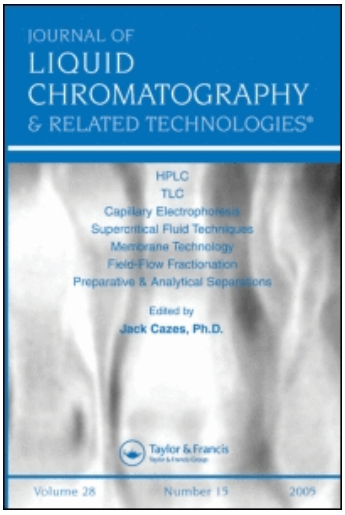

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Resveratrol Analysis and Degradation Study in Blueberry Samples by HPLC with Fluorescence Detection

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Abstract: Separation and quantification of *trans*-resveratrol in blueberry extracts were achieved using high performance liquid chromatography (HPLC). *Trans*-resveratrol in blueberry juice was found to be at a high level of $16.60 \pm 0.19\,\mu g/mL$. This antioxidant polyphenolic compound was also quantified, in two brands of blueberry powder, to be $236.4 \pm 7.5\,\mu g/g$ or $269.9 \pm 5.7\,\mu g/g$. Degradation of *trans*-resveratrol was observed in standard solutions and in blueberry samples (juice and powder extracts). It was found that degradation of *trans*-resveratrol was more severe when it was exposed to room light at room temperature. Therefore, it is advisable to store blueberry juice at 4°C in the dark, and consume it within 1–3 days in order to benefit from adequate intake of the antioxidant in the juice.

Keywords: Analysis, Blueberries, Degradation, Resveratrol

INTRODUCTION

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring stilbene which is present in a number of plant species.[1] Resveratrol belongs to the subclass of phytochemicals known as phytoalexins which are defense

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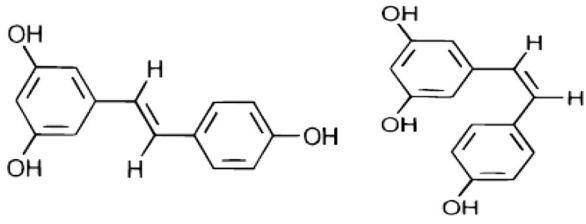

Figure 1. Chemical structures of (a) trans-resveratrol and (b) *cis*-resveratrol.

compounds synthesized in response to pathogens (e.g., *B. cinera*) and abiotic stress (e.g., UV light).[2] In recent years, biochemical and medical investigations on resverarol have revealed its many potential biological effects to human health. These include antioxidant activity,[3,4] anticancer effect,[5,6] inhibition of platelet aggregation,[7] phytoestrogenic effect,[8] and anti-inflammatory activity.[9]

Resveratrol was first discovered in grapes in 1976.[10] It exists as the *trans* and *cis* isomeric forms (Fig. 1). In grapes (i.e., *Vitis vinifera*), *trans*-resveratrol is the major isomer and is mainly found in the fruit skin, i.e., 50–100 μg/g.[11] It is known that the *trans* isomer is converted from the *cis* isomer by fungal infection.[12,13]

On the other hand, it was reported that *trans*-resveratrol could be irradiated by UV to undergo partial isomeric conversion to *cis*-resveratrol, resulting in a mixture of *trans* and *cis*-resveratrol.[12] In any event, the bioactivity of *cis*-resveratrol is reported to be equal to that of the *trans* isomer, e.g., in protein kinase inhibition[14,15] and platelet aggregation.[7]

The resveratrol content has been analyzed in a variety of red and white wines using HPLC.[16–20] For instance, up to 6.8 μg/mL *trans*-resveratrol was found in red wine samples.[20] *Cis*-resveratrol was also found in wines, but in a lower amount, i.e., 0.1–2.6 μg/mL.[20] However, the compound was only slightly detectable in grapes.[12] Recently, *trans*-resveratrol was also found in blueberries.[22,23] But, the determination of resveratrol content in blueberry products has not been widely reported.

The purpose of this study is to quantify resveratrol in blueberry juice and blueberry powder in capsules using HPLC with fluorescence detection. In addition, studies of the degradation of resveratrol in pure standard, blueberry juice, and blueberry extract (from powder) have been conducted. Although there have been reported analysis and studies of resveratrol in grapes or wines, we present the first systematic study of the antioxidant in blueberry juice and extracts, which have now gained more importance because of the presence of a high content of resveratrol in blueberry.

