JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Positive Correlation between High Levels of Ochratoxin A and Resveratrol-Related Compounds in Red Wines

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The natural occurrence of ochratoxin A in red wines has been widely reported by several authors, as well as a that of group of stilbenes including *cis*- and *trans*-resveratrols and related glucosylated forms. In the present study 112 samples of retail red wines were collected from northern (17), central (46), and southern (49) Italy and were analyzed for both ochratoxin A and resveratrol-related stilbenes. The mean levels of total resveratrols and total piceids were 3.14 and 5.80 mg/L, respectively, whereas the ochratoxin A mean level was 0.64 μg/L. The Merlot wines showed the highest mean value of total stilbenes, followed by Negroamaro and Negroamaro blend, Aglianico, and Syrah, all with mean levels of >10 mg/L. Ochratoxin A was detected in 70, 59, and 100% of wine samples from northern, central, and southern Italy, with mean levels of 0.12, 0.07, and 1.36 μg/L, respectively. The highest values of ochratoxin A were recorded in Negroamaro- and Primitivo-based wine samples from southern Italy, showing also the highest content of stilbenes. In wine samples from southern Italy, a positive correlation was obtained between levels of ochratoxin A and total stilbenes ($r = 0.74$) as well as between ochratoxin A and total resveratrols ($r = 0.50$) and between ochratoxin A and total piceids ($r = 0.74$). These results suggest that toxic levels of ochratoxin A in red wine may be, at some extent, counterbalanced by the beneficial effects of resveratrol derivatives. Further investigation should be warranted in this regard.

KEYWORDS: Stilbenes; *trans*- and *cis*-resveratrol; *trans*- and *cis*-piceid; ochratoxin A; wine; mycotoxins

INTRODUCTION

The natural occurrence of ochratoxin A (OTA), a nephrotoxic, hepatotoxic, and carcinogenic compound, has been frequently reported in red wines during the past decade (*1*−*4*). On the other hand, resveratrol, a stilbenoid compound exhibiting a number of beneficial effects, for example, prevention of atherosclerosis and anticarcinogenic activity, has also been shown to occur naturally in red wines both in its free forms (*cis* and *trans*) and in the related glucosylated forms (*cis*- and *trans*-piceids) (*5*, *6*). The toxic effects of ochratoxin A (**Figure 1**), a mycotoxin produced by *Aspergillus* and *Penicillium* species, have been well documented (*7*). Exposure to ochratoxin A through the diet has been somewhat associated with Balkan endemic nephropathy (BEN) and to the incidence of and mortality from urothelial urinary tract tumors; the International Agency for Research on Cancer (IARC) has classified ochratoxin A as a group 2B carcinogen, that is, a possible carcinogen to humans (*7*).

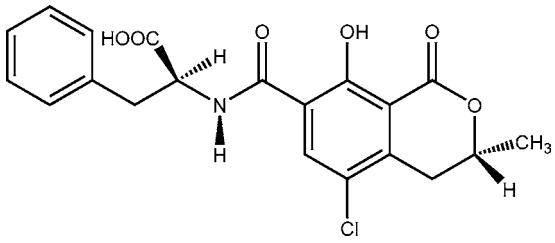

Figure 1. Chemical structure of ochratoxin A.

A recent data collection, aimed at assessing the risk of dietary intake of ochratoxin A by the population of EU Member States, showed that 59% of 1470 analyzed wine samples were contaminated. Wine represented, after cereals, a major source of daily ochratoxin A intake for the European populations. Wines from southern and warmer regions of Europe showed incidences and levels of contamination (72.3%, mean value = 0.636 μg/L, $n = 635$) higher than those from the northern European area (incidence of contamination = 50.3%, mean value = 0.181 μg/L, $n = 835$) (*8*). In addition, wines produced in southern Italy, where the climatic conditions favor the growth of ochratoxin A producing fungi in grapes, generally show

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10.1021/jf070993l CCC: $37.00
Published on Web 07/11/2007

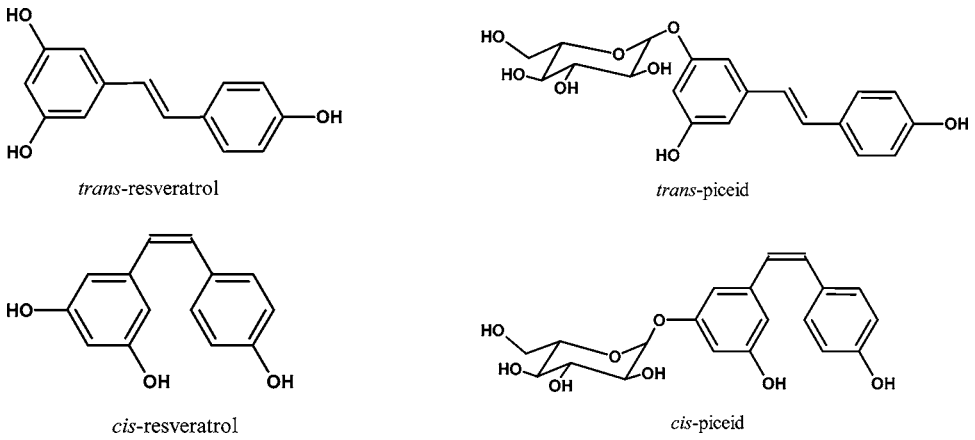

Figure 2. Chemical structures of *trans*- and *cis*-resveratrol and their piceid isomers.

incidences and levels of contamination higher than those of wines produced in northern and central Italy (*2*, *4*).

