

Comparison of the Antioxidative Effects and Content of Anthocyanin and Phenolic Compounds in Different Varieties of *Vitis vinifera* Ethanol Extract

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Abstract

This study was a quantitative HPLC analysis of four anthocyanins and five phenolic compounds contained in the skins, vines and seeds of the Campbell Early, Muscat Bailey A and Neo Muscat grape varieties. In the phenolic analysis, the seeds of the Campbell Early were found to contain 1.9, 1.8 and 1.6 times higher quantities of gallic acid, catechin and epicatechin relative to other grape seeds. Three anthocyanins, cyanidin, peonidin and pelargonidin, were also found to be higher in the skins of the Campbell Early relative to other grape skins. Therefore, the Campbell Early is the most useful grape variety with regard to the extraction of these six compounds from these grape seeds and skins. The free radical scavenging effects of grape seeds were also compared, and the results indicated that the Campbell Early seeds were most effective among them.

Key words: anthocyanin, Campbell Early, catechin, grape, antioxidant

INTRODUCTION

The grape (*Vitis vinifera*) is a deciduous voluble plant of the Vitaceae family in the order Rhamnales and is one of Korea's principal crops, accounting for 20% of domestic fruit production. It is also known to harbor an abundance of polyphenol compounds, which have a variety of bio-regulatory functions (1-3). The major varieties of grape produced in the country are the Campbell Early, Muscat Bailey A, Neo Muscat, Kyoho and Sheridan grape. The production volume of the Campbell Early in 2006 amounted to approximately 330,000 tons, which accounts for 70% of the entire grape output (4,5). The waste parts of grapes, excluding the flesh, include the seeds and skins. The seeds represent approximately 3% of the weight of the raw fruit and the skins represent approximately 15%. A variety of physiological properties of phenolic compounds and anthocyanins have recently been identified, including the antioxidant and anti-carcinogenic effects, and a number of studies have been conducted regarding the appropriate use of grape waste components (3,6-11).

As part of these studies, Iacopini et al. (12) has conducted a quantitative analysis of five phenolic compounds in the skins and seeds of 10 varieties of grape grown in Italy, while Anastasiadi et al. (13) conducted a quantitative analysis of 18 phenolic compounds in the waste parts of four varieties of grape grown in Greece. Quantitative analyses on the waste parts of domestic

grape varieties are rare, with the exception of one report conducted in 2003 on the resveratrol content of the various constituent parts of a grape variety (1). This study reported the resveratrol contents of the skins, vines and seeds of domestic grape varieties, including the Campbell Early. However, the numbers of varieties and compounds are rather small, and thus an additional comparative quantitative study on anthocyanins and phenolic compounds is required to facilitate the efficient use of these byproducts. Therefore, this study involved a comparative quantitative analysis of four anthocyanins and five phenolic compounds contained in the skins, vines and seeds of the Campbell Early, Muscat Bailey A and Neo Muscat varieties, in an effort to identify the variety containing the greatest concentration of these chemicals. Moreover, the free radical scavenging effects of grape seeds were also assessed.

MATERIALS AND METHODS

Sample preparation

The Campbell Early specimens used in this study were harvested in late July 2007 from Gimcheon, Gyeongsangbuk-do; the Neo Muscat specimens were harvested in late July 2007 in Haman-gun, Gyeongsangnam-do; and the Muscat Bailey A specimens were harvested in late mid-September 2007 in Yeongcheon, Gyeongsangbuk-do. The collected samples were washed in distilled water and the skins, vines and seeds were separated and

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crushed, followed by extraction five times with 80% ethanol at room temperature using an ultrasonic extractor (Branson, Danbury, CT, USA) for one hour, in triplicate. Ethanol extract can be used on raw materials of food and cosmetics. The extract was filtered through filter paper and then concentrated completely using a rotary evaporator (Eyela, Tokyo, Japan) to prepare the skin, vine and seed extracts of the respective grape varieties. For the sample solution employed in the analysis, these extracts were dissolved with 10 mg/mL of methanol and filtered with a 0.45 μm membrane filter (Millipore, Billerica, MA, USA).

