Resveratrol Content in Strawberry Fruit Is Affected by Preharvest Conditions

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This study investigated the occurrence of resveratrol in Fragaria × ananassa Duchesne and the effect of preharvest conditions on resveratrol content. Both cis- and trans-resveratrol were detected in strawberry achenes (seeds) and pulp (receptacle tissue). Resveratrol was found to be higher in achenes than in fruit pulp. The levels of resveratrol were affected by genotype variations, fruit maturation, cultural practices, and environmental conditions. High growing temperature (25 and 30 °C) or enriched CO2 in the atmosphere significantly enhanced resveratrol content of strawberries. Advancing maturation also increased resveratrol content. The mature pulp and achenes contained higher amounts of resveratrol than the immature fruit. Adding compost as a soil supplement or preharvest application of methyl jasmonate (MJ) also significantly enhanced the level of resveratrol in strawberry fruit. Among the plants grown in hill plasticulture, fruits of ‘Ovation (B28)’, ‘Mohawwwk’, ‘Earliglow’, and ‘B35’ had higher amounts of resveratrol than fruits of other genotypes. ‘Ovation’ contained the highest amount of resveratrol among strawberryreis grown in matted row, whereas ‘Latestar’ contained the least. Ten of 14 tested genotypes (all except ‘Allstar’, ‘Delmarvel’, ‘Northeastar’, and ‘MEUS 8’) had higher amounts of resveratrol when grown in hill plasticulture compared to matted row.

KEYWORDS: Strawberry (Fragaria × ananassa Duch.); resveratrol; growth temperature; compost; methyl jasmonate; cultural system; carbon dioxide; temperature

INTRODUCTION

Resveratrol (trans-3,5,4′-trihydroxystilbene) (Figure 1) is a polyphenolic compound and is synthesized from cinnamic acid derivatives. Several beneficial biological effects of resveratrol on human health have been discovered in recent years. The biological and pharmacological activities of resveratrol are thought to be due to its strong antioxidant property (1, 2). Resveratrol effectively scavenges free radicals and other oxidants (3) and inhibits low-density lipoprotein (LDL) oxidation (1, 4). Resveratrol has been shown to exhibit cardioprotection (5), inhibition of platelet aggregation, induction of vasorelaxation (6–9), antiinflammatory property (10) and anticancer activity (7, 11).

Resveratrol is found in grapes (12), grape products such as wine (13), and some other botanical sources, including peanuts (14), cranberries (15), and Vaccinium berries such as blueberries, sparkleberries, lingonberries, partridgeberries, bilberries, and deerberries (16). The amount of resveratrol in various berries

and fruits can be affected by different treatments or stresses such as UV irradiation (17) and application of abiotic stresses (18).

To the best of our knowledge, the presence of resveratrol in strawberries has not been reported. The purpose of this study is to investigate the occurrence of resveratrol in strawberries and to determine if its content is affected by preharvest factors such as genotype, fruit maturity, cultural practices, and environmental conditions.

MATERIALS AND METHODS

Chemicals. trans-Resveratrol standard was purchased from Sigma Chemical Co. (St. Louis, MO). Conversion of trans-resveratrol to the cis form in standards was accomplished by exposing samples to a UV lamp (366 nm, model UVL-S6, UVP, Inc., San Gabriel, CA) for 5 min (13). Water, acetone, and acetonitrile were of optima grade and purchased from Fisher Scientific (Pittsburgh, PA). Formic acid was of mass spectrometry grade and purchased from Sigma Chemical Co.