In the past few years, great interest has been directed to the antioxidant activity of stilbenes, in particular, resveratrol (3,5,4′-trihydroxystilbene), a nonflavonoid polyphenol commonly found in a number of plant families (*5*, *9*). Resveratrol occurs in the *cis* and *trans* configurations as well as the glucosylated forms (piceids: *cis*- and *trans*-3,5,4′-trihydroxystilbene-3-β-mono-D-glucoside) (**Figure 2**). The antioxidant and anti-inflammatory activities of resveratrol have been reported to be responsible for the beneficial effects of red wine consumption in the prevention of atherosclerosis and coronary heart disease (*6*). Antioxidant, anticlotting, and anti-inflammatory activities have been reported also for the *trans*-piceid isomer (*10*).

The presence of stilbenes in wine has been widely investigated, and their concentrations vary considerably (from <0.1 to 20 mg/L) depending on multiple factors such as grape variety, fungal infections, winemaking procedures, and climatologic conditions (*5*, *6*, *11*). Red wines usually contain higher stilbene concentrations than rosé or white wines. This depends on the more prolonged skin contact of must during fermentation and the high phenolic content of red grape cultivars (*12*). In vivo experiments with animals pretreated with antioxidants have shown a significant reduction of genotoxic damages induced by ochratoxin A (*13*). A significant reduction of hepatic and renal damages caused by ochratoxin A was also observed in mice when OTA administration was performed through contaminated grape juice (*14*).

Although the occurrence of ochratoxin A and resveratrol-related stilbenes in red wines is widely documented, no reports are available about a possible correlation between these toxic and beneficial compounds, respectively. On the basis of results from in vitro trials, Bavaresco et al. (*15*) hypothesized a different genetic capability to trigger stilbene synthesis by several ochratoxin A producing *Aspergillus* species and found a positive correlation between ochratoxin A and *trans*-resveratrol levels in grapevine berries.

The aim of this study was to verify if a positive correlation exists between the levels of ochratoxin A and resveratrol-related compounds in red wines that could result from the elicitation of stilbene synthesis in grapes after the infection of ochratoxigenic fungi in the field. Data on the distribution of ochratoxin A and resveratrol-related compounds in retailed Italian red wines from different appellations and vintages were gathered.

MATERIALS AND METHODS

Chemicals. β-D-Glucosidase, *trans*-resveratrol, and ochratoxin A were purchased from Sigma-Aldrich s.r.l. (Milan, Italy). HPLC grade acetonitrile, methanol, toluene, and 2-propanol were obtained from Carlo Erba (Milan, Italy). Analytical reagent grade formic acid and acetic acid were also supplied by Carlo Erba. Ultrapure water was produced by a Milli-Q system (Millipore, Bedford, MA). *cis*-Resveratrol was prepared from the *trans*-isomer solution by UV irradiation as previously described by Trela and Waterhouse (*16*).

Wine Samples. One hundred and twelve red wine samples (70 monovarietal wines and 42 blends from different varieties) were purchased from local retailers in Lazio and Apulia (Italy) and selected on the basis of their geographic origin, representing either Denomination of Controlled Origin (DOC) or Identification of Geographical Tipicity (IGT) Italian wines. All wine samples used in this study were obtained from grapes cultivated in different Italian regions from 1997 to 2002, namely, 17 from the north, 46 from central Italy, and 49 from the south. Further information on the geographical origin, varieties, and vintage year are reported in **Table 1**. All wines were analyzed for ochratoxin A and stilbenes (*cis*-, *trans*-resveratrol and *cis*-, *trans*-piceid) immediately after bottle opening, and three analyses per bottle were performed.

Ochratoxin A Analysis. OTA analyses were performed according to the AOAC Official Method 2001.01 (*17*). Ten milliliters of wine samples were diluted with 10 mL of a solution containing 1% polyethylene glycol (PEG 8000) and 5% sodium bicarbonate and filtered through glass microfiber filters. Ten milliliters of filtered extract was cleaned up through an OchraTest immunoaffinity column (VICAM, Watertown, MA) at a flow rate of about 1 drop per second. The column was washed with 5 mL of solution containing sodium chloride (2.5%) and sodium bicarbonate (0.5%) followed by 5 mL of distilled water at a 1−2 drops per second flow rate. Ochratoxin A was eluted from the column with 2 mL of methanol, and the eluted extract was evaporated under air stream at ca. 50 °C and reconstituted with 500 μL of the HPLC mobile phase. One hundred microliters of reconstituted extract was injected into the chromatographic apparatus by a full loop injection system. The toxin was determined by HPLC/fluorescence detector (Agilent 1100 Series, Agilent Technology) set at an excitation wavelength of 333 nm and an emission wavelength of 460 nm. The analytical column was a Symmetry C18 (150 × 4.6 mm, 5 μm, Waters, Milford, MA) preceded by a 0.5 μm Rheodyne guard filter. The mobile phase consisted of a mixture of acetonitrile/water/acetic acid (99:99:2 v/v/v) eluted at a flow rate of 1.0 mL/min. The detection limit (LOD) for OTA, defined as a signal-to-noise of 3:1, was 0.01 μg/L; the limit of quantification was 0.02 μg/L.

