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ORIGINAL RESEARCH

# Contents of *Trans*-resveratrol in Korean Grape Cultivars, including “Kyoho”

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**Abstract** Concentrations of *trans*-resveratrol were analyzed in 36 Korean-grown grape cultivars, including cv. Kyoho. This cultivar has large irregular berries and a deep-black skin and accounts for >15% of all grapevines grown in Korea. The content of *trans*-resveratrol is an important quality parameter, and its pattern of variability increased gradually until the time of harvest. Its distribution also fluctuated significantly among leaves, seeds, and exocarps, with the highest amount (1,477 μg $g^{-1}$ dry weight) occurring in the leaves. Among all cultivars evaluated here, trans-resveratrol contents were significantly higher in “Cheongsoo” and “High Bailey,” their levels being 50% above the overall mean (i.e., 2.0-mg $g^{-1}$ dry weight), whereas 14 cultivars had contents that were 10% less than that mean value

**Keywords** Grape · “Kyoho” · Resveratrol

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Fruit consumption is important to the protection of human health. This benefit is mainly associated with the antioxidant activity of phenolic compounds that are largely present in fruits and in the beverages made from them. These effects are due to the properties of antioxidants that act as reducing agents by donating hydrogen, quenching singlet oxygen, serving as chelators, and trapping free radicals (Iacopini et al. 2008). Therefore, fruits are a critical source of antioxidants such as vitamins and phenolic phytochemicals (Tsao and Yang 2003).

Interest has been heightened in the use of naturally occurring compounds as potential cancer chemopreventive agents in human populations. A significant correlation has been found between dietary intake and many types of cancer, based on global epidemiological data; in animal experiments, numerous dietary substances have been documented to have anticancer properties. These include green tea catechins, lycopene, soy isoflavones, pomegranate phenolics, selenium, vitamins E and D, curcumin, silibinin, and resveratrol (Athar et al. 2007; Kim et al. 2007).

Resveratrol (3,5,4′-trihydroxystilbene) is a stilbene phytoalexin and a natural component of grape plant organs (Woo et al. 2005). It has received much attention as a heart-healthy anticancer compound, and grapes have been promoted in the press as its best source (Cseke and Kaufman 1999). Because *cis*-resveratrol has not been detected in grape skins or juice, it appears to be formed from the isomerization of *trans*-resveratrol or the breakdown of resveratrol polymers during fermentation (Soleas et al. 1995; Roggero 1996). Focus has been growing because of claims that it also protects against coronary heart disease. This potential effect could be due to the ability of resveratrol to inhibit low-density lipoprotein oxidation, synthesize eicosanoids, and block platelet aggregation, a process that plays an important role in the progression of atherosclerosis, as well as the final occlusive events that lead to myocardial infarction and stroke. This compound also reduces levels of triacylglycerol and protects the liver from lipid peroxidation. Other attributes include action as a significant antioxidant, anti-inflammatory, and anticancer properties (Signorelli and Ghidoni 2005), inhibition of the development of preneoplastic lesions in carcinogen-treated mouse mammary glands in culture, and blocked tumorigenesis in a mouse skin cancer model.

Grape wine is believed to be a very healthy beverage because resveratrol can be extracted from the skins, thus resulting in a so-called French Paradox (Cho et al. 2005). Grapefruits have some of the highest content of that compound and abundant phenolics are present in the skin, pulp, and seeds (Revilla and Ryan 2000).

The “Kyoho” grape cultivar studied here is grown mainly in Korea, accounting for more than 15% of the total grapevine region compared with the better-known “Campbell Early,” which encompasses 67% of that growing area. “Kyoho” has compact medium-to-large bunches with large irregular berries and a deep-black-colored skin. Our objective was to derive basic data for the chemotaxonomy of various grape cultivars.

## Materials and Methods

### Plant Material

We investigated the resveratrol profiles from leaves, exocarps, and seeds of 36 grape cultivars, including the tetraploid grape “Kyoho” (*Vitis vinifera* L. × *Vitis labrusca* L.). Vines were sampled during the 2005 and 2006 growing seasons in the vineyard of Cheonan, Korea. Fruits were collected in August, when most were ripening, and leaves were sampled from August to September. All tissues were immediately freeze-dried and stored at −60°C.