Quantitative analysis of anthocyanin content

Among the anthocyanins, which are grape pigments, the cyanidin, peonidin, pelargonidin and malvidin contents were quantified using an HPLC system (Jasco, Tokyo, Japan), with a bond-pack C18 (4 μm , 300 \times 3.9 mm) column. The standard chemicals used herein were purchased from Sigma-Aldrich (St. Louis, MO, USA), and the analytical conditions consisted of a mobile phase of distilled water containing 10% formic acid (solvent A) and acetonitrile containing 10% formic acid (solvent B), with a gradient elution of 27% after 15 minutes and 100% after 45 minutes, from 10% of the initial solvent B and an elution speed of 1.7 mL/min. The column temperature was maintained at 40°C, and the samples were detected at 530 nm (14,15).

Quantitative analysis of phenolic ingredients

Five phenolic ingredients (gallic acid, catechin, epicatechin, resveratrol, and quercetin) were purchased from Sigma-Aldrich and used as standard chemicals for quantitative analysis. Analysis was conducted using an HPLC system (Jasco) with a bond-pack C18 (4 μm , 300 \times 3.9 mm) column, with the mobile phase of distilled water containing 2% acetic acid (solvent A) and 50% acetonitrile containing 0.5% acetic acid (solvent B), and with a gradient elution of 80% after 70 minutes from 10% of the initial solvent B and an elution speed of 0.8 mL/min. The column's temperature was maintained at 40°C, and the samples were detected at 280 nm (16,17).

DPPH radical scavenging activity

Each 0.2 mL of grape seed extract dissolved in 80% ethanol was added to 3 mL of ethanol, to which 0.8 mL of 4×10^{-4} M DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma-Aldrich) dissolved in ethanol was subsequently added. This was vortexed for 10 seconds and maintained for 10 minutes at room temperature, after which absorbance was measured at 517 nm (18,19). The DPPH radical scavenging activity was expressed as a percentage of the absorbance of the group to which no DPPH was added.

Vitamin C was employed as a positive control.

ABTS radical scavenging activity

The ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] radical scavenging activity of the grape seed extract was measured via a slightly modified version of the method proposed by van den Berg et al. (20). To 0.1 M PBS (pH 7.4), 2.5 mM ABTS and 1.0 mM AAPH [2,2'-azobis(2-methylpropionamide) dihydrochloride] were added and maintained at 68°C for 12 minutes in a dark room, then quickly cooled to prepare an ABTS⁺ solution. 20 μL of grape seed extracts dissolved in PBS was added to 980 μL of ABTS⁺ solution and incubated for 10 minutes at 37°C, whereupon absorbance was measured at 734 nm.

Statistical analysis

Each experimental result was expressed as the mean \pm SD. Statistical comparisons between different treatments were calculated via ANOVA.

RESULTS

Yield of extract by variety and by part

The respective weights of the skins following separation from the raw grapes (10 kg) were 2.48 kg for the Campbell Early, 1.68 kg for the Neo Muscat and 1.58 kg for Muscat Bailey A. Although the heaviest skin was that of the Campbell Early, the yield of the extract obtained from the skins using 80% ethanol was found to be highest in the Muscat Bailey A, at 8.7%, and thus the final weight of the obtained skin extract was in the range of 96~137 g, without any significant difference. The weights and yields of the extracts obtained from the separated vines were found to be similar among the three varieties, and the weights of the separated seeds were 0.23, 0.29 and 0.33 kg for the Neo Muscat, Campbell Early and Muscat Bailey A varieties, respectively. The yields of the seed extract were highest in the opposite order of Neo Muscat, Campbell Early and Muscat Bailey A, the weight of the seed extract finally obtained was in the range of 14~25 g (Table 1).