HPLC Analysis of Stilbenes. Analyses were performed with a Shimadzu LC-10A$_{VP}$ system consisting of an SCL-10A$_{VP}$ system controller, two LC-10AD$_{VP}$ solvent delivery units, and a photodiode array detector SPD-M10A equipped with a semi-microcell. Chromatographic separations and quantification were carried out according to a procedure reported by Careri et al. (*18*) with the following modification. The column was a Polaris C18A (150 × 2.0 mm i.d., 5 μm, Varian Inc., Lake Forest, CA) in conjunction with a C18 (30 × 2 mm, 5 μm) guard cartridge column. Aliquots of wine samples were directly injected into the HPLC column without further treatment and separated by

Table 1. Italian Red Wine Samples Grouped by Geographical Origin and Variety

geographical origin	Italian region	wine variety (no. of tested samples)	vintage years
northern Italy ($n = 17$)	Piemonte	Barbera (1);[a] Nebbiolo (1);[a] Dolcetto (1)[a]	2001–2002
	Lombardia	Cabernet Sauvignon (1); Barbera (1);[a] Cabanon (1)[a]	2000–2002
	Toscana	Sangiovese (1);[a] Cannaiolo Nero (3)	2000–2002
	Trentino	Merlot (1); Marzemino (1)[a]	2001–2002
	Friuli	Merlot (2); Cabernet Sauvignon (1)	2002
	Veneto	Cabernet Sauvignon (1); Corvina Veronese (1)[a]	1999; 2002
central Italy ($n = 46$)	Lazio	Merlot (14); Syrah (10); Cabernet Sauvignon (4); Cesanese (5); Montepulciano blend (4); Vignanello (1);[a] Rufus (1);[a] Petit Verdot (4)	1997–2002
	Abruzzo	Montepulciano blend (1)	2002
	Sardegna	Cannonau (1);[a] Monica (1)[a]	2000; 2002
southern Italy ($n = 49$)	Puglia	Primitivo (20); Negroamaro (7); Negroamaro blend (12); Montepulciano blend (3); Leverano (1);[a] Aleatico (1)[a]	1997–2002
	Campania	Aglianico (3)	2000–2002
	Calabria	Gaglioppo (1)[a]	2002
	Sicilia	Nero d'Avola (1)[a]	2001

[a] Samples grouped as "other varieties" not considered for statistical analysis.

isocratic elution with a water/acetonitrile/2-propanol mixture (80:15:5 v/v/v) containing 0.5% (v/v) formic acid at a flow rate of 0.2 mL/min. Chromatograms were recorded at two different wavelengths, 285 and 306 nm, corresponding to the absorbance maxima of *cis*- and *trans*-isomers, respectively. Identification of *trans*-resveratrol was performed by comparison of both retention time and UV spectrum (from 220 to 400 nm) with those obtained with the standard. A calibration curve was obtained for *trans*-resveratrol at 306 nm. *cis*-Resveratrol was identified by comparing retention time and UV spectrum with the new peak appearing after UV irradiation of *trans*-resveratrol standard solution. As *cis*-resveratrol is not commercially available, its calibration curve was obtained at 285 nm using the same solutions as for the *trans*-isomer, after exposure of the solution to UV light at 366 nm for 30 min. This time of exposure was necessary to determine at least 90% of the *trans*- to *cis*-isomer conversion that was ascertained on the basis of the decrease in the *trans*-isomer (*16*). The concentrations of *cis*-resveratrol standard solutions were obtained from *trans*-resveratrol concentrations corrected by the relevant conversion factor (*19*). Calibration curves were linear, with a correlation coefficient of 0.9999 over the ranges of 0.07−53.50 mg/L for *trans*-resveratrol and 1.25−49.50 mg/L for *cis*-resveratrol. LODs were 0.02 mg/L for *trans*-resveratrol and 0.03 mg/L for *cis*-resveratrol.

For the analysis of piceids, wine samples (1 mL aliquots) were subjected to hydrolysis by digestion with β-D-glucosidase (30 °C for 18 h under darkness) after the pH had been adjusted to 6.0 with 0.1 N NaOH (*19*). Piceids were identified by comparison of the HPLC chromatograms of wine samples before and after enzymatic hydrolysis with β-D-glucosidase, as reported elsewhere (*20*). As is known from the literature (*24*, *28*, *29*), the UV spectra of the resveratrol glucosides are very close to those of resveratrol aglycones; therefore, the quantification of *trans*- and *cis*-piceids was based on the assumption of identical molar extinction coefficients of *trans*- and *cis*-resveratrol at 306 and 285 nm, respectively. Peak purity was checked to exclude any contribution from interfering peaks.

Statistical Analysis. Data analysis was performed with Statistica software package for Windows (StatSoft, Tulsa, OK). Significant differences ($P < 0.05$) between mean levels of ochratoxin A, piceids (*cis*- and *trans*-isomers), and resveratrols (*cis*- and *trans*-isomers) in wine samples obtained from different cultivars and with different geographical origins (northern, central, and southern Italy) were identified by the General Linear Model (GLM) procedure with Duncan's test. Regression analyses of ochratoxin A versus stilbenes levels were performed both on OTA positive wine samples ($n = 88$, ochratoxin A ≥ 0.02 μg/L) and on wine samples originating from southern Italy ($n = 49$).