EXPERIMENTAL

Reagents and Chemicals

Trans-resveratrol (CAS 501-36-0, MW = 228.25) was obtained from Sigma-Aldrich. Acetonitrile, methanol, and acetic acid were obtained from BDH Co. (Toronto, ON). Pure blueberry juice (Bremner, 100%) was purchased from London Drugs (Surrey, BC, Canada). It was labeled not to contain additional water, sugar, additives, and preservatives. Blueberry powder capsules (WellQuest or WQ and Webber Naturals or WN) were obtained from Pharmasave (Burnaby, BC, Canada). Although they were labeled to contain anthocyanins, there was no information on their resveratrol contents.

Equipment and HPLC Conditions

An HPLC system (Bio-Rad 1330) was used in this study. Separation was performed using a reverse-phase column (Waters, Nucleosil C18, 4.6×250 mm, 5 μm). Isocratic elution using the mobile phase of 2% acetic acid:acetonitrile (A:B) of various compositions (i.e., 30–60% B) was conducted at a flow rate of 1.0 mL/min. An injection volume of 25 μL was employed. Using a fluorescence detector (HP 1046A), the eluate was monitored at the excitation wavelength of 330 nm and emission wavelength of 374 nm, as used previously.[13,21]

Sample Preparation

Blueberry juice was filtered through a syringe filter (Acordisc 0.45 μm) before HPLC analysis. The remainder of the sample was kept in the refrigerator at 4°C until further use. Blueberry powder in capsules was dissolved in the mobile phase and gravity filtered prior to direct HPLC injection.

Trans-resveratrol was dissolved in methanol to produce a stock solution at a concentration of 1 mg/mL, to be kept at 4°C in the dark. Then, 1 mL of the stock was diluted to 10 mL using the mobile phase. Serial dilutions were conducted to obtain the concentrations of 0.002, 0.004, 0.02, and 0.1 mg/mL for calibration.

For the degradation study, two samples of blueberry juice were stored in the dark, one at room temperature for 1 day, and the other at 4°C for a duration of 6 days. The two solution extracts of blueberry powder were left at room temperature and exposed to room light for a duration of up to 7 days. Two *trans*-resveratrol standard solutions were

left at room temperature, one in the dark and one under room light conditions for a duration of up to 21 days. The solutions were sampled regularly for HPLC analysis to quantify the amount of *trans*-resveratrol.

RESULTS AND DISCUSSION

Analysis of *trans*-Resveratrol

The contents of *trans*-resveratrol in various blueberry samples were quantified in this study. First, the HPLC conditions were optimized using the blueberry juice sample. Different mobile phase compositions of acetic acid (2%)/acetonitrile were employed for separation (see Fig. 2). The use of the 50:50 composition has resulted in adequate separation. The resveratrol peak was confirmed by spiking (see the inset of Fig. 2). The broad peaks could be the result of hydroxyl group dissociation, as previously noted in wine samples.[21] Thereafter, different percentages of acetic acid in the mobile phase were tested (see Fig. 3), confirming that 2% acetic acid produced the best result. The optimal composition of mobile phase was thus found to be 50:50 (2% acetic acid:acetonitrile). In addition, the analysis was completed at a reasonably short retention time of 250 sec (or ~4 min). Under these conditions, the amount of *trans*-resveratrol

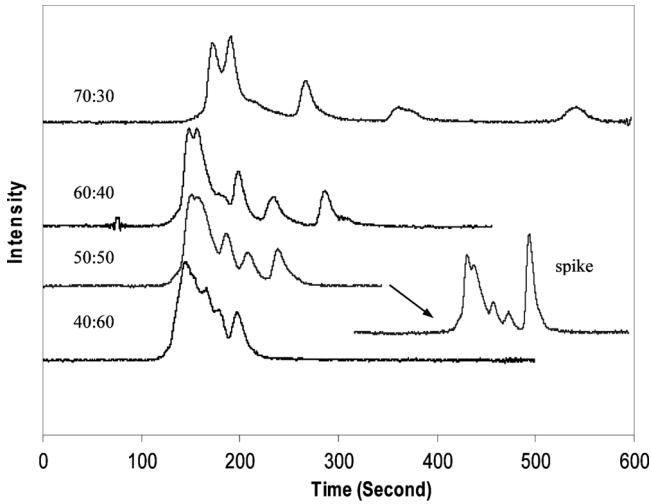

Figure 2. HPLC analysis of blueberry juice at different compositions of acetic acid (2%)/acetonitrile mobile phase. For other HPLC conditions, see experimental section. Spiking of resveratrol into blueberry juice analyzed at a mobile phase composition of 50:50 is shown in the inset.