Plant Growth Temperature. One-year-old plants uniform in size of ‘Earliglow’ and ‘Kent’ cultivars were used. The plants were propagated by runner-tip cuttings in June, and plants were grown in 2 L plastic pots (15.0 × 12.0 cm) containing Pro Mix BX (Premier Brands Inc., Stamford, CT) in a greenhouse. Radiation sources in the greenhouse consisted of natural daylight and Watt-Miser incandescent lamps (Nela Park, Cleveland, OH) that provided a PAR 700 µmol m−2 s−1 for 14 h/d (6:00 a.m.–8:00 p.m.). Temperatures were set at 25 °C
for the day and 20 °C at night. During the growing season, all plants were watered daily and fertilized biweekly with 150 mL/plant of Peters fertilizer (20–20–20, N/P/K). Plants with at least 10 fruits per plant at their green fruit stage were selected for the growth chamber experiments. Forty plants each of ‘Earliglow’ and ‘Kent’ were removed from the greenhouse in March and divided into lots of 10 plants. One lot of each cultivar was randomly placed in each of four growth chambers set at day/night temperatures of 18/12, 25/12, 25/22, and 30/22 °C. The plants were kept in growth chambers for 1.5 months. The photoperiod for each growth chamber was 14 h (6:00 a.m.–8:00 p.m.) with a PAR 700 µmol m⁻² s⁻¹ at plant height. Firm red-ripe fruits free from defects or decay were harvested from each growth chamber for each cultivar during the fruiting stage and then used for analyses.

Fruit Maturation. Strawberries free of damage were selected and harvested at different maturity stages according to the red color development (20, 50, 80, and 100%). Pulp and achenes were separated and then used for analyses.

Use of Compost as a Soil Supplement. One hundred and twenty plants each of ‘Allstar’ and ‘Honeoye’ strawberry were grown on four soil treatments: (a) 100% soil, (b) 50% soil plus 50% sand, (c) 50% soil plus 50% compost, and (d) 100% compost. The compost was obtained from the USDA Compost Center at the Beltsville Agricultural Research Center, Beltsville, MD (19). The soil samples and compost used in this study were analyzed for nutrients and other soil characteristics through the Soil Testing Laboratory, University of Maryland, College Park, MD, and the results have been reported (20). Plants were placed in pots (15.0 × 12.0 cm) and grown in the greenhouse. Each soil treatment was subdivided into two groups: (i) control (no fertilizer, water only) and (ii) fertilized biweekly with a half-strength of Peter’s nutrition solution (20–20–20, N/P/K) (50% fertilizer). The firm-ripe fruits were harvested from each cultivar in each growth chamber for each treatment during the fruiting stage for chemical analyses.

Preharvest Application of Methyl Jasmonate (MJ). Forty plants of 1-year-old ‘Honeoye’ were used. The plants were propagated by runner-tip cuttings in June, and plants were grown in 2 L plastic pots in a greenhouse. Four sets of 10 plants were randomly selected, and the treatments were designated (i) control, (ii) 0.1 mM MJ, (iii) 0.2 mM MJ, and (iv) 0.3 mM MJ. MJ plus 0.05% Tween-20 was applied as a foliage–berry spray to runoff when berries were in early light pink stage and sprayed twice more at four-day intervals. The control plants were sprayed with 0.05% Tween-20. Three 100 g undamaged ripe berries were randomly selected around 9:00 a.m. from each treatment and used for chemical analyses.

Genotypes and Cultural Systems. Fourteen strawberry cultivars and selections were grown on the North Farm of the Henry A. Wallace Agricultural Research Center at Beltsville, MD, in either flat, matted row beds without plastic mulch or on hill pluiculture beds covered with black polyethylene mulch. Each type of planting was supplied with drip irrigation tubing down the center of each row. The plants received 34 kg ha⁻¹ N–P–K (10–10–10) at planting time, 45 kg ha⁻¹ N (NH₄NO₃) via the drip irrigation line in the summer and fall, and 17 kg ha⁻¹ N (NH₄NO₃) via the drip irrigation line in the spring. During the fruiting season, the average maximum temperature was 15.5 °C, the minimum temperature was 8.5 °C, and the rainfall was 5.84 mm. The mature fruits were harvested from four plots of a replicated complete block design (RCB) for the matted row field and from four plots of the RCB for the double-hill field. Twenty ripe fruits with well-developed red color were selected around 9:00 a.m. from each cultivar in each plot and were used for the analyses.

