for peak areas, RSD values were 0.365 and 0.423% for *trans*- and *cis*-resveratrol, respectively. These results show that the developed method has good precision.

Recovery

The recovery, specificity and selectivity of the method were evaluated by spiking a wine sample with each of five increasing concentrations of standard solutions within the concentration range, in duplicate. The percentage of recovery varied from 95.6 to 100.4% for *trans*-resveratrol and from 98.2 to 101.8% for *cis*-resveratrol.

LODs

The LODs were determined starting from the principle that a peak, to be detected, must have an S/N ratio > 3. The LODs were 0.004 mg/L for *trans*-resveratrol and 0.02 mg/L for *cis*-resveratrol.

Study of the changes of trans-resveratrol caused by UV light

UV light exposure to *trans*-resveratrol is a common way of transforming *trans*-resveratrol into the *cis* form. At irradiation through glass, it was observed that the derivative *cis* is formed from the *trans* form. Figure 2 shows model chromatograms at 306 nm obtained after 2, 4, 6, 8 and 10 h of exposure through glass, and Figures 3 and 4 illustrate the spectra of *trans*- and *cis*-resveratrol, respectively, which were similar to those reported in previous papers.

The irradiation of *trans*-resveratrol through quartz determined the decrease of both *cis* and *trans* isomer concentrations and induced the formation of two unknown compounds. The major transmission difference between glass and quartz is that quartz transmits both UV and infrared well, while glass is

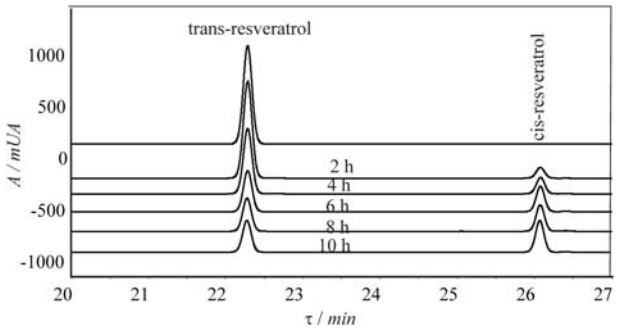

Figure 2. Chromatograms obtained during irradiation of *trans*-resveratrol through glass.

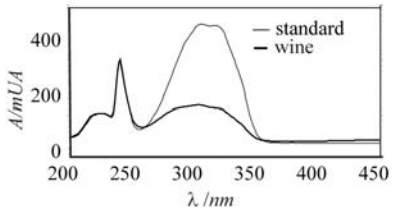

Figure 3. Absorption spectra of *trans*-resveratrol.

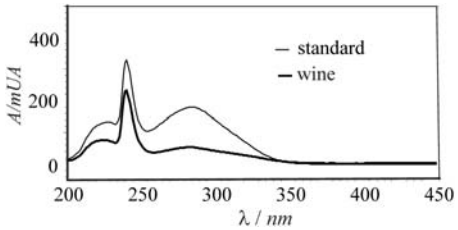

Figure 4. Absorption spectra of *cis*-resveratrol.

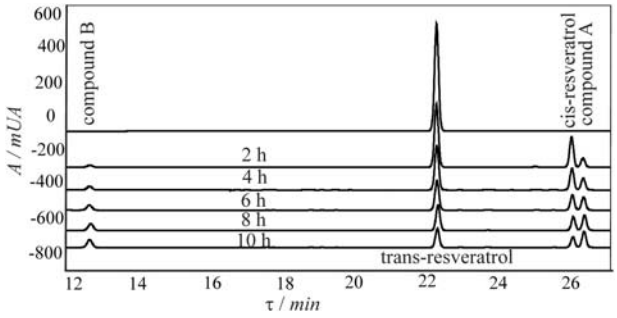

Figure 5. HPLC chromatograms obtained during irradiation of *trans*-resveratrol through quartz.

relatively opaque to UV and infrared. Both quartz and glass transmit visible light.

Figure 5 shows model chromatograms at 306 nm obtained after 2, 4, 6, 8 and 10 h of exposure through quartz, while Figure 6 illustrates the spectra of the two unknown compounds that were generated.