Comparative analysis of anthocyanin content

The results of the quantitative analysis of the cyanidin, peonidin, pelargonidin and malvidin content of the anthocyanins in the pigments of the grape skins is shown in Table 2. Of the three varieties used, the Campbell Early skins displayed the highest cyanidin, peonidin and pelargonidin contents, with the cyanidin content, in particular, being more than 2.8 times that of Muscat Bailey A. None of the aforementioned four compounds were detected in the Neo Muscat variety of green grape. On

Table 1. Yields of grape skin, vine and seed extracts (%)

Varieties	Components	Fresh weights ¹⁾	Yields of 80% ethanol extract ²⁾
Campbell Early	Skin	24.8	0.97
	Vine	2.1	0.12
	Seed	2.9	0.16
Muscat Bailey A	Skin	15.8	1.37
	Vine	2.4	0.17
	Seed	3.3	0.14
Neo Muscat	Skin	16.8	0.96
	Vine	2.0	0.14
	Seed	2.3	0.25

¹⁾The respective weights % of the parts from the 10 kg of total fresh grape.

²⁾Each value represents a % of the total fresh grape (w/w).

the other hand, the malvidin content was highest in the Muscat Bailey A. According to a report by Oh et al (21), total anthocyanin content in Muscat Bailey A grape juice was higher than that in Campbell Early grape juice.

Comparative analysis of phenolic compounds

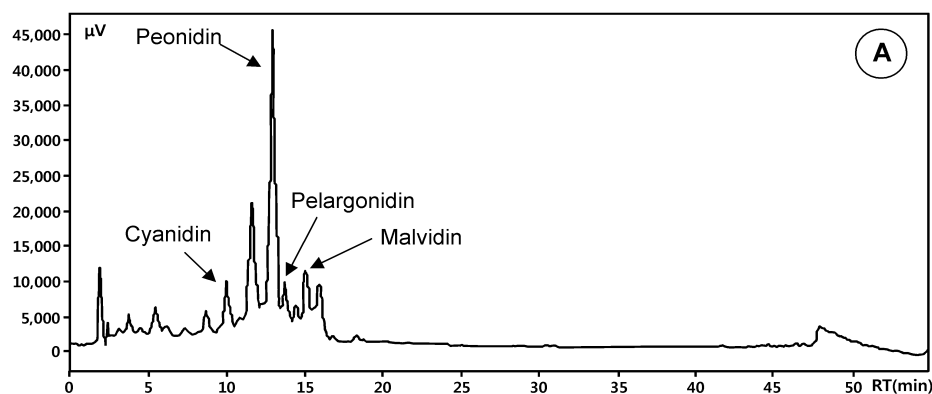
Five phenolic compounds (gallic acid, catechin, epicatechin, resveratrol and quercetin), which are known to be present in grape skins, vines and seeds, were analyzed by grape variety, as follows. In the seeds, the gallic acid, catechin and epicatechin contents of the Campbell Early were found to be 1.9, 1.8 and 1.6 times those of the other

varieties. Also, as regards the grape skins, the Campbell Early was found to have a catechin content 2.4 times that of the other varieties and an epicatechin content 2.3 times as high. With regard to the grape waste parts, all three varieties evidenced higher gallic acid, catechin and epicatechin contents in the seeds than in either the skins or the vines. Guendez et al. (22) reported that gallic acid, catechin and epicatechin content in grape seed of nine Hellenic native and international *Vitis vinifera* varieties cultivated in Greece were averaged 4.8, 189.0 and 98.6 mg/100 g, respectively.

The resveratrol content was higher in the stems than in the other parts, which was consistent with the finding of Cho et al. (1) that resveratrol is present at higher concentrations in the grape vines. However, its content was only 0.002~0.02%, which is much lower than the gallic acid, catechin, or epicatechin contents (Table 3). In addition, these five phenolic compounds were not found in flesh of all three varieties (data not shown).

