Identification and Quantification of Flavonoids in Korean Wild Grape (Meoru grape, *Vitis coignetiae*) and Its Pomace

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Abstract - Large quantities of Korean wild grape (*Vitis coignetiae*, KWG) pomace, a by-product of grape juice and wine manufacture, are generated annually, and disposal cost of KWG pomace is now increasing. Therefore, this study was conducted to determine total polyphenol, flavonoid, and anthocyanin content in whole KWG, KWG skin and KWG pomace and to identify and quantify flavonoids found in whole KWG and KWG pomace using LC/MS/MS. Spectral analysis showed high total polyphenol and flavonoid in KWG skin extracted with 75% ethanol ($p < 0.05$). KWG pomace had higher amount of total polyphenol, flavonoid and anthocyanin than whole grape ($p < 0.05$). Sixteen flavonoids were identified, but only 10 flavonoids were quantifiable from whole KWG and KWG pomace. Both epicatechin and rutin were the major flavonoids, and 521 or 147 ng/g of epicatechin ($p < 0.05$) and 305 or 110 ng/g of rutin ($p > 0.05$) were found in whole KWG and KWG pomace, respectively. The results show that KWG pomace is a very rich source of flavonoids, thus KWG pomace can be used as a functional food additive. Plans to include KWG pomace in food production are necessary.

Key words - By-product, Ethanol, Korean wild grape skin, LC/MS/MS, Polyphenol

Introduction

As consumers' demand for health benefits *via* food consumption increases, interests for phytochemicals of fruits and vegetables are elevated. The polyphenols, a group of phytochemical containing more than one phenolic group, have anti-microbial, anti-oxidant and anti-inflammatory effects (Ruberto *et al.*, 2007; Stevenson and Hurst, 2007; Shin *et al.*, 2011; Gong *et al.*, 2012; 2014), and it seems that they improve foods quality and then consumers' health as a result. Shin *et al.* (2011) reported that the addition of sorghum bran with vitamins C and E in chicken thigh meat sausages decelerates the lipid oxidation. Either or both hydrophilic and/or hydrophobic polyphenols of sorghum bran seems to contain major compounds providing an electron to reactive oxygen species (ROS) directly or an electron to pro-vitamins C and E

for electron restoration. In addition, based on the literature evidences, both kaempferol and resveratrol, flavonoids or stilbenoids of grapes, seem to attenuate allergic responses when either was offered to BALB/c up to 10-20 mg/kg (Gong *et al.*, 2012, 2014; Han *et al.*, 2013). Therefore, efforts to investigate polyphenol content of fruits and vegetables are now progressing, and making polyphenol identification and quantification of traditional Korean wild grape (Meoru grape, *Vitis coignetiae*, KWG) is necessary.

Grape is one of the world's most common fruits, containing high amounts of polyphenols, include flavonoids, and about 67 million tons are produced each year due to the FAOSTAT database (FAO STAT, 2014). As grapes are harvested, they are used for juice or wine production in Asia and Europe. Around 20% of the grapes particularly seeds and skins, remains as a residue after grapes are crushed and pressed during juice and wine manufacture (Amico *et al.*, 2004; Llobera and Cañellas, 2007; Ruberto *et al.*, 2007). Disposal cost of

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grape residues which are now called grape pomace, is increasing. To control these costs, some of the grape pomace is disposed as soil compost and animal feed (Negro *et al.*, 2003; Kammerer *et al.*, 2005; Khanal *et al.*, 2010). Furthermore, in spite of the high content of polyphenols in grape pomace, only a small amount of the grape pomace is used as food materials in the manufacture of vinegar and grappa (Lu and Foo, 1999; Özkan *et al.*, 2004; Ruberto *et al.*, 2007; Monrad *et al.*, 2010). Qualitative and quantitative polyphenols of grape pomace are important to produce quality vinegar and grappa, and different qualities of the pomace depend on the grape variety, cultivar, and cultivation district (Tourtoglou *et al.*, 2014). Both qualitative and quantitative polyphenols of grape pomace should be determined before creating KWG pomace as a novel food material.

Therefore, as the first step, this study focused on the identification of flavonoids and non-flavonoids in Korean wild grape (KWG) pomace and when possible, quantification of major flavonoids through LC/MS/MS was performed. Also, spectral analysis for total polyphenol, flavonoid and anthocyanin contents of KWG pomace was examined to determine the nutritional value of grape pomace as a food material.