RESULTS

Stilbenes and Ochratoxin A Contents in Red Wines. The method used herein for stilbenes analysis allowed identification of stilbenes in different types of wines by direct injection into the HPLC apparatus, without any prior purification of the sample. The reproducibility of the method, determined by injecting the same sample for four consecutive days, showed a coefficient of variation of 1.36%. The accuracy of the method was evaluated by triplicate analyses of a wine sample spiked with amounts of *trans*-resveratrol corresponding to 10, 30, and 50% of the concentration previously determined in the same sample. Recovery values ranged between 97.8 and 98.6%

Stilbenes and OTA contents in wine samples, grouped according to wine variety and geographical origin, are summarized in **Tables 2** and **3**, respectively. A great variability of resveratrol and piceid levels was observed among the tested wine samples. The mean content of total stilbenes ranged from 5.39 to 13.67 mg/L with a mean level of 8.94 mg/L. The highest mean value of 13.67 mg/L found in Merlot wines was significantly higher ($P < 0.05$) than that found in the other cultivars with the exception of Negroamaro and Negroamaro blend, Aglianico, and Syrah, all containing mean levels of >10 mg/L. Mean levels of total resveratrols (*cis*- and *trans*-isomers) ranged from 1.58 mg/L (Cabernet Franc blend) to 7.25 mg/L (Merlot) (**Table 2**). Levels of *cis*- and *trans*-resveratrol in Merlot wine samples were significantly higher ($P < 0.05$) than those found in Primitivo, Cesanese, Aglianico, Cannaiolo Nero, and all blend varieties ($P < 0.05$). A significance was also observed between *cis*-resveratrol levels in Merlot and Cabernet Sauvignon ($P < 0.05$), Negroamaro ($P < 0.001$), and Petit Verdot wines ($P < 0.001$). Although high levels of resveratrol were found in wine samples from Apulia (up to 7.57 mg/L in Negroamaro blend), mean levels in samples from southern Italy (2.23 mg/L) were significantly lower ($P < 0.05$) than those found in samples from central Italy (4.04 mg/L) (**Table 3**). Levels of total piceids in wines from different grape cultivars ranged from 1.15 to 15.62 mg/L, with averages ranging from 1.97 mg/L (Petit Verdot) to 9.58 mg/L (Negroamaro blend) (**Table 2**). Wine samples from southern regions (mainly Negroamaro and Primitivo from Apulia) showed total piceids contents (mean = 7.63 mg/L) significantly higher ($P < 0.01$) than those from northern (4.91 mg/L) or central regions (4.17 mg/L) (**Table 3**). Ratios between total piceids and total resveratrols levels in wine samples from southern Italy were higher than those observed in wines from central and northern Italy ($P < 0.01$); in particular, a mean ratio of 5.58 (range = 0.96−27.27) was observed in southern wines, whereas mean ratios were 2.33 (range = 0.30−

Table 2. Stilbenes and Ochratoxin A Levels in Italian Red Wines

	ochratoxin A (μg/L)		*trans*-resveratrol (mg/L)		*cis*-resveratrol (mg/L)		*trans*-piceid (mg/L)		*cis*-piceid (mg/L)		total stilbenes (mg/L)
wine variety[a]	min–max	mean	min–max	mean	min–max	mean	min–max	mean	min–max	mean	mean
Primitivo (20)	0.42–3.91	1.41bc[b]	0.11–3.43	1.25a	nd[c]–1.88	0.59ab	1.59–5.39	2.97cde	1.99–8.57	3.92bc	8.73abc
Negroamaro (7)	0.38–4.54	1.28abc	1.08–4.72	2.52abc	0.18–2.77	1.35abc	0.98–7.87	3.85de	0.10–7.75	3.67bc	11.40bcd
Merlot (9)	nd–0.15	0.04a	2.38–5.87	3.83c	2.57–4.32	3.42e	1.13–4.95	2.57bcd	1.82–5.94	3.85bc	13.67d
Cabernet Sauvignon (4)	0.02–0.05	0.03a	1.32–4.44	2.54abc	1.37–3.28	2.10cd	0.66–2.38	1.24abc	1.57–2.29	2.01ab	7.89abc
Syrah (4)	nd–0.03	0.01a	1.24–6.74	3.97c	1.25–4.13	2.71de	0.73–1.31	0.94ab	2.14–4.27	2.83ab	10.44abcd
Cesanese (5)	nd–0.23	0.08a	0.98–3.46	1.85ab	0.61–1.99	1.45abc	0.50–2.51	1.60ab	0.65–3.46	2.36ab	7.26abc
Aglianico (3)	0.02–0.73	0.28ab	0.47–2.32	1.39a	nd–0.81	0.27a	2.49–5.98	3.94de	4.27–5.82	5.11c	10.71bcd
Petit Verdot (4)	nd–0.02	0.01a	0.84–5.60	3.34bc	0.64–2.72	1.50bc	0.21–0.75	0.56a	1.13–1.68	1.41a	6.81ab
Cannaiolo Nero (3)	0.05–0.31	0.21ab	0.95–1.60	1.29a	nd–1.18	0.50ab	1.63–2.64	2.20abcd	1.84–3.54	2.67ab	6.66ab
main variety of blend wines											
Negroamaro (12)	0.31–4.93	2.00c	0.70–6.51	2.04ab	nd–2.68	0.69ab	2.22–7.31	4.48a	2.56–7.89	5.10c	12.31cd
Merlot (8)	nd–0.22	0.07a	0.27–3.73	1.90ab	nd–3.61	1.90cd	0.45–3.22	1.61abc	1.44–4.86	2.84ab	8.25abc
Montepulciano (8)	nd–0.80	0.26ab	0.19–2.81	1.20a	nd–1.83	0.73ab	1.54–4.64	2.84cde	1.69–5.18	3.02abc	7.80abc
Syrah (5)	nd–0.02	0.01a	0.62–3.02	1.70ab	nd–2.23	1.20abc	0.50–1.73	0.64a	1.06–2.91	1.83ab	5.37a
Cabernet Franc (3)	nd–0.54	0.25ab	0.78–1.42	1.05a	0.16–1.16	0.53ab	1.42–2.27	1.81abc	2.69–3.22	2.93ab	6.32ab
other varieties (17)	nd–1.38	0.28	0.14–2.80	1.26	nd–2.76	0.69	0.65–2.02	1.98	1.12–4.94	2.69	6.62
total (112)	nd–4.93	0.64	0.11–6.74	1.93	nd–4.32	1.21	0.21–7.87	2.49	0.09–8.57	3.31	8.94