Trials with Increased Carbon Dioxide Concentration. Sixteen 1-year-old “Honeoye” strawberry plants were transplanted into each of six open-topped clear acrylic chambers 1.8 m in height, each covering an area of 1.1 m². A blower pulled air out of each chamber at the base, at a rate of 6 m³/min⁻¹. Carbon dioxide was introduced into four of the chambers at the inlets of mixing fans positioned above the canopies. Flow rates of CO₂ were such that two chambers had a CO₂ of 300 ± 50 µmol mol⁻¹ above that of outside air and two chambers had a CO₂ of 600 ± 50 µmol mol⁻¹ above that of outside air, whereas two chambers received no supplemental CO₂. Samples of air from each CO₂ treatment were pumped sequentially through an absolute infrared analyzer in an adjacent air-conditioned shelter, and CO₂ air temperatures, and photosynthetic photon flux density (PPFD) were logged every 5 min. The chambers transmitted 90% of the PPFD, and chamber air temperatures averaged 1 °C above those of outside air. The mean CO₂ concentration of ambient air was 353 µmol mol⁻¹ during the day and 400–600 µmol mol⁻¹ at night. The ventilation of the chambers kept the humidity for all of the chambers the same as that of outside air. The duration of the entire experiment was 28 months. Full details of this experimental plan were described in a previous publication (21).

Sample Preparation. Triplicate 10 g of whole fruit or pulp samples were extracted twice with 30 mL of 80% acetonitrile using a Polytron (Brinkmann Instruments, Inc., Westbury, NY) for 1 min, and triplicate achenes (1.0 g) were extracted twice with 15 mL of 80% acetonitrile using a mortar and pestle. Extracts (30 mL) were combined and concentrated to 1 mL using a Buchler Evapomix (Buchler Instruments, Fort Lee, NJ) in a water bath at 35 °C. The concentrated sample was dissolved in 10 mL of acidified water (3% v/v of formic acid) and then passed through a C₁₈-Sep-Pak cartridge (Waters Corp., Milford, MA), which had been previously activated with methanol followed by water and 3% aqueous formic acid. Resveratrol was adsorbed onto the column, whereas sugars, acids, and other hydrophilic compounds were eluted with 10 mL of 3% aqueous formic acid. The resveratrols were then recovered with 2.0 mL of ethyl acetate. The ethyl acetate extract was concentrated, dissolved in 1.0 mL of methanol, and passed through a 0.45 µm membrane filter (Millipore, MBI, Westboro, MA), and 20 µL was analyzed by HPLC.

Isolation, Identification, and Quantitation of Resveratrol Using HPLC-Photodiode Array. High-performance liquid chromatography (HPLC) was used to separate and determine resveratrol in strawberry fruit samples. The samples were analyzed using a Waters HPLC system equipped with two pumps (600 E system controller; Waters) coupled with a photodiode array detector (Waters 991 series). Samples were injected at ambient temperature (20 °C) onto a reverse phase Nova-Pak C₁₈ column (150 × 3.9 mm, particle size = 4 µm) with a guard column (Nova-Pak C₁₈, 20 × 3.9 mm, particle size = 4 µm) (Sentry guard universal holder) (Waters Corp.). The mobile phase was acidified water containing 2.5% formic acid (A) and acetonitrile (B). The gradient program is as follows: a linear gradient from 5 to 20% B in the first 15 min, followed by a linear gradient from 20 to 30% B in 5 min, then held at 30% B for 5 min, followed by a linear gradient from 30 to 90% B for 5 min, and held at 90% B for 2 min before returning to the initial conditions. The flow rate was 1.0 mL/min and the injection volume was 20 µL. The wavelengths of detection were set at 288 and 320 nm, where cis- and trans-isomers have absorbance maxima, respectively. Scanning between 210 and 400 nm was performed, and data were collected by the Waters 991 3-D chromatography data system. Retention time and spectrum were compared to the pure standards. Results were expressed as micrograms per gram of dry weight. A standard curve for trans-resveratrol in 10% ethanol was generated to allow quantitation of resveratrol in strawberry fruit extracts.