Materials and Methods

Sample preparation

De-stemmed KWGs (Yeongdong meoru grape, *Vitis coignetiae*), which are native and widely grown in Republic of Korea, were purchased from a local nonhyup-mart in Jeonju (September 2013). Some whole KWGs were immediately freeze-dried, powdered and stored at -20°C for analysis. Others were processed to generate grape skin and grape pomace. Grape skins or grape pomaces (grape skins with seeds only) without grape stems, were pressed and collected after juicing (Hurom Group Co., Gimhae, Gyeongnam, Korea). Each grape skin or grape pomace was then vacuum freeze-dried (Ilshinbiobase Co. Ltd., Yangju, Gyeonggi, Korea) for 2 days, powdered by a grinder (Ya Hong Electronic Co., Guangdong, China) for 1min and then stored at -20°C in a freezer (LG electronics., Seoul, Korea). Three different samples of whole grape, grape skin, and grape pomace, were collected and

provided for analysis. Individual sample was analyzed on a duplicate basis.

Determination of phenolic compounds

10 g of whole grape, grape skin and grape pomace powder were sampled for total polyphenol determination. The total soluble polyphenols was extracted with 90 ml of either 100% or 75% ethanol at 4°C for 24 hr with continuous shaking at 200 rpm (Vision Scientific Co., Daejeon, Korea). Each extract was filtered, and 0.1 ml of each sample extract was assigned to individual tube containing 1.0 ml of Folin-Ciocalteu reagent (1:10 v/v with water) (Anesini *et al.*, 2008; Park *et al.*, 2012). Then, 0.3 ml sodium carbonate (75 g/L) was added. Each tube was then incubated at room temperature for 90 min, and the absorbance at 765 nm was read against a blank containing respective solvent. All results were then compared to gallic acid (mg/g) as a standard.

0.5 ml of whole grape, grape skin or grape pomace extract prepared as discussed above, was mixed with 1 mL of 10 mM Tris HCl (pH 7.0) and 75 µl of 5% sodium nitrite (Juan and Chou, 2010). After incubation for 6min at room temperature, 150 µl of 10% aluminum chloride, 500 µl of 1 M sodium hydroxide and 275 µl of double distilled water (DDW) were added and thoroughly mixed. The spectrometric absorbance of each sample solution was measured at 510 nm, and the total flavonoid of whole grape, grape skin and grape pomace was calibrated using quercetin (mg/g) as a standard.

0.1 g freeze-dried whole grape, grape skin, and grape pomace were mixed with 10 ml of ethanol:DDW:HCl (85:13:2, v:v:v) (Jang *et al.*, 2008). The sample mixture was incubated at 15°C for 24 hr with continuous shaking at 220 rpm and then filtered. The absorbance of the whole grape, grape skin and grape pomace was measured at 535 nm. The amount of anthocyanin was then calculated as follows:

Total anthocyanin contents ($\mu\text{g/g}$) = $[\text{Absorbance}_{535} \times \text{Extractant (ml)}] / [\text{Sample (g)} \times [1/65.1] (\text{Absorption Coefficient})]$

Determination of water content and pH

Whole KWG and grape pomace were tested for water content and pH. Water content of whole grape and grape pomace was obtained by finding the weight difference before and after lyophilization (%). 10 g of whole grape or grape

pomace powder in 90 ml DDW was homogenized, filtered, and tested with a pH meter (Corning Inc., NY, USA), which had been calibrated with a standard buffer of pH 7. Duplicate readings for each sample were conducted, and the average was reported.

LC/MS/MS analysis of flavonoid compounds

Sample preparation for flavonoid determination was performed by addition of 90 ml ethanol (100 or 75%) to 10 g of whole grape, grape skin, and grape pomace powder in a brown colored glass bottle. The mixture was incubated at 15°C for 24 hr with continuous shaking at 150 rpm and then filtered. The aqueous mixture was concentrated on the rotary evaporator under reduced pressure at room temperature. The extract was then passed through an activated C18 (60 cm³/10 g) Mega Bond Elute cartridge (Supelco, Bellefonte, PA, USA). The cartridge was rinsed with water to remove sugars and simple organic acids. The phytochemicals retained, were then eluted with 100% ethanol. Individual 5 µl liquid samples were directly injected to the liquid chromatography (Agilent Technologies, Palo Alto, CA, USA) system coupled with an electrospray ionization-mass spectrometer (ESI-MS/MS) detector. The chromatographic separation was achieved with a Phenomenex synergy Hydro-RP (2.1 mm i.d. × 150 mm, Phenomenex, Torrance, CA, USA) followed by 0.2 ml/min of flow under 400 psi at 30°C. The gradient elution composed of 0.1% formic acid (A) in water and 0.1% formic acid acetonitrile (B), 5% B linear gradient (min 0-1); 5-100% B linear gradient (min 1-10); 100% B (min 10-11); 100-5% B linear gradient (min 11-14); 5% B (min 14-20). The column was re-conditioned for 15 min before the next run.

