Direct Analysis of Resveratrol in Wine by Micellar Electrokinetic Capillary Electrophoresis

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Resveratrol is an antioxidant found in grapes and other plants. It has been reported to have health benefits including anticarcinogenic activity and protection against coronary heart disease. Previous methods for its quantification in wines have relied on HPLC and GC. However, an alternative method utilizing capillary electrophoresis (CE) has been developed in our laboratory. The CE procedure resolves *cis*- and *trans*-resveratrol isomers, using a micellar mode of separation. The detection limit for resveratrol was 1.25 μM using UV detection at 310 nm. This procedure was employed for the direct analysis of *trans*-resveratrol in wines. The direct analysis method used was advantageous because the analysis time was greatly reduced and sample preparation procedures that might result in resveratrol isomerization were avoided. The CE procedure was used to determine the level of *trans*-resveratrol in several California red wines, and the values obtained were in close agreement with those reported using other methods.

Keywords: *Resveratrol; capillary electrophoresis; wine*

INTRODUCTION

Resveratrol is an antioxidant compound belonging to the family of stilbenes. It is found in a number of plants including grapes, in which it is primarily produced as an antifungal agent (Langcake and Pryce, 1976). Antimicrobial compounds produced in plants upon infection are called phytoaxelins. Resveratrol is mainly located in the plant leaves (Langcake and Pryce, 1976) and grape skin (Creasy and Coffee, 1988). It is produced rapidly in response to infection and therefore can be found in healthy grapes as well as infected ones (Jeandet et al., 1995a). However, fruit heavily infected with *Botrytis* remarkably contained little or no resveratrol (Pezet et al., 1991). This was attributed to degradation by a laccase-like stilbene oxidase of fungal origin.

Resveratrol content in wines can vary as a function of viticultural and enological practices (Jeandet et al., 1995b). Wines produced with long maceration times were found to be much higher in resveratrol content. Red wines contained higher levels of resveratrol than white wines regardless of maceration duration. It was concluded that this was due to differences in the abilities of grape varieties to synthesize resveratrol in response to fungal infection (Jeandet et al., 1995b).

Human consumption of antioxidants has many alleged health benefits, including protection against cardiovascular diseases (Kushi et al., 1995) and most recently cancer (Hertog et al., 1995; Jang et al., 1997). Red wines contain a variety of polyphenolic antioxidants, which have been discovered to inhibit the oxidation of low-density lipoprotein in humans (Frankel et al., 1995). Cardiovascular disease is usually associated with high intake of saturated fat in the diet, but this was not seen to be the case in France (Renaud and Lorgeril, 1992). It is thought that the consumption of red wines by the French could provide an explanation for the low cardiovascular disease mortality rate encountered in that country. In addition, traditional Oriental medicines such as Kojo Kon, which proportedly provide protection against arteriosclerosis, has also been found to contain resveratrol (Goldberg et al., 1995a).

A variety of methods have been developed for the quantification of resveratrol including GC (Goldberg et al., 1995b; Soleas et al., 1995; Lamikanra et al., 1996) and HPLC (Jeandet et al., 1995b, 1997; Pezet et al., 1994; Goldberg et al., 1995c; Lamuela-Raventos et al., 1995). These methods generally involve extraction and derivatization requiring extensive precautions such as exclusive nitrogen environments and protection from UV light. However, they can resolve both isomers of resveratrol and are quite sensitive with a 10^{-8} M limit of detection (Lamuela-Raventos et al., 1995; Jeandet et al., 1995b; Goldberg et al., 1995c). HPLC methods have also been reported for direct analysis of resveratrol when the separation is coupled with either fluorescence (Pezet et al., 1994) or electrochemical detection (McMurtrey et al., 1994).

GC methods have also been used successfully for resveratrol determination in wine samples (Goldberg et al., 1994, 1995b; Soleas et al., 1995; Jeandet et al., 1995b). These methods can separate both isomers and, when coupled with mass spectrometric analysis, are very sensitive with reported detection limits of 10^{-9} M (Soleas et al., 1995). A direct injection GC method that separated underivatized *trans*-resveratrol following preconcentration on a C_{18} cartridge was reported by Goldberg et al. (1994). The technique when coupled with mass spectrometric detection was able to determine *trans*-resveratrol at 10^{-8} M in wine samples (Goldberg et al., 1994).