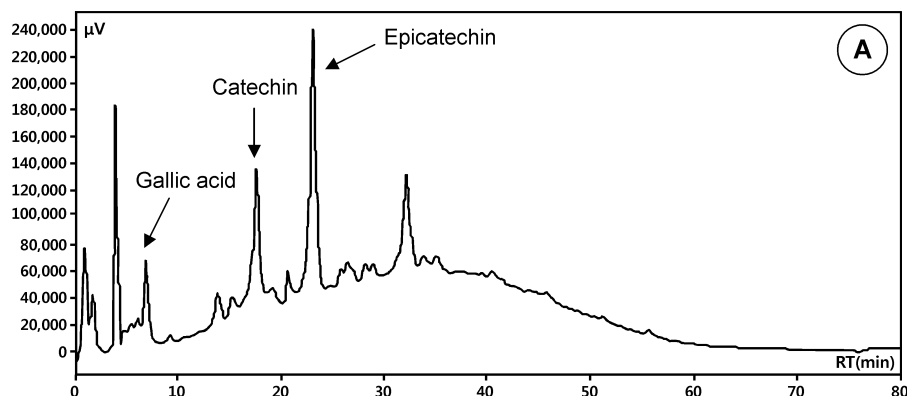
Radical scavenging activity

Free-radical species can induce oxidative damage to tissue and DNA nucleic acid sequences. DPPH and ABTS assays were conducted to determine whether grape seed extracts can scavenge free radicals. DPPH and ABTS radicals can be employed in preliminary screen-

Table 2. Quantitative analysis of anthocyanin in grape skin, vine and seed extracts

Varieties	Components	Cyanidin	Peonidin	Pelargonidin	Malvidin
Campbell Early	Skin	0.048 ± 0.002	0.226 ± 0.005	0.082 ± 0.01	0.153 ± 0.006
	Vine	ND	ND	ND	ND
	Seed	ND	ND	ND	ND
Muscat Bailey A	Skin	0.017 ± 0.001	0.061 ± 0.002	0.051 ± 0.001	0.216 ± 0.008
	Vine	ND	ND	ND	ND
	Seed	ND	ND	ND	ND
Neo Muscat	Skin	ND	ND	ND	ND
	Vine	ND	ND	ND	ND
	Seed	ND	ND	ND	ND

Each value represents a % (w/w) in the extracts. A: Campbell Early skins. ND: not detected. Data are the mean ± SD of the three replicates.

Table 3. Quantitative analysis of the phenolic compounds in grape skin, vine and seed extracts

Varieties	Components	Gallic acid	Catechin	Epicatechin	Resveratrol	Quercetin
Campbell Early	Skin	0.005 ± 0.000	0.076 ± 0.001	0.059 ± 0.001	0.003 ± 0.000	ND
	Vine	0.025 ± 0.001	0.282 ± 0.012	0.787 ± 0.014	0.020 ± 0.001	ND
	Seed	0.384 ± 0.07	2.055 ± 0.042	4.642 ± 0.079	ND	ND
Muscat Bailey A	Skin	0.003 ± 0.000	0.031 ± 0.000	0.025 ± 0.000	0.003 ± 0.000	ND
	Vine	0.021 ± 0.001	0.350 ± 0.005	0.231 ± 0.008	0.019 ± 0.001	ND
	Seed	0.094 ± 0.002	1.093 ± 0.033	2.375 ± 0.052	ND	ND
Neo Muscat	Skin	0.004 ± 0.000	0.007 ± 0.000	0.011 ± 0.000	ND	ND
	Vine	0.040 ± 0.000	0.182 ± 0.006	0.527 ± 0.011	0.002 ± 0.000	ND
	Seed	0.195 ± 0.003	0.568 ± 0.010	2.886 ± 0.027	ND	ND

Each value is expressed as a % (w/w) of the extracts. A: Campbell Early seeds. ND: not detected. Data are expressed as the means ± SD of three replicates.