LC-MS Confirmation. Mass spectrometry was carried out by using a Micromass Quattro Micro triple-quadrupole mass spectrometer (Waters/Micromass, Milford, MA). Electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode was performed in positive ion mode. In MRM mode, quadrupole 1 was fixed at a set parent ion (m/z 229 for resveratrol), quadrupole 2 was used as a collision chamber.
Resveratrol Content in Strawberries


Figure 2. HPLC and LC-MS chromatograms of an extract of strawberry achenes: (A) HPLC-UV chromatogram; (B) LC-MS chromatogram with single ion monitoring of m/z 229 for strawberry achene extract showing detection of protonated molecules of cis- and trans-resveratrol.

to induce fragmentation, and quadrupole 3 was fixed at a set daughter ion (m/z 183). MRM is the preferred mode for known analyte because it usually achieves the best possible specificity and signal-to-noise ratio (S/N) for a given analyte. The mass spectrometer conditions used were as follows: The desolvation gas (N2) was 20 µL of 650 L/h, the cone gas (N2) was 50 L/h, and the collision gas (Ar) was tuned to a pressure of 2.3 × 10^-3 torr. Source temperature was set at 100 °C, and the desolvation temperature was set at 280 °C. For the electrospray source, the capillary voltage was set at 3.5 kV; the extractor voltage was set at 3 V; and the RF lens voltage was set at 0.3 V. For the analyzer, the LM resolution 1 and HM resolution 1 and 2 were 15 V and ion energy 1 was 0.5 V; the entrance was 0 V and the exit was 1 V; the LM resolution 2 and HM resolution 2 were 15 V and ion energy 2 was 5 V. The multiplier voltage was 700 V. The cone voltages and collision energies, respectively, for resveratrol were -25 V and 20 V.

Statistical Analysis. Data presented are the mean ± SE values. All statistical analyses were performed with NCSS Statistical Analysis System (Statistical Analysis and Graphics, Kaysville, UT) (22). One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered to be significant at P ≤ 0.05.

RESULTS

Occurrence of Resveratrol in Strawberries. LC/MS-MRM was used to identify trans- and cis-resveratrol peaks from the complex matrix of strawberry extract. Using the same HPLC conditions as the LC/UV method, the MS-MRM detection, combined with the retention times obtained using the standards, successfully identified the trans- and cis-resveratrol peaks. The peaks for trans- and cis-resveratrol in strawberries were detected at retention times of 17.06 and 18.77 min, respectively. The results were also confirmed by co-injection with authentic resveratrol standards using LC/MS-MRM. The LC/MS-MRM results were then used to find the correct peak in the corresponding LC/UV chromatogram (Figure 2). In addition to the HPLC retention times, the trans- and cis-resveratrol molecules in strawberries were also distinguished by their different absorption maxima of 320 and 288 nm, respectively.

The cis- and trans-resveratrols were detected in strawberry achenes (seeds) and pulp (receptacle tissue). More resveratrol was found in achenes than in fruit pulp. trans-Resveratrol content of ‘Allstar’ strawberry pulp was 830.5 ± 20.6 ng g^-1 of dry weight (90.5 ± 11.8 ng g^-1 of fresh weight) and 1.64 ± 0.08 µg g^-1 of dry weight (1.51 ± 0.05 µg g^-1 of fresh weight) in achenes. cis-Resveratrol content of strawberry pulp was 227.3 ± 11.4 ng g^-1 of dry weight (24.9 ± 2.7 ng g^-1 of fresh weight) and in achenes was 2.03 ± 0.09 µg g^-1 of dry weight (1.87 ± 0.08 µg g^-1 of fresh weight).

Effect of Plant Growth Temperature on Resveratrol Content. The presence of resveratrol in ‘Kent’ and ‘Earliglow’ fruit extracts ranged from 0.68 to 1.48 (µg/g of dry weight) (Figure 3). High-temperature growing conditions (25 and 30 °C) significantly enhanced total resveratrol contents of fruit in both cultivars. Fruit on plants grown in the cool day and night temperature (18/12 °C) treatment generally had the lowest resveratrol. An increase in night temperature from 12 to 22 °C, with the day temperature kept constant at 25 °C, resulted in a significant increase in resveratrol contents of ‘Earliglow’ and ‘Kent’ fruit. The highest day/night temperatures (30/25 °C) yielded fruit with the highest amount of resveratrol. ‘Earliglow’ strawberry had higher values of resveratrol compared to ‘Kent’ (Figure 3).