Capillary electrophoresis (CE), a new separation technique, has unique advantages including high efficiency and ability to analyze many types of compounds (Vesterberg, 1989; Zeece, 1992; Cancalon, 1995). The small-diameter column results in high separation ef-

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ficiency because high field strengths can be applied without heating problems. CE is also advantageous because of the small sample size required and separations generally use little or no organic solvents.

The application of CE to the separation of phenolic compounds has been previously reported (Ong et al., 1990; Jen et al., 1996; Martinez et al., 1996). CE has been used to separate typical food antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Hall et al., 1994). However, a CE method for the determination of resveratrol in wine has not been reported. Therefore, we have developed a CE method for separation and quantification of this important compound.

MATERIALS AND METHODS

Chemicals. Sodium phosphate, sodium borate, and SDS were obtained from Sigma Chemical Co. The compound 2-[(2-aminoethyl)amino]-5-nitropyridine hydrochloride, also obtained from Sigma, was used as an internal standard for quantification of resveratrol. This compound was further purified by dissolving 50 mg in 1.0 mL of water and adjusted to pH 7.0 with 0.1 M NaOH. The precipitate was removed by centrifugation at 14000*g* for 5 min. The supernatant was diluted with water to a concentration of 10 mM. *trans*-Resvereatrol was also purchased from Sigma. A mixture of *cis* and *trans* forms was produced by exposing 1.0 mL of a 5 mM stock solution in 50% acetonitrile to UV light from a gel trans illuminator for 2−4 min. Distilled water purified by reverse osmosis treatment to 18 mΩ was used in all experiments.

Wines Analyzed. Four California wines were obtained locally and analyzed for resveratrol level using the procedures described. All wines were stored in the dark at 4 °C, and each was opened immediately prior to analysis. Wines were prepared for CE by mixing a 30 μL aliquot with 5 μL of the internal standard.

Instrumentation. Instruments used for the analysis were ISCO model 3850 and Beckman P/ACE system 5510. Data collection and analysis were performed using P/ACE System Gold software or Caesar (version 4.01) for Beckman and ISCO instruments, respectively. The capillary was fused silica with 50 μm internal diameter and approximately 30 cm in length to the detector.

Capillary Electrophoresis. CE was performed in 25 mM sodium borate, 25 mM sodium phosphate, and 75 mM SDS, pH 9.0. The buffer was filtered through a 0.45 μm microfilter before use. Separations were conducted with fused silica capillaries (50 μm i.d. × 30 cm effective length) at 16 kV and 20 °C (Beckman Instrument) or 20 kV and 20−25 °C (ISCO instrument). Wine samples were filtered through a 0.45 μm filter and injected without further treatment into the capillary.

RESULTS AND DISCUSSION

CE Separation of *trans*- and *cis*-Resveratrol. Previous work concerning CE separation of phenolic antioxidants has shown that aromatic compounds can be satisfactorily separated using an SDS micellar mode (Hall et al., 1994). The separation of other phenolic compounds by micellar electrokinetic chromatography (MEKC) (Martinez et al., 1996) also suggests the suitability of this method to achieve resolution of resveratrol isomers.

Both *trans*- and *cis*-resveratrol isomers have strong absorbance maxima at ≈210 nm. These isomers have secondary maxima at 305−330 and 280−295 nm, respectively (Trela and Waterhouse, 1996). A mixture of *trans* and *cis* forms was obtained by short exposure of the *trans* form to UV irradiation and then separated in the Beckman P/ACE instrument using diode array detection (Figure 1).