The peaks were identified by comparison of retention time (RT) and mass spectra (MS) with references. All reference compounds including apigenin (lot no. SLBG8924V), benzoic acid (lot no. MKBL6689V), caffeic acid (lot no. MKBQ5343V), catechin (hydrate) (lot no. BCBK7891V), cyanin chloride (lot no. BCBL3222V), epicatechin (lot no. SLBH5965V), epigallocatechin (lot no. SLBG2332V), epigallocatechin gallate (lot no. SLBG6681V), gallic acid (lot no. SLBF8212V), gallocatechin (lot no. SLBH7895V), isorhamnetin (lot no. BCBK2503V), isovanillic acid (lot no. 1419575V), kaempferol (lot no. BCBM7287V), luteorin (lot no. 083M4090V), myricetin

(lot no. BCBL4930V), naringenin (lot no. BCBJ2179V), pelargonidin chloride (lot no. SLBF4330V), quercetin (lot no. SLBD8415V), quercetin 3-D-galactoside (lot no. BCBH6299V), rosmarinic acid (lot no. SLBG3402V) and rutin (trihydrate) (lot no. 1380505V), were purchased from Sigma Chemical Company. Multiple reactions monitoring (MRM) transition of each reference was used for quantitative analysis of compounds in whole grape and grape pomace. Each reference compound was used for preparation of calibration at concentrations between 1 and 1000 ng/ml. The grape skin was employed for kaempferol determination only (Table 3).

Statistical analysis

Statistical analysis was conducted using a SAS program (Version 6.12, Cary, NC, USA, 1998) (SAS, 1998), and significant difference was determined by an Analysis of Variance (ANOVA) using GLM procedure, followed by a Duncan's Multiple Range Test with a predetermined significance level of $p < 0.05$.

Results and Discussion

Phenolic composition of whole grape, grape skin and grape pomace

Total polyphenols and flavonoids expressed as mg/g of KWG skin, were determined by the Folin-Ciocalteu reagent, and the results are presented in Table 1. From comparison of the literature on the yield of phenols from grape, phenols were high when ~30% water was mixed with ethanol and then used as a solvent (Spigno *et al.*, 2007; Monrad *et al.*, 2010) unless both flavonols and anthocyanins are less soluble in water than in an organic media (Corrales *et al.*, 2008). As evidenced, both total polyphenols and flavonoids were significantly higher when extracted in 75% ethanol solvent (1.15 or 1.08 mg/g of grape skin, respectively) ($p < 0.05$). Most polyphenols of grape skin are flavonoids, covering 94% of polyphenols. Actual flavonoid versus polyphenol ratio of KWG was high in comparison to that of Bulgarian whole black grape (*Vitis vinifera*), which Marinova *et al.* (2005) found to be 0.36. 75% ethanol showed lower kaempferol than 100% ethanol extractant albeit the content was not significantly different ($p > 0.05$). The amount of anthocyanin detected, was

less than total polyphenols and flavonoids, and it is similar to the results demonstrated by Negro *et al.* (2003), showing 1.40, 1.31 and 0.33 g/L of total polyphenol, flavonoid and anthocyanin in red grape, respectively.

When whole grape and grape pomace were freeze-dried, moisture content was 79.46 and 54.41%, respectively ($p < 0.05$) (Table 1), and these are lower than the moisture of whole grape (96.19%) and grape pomace (73.65-80.12%) of *Vitis vinifera* ssp. reported by Corrales *et al.* (2008). The pH value of whole grape was 4.00 and then elevated to 4.41 when whole grapes were pressed and turned to grape pomace ($p < 0.05$).

All polyphenols were significantly higher in grape pomace than in whole grape ($p < 0.05$) (Table 1). The amount of polyphenols and flavonoids was elevated in grape pomace and is ~2.30 time higher than that of whole grape, which is in agreement with Butkhup *et al.* (2010). Unexpectedly, grape pomace posed high concentration of anthocyanins when exactly equal amount of whole grape and grape pomace were weighed and analyzed. Khanal *et al.* (2009) reported that anthocyanin of whole grape is lower during juice mashing steps and substantial amounts of anthocyanins are lost due to elution as a form of grape juice (Kammerer *et al.*, 2004; Wu *et al.*, 2006; Monrad *et al.*, 2010). However, Maier *et al.* (2009) and Pinelo *et al.* (2006) suggested that anthocyanin deficiency is depending on the pectin and/or sugar of the grape, and it is clear that the anthocyanin of grape pomace seems closely related to water loss due to grape cell wall damage. Without complete destruction of pectin structure and/or loss of phenol-sugar binding, anthocyanin may be eluted as a form of grape juice less than expected (Kennedy and Matthews, 2002; Pinelo *et al.*, 2006; Maier *et al.*, 2009). Anthocyanin of grape pomace was not the lost and retained 12.42 $\mu\text{g/g}$, indicating grape itself, being deprived of grape skin and seed, may not be a good source of polyphenol due to high content of polyphenols in grape skin and seed (Pinelo *et al.*, 2006). According to the study of Ruberto *et al.* (2007), general Italian grape possessed greater total anthocyanin than flavonoid. However, the amount of anthocyanin was not greater than flavonoid in KWG. The amount of flavonoid and anthocyanin of KWG pomace are 6.54 mg/g and 12.42 $\mu\text{g/g}$, respectively.