[a] Number of samples in parentheses. [b] Values with different letters in the same column are significantly different ($P < 0.05$). [c] Not detected.

Table 3. Stilbenes, Ochratoxin A Levels, and Total Piceids/Total Resveratrols Ratios in Italian Red Wines from Different Geographical Origins

	ochratoxin A (μg/L)		total resveratrol (*cis* + *trans*) (mg/L)		total piceids (*cis* + *trans*) (mg/L)		total stilbenes (mg/L)		total piceids/ total resveratrols	
geographical origin[a]	min–max (median)	mean	min–max	mean	min–max	mean	min–max	mean	min–max	mean
north (17)	nd[b]–0.54 (0.05)	0.12a[c]	0.53–9.87	3.32a	1.86–8.08	4.91a	3.14–14.36	8.23a	0.30–4.92	2.33a
central (46)	nd–0.80 (0.02)	0.07a	0.19–10.28	4.04a	1.15–10.89	4.17a	2.79–16.95	8.21a	0.26–24.6	2.05a
south (49)	0.02–4.93 (1.03)	1.36b	0.14–7.57	2.23b	3.23–15.62	7.63b	3.93–20.83	9.86a	0.96–27.07	5.58b

[a] Number of samples in parentheses. [b] Not detected. [c] Values with different letters in the same column are significantly different ($P < 0.05$).

4.92) for northern and 2.05 (range = 0.26−7.45, except one sample at 24.63) for central Italy wine samples.

Incidences of ochratoxin A contamination were 70, 59, and 100% of wine samples from northern, central, and southern Italy, with mean (and median) levels of 0.12 μg/L (0.05), 0.07 μg/L (0.02), and 1.36 μg/L (1.03), respectively. Levels in wines from southern Italy were significantly higher ($P < 0.0001$) than those found in the rest of Italy, with maximum and mean values of 4.93 and 2.00 μg/L, respectively, recorded in Negroamaro blend from Apulia (**Table 2**). In particular, 18% of wines from southern Italy contained OTA levels of >2.00 μg/L, the European Union (EU) regulatory limit set for wine and derived products (*22*). It has to be noted, however, that all wine samples analyzed in this study were from vintage years (1997−2002) prior to the enforcement of the EU regulation in 2005.

Correlation between Ochratoxin A and Stilbenes in Red Wines. When all OTA-positive wine samples ($n = 88$, assuming 0.02 μg/L as the limit of quantification) were considered for statistical analysis, positive correlations were found between ochratoxin A and total stilbenes ($r = 0.62$) as well as between ochratoxin A and *trans*-piceids ($r = 0.70$), *cis*-piceids ($r = 0.66$), and *trans*-resveratrol ($r = 0.25$), whereas no correlation was found with *cis*-resveratrol. The low correlation with resveratrols was mainly attributable to the low OTA levels recorded in wine samples from northern (median = 0.05 μg/L) and central Italy (median = 0.02 μg/L) that were poorly scattered (mostly grouped at levels close to the detection limit) and did not allow an appropriate statistical evaluation. A good scattering of OTA levels was found instead in wine samples from southern Italy, ranging from 0.02 to 4.93 μ/L, with a mean level at 1.36 μg/L. Statistical analysis performed on southern wine samples ($n = 49$) resulted in positive correlation between ochratoxin A and all resveratrol-related compounds, either individually or grouped. In particular, correlation coefficients (r) for OTA versus *trans*-resveratrol (0.52) and *cis*-resveratrol (0.29) were considerably higher than in all OTA-positive samples, whereas results were similar for *trans*-piceid (0.66) and *cis*-piceid (0.67). **Figure 3** shows the linear regression of OTA versus total stilbenes ($r = 0.74$), total piceids ($r = 0.74$), and total resveratrols ($r = 0.50$). Moreover, similar correlation coefficients (r) between *trans*-resveratrol and ochratoxin A were found within wine samples of the same variety grown in southern Italy; they were 0.47 and 0.53 for Negroamaro and Primitivo, respectively. The six samples with the highest values of ochratoxin A (from 3.17 to 4.93 μg/L) were also those containing the highest levels of stilbenes (from 15.65 to 20.83 mg/L).