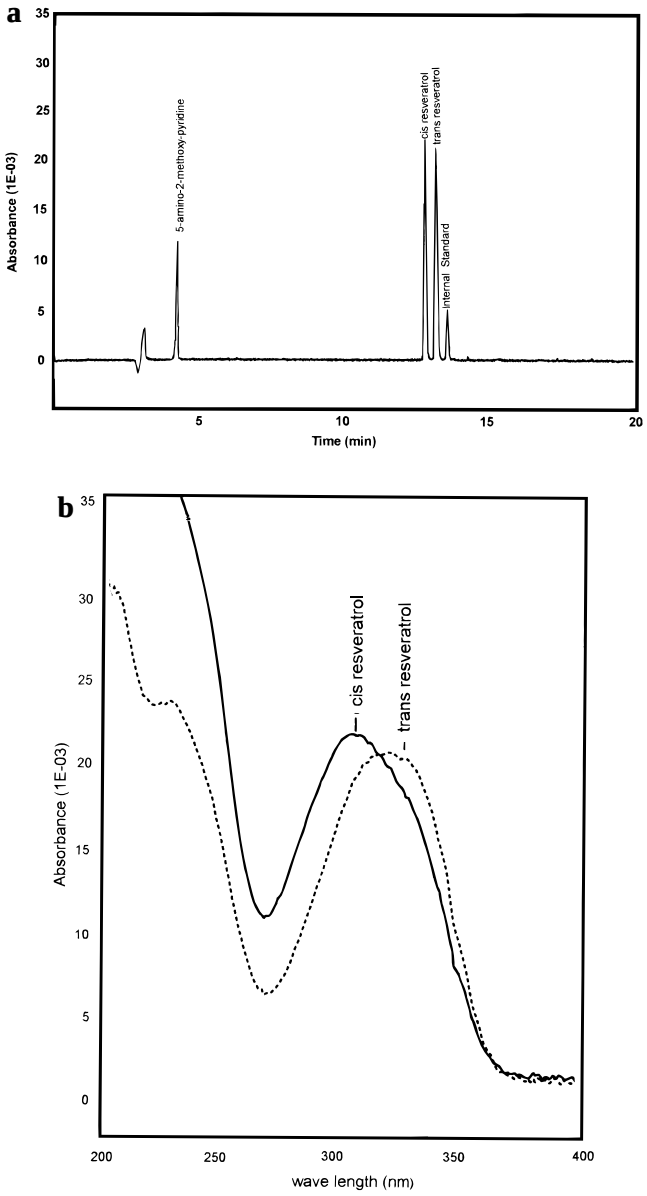

Figure 1. Electropherogram of *cis*- and *trans*-resveratrol: (a) Separation of the mixture of *cis*- and *trans*-resveratrol produced by UV irradiation was performed in 25 mM sodium borate, 25 mM sodium phosphate, and 75 mM SDS, pH 9.0, with a fused silica column (50 μm × 28 cm effective length) at 16 kV using a Beckman P/ACE 5510 instrument, equipped with diode array detector. The position of the internal standard used for quantitation, 2-[(2-aminoethyl)amino]-5-nitropyridine hydrochloride, is labeled as internal standard in this figure. The position of 5-amino-2-methoxypyridine, an early migrating compound also suitable for use as an internal standard, is indicated. (b) Spectral scan (200−400 nm) of the *cis* and *trans* peaks shown in part a.

Resveratrol *trans* and *cis* isomers were well resolved under micellar conditions employing 25 mM sodium phosphate−sodium borate, pH 9.0, containing 75 mM SDS (Figure 1a). Baseline resolution of the two forms can be seen in this electropherogram with detection at 310 nm. This figure shows that the UV treatment (2 min) of *trans*-resveratrol resulted in ≈50% conversion to the *cis* form, on the basis of relative peak areas. Longer exposure times resulted in a significant increase in degradation products. The migration time of the internal standard, 2-[(2-aminoethyl)amino]-5-nitropy-

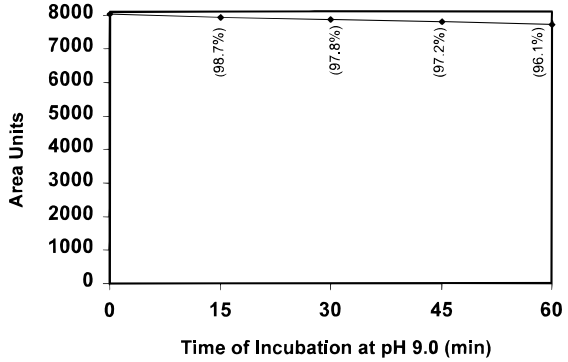

Figure 2. Stability of *trans*-resveratrol at pH 9.0. A 1.0 mM solution of *trans*-resveratrol containing 25 mM sodium borate, 25 mM sodium phosphate, and 75 mM SDS, pH 9.0, was incubated at 20 °C. Aliquots of the solution were analyzed as described in Figure 1 after 0, 15, 30, 45, and 60 min of incubation and *trans*-resveratrol peak area plotted vs time of incubation.

ridine, was slightly longer than that of *trans*-resveratrol and thus did not interfere with the separation of either isomer. Another compound (5-amino-2-methoxypyridine) also present in this separation (Figure 1a) was included as an additional internal standard. Its migration time was ~4 min and had an absorption maximum near 300 nm. However, it was not used in subsequent analyses of wines.