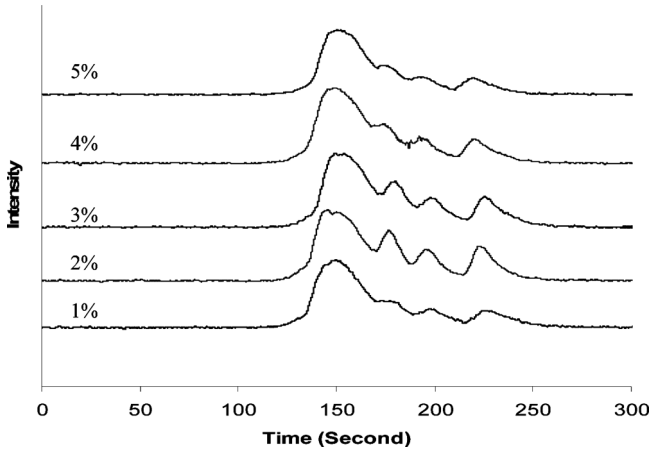

Figure 3. HPLC analysis of blueberry juice at different acid compositions of acetic acid/acetonitrile (50:50) mobile phase. For other HPLC conditions, see experimental section.

in blueberry juice was quantified to be $16.6 \pm 0.19\,\mu g/mL$ using the calibration curve in Fig. 4.

Previous studies have reported various amounts of resveratrol in grape, cranberry, and wine samples. For instance, the total resveratrol concentration was determined to be 1.56 nmol/g (or $0.36\,\mu g/g$) in grape juice,[11] 1.07 nmol/g (or $0.24\,\mu g/g$) in cranberry juice,[11] and $5.01\,\mu g/g$ in red wine.[22] In our study, a high level of $16.6\,\mu g/mL$ of resveratrol was found in blueberry juice. This is a much higher concentration as compared to a study reporting the amount of up to $0.853\,\mu g/g$ of resveratrol in dry samples of blueberry fruit.[23]

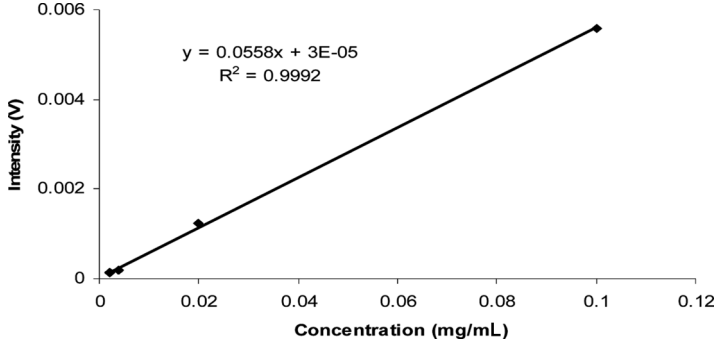

Figure 4. Calibration curve of *trans*-resveratrol. Mobile phase:acetic acid (2%)/acetonitrile (50:50). For other HPLC conditions, see experimental section.

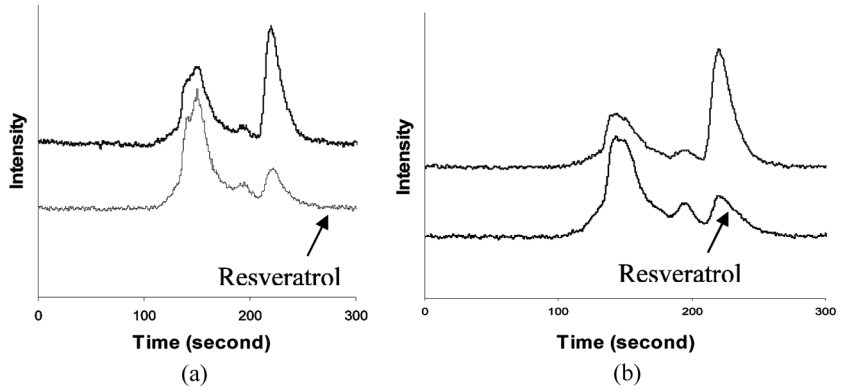

Figure 5. HPLC analysis of the extracts of blueberry powder (a) WQ, (b) WN. In each case, the bottom curve indicates the sample and the top curve indicates the sample spiked with the resveratrol standard.