### Chemicals

The *trans*-resveratrol was purchased from Sigma Chemical Co. All solvents used for extraction and high-performance liquid chromatography (HPLC) analysis were obtained from Fisher Scientific (Santa Clara, CA, USA) and Merck (Darmstadt, Germany). HPLC-grade water was prepared by redistillation.

### Preparation of Crude Resveratrol Extracts

Extraction and primary purification were conducted according to the method described by Rodriguez-Delgado et al. (2002). Freeze-dried leaves or exocarps (1 g) were ground at high speed in a blender and extracted for 2 h in 50% aqueous ethanol at room temperature. Afterward, the slurry was sonicated for 30 min, centrifuged for 20 min, and filtered through Whatman no. 1 filter paper in a Buchner funnel. The extract was then freeze-dried and stored at −4°C.

### Analysis of *Trans*-resveratrol

HPLC for *trans*-resveratrol analysis was performed with a UV dual-pump system (Model SCA-10A; Shimadzu, Japan) and Shimadzu Shim-pack VP-ODS C-18 column (4.6 mm×250 L). The detection wavelength was set at 307 nm on 0.5-1 a.u.f.s. sensitivity. A multistep gradient method was applied, using a methanol/water/acetic acid (10:90:1; *v*/*v*/*v*) mixture as solvent A and a methanol/water/acetic acid (90:10:1; *v*/*v*/*v*) mixture as solvent B with a flow rate of 3-ml $min^{-1}$ gradient elution for a 35-min cycle and 100–30% solution B in solution A. All solvents were of HPLC grade and were filtered and degassed before use. Resveratrol peaks were identified and quantified by a calibration curve of standards. Regression analysis was conducted with mean values from three replicates on each of three sampling dates.

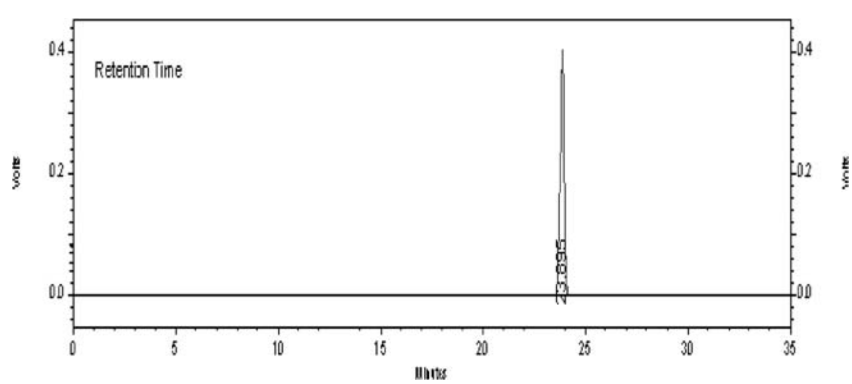


**Fig. 1** HPLC data of the resveratrol standard in 35 min cycle

## Results and Discussion

Grapefruits have distinct phenolic compositions (de Villiers et al. 2004; Ortega-Regules et al. 2006). Although these are genetically determined, their expression throughout the ripening process is modulated by agricultural and environmental factors (Delgado et al. 2004; Xiaodong et al. 2006).

The *trans*-resveratrol content is an important quality parameter that appears in the fruit and the wine. Apart from plant genetic background, the maturation and ripening stages and water status can impact the level of resveratrol. Its production may also increase in response to environmental stress or fungal infection, thus demonstrating that cultural practices can be used to modify composition. In our experimental conditions, we tried to minimize these mitigating factors by collecting samples from the same experimental field and at the same ripening stage. Our chromatogram revealed a peak for the *trans*-resveratrol signal at 23.895 min (Fig. 1). That value was used in the regression analysis of each cultivar.

Fruit ripening was assessed over 3 months of growth by measuring berry weights, widths, and lengths. Data from each cultivar were then compared with information from "Kyoho" at each sampling date (Fig. 2). Throughout the ripening process, the increase in weight was gradual for all cultivars except "Kyoho," which eventually became moderate. However, a significant increase was found from June to July. Berry size is of fundamental importance because anthocyanins are synthesized in the skin and each grape variety has a particular potential for the biosynthesis of those phenolic compounds.