Flavonoids and non-flavonoids identification

Flavonoid and non-flavonoid profiles of whole grape and grape pomace were checked and identified (Table 2). Twenty-one peaks were identified and were mostly flavan-3-ols, flavanone, flavone, flavonol and phenolic acids (non-flavonoids). In whole grape and grape pomace, catechin (hydrate), epigallocatechin, epicatechin, epigallocatechin gallate, gallo-catechin were recognized as a group of flavan-3-ols (Yilmaz and Toledo, 2004), which are generally from grape skin and seed (Lee *et al.*, 2003; Kammerer *et al.*, 2004; Pinelo *et al.*, 2006). Naringenin which reduces plasma and hepatic cholesterol by suppressing HMG-CoA reductase activity (Panoutspopoulos and Beedham, 2005; Chang *et al.*, 2011), was the only flavanone confirmed in both whole grape and grape pomace. As sub-groups of anthoxanthins, 8 flavones and flavonols were identified, (flavones: apigenin, luteolin, quercetin 3-D-galactoside and rutin; flavonols: myricetin, isorhamnetin, quercetin and kaempferol, respectively). Isovanillic acid, a non-flavonoid and a metabolized form of isovanillin by aldehyde dehydrogenase (Revilla and Ryan, 2000), was distinguished. Moreover, benzoic acid, caffeic acid, gallic acid, and rosmarinic acid, non-flavonoids, were found in either whole grape or grape pomace, which is similar to those found in the *Vitis vinifera* L. of Germany and USA and Shiraz red grape of Thailand (Lee *et al.*, 2003; Kammerer *et al.*, 2004; Butkhup *et al.*, 2010).

Flavonoids quantification

The chromatographic fingerprint of flavonoids from whole grape and grape pomace was analyzed by LC/MS/MS. Using 16 different flavonoids as standards, 10 flavonoids were quantified by LC/MS/MS in both or either whole grape (Fig. 2) and grape pomace (Fig. 3). Those were cyanin chloride, epigallocatechin, epicatechin, gallo-catechin, naringenin, quercetin 3-D-galactoside, rutin (trihydrate), isorhamnetin, myricetin, and quercetin. Among them, epicatechin, rutin and myricetin were the main flavonoids quantified by LC/MS/MS (Fig. 1), which is not consistent with Amico *et al.* (2004). Amico *et al.* (2004) reported that quercetin was the main flavonoid of Nerello Mascalese grape pomace followed by isorhamnetin. Both whole grape and grape pomace contained 521.69 and 147.58 ng/g of epicatechin and 305.45 and 110.94

Fig. 1. Three different major flavonoids observed in both whole Korean wild grape and grape pomace.

Fig. 2. Flavonoids quantification of whole Korean wild grape using LC/MS/MS.

*Cyanin chloride (610.9-287.0;8.59), epigallocatechin (306.9-138.9;4.792), epicatechin (290.9-138.9;9.665), galocatechin (306.9-39.0;8.249), naringenin (272.9-153.0;11.879), quercetin 3-D galactoside (464.9-302.9;10.095), rutin (611.0-302.8;9.865), isorhamnetin (316.9-152.9;12.231), myricetin (318.9-152.9;10.552), quercetin (302.9-152.9;11.527).

**Naringenin = Not detected.

Fig. 3. Flavonoids quantification of whole Korean wild grape pomace using LC/MS/MS.

*Cyanin chloride (610.9-287.0;8.546), epigallocatechin (306.9-138.9;4.864), epicatechin (290.9-138.9;9.656), galocatechin (306.9-139.0;8.052), naringenin (272.9-153.0;11.862), quercetin 3-D-galactoside (464.9-302.9;10.048), rutin (611.0-302.8;9.821), isorhamnetin (316.9-152.9;12.21), myricetin (318.9-152.9;10.548), quercetin (302.9-152.9;11.482).

**Epigallocatechin, Quercetin 3-D-galactoside = Not detected.

ng/g of rutin, respectively, and these were mainly from grape seed or skin (Lacopini *et al.*, 2008, Ratnasooriya *et al.*, 2010). The content of epicatechin and rutin of KWG was