DISCUSSION

Stilbenes are known as phytoalexins, that is, antioxidant compounds with a significant role in the protection of crop plants, the biosynthesis of which is induced by both biotic (e.g., fungal or bacteria attacks) and abiotic elicitors (e.g., UV irradiation, aluminum chloride, ozone, etc.) (*23*). The elicitation of stilbene biosynthesis in grapevines by attacks of *Botrytis cinerea*, *Plasmopara viticola*, and other parasitic fungi is well documented (*24*−*26*), whereas limited data are available on the interaction between ochratoxigenic fungi and grapevine (*15*). Red wines usually contain higher stilbene concentrations than rosé or white wines. In agreement with previous papers (*5*, *6*)

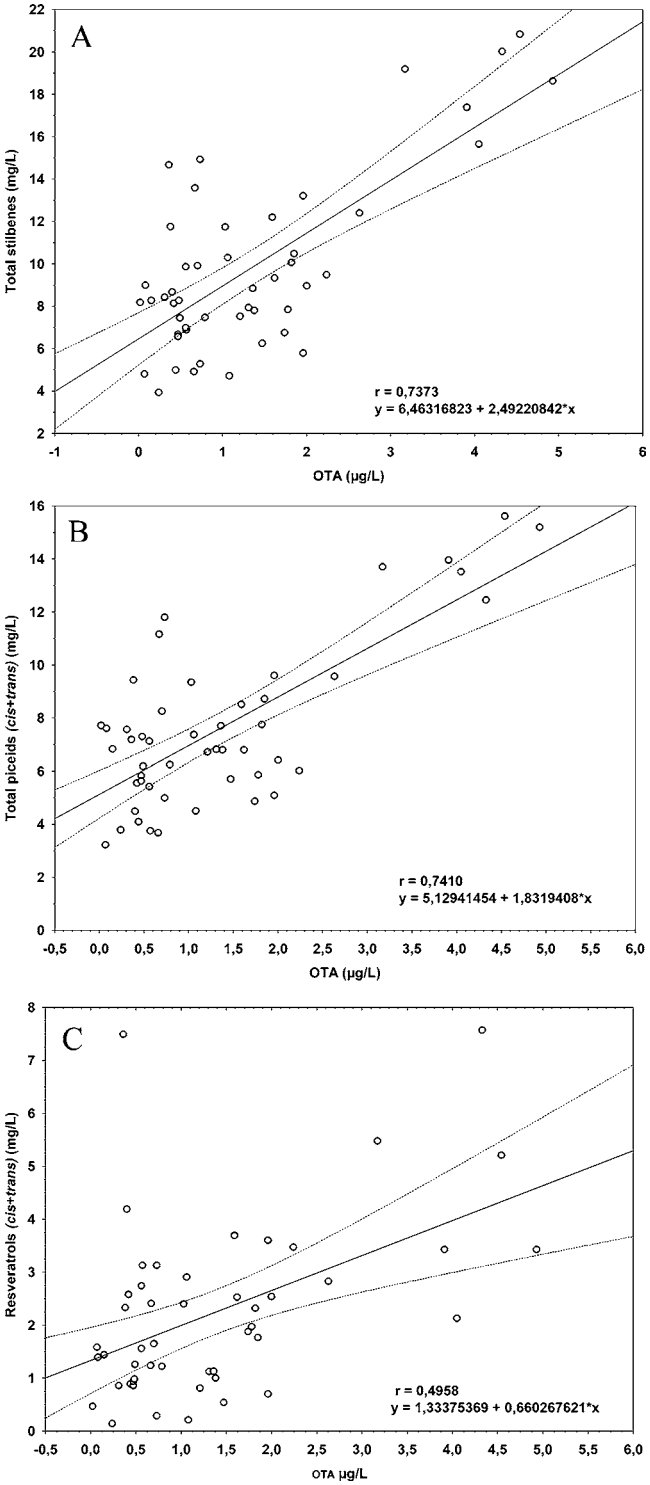

Figure 3. Regression analysis of ochratoxin A versus stilbenes (**A**), piceids (**B**), and resveratrols (**C**) in red wine samples from southern Italy.

our results support the evidence that there is a great variability of resveratrol content in wines from different grape varieties. This variability can be explained not only in terms of intrinsic physiological properties of the specific cultivar and grape maturation level but also in terms of exogenous factors such as climatic conditions and degree of fungal infection (*25*, *27*). Wines obtained from the Merlot grape variety were shown to contain the highest amounts of resveratrol (*cis* + *trans*) in the present study as well as in several previous surveys (*5*, *12*, *28*).

Our results highlight the occurrence of higher levels of piceids with respect to free resveratrols in wines obtained from the warm and dry regions of southern Italy (Negroamaro, Negroamaro blend, Primitivo, and Aglianico). This is in agreement with similar data that have been reported for some wines from warm and dry regions of Spain, Portugal, and South America (*5*, *28*, *29*), whereas most wines from cooler areas, such as Burgundy, Bordeaux, Pinot Noir, or Merlot, exhibit profiles with higher amounts of resveratrol in its free form with respect to piceids. Previous studies indicated that resveratrol is mostly bound as *cis*- and *trans*-glucoside in grape berry skins of grapevine cultivars grown in warm regions (*20*, *21*, *27*). The different piceids concentrations in wines were suggested to depend on the enzymatic activity of β-D-glucosidase and the relevant presence of the enzyme in musts from different grape varieties (*5*).