Resveratrol extraction and HPLC determination of *trans*-resveratrol were performed for leaves, exocarps, and seeds from grapes collected at maturity. Unlike for other species,

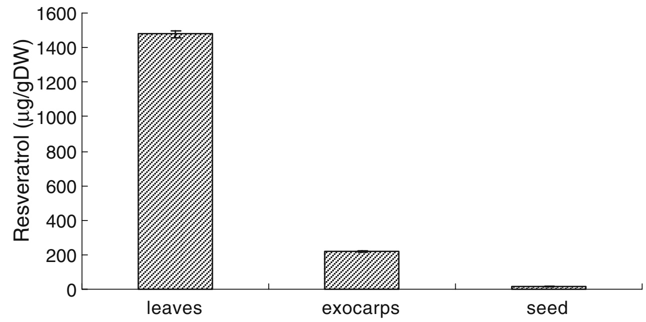


**Fig. 3** Resveratrol contents (µg $g^{-1}$ DW) in seeds, leaves, and exocarps from "Kyoho" grapes sampled in August. Values from HPLC analysis are means of three replicates

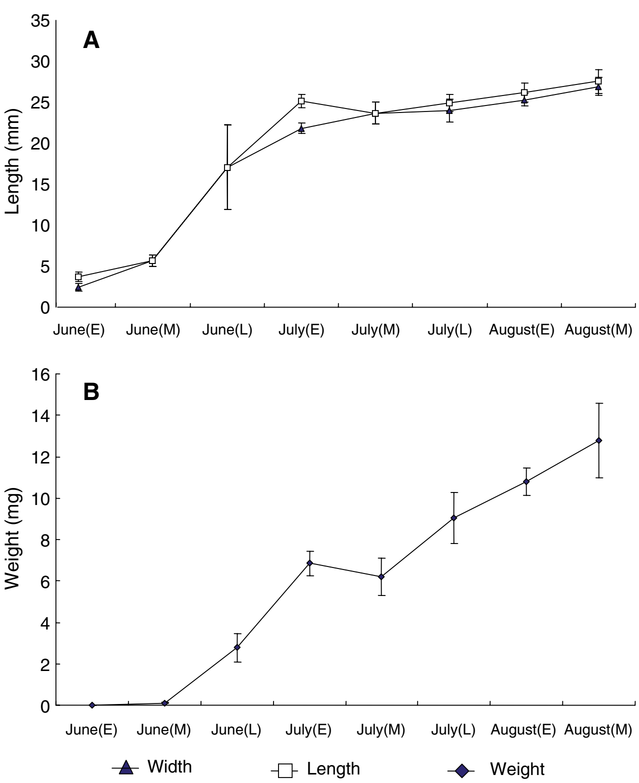


**Fig. 2** Growth curve from June to August (*E* early, *M* middle, *L* late) for width and length (**a**) and weight increase (**b**) of "Kyoho" grape fruits