In addition to blueberry juice, two different kinds of blueberry powder capsules (WQ and WN) were analysed for *trans*-resveratrol, and its presence was confirmed by spiking (Fig. 5). It was determined that WQ powder contained $236.4 \pm 7.5\,\mu g/g$ and WN powder contained $269.9 \pm 5.7\,\mu g/g$ of *trans*-resveratrol. It was previously reported that 5.04–1401.92 nmol/g (or 1.15–319.99 μg/g) was present in grape powders.[11]

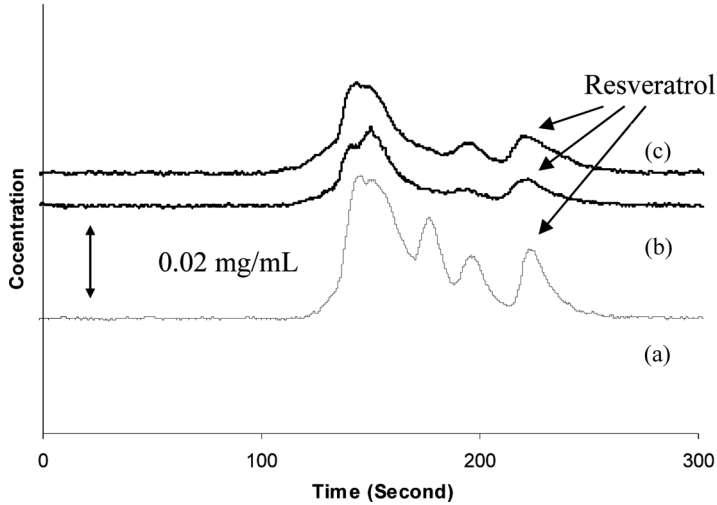

Figure 6. Comparison of HPLC analysis of blueberry samples. (a) Blueberry juice, (b) extract of WQ powder, (c) extract of WN powder.

For direct comparison, the HPLC analysis of resveratrol in all blueberry samples used in this study are displayed in Fig. 6. The concentrations of *trans*-resveratrol are 16.6 μg/mL in blueberry juice, and 5.9 and 9.0 μg/mL in WQ extract and WN extract, respectively.

Degradation of *trans*-Resveratrol

During the analysis of *trans*-resveratrol, its presence in the blueberry juice was found to disappear after a week of storage of the juice in the refrigerator. To study the degradation of *trans*-resveratrol in the juice, the blueberry juice stored at room temperature and at 4°C in the refrigerator were analyzed. Moreover, the standard solutions of *trans*-resveratrol stored at room temperature under light and no light conditions were analyzed.