López-Hernández *et al.*, in a study of the changes of *trans*-resveratrol caused by UV light (41), observed the formation of

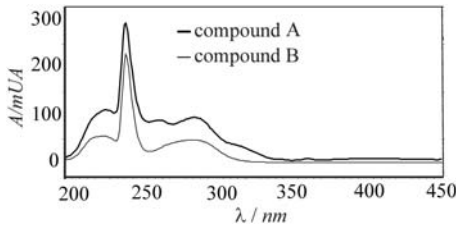

Figure 6. Absorption spectra of compounds A and B.

a third compound whose concentration increased as the light exposure time increased. This new compound seemed to be generated from the *cis*-resveratrol and presented strong fluorescence and weak UV absorption. This unknown compound appeared in a higher concentration when the initial concentration of the *trans*-resveratrol solution was low. Roggero also reported the formation, at the irradiation of *trans*-resveratrol, of a third compound that was highly fluorescent in the UV range (71), but the detection of a fourth compound at the irradiation of *trans*-resveratrol was not reported in the literature.

Taking into account that the conditions of irradiation greatly influence the conversion and generation of other compounds, care must be taken for the correct quantification of *cis*-resveratrol.

Analysis of wine samples

The results obtained from wine samples are given in Table II. All determinations were carried out in triplicate. Typical chromatograms at $\lambda = 306$ nm are shown in Figure 7.

The use of a diode array detector allowed us to confirm the identity of the peak not only by its migration time, but also by the overlay of the UV-VIS spectra with a standard.

The concentration of *trans*-resveratrol ranged from 0.287 to 7.188 mg/L with a mean value of 2.246 ± 1.55 mg/L, while the content of *cis*-resveratrol ranged from 0.718 to 6.587 mg/L. Mark *et al.* (69) found from 0.1 to 14.3 mg/L *trans*-resveratrol in Hungarian red wines, while Paulo *et al.* (67), after analyzing 186 Portuguese red wines from different regions, grape varieties and vintages, found that the content of *trans*-resveratrol in red wines ranged from 0.05 to 10.9 mg/L and the concentrations of *cis*-resveratrol ranged from 0.04 to 8.71 mg/L.

Interesting findings arise by comparing our results with those reported by Stervbo *et al.* in a review of the resveratrol content in red wine based on relevant published data (72). The purpose of his review was to compare the levels of resveratrol in different red wines from a single grape variety (monovarietal red wines) and to compare the resveratrol content of red wines from different regions. The average value of *trans*-resveratrol in Romanian red wines (Oltenia region) appeared to be higher than the average value reported by Stervbo *et al.* (1.9 ± 1.7 mg/L) for all mono-varietal red wines. Also, the average value of *trans*-resveratrol in Romanian red wines (Oltenia region) appeared to be higher than those reported for Serbian (55) and Hungarian (68) red wines.

Generally, in the literature, there is a considerable variability in resveratrol concentrations. This is due to several factors, including climate. Other factors, such as the geographical area of cultivation, the development conditions of the grape, the wine

Table II
Concentration of *Trans*- and *Cis*-Resveratrol in Romanian Red Wines (mg/L)