In agreement with previous surveys indicating that warm climatic conditions favor ochratoxin A production in grapes (*1*, *3*, *4*), our results showed contamination to be predominant in wines from southern Italy with an increasing trend "north to south". Battilani et al. (*30*) found, in laboratory experiments, a different susceptibility to *Aspergillus carbonarius* infection and OTA accumulation by different grapevine varieties, indicating Primitivo and Negroamaro among the susceptible ones. Our results support these data showing that high levels of OTA contamination occur in red wines obtained from Primitivo and Negroamaro cultivars. Moreover, for these wines containing relevant amounts of ochratoxin A, a positive correlation was found between OTA and total stilbenes levels, supporting the hypothesis that stilbene biosynthesis elicitation could take place in the field during the grape-ripening stage due to the presence of ochratoxigenic fungi on grape berries. Although resveratrol content in grapes depends on several biotic and abiotic factors, including grape variety and geographical location, our findings suggest that, for the same grape varieties in the same geographical location (southern Italy), resveratrol formation is to a certain extent related to the infection of black aspergilli. The effect of such infection on resveratrol response is, however, relevant when high levels of OTA are formed, that is, when the fungus has already developed at considerable extent. This hypothesis is in agreement with previous results on the low fungicidal activity of black aspergilli (*15*) and on OTA accumulation in grape berries only when high contaminations by black aspergilli ($>10^6$ CFU) occur in the field (*31*). Our results provide a stronger evidence for the positive correlation between OTA and *trans*-resveratrol in red wine ($r = 0.52$) with respect to the similar correlation ($r = 0.20$) previously reported by Bavaresco et al. (*15*) for grapevine berries.

Further studies, involving field trials and vineyard monitoring at different grape maturity stages, would be required to assess the direct effect of ochratoxigenic strains of *A. carbonarius* on ochratoxin A production in different grapevine varieties also in relation to their capability to synthesize stilbenes in response to the specific fungal attack.

Jeswal (*14*) pointed out that concurrent administration of berry and leaf juice of common grapes to mice, together with OTA, significantly reduces the hepatic and renal damage caused by ingestion of the mycotoxin. This is the first study showing a positive correlation between ochratoxin A contamination and resveratrol content of red wine in retail samples. Further studies should be warranted using effective in vitro and in vivo tests to understand the possible anticancer/antioxidant role of resveratrol in impairing OTA toxicity toward animals and human. The toxic risk related to OTA exposure due to red wine consumption could be reconsidered in view of the beneficial effect of the relevant content of resveratrol.

ACKNOWLEDGMENT

We thank G. Panzarini for technical help in the ochratoxin A analysis.

LITERATURE CITED

(1) Zimmerli, B.; Dick, R. Ochratoxin A in table wine and grape juice: occurrence and risk assessment. *Food Addit. Contam.* **1996**, *13*, 655−668.

(2) Visconti, A.; Pascale, M.; Centonze, G. Determination of ochratoxin A in wine by means of immunoaffinity column clean-up and high-performance liquid chromatography. *J. Chromatogr., A* **1999**, *864*, 89−101.

(3) Otteneder, H.; Majerus, P. Occurrence of ochratoxin A (OTA) in wines: influence of the type of wine and its geographical origin. *Food Addit. Contam.* **2000**, *17*, 793−798.

(4) Pietri, A.; Bertuzzi, T.; Pallaroni, L.; Piva, G. Occurrence of ochratoxin A in Italian wines. *Food Addit. Contam.* **2001**, *18*, 647−654.

(5) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Resveratrol: a molecule whose time has come and gone? *Clin. Biochem.* **1997**, *30*, 91−113.

(6) Frémont, L. Biological effects of resveratrol. *Life Sci.* **2000**, *66*, 663−673.

(7) IARC. *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*; Monograph on the Evaluation of Carcinogenic Risks to Humans 56; International Agency for Research on Cancer: Lyon, France, 1993; pp 489−521.

(8) European Commission. 2002. EC—Directorate-General Health and Consumer Protection. Assessment of dietary intake of ochratoxin A by the population of EU Member States; reports on tasks for scientific cooperation, 153 pp; http://europa.eu.int/comm/food/fs/scoop/3.2.7_en.pdf (accessed March 30, 2007).

(9) Hart, J. H. Role of phytostilbenes in decay and disease resistance. *Annu. Rev. Phytopathol.* **1981**, *19*, 437−458.

(10) Kimura, Y.; Okuda, H. Effects of naturally occurring stilbene glucosides from medicinal plant and wine, on tumour growth and lung metastasis in Lewis lung carcinoma-bearing mice. *J. Pharm. Pramacol.* **2000**, *52* (10), 1287−1295.

(11) Goldberg, D. M.; Ng, E.; Yan, J.; Karumanchiri, A.; Soleas, G.; Diamandis, E. Regional differences in resveratrol isomer concentrations of wines from various cultivars. *J. Wine Res.* **1996**, *7*, 13−24.

(12) Mattivi, F.; Nicolini, G. Influenza della tecnica di vinificazione sul contenuto di resveratrolo dei vini. *L'Enotecnico* (*luglio/agosto*) **1993**, 81−88.