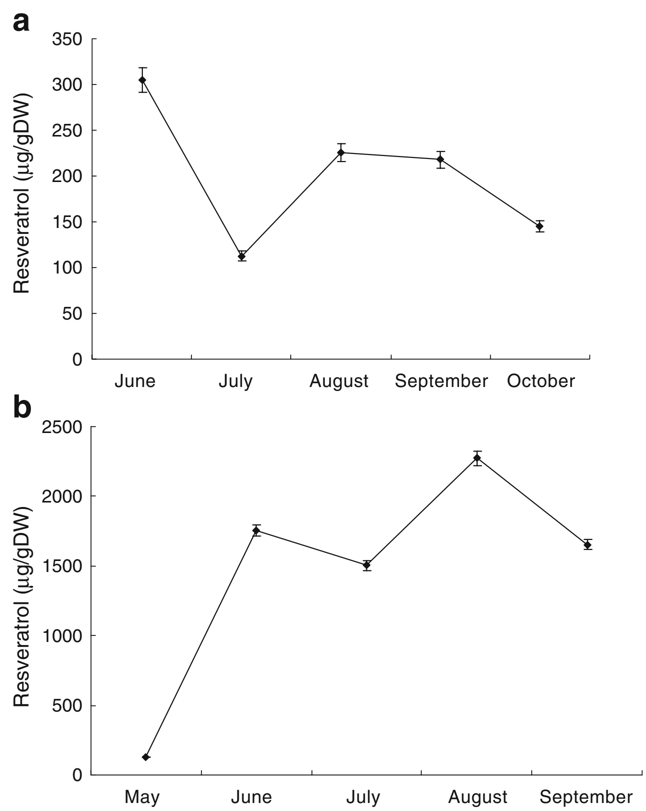


**Fig. 4** Resveratrol contents (µg $g^{-1}$ DW) in exocarps (**a**) and leaves (**b**) of "Kyoho" grape at early stage of fruit development, from May through August. Values are means of three replicates

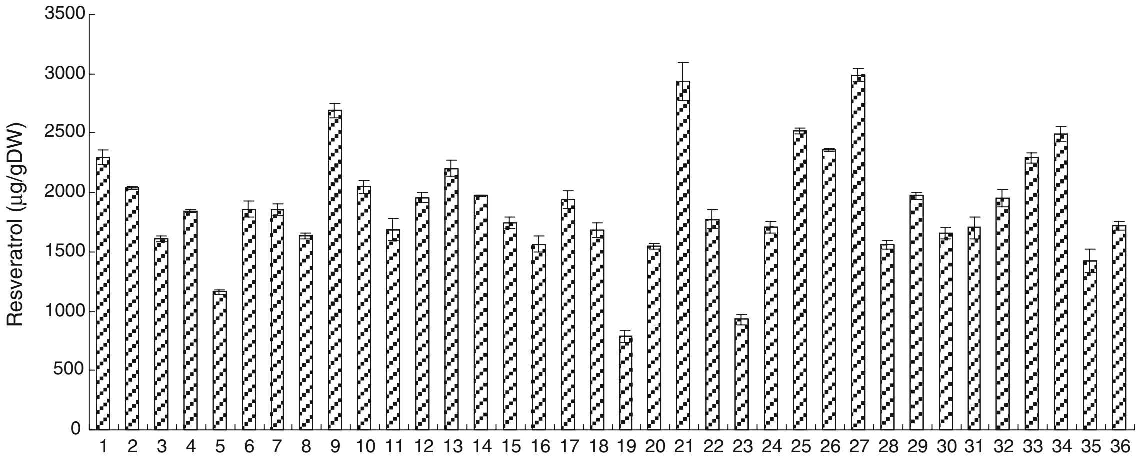


**Fig. 5** Resveratrol contents (μg $g^{-1}$ DW) in leaves from 36 grapevine cultivars at complete maturation stage from August to September. Values are means of three replicates. *1*, "Kyoho"; *2*, "Ryogyoku"; *3*, "Takasumi"; *4*, "Takasago"; *5*, "Ohtsubu Campbell Early"; *6*, "Daebong"; *7*, "Tobu Kyoho"; *8*, "Red Queen"; *9*, "Rizamat"; *10*, "Fuen"; *11*, "Black Olympia"; *12*, "Seto Giants"; *13*, "Schuyler"; *14*, "Arehonsurebara"; *15*, "Aki Queen"; *16*, "Kyoho Sel."; *17*, "Lizuka Kyoho"; *18*, "Shigyoku"; *19*, "Wase Campbell Early"; *20*, "Tianshui"; *21*, "Cheongsoo"; *22*, "Jingzao"; *23*, "Campbell Early"; *24*, "Pione"; *25*, "Honey Black"; *26*, "Honey Seedless"; *27*, "High Bailey"; *28*, "Benifiji Sel."; *29*, "Beniyamabiko"; *30*, "Benizuiho"; *31*, "Beniizu"; *32*, "Fujiminori"; *33*, "Heukok"; *34*, "Himrod Seedless"; *35*, "Flame Seedless"; *36*, "Muscat Bailey A"

stem portions may also be a source of resveratrol. Cho et al. (2003) have reported that the peduncle, or fruit stem, contains 170 to 440-μg $g^{-1}$ dry weight (DW) and that this compound is nearly undetectable in the flesh. Here, we found significant variations among organs, with the highest amount (1,477-μg $g^{-1}$ DW) occurring in the leaves (Fig. 3). There, the resveratrol content was about 80 times greater than that measured in the seeds and about sevenfold higher than in the exocarps.