Wine	Vintage	Wine producer	*Trans*-resveratrol	*Cis*-resveratrol	Total resveratrol content
Cabernet Sauvignon (1)	2008	Domeniile Coroanei, Segarcea, Dolj	0.289 ± 0.03	1.099 ± 0.04	1.388 ± 0.03
Cabernet Sauvignon (2)	2008	Domeniile Coroanei, Segarcea, Dolj	0.334 ± 0.03	1.439 ± 0.04	1.773 ± 0.03
Cabernet Sauvignon (1)	2009	Starmina, Mehedinti	1.933 ± 0.04	2.037 ± 0.04	3.971 ± 0.03
Cabernet Sauvignon (2)	2009	Starmina, Mehedinti	1.198 ± 0.03	1.078 ± 0.04	2.276 ± 0.03
Cabernet Sauvignon (1)	2010	Starmina, Mehedinti	3.664 ± 0.05	2.293 ± 0.04	5.957 ± 0.04
Cabernet Sauvignon (2)	2010	Starmina, Mehedinti	1.180 ± 0.04	1.008 ± 0.03	2.188 ± 0.03
Cabernet Sauvignon	2010	Vanju Mare, Mehedinti	1.140 ± 0.03	1.733 ± 0.02	2.873 ± 0.02
Cabernet Sauvignon	2010	Corcova, Mehedinti	2.362 ± 0.03	2.290 ± 0.04	4.652 ± 0.03
Mean ± standard deviation			1.513 ± 1.12	1.622 ± 0.54	3.135 ± 1.58
Merlot	2007	Domeniile Coroanei, Segarcea, Dolj	0.287 ± 0.04	0.913 ± 0.03	1.200 ± 0.03
Merlot (1)	2008	Domeniile Coroanei, Segarcea, Dolj	0.437 ± 0.04	0.945 ± 0.05	1.382 ± 0.05
Merlot (2)	2008	Domeniile Coroanei, Segarcea, Dolj	3.442 ± 0.02	0.659 ± 0.04	4.101 ± 0.03
Merlot	2009	Domeniile Coroanei, Segarcea, Dolj	1.456 ± 0.04	3.886 ± 0.04	5.342 ± 0.03
Merlot (1)	2009	Corcova, Mehedinti	3.027 ± 0.02	2.845 ± 0.04	5.872 ± 0.03
Merlot (2)	2009	Corcova, Mehedinti	3.010 ± 0.03	2.464 ± 0.03	5.474 ± 0.03
Merlot	2010	Vanju Mare, Mehedinti	7.188 ± 0.04	3.882 ± 0.04	11.07 ± 0.04
Merlot (1)	2010	Starmina, Mehedinti	3.761 ± 0.04	2.465 ± 0.03	6.226 ± 0.03
Merlot (2)	2010	Starmina, Mehedinti	3.546 ± 0.03	2.453 ± 0.04	5.999 ± 0.03
Merlot (3)	2010	Starmina, Mehedinti	2.470 ± 0.02	2.162 ± 0.03	4.632 ± 0.02
Merlot (4)	2010	Starmina, Mehedinti	3.755 ± 0.04	2.406 ± 0.05	6.161 ± 0.04
Mean ± standard deviation			2.944 ± 1.89	2.280 ± 1.09	5.224 ± 2.64
Pinot Noir	2008	Domeniile Coroanei, Segarcea, Dolj	1.360 ± 0.04	2.546 ± 0.04	3.906 ± 0.03
Pinot Noir	2009	Domeniile Coroanei, Segarcea, Dolj	2.274 ± 0.03	5.935 ± 0.04	8.209 ± 0.03
Pinot Noir	2010	Corcova, Mehedinti	2.943 ± 0.04	2.615 ± 0.03	5.558 ± 0.04
Mean ± standard deviation			2.192 ± 0.79	3.699 ± 1.94	5.891 ± 2.17
Feteasca Neagra	2007	Banu Maracine, Dolj	0.954 ± 0.04	0.718 ± 0.03	1.672 ± 0.03
Feteasca neagra	2008	Domeniile Coroanei, Segarcea, Dolj	1.304 ± 0.02	1.820 ± 0.03	3.124 ± 0.02
Feteasca Neagra	2009	Starmina, Mehedinti	3.737 ± 0.03	3.211 ± 0.03	6.948 ± 0.03
Mean ± standard deviation			1.998 ± 1.52	1.916 ± 1.25	3.915 ± 2.73
Syrah	2009	Domeniile Coroanei, Segarcea, Dolj	0.813 ± 0.03	2.331 ± 0.03	3.144 ± 0.02
Syrah	2010	Corcova, Mehedinti	4.738 ± 0.04	2.503 ± 0.03	7.241 ± 0.03
Mean ± standard deviation			1.817 ± 2.77	4.241 ± 0.12	6.058 ± 2.89
Cabernet Sauvignon + Merlot	2008	Corcova, Mehedinti	1.582 ± 0.03	2.417 ± 0.03	3.999 ± 0.03
Cabernet Sauvignon + Merlot	2009	Corcova, Mehedinti	1.633 ± 0.03	2.277 ± 0.03	3.910 ± 0.03
Mean ± standard deviation			1.608 ± 0.04	2.347 ± 0.10	3.955 ± 0.06
Negru de Dragasani	2010	Starmina, Mehedinti	1.569 ± 0.04	2.552 ± 0.04	4.121 ± 0.04