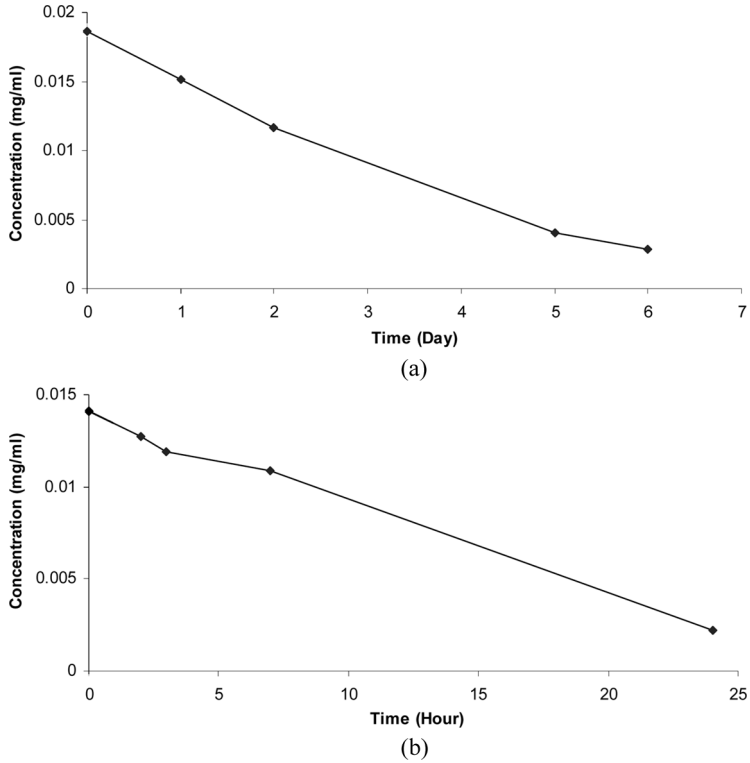

Figure 7. Degradation of *trans*-resveratrol in blueberry juice stored in the dark, and (a) in the refrigerator (b) at room temperature. The resveratrol content was analyzed using the calibration curve shown in Fig. 4.

The degradation of *trans*-resveratrol in blueberry juice stored in the refrigerator was shown in Fig. 7a. The resveratrol content degraded to half after ~3 d. It is shown to degrade less rapidly relative to the one stored at room temperature (Fig. 7b), i.e., a half life of ~15 h. Therefore, in order to get the maximum benefits from resveratrol, in consuming the juice, it is better to store the juice in the refrigerator after it is opened, and finish consuming it within 3 d. Degradation of *trans*-resveratrol in blueberry powder extracts was also studied. A similar solution degradation rate was observed, though this was of no concern for the users because the powder capsules were stored dry before consumption.

A degradation study was also conducted using a solution made from the *trans*-resveratrol standard. When it was exposed to light, degradation of *trans*-isomer was observed to be faster, as compared to the no-light condition (Fig. 8a). Moreover, a second peak appeared after 6 d (Fig. 8b), due to the conversion to the *cis* isomer, as previously reported

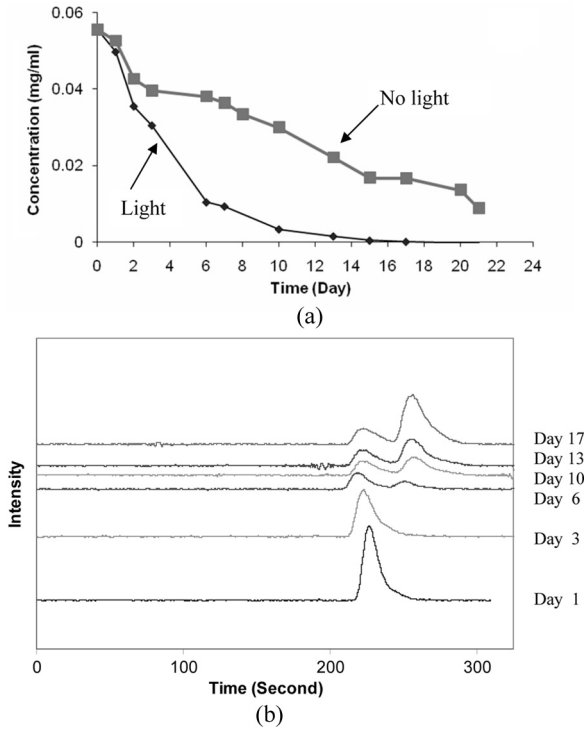

Figure 8. Degradation of a *trans*-resveratrol standard stored at room temperature. (a) when it was exposed to room light (light) and not exposed to light (no light). (b) when it was exposed to room light for 1–17 days.

in wine samples.[24] However, isomerization of the standard that was not exposed to light was not apparent until after day 21 (data not shown). It clearly shows that light is an important factor causing degradation and isomerization to occur. Although degradation of *trans*-resveratrol was also observed in blueberry juice and powder extract, isomerization was not observed. Some other compounds could be present, or some chemical reactions could have taken place, in the blueberry juice and extract. In one reported study in red wine, after isomerization of the *trans* isomer to the *cis* isomer, it was subsequently polymerized to form resveratrol oligomers.[24]

CONCLUSION

An HPLC method was employed to separate and detect *trans*-resveratrol in blueberry juice and powder extracts within ~4 min. In addition, degradation of pure *trans*-resveratrol was studied under room temperature and light conditions. Degradation of this antioxidant in blueberry juice suggests that it should be consumed within a short duration in order to benefit from the intake of sufficient resveratrol. This work reports the first study of *trans*-resveratrol quantification and degradation in blueberry samples.

ACKNOWLEDGMENT

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