Navarro et al. (2008) have shown that bloom time is usually the standard for gathering leaves from grape plants. However, the short duration of that stage often makes adequate collection and sample analysis difficult. Therefore, veraison (i.e., when berries soften and change color) may be the most suitable when evaluating the nutritional status of grapevines because the leaf mineral composition is more stable at that phonologic stage.

Variations in *trans*-resveratrol contents were found in the exocarps or leaves of "Kyoho" analyzed at five stages: bloom, postbloom, veraison, preharvest, and harvest (Fig. 4). Concentrations in the exocarps were greatest at the beginning of the sampling period (June), then declined in July, increased in August, and progressively decreased until harvest. The trend for leaf contents was similar.

All of these results suggest that this drop in *trans*-resveratrol levels is mainly due to the translocation of mobile macroelements to different sink organs, e.g., the fruits, that are developing during that period. For example, prior to veraison, grape berries are more resistant to fungal infection than in the last maturation phase. This resistance has been directly correlated with the synthesis of resveratrol (Bais et al. 2000; Fornara et al. 2008). Moreover, Fornara et al. (2008) have demonstrated that the level of stilbene synthase messenger RNA does not appear to translate into high resveratrol contents in 16-week-old berry skin compared with the amounts detected in 5-week-old fruits. Likewise, the

**Table 1** Resveratrol contents in individual grape cultivars compared with the overall mean (2.0 mg $g^{-1}$ DW)

| Resveratrol contents (more/less than overall mean) | Cultivar |
|---|---|
| <10% | Benizuiho, Beniizu, Takasumi, Aki Queen, Red Queen, Black Olympia, Muscat Bailay A, Tianshui, Kyoho Sel., Flame Seedless, Ohtsubu Campbell Early, Campbell Early, Wase Campbell Early, Pione |
| >10% | Kyoho, Schuyler, Honey Seedless, Heukok |
| >20% | Honey Black, Himrod Seedless |
| >40% | Rizamat |
| >50% | Cheongsoo, High Bailey |

increase in levels of structural and nonstructural leaf compounds that are produced from spring to autumn contributes to a reduction in macroelement contents, as was also observed in our analysis (Navarro et al. 2008). Although the pattern of variation in *trans*-resveratrol increased gradually until harvest time (Fig. 4b), accumulations after veraison were highly significant until 2 weeks before harvest, after which these compounds remained virtually stable. This profile is in agreement with one previously reported for various grape cultivars (Delgado et al. 2004; Navarro et al. 2008).

The biosynthesis of resveratrol, a phenolic phytoalexin of the stilbene family, increases in response to different biotic and abiotic stresses that affect the vines (Romero-Pérez et al. 2001). Stilbenes are transferred from skins to musts during maceration, but the final resveratrol concentration in wine depends on numerous environmental factors. We characterized the composition of phenolics from 36 grape cultivars during the ripening process (Fig. 5, Table 1). Here, *trans*-resveratrol contents were significantly higher in "Cheongsoo" and "High Bailey" with values that were 50% higher than the overall mean of 2.0 mg $g^{-1}$ DW. In contrast, 14 cultivars had contents that were 10% lower than that mean. Thus, we can conclude that polyphenol evolution depends on cultivars and that these natural products decrease significantly in ripe fruits. Because environmental conditions and management practices were identical among our sampling plots, these data clearly demonstrate that resveratrol biosynthesis is closely linked to the genetic characteristics of each cultivar. This finding is in agreement with previous grape analysis (Chafer et al. 2005; Gerogiannaki-Christopoulou et al. 2006; Moreno et al. 2008; Navarro et al. 2008). Therefore, our results provide a database for resveratrol contents from grapevines cultivated in Korea.

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