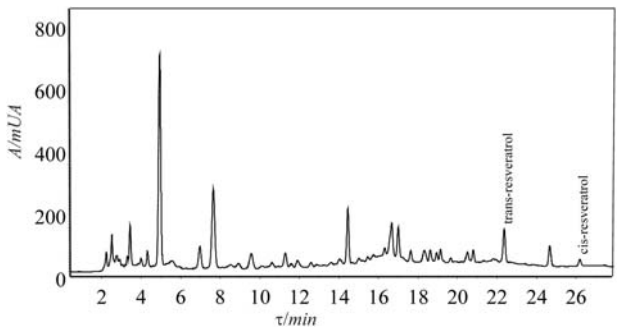

Figure 7. Chromatogram at λ = 306 nm of the red wine Sirah (Corcova, Mehedinti, 2010).

making techniques and the storage conditions, also have great influence on the content of resveratrol in wines (41). Stervbo *et al.* also found a great variance in the levels of resveratrol across variety. Nevertheless, our average values for *trans*-resveratrol in red wines made from Merlot, Cabernet Sauvignon and Syrah varieties (Table II) were very close to those presented by Stervbo *et al.* (2.8 ± 2.6 mg/L for Merlot, 1.7 ± 1.7 mg/L for Cabernet Sauvignon and 1.8 ± 0.9 mg/L for Syrah).

As shown in Table II, the highest amount of *trans*-resveratrol was found in Merlot from Vanju Mare, Mehedinti (7.188 mg/L), followed by Sirah from Corcova, Mehedinti (4.738 mg/L), both from the 2010 harvest. Atanacković *et al.*, in a study of the influence of winemaking techniques and cultivars on the resveratrol content of wines made from the cultivars Merlot, Cabernet Sauvignon, Pinot Noir and Prokupac, also found that Merlot wines had the highest average resveratrol content (60).

In a study of *trans*- and *cis*-resveratrol content of 20 different wine samples from Romania, Vlase *et al.* found for dry red wines an average concentration of 4.90 ± 3.74 mg/L *trans*- plus *cis*-resveratrol (73), close to 4.48 ± 2.26 mg/L obtained in our study. They also found in all red wines that the *trans*-resveratrol content is higher than the *cis*-resveratrol content, which was not a rule in our findings. Indeed, we found an average of 2.23 ± 1.09 mg/L *cis*-resveratrol, very close to the average content of 2.24 ± 1.55 mg/L *trans*-resveratrol.

Because the wines analyzed in our study were not from a single vintage, no conclusions can be made for the role of age regarding *trans*-resveratrol concentration in red wines. However, we observed that the concentrations of *trans*-resveratrol of the red wines harvested in 2010 exceed 1.140 mg/L *trans*-resveratrol.

Conclusions

In the past few years, *trans*- and *cis*-resveratrol have attracted great attention due to their health properties, and several papers have reported methods to determine these compounds, but few have described the formation of derivatives from *trans* or *cis*

isomers. The formation of new compounds from *trans*- and *cis*-resveratrol can occur at strong UV irradiation and may undergo errors in the quantification if is not taken into account.

An RP-HPLC method with DAD was developed for the quantitative determination of *trans*- and *cis*-resveratrol in wines. The results concerning linearity, recovery, precision and sensitivity were highly satisfactory and comparable to those obtained by the proposed methods in the literature. No sample pretreatment was needed. The developed method allows the determination of *cis*- and *trans*-resveratrol at low levels with LOD of 0.02 mg/L for *cis*-resveratrol and 0.004 mg/L for *trans*-resveratrol.

Thirty commercial red wines produced in the southern region of Romania were analyzed and significant concentrations of *trans*- and *cis*-resveratrol were found. The concentration of these substances seems to vary considerably, depending on diverse factors such as cultivar, climate and winemaking technology.

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