A spectral scan of each of the resveratrol peaks in Figure 1a showed secondary absorbance maxima for *trans*- and *cis*-resveratrol at approximately 295 and 310 nm, respectively (Figure 1b). This mode of detection could potentially be used to identify both *cis* and *trans* isomers of resveratrol in separation of wine samples. However, in the interest of maximizing sensitivity, a UV detector was employed (at 310 nm) for investigations of resveratrol in wine samples.

The stability of resveratrol under the conditions used for these separations was examined in light of the reported degradation of resveratrol at pH 10 (Trela and Waterhouse, 1996). These authors found that *trans*-resveratrol was more susceptible to degradation than the *cis* isomer and exhibited half-lives of 1.6 and 63.7 h, respectively. Incubation of the more sensitive *trans*-resveratrol form in the separation buffer employed here, containing sodium phosphate and borate at pH 9.0, resulted in a loss of only 3.9% after 60 min (Figure 2). Because resveratrol separations, and thus exposure to pH 9.0, were accomplished in <15 min, the loss (≈1.2%) was considered to be acceptable.

A standard curve for *trans*-resveratrol was established using 2-[(2-aminoethyl)amino]-5-nitropyridine as the internal standard. The ratio of peak areas for *trans*-resveratrol and 2-[(2-aminoethyl)amino]-5-nitropyridine was plotted versus *trans*-resveratrol concentration. The curve was linear in the range of 1.25−25 μM, with a linear correlation of 0.997 (r^2). The lower end of this curve also represents the limit of detection.

CE Separation of Resveratrol in Wine. A typical electrophoretic separation of California wine (Cabernet Sauvignon, 1993) can be seen in Figure 3. The separation was performed with the ISCO instrument using a UV detector set at 310 nm. This electropherogram contains many components, some of which are clustered together in the 5−8 min region. However, resveratrol migrated near the end of the separation and was resolved from other components.

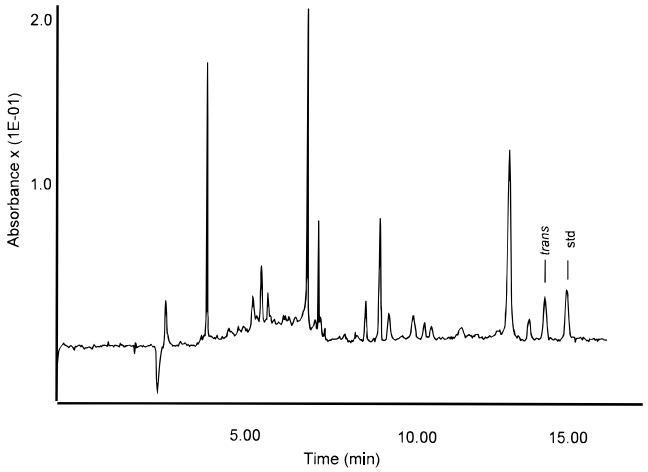

Figure 3. Electropherogram of California Cabernet Sauvignon (1993) wine. Separation was performed as in Figure 1 with a fused silica (50 μm × 30 cm effective length) at 20 kV using an ISCO model 3850 instrument equipped with UV detector set at 310 nm. The positions of *trans*-resveratrol *(trans)* and internal standard 2-[(2-aminoethyl)amino]-5-nitropyridine hydrochloride (std) are indicated.

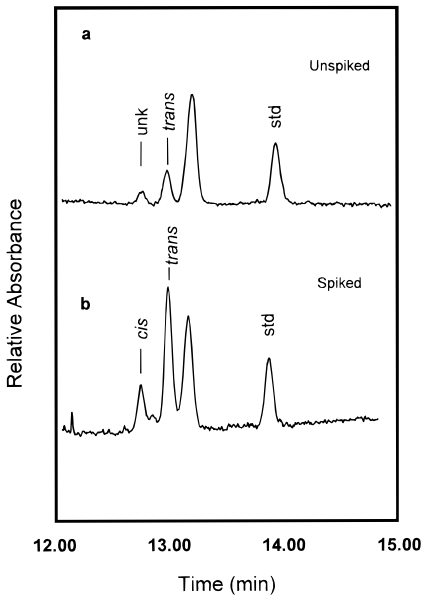

Figure 4. Identification *trans*-resveratrol in electropherogram of California Cabernet Sauvignon (1994) wine: (a) unspiked Cabernet Sauvignon (1994) wine; (b) Cabernet Sauvignon (1994) wine spiked with a mixed resveratrol standard produced by UV irradiation. Separations and conditions were performed as indicated in Figure 3.