Effect of Maturation on Resveratrol Content in Achenes and Pulp of Strawberries. At different stages of maturity, the contents of resveratrol in achenes and pulp were different (Figure 4). The pulp and achenes from mature fruit contained...
higher amounts of resveratrol than those from immature fruit. The achenes also contained a higher amount of resveratrol than the pulp (Figure 4).

Effect of Compost on Resveratrol Content. Compost as a soil supplement significantly enhanced the level of total resveratrol content in fruits of two strawberry cultivars, ‘Allstar’ and ‘Honeoye’ (Figure 5). Fruit of plants grown in plots amended with compost as a soil supplement. Different letters indicate statistically significant differences at \( P \leq 0.05 \).

Preharvest Application of MJ Affected Resveratrol Content. MJ treatments significantly enhanced total resveratrol content in fruit of strawberry ‘Honeoye’ (Figure 6). The level of resveratrol increased with increasing MJ concentration from 0.1 to 0.3 mM. Resveratrol concentration increased from 1.05 \( \mu g/g \) of dry weight in untreated grown strawberries to 1.67 \( \mu g/g \) of dry weight in 0.3 mM MJ treated strawberries (Figure 6).

Genotypes and Cultural Practices Affected Resveratrol Content. Among plants grown in hill plasticulture, fruit from ‘Ovation (B28)’, ‘Mohawk’, ‘Earliglow’, and ‘B35’ had higher amounts of total resveratrol than fruit of other genotypes. ‘Ovation (B28)’ contained the highest amount of resveratrol among those grown in matted row, whereas ‘Latestar’ contained the least (Figure 7). Ten of 14 genotypes (all except ‘Allstar’, ‘Delmarvel’, ‘MEUS 8’, and ‘Northeaster’) had higher amounts of resveratrol when grown in hill plasticulture compared to in matted row. The mean resveratrol contents in strawberry extracts were 1.46 and 1.94 \( \mu g/g \) of dry weight for matted row and hill plasticulture grown strawberries, respectively.

Elevated Carbon Dioxide Affected Resveratrol Content. Strawberries had higher concentrations of total resveratrol when plants were grown under enriched CO\(_2\) environment than in air (Figure 8). An increase of CO\(_2\) level (ambient + 300, and ambient + 600 \( \mu \text{mol mol}^{-1} \) CO\(_2\)) resulted in an increase in resveratrol in strawberries. Resveratrol concentration increased from 1.12 \( \mu g/g \) of dry weight in ambient atmosphere grown strawberries to 1.75 \( \mu g/g \) of dry weight in 600 \( \mu \text{mol mol}^{-1} \) CO\(_2\) enriched environment.

DISCUSSION

Resveratrol exists naturally in cis and trans configurations in certain plants including grapes, cranberries, blueberries, and peanuts (15, 23). Resveratrol is synthesized from \( p \)-coumaroyl CoA and malonyl CoA, and its synthesis is enhanced by stress,
injury, infection, or UV irradiation (24). In recent years, research on resveratrol has revealed several beneficial biological effects of this compound on human health including antioxidant and anticancer activities, anti-inflammatory activity, cardioprotection, and inhibition of platelet aggregation (1, 2, 5, 6, 10, 11). However, most of the biomedical research is focused on trans-resveratrol; therefore, the role of cis-resveratrol is less clear.