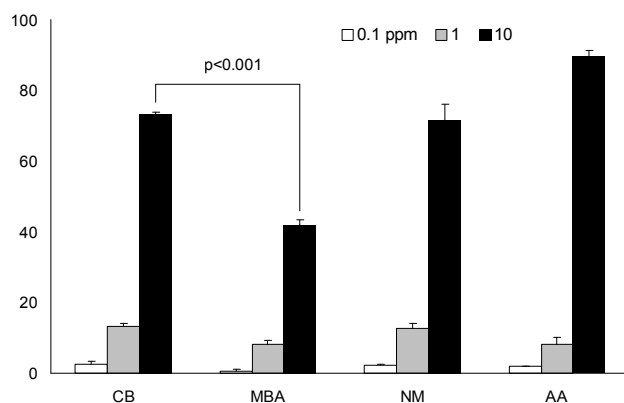


Fig. 1. DPPH radical scavenging activity of grape seed extracts. Data are expressed as the mean ± SD of three experiments. CB: Campbell Early, MBA: Muscat Bailey A, NM: Neo Muscat, AA: ascorbic acid. Ascorbic acid was used as a positive control.

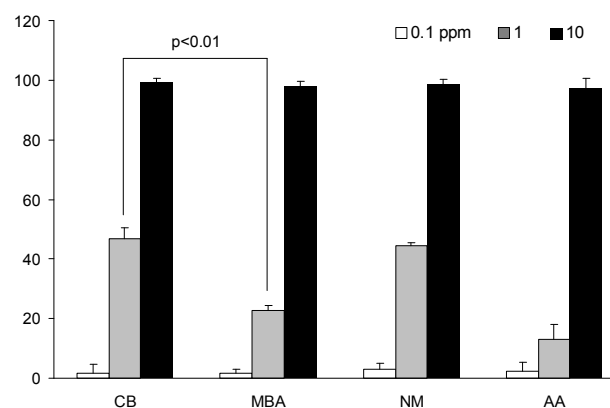


Fig. 2. ABTS radical scavenging activity of grape seed extracts. Data are expressed as the mean ± SD of three experiments. CB: Campbell Early, MBA: Muscat Bailey A, NM: Neo Muscat, AA: ascorbic acid. Ascorbic acid was used as a positive control.

ings of antioxidative agents. The measurement of DPPH scavenging activity indicated that the Campbell Early seeds were the most effective scavenger among them. The DPPH scavenging activity of the Campbell Early, Neo Muscat and MBA seeds at 10 ppm were found to be 73.3%, 71.6% and 41.9%, respectively (Fig. 1). Similarly, Min et al. (23) reported that the dose required for a 50% inhibition against 300 μM DPPH radical of 80% ethanol extract of Campbell Early seed was 21.9

ppm. In the ABTS assay, although the Campbell Early and Neo Muscat seeds evidenced similar levels of activity, the radical scavenging activity of the Campbell Early seeds was also stronger than those of other grape seeds (Fig. 2).

DISCUSSION

Three grape varieties, which are produced domestically in great quantities and evidence distinct varietal

characteristics, namely, the Campbell Early, Muscat Bailey A (a hybrid variety consisting of a cross with a wild grape variety) and Neo Muscat (green grape), were selected and quantitatively analyzed for the presence of four anthocyanins and five phenolic compounds in their skins, vines and seeds. The Campbell Early was found to have the highest cyanidin, peonidin, pelargonidin, gallic acid, catechin and epicatechin contents in its skins and seeds. Moreover, the Campbell Early seed exhibits the most effective free radical scavenging effects when compared to the MBA and Neo Muscat seed. The Campbell Early accounts for approximately 70% of domestic grape production, and thus its skins and seeds, enormous quantities of which are discarded each year, are regarded as potentially excellent materials for use in the extraction of effective active ingredients including cyanidin, peonidin, pelargonidin, gallic acid, catechin and epicatechin.

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