The identity of resveratrol in electropherograms was confirmed by spiking wines with a mixed resveratrol standard similar to that shown in Figure 1. A comparison of the spiked and unspiked samples shows that *trans*-resveratrol was readily identified in the separation of Cabernet Sauvignon (1994) (Figure 4). However, the identification of a peak corresponding to *cis*-resveratrol in this wine was ambiguous. A peak was observed to migrate in the region of *cis*-resveratrol in the unspiked sample of Cabernet Sauvignon (Figure 3). The corresponding spiked separation contained an additional peak in the valley between the *cis* and *trans* peaks. Similar difficulties with comigration and low concentration were encountered with Merlot and Pinot Noir wines. Therefore, no attempt was made to quantitate *cis*-resveratrol in these wines.

Table 1. Quantification of *trans*-Resveratrol in Selected California Wines at pH 9.0[a]

wine	*trans*-resveratrol concn (μM)
Pinot Noir	5.3 ± 0.4
Merlot	15.3 ± 0.7
Carbernet Sauvignon, 1993	1.9 ± 0.5
Cabernet Sauvignon, 1994	8.9 ± 1.6

[a] Values represent means (±SD) of three determinations in these wines.

Determination of *trans*-Resveratrol in Wine. Four wines were investigated for resveratrol content in this study. The wines surveyed varied greatly in *trans*-resveratrol content (Table 1). However, the values shown in Table 1 are in the same concentration range (low micromolar) as reported by others using GC and HPLC methods (McMurtrey et al., 1994; Frankel et al., 1995; Goldberg et al., 1995b; Lamikanra et al., 1996).

The level of *trans*-resveratrol found in Merlot samples in these studies (15.3 μM) was in the same range (4−16 μM) as previously reported using other methods of analysis (Soleas et al., 1995; Lamuela-Raventos et al., 1995; Lamikanra et al., 1996).

As mentioned above, the separation did not permit unambiguous determination of *cis*-resveratrol in these wine samples. It could be estimated from relative peaks areas that *cis*-resveratrol concentration was somewhat <1 μM in the wines tested. However, this result is in disagreement with other studies that reported concentrations of *cis*-resveratrol between 2.5 and 6.2 μM for Pinot Noir (Jeandet et al., 1995b; Soleas et al., 1995). It is unlikely the *cis* form of resveratrol was degraded as a result of exposure to pH 9.0 during the separation as it has been shown to be much more stable than the *trans* form (Trela and Waterhouse, 1996). The *trans*-resveratrol degradation was shown (Figure 2) to be ≈1% after a period of incubation at pH 9.0 equivalent to the separation time (15 min). A possible explanation for the discrepancy in *cis* resveratrol concentrations may be that its level in these wines was just below that required for reliable detection by this CE method.

This difficulty in the analysis of resveratrol isomers in wines by CE further illustrates the need for methods of increased sensitivity. One approach to enhancing the sensitivity could be provided through the application of on-line preconcentration devices, as described by Tomlinson et al. (1995).

Conclusions. This work demonstrates the first effort in developing a capillary electrophoretic procedure for the analysis of resveratrol in wine. The method can separate pure forms of *cis* and *trans*-resveratrol. Resveratrol separations were performed in less <15 min with a detection limit of 1.25 μM. The separation method was applied to the direct determination of resveratrol in wines. Values for *trans*-resveratrol found in the wines tested were in close agreement with those reported for other methods. However, *cis*-resveratrol could not be unequivocally identified and measured. In summary, these results demonstrate the potential usefulness of CE, in conjunction with direct analysis, for the determination of resveratrol in wines and other materials.

ABBREVIATIONS USED

CE, capillary electrophoresis; GC, gas chromatography; HPLC, high-performance liquid chromatography; SDS, sodium dodecyl sulfate.

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