This study showed for the first time the occurrence of resveratrol in strawberry (Fragaria × ananassa Duchesne). We found that strawberry achenes had higher contents of resveratrol than fruit pulp. Resveratrol concentrations in grapes have been reported to range from 0.356 to 237.83 µg/g of powder (15), which is 10–75-fold higher than the highest concentration of resveratrol detected in blueberries or bilberries. The content of resveratrol in strawberries tested in our study was comparable with that of bilberries, deerberries, and peanut (14, 16). Although the concentrations of resveratrol in strawberries are relatively low compared to the levels reported in grape and cranberry juices (15), strawberries along with blueberries and bilberries may still serve as another dietary source of resveratrol.

Growth temperatures affected strawberry fruit resveratrol content. Our previous study showed that high-temperature conditions significantly enhanced the content of flavonoids and antioxidant capacities against ROO’, O2•−, H2O2, OH•, and O2 radicals in strawberry fruit (25). Likewise, high growth temperature also promoted resveratrol levels. The increase of resveratrol content in strawberries might have also contributed to the high free radical scavenging capacity.

Composting is a key technology for recycling and building soil organic matter as part of sustainable agriculture. Composting offers a number of benefits to farmers, including flexible manure management, potential added income, increased odor control, weed control, and reduced pollutants and diseases (26). In our study, compost as a soil supplement significantly enhanced the level of resveratrol content in strawberry fruit. It is possible that compost causes changes in soil chemical and physical characteristics, increases beneficial microorganisms activity (27), and enhances nutrient availability and uptake (26, 27), thus resulting in the high levels of antioxidants including resveratrol in fruit of strawberry.

Jasmonic acid (JA), 3-oxo-2-(2-cis-pentenyl cyclopentane-1-acetic acid), and its methyl ester (MJ) are a class of oxylipins derived from lipoxygenase-dependent oxidation of fatty acids, which occur naturally in a wide range of high plants (28). JA and MJ play key roles in plant growth and affect a great diversity of physiological and biochemical processes. MJ treatments significantly enhanced resveratrol content in strawberries. It has also been reported that MJ stimulates anthocyanin content during in vitro strawberry ripening (29). MJ has been shown to stimulate ethylene synthesis and β-carotene synthesis (30, 31). It has been observed that ethylene causes an increase in anthocyanin contents by stimulating enzymes involved in phenolic biosynthesis, and particularly phenylalanine ammonia-lyase activity (32, 33). Phenylalanine ammonia-lyase activity plays an important role in the biosynthesis of resveratrol (34). Therefore, the increase of resveratrol content in MJ-treated strawberries may be exerted through the enhancement of the phenylalanine ammonia-lyase activity.

Different genotypes contain different amounts of resveratrol in strawberries. In our study, fruits of ‘Ovation’, ‘Mohawk’, ‘Earliglow’, and ‘B35’ had higher amounts of resveratrol than fruits of other genotypes. ‘Ovation’ contained the highest amount of resveratrol among strawberries grown in matted row, whereas ‘Latestar’ contained the least. In general, strawberries had higher amounts of resveratrol when grown in hill plasticulture than in matted row. Different cultural practices using different mulches probably led to differences in canopy temperature, soil temperature, moisture content, and the quantity and quality of light transmitted, reflected, or absorbed. These differences in turn could have affected plant growth, development, fruit quality, carbohydrate metabolism, and resveratrol content in strawberries. Our results indicate that genetic factors are important in determining resveratrol content, and cultural practices could also affect its level in strawberries. However, genetic factors often overshadow the impact of cultural practices.

Carbon dioxide is one of the limiting factors in photosynthesis. Improving photosynthesis in crops through CO2 enrichment has fascinated agriculturists for many years. CO2 enrichment has been shown to increase plant growth, development, and yield of agricultural crops, and this response is a function of CO2 concentration and duration (35, 36). We found that strawberry fruit had a higher concentration of resveratrol when the plants were grown under enriched CO2 environments than when they were grown in air. The increase in resveratrol in elevated CO2-treated strawberries should help in quenching the excited state of active oxygen species and reducing free radical activity.

In conclusion, resveratrol was detected in strawberries. This suggests that strawberries may serve as an alternative dietary source for the antioxidant resveratrol. Preharvest factors including genotypes, fruit maturity, cultural practices, and environmental conditions might affect levels of resveratrol in strawberries.

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