(13) Grosse, Y.; Chekir-Ghedira, L.; Huc, A.; Obrecht-Pflumio, S.; Dirheimer, G.; Bacha, H.; Pfohl-Leszkowic, A. Retinol, ascorbic acid and α-tocopherol prevent DNA adduct formation in mice treated with the mycotoxins ochratoxin A and zearalenone. *Cancer Lett.* **1997**, *114*, 225−229.

(14) Jeswal, P. Antidotal effect of grape juice (*Vitis vinifera*) on ochratoxin A caused hepatorenal carcinogenesis in mice (*Mus musculus*). *Cytobios* **1998**, *93*, 123−128.

(15) Bavaresco, L.; Vezzulli, S.; Battilani, P.; Giorni, P.; Pietri, A.; Bertuzzi, T. Effect of ochratoxin A-producing *Aspergilli* on stilbenic phytoalexin synthesis in grapes. *J. Agric. Food Chem.* **2003**, *51*, 6151−6157.

(16) Trela, B. C.; Waterhouse, A. A. Resveratrol: isomeric molar absorptivities and stability *J. Agric. Food Chem.* **1996**, *44*, 1253−1257.

(17) Visconti, A.; Pascale, M.; Centonze, G. Determination of ochratoxin A in wine and beer by immunoaffinity column clean-up and liquid chromatographic analysis with fluorometric detection: collaborative study. *J. AOAC Int.* **2001**, *84*, 1818−1827.

(18) Careri, M.; Corradini, C.; Elviri, L.; Nicoletti, I.; Zagnoni, I. Direct HPLC analysis of quercitin and trans-resveratrol in red wine, grape, and winemaking byproducts. *J. Agric. Food Chem.* **2003**, *51*, 5226−5231.

(19) Goldberg, D. M.; Ng, E.; Karumanchiri, A.; Yan, J.; Diamandis, E. P.; Soleas, G. J. Assay of resveratrol glucosides and isomers in wine by direct-injection high-performance liquid chromatography. *J. Chromatogr., A* **1995**, *780*, 89−98.

(20) Gobbi, L.; Nicoletti, I.; Tarola, A. M.; Corradini, C. Evaluation of the level of *cis*- and *trans*-resveratrol and their glucosides in red wines produced in Lazio Region (area). *J. Commod. Sci.* **2004**, *43*, 173−190.

(21) Waterhouse, A. L.; Lamuela-Raventòs, R. M. The occurrence of piceid, a stilbene glucoside, in grape berries. *Phytochemistry* **1994**, *37*, 571−573.

(22) European Commission Directive 123/2005 of Jan 26, 2005.

(23) Adrian, M.; Jeandet, P.; Douillet-Breuil, A. C.; Tesson, L.; Bessis, R. Stilbene content of mature *Vitis vinifera* berries in response to UV-C elicitation. *J. Agric. Food Chem.* **2000**, *48*, 6103−6105.

(24) Adrian, M.; Jeandet, P.; Veneau, J.; Weston, L. A.; Bessis, R. Biological activity of resveratrol, a stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. *J. Chem. Ecol.* **1997**, *23*, 1689−1702.

(25) Jeandet, P.; Douillet-Breuil, A. C.; Bessis, R.; Debord, S.; Sbaghi, M.; Adrian, M. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *J. Agric. Food Chem.* **2002**, *50*, 2731−2741.

(26) Filip, V.; Plocková, M.; Smidrkal, J.; Špičková, Z.; Melzoch, K.; Schmidt, Š. Resveratrol and its antioxidant and antimicrobial effectiveness. *Food Chem.* **2003**, *83*, 585−593.

(27) Roldán, A.; Palacios, V.; Caro, I.; Pérez, L. Resveratrol content of *Palomino fino* grapes: influence of vintage and fungal infection. *J. Agric. Food Chem.* **2003**, *51*, 1464−1468.

(28) Lamuela-Raventòs, R. M.; Romero-Pèrez, A. I.; Waterhouse, A. L.; de la Torre-Boronat, M. C. Direct HPLC analysis of *cis*- and *trans*-resveratrol and piceid isomers in Spanish red *Vitis vinifera* wines. *J. Agric. Food Chem.* **1995**, *43*, 281−283.

(29) Moreno-Labanda, J. F.; Mallavia, R.; Pérez-Fons, L.; Lizama, V.; Saura, D.; Micol, V. Determination of piceid and resveratrol in Spanish wines deriving from *Monastrell* (*Vitis vinifera* L.) grape variety. *J. Agric. Food Chem.* **2004**, *52*, 5396−5403.

(30) Battilani, P.; Logrieco, A.; Giorni, P.; Cozzi, G.; Bertuzzi, T.; Pietri, A. Ochratoxin A production by *Aspergillus carbonarius* on some grape varietes grown in Italy. *J. Sci. Food Agric.* **2004**, *84*, 1736−1740.

(31) Cozzi, G.; Pascale, M.; Perrone, G.; Visconti, A.; Logrieco, A. Effect of *Lobesia botrana* damages on black aspergilli rot and ochratoxin A content in grapes. *Int. J. Food Microbiol*. **2006**, *111* (F), S22−S27.

Received for review April 4, 2007. Revised manuscript received May 30, 2007. Accepted May 31, 2007. This research was supported by the Italian Ministry of Education, University and Research (MIUR), Project 12818 − SIVINA (DD 1801 − 31/12/2004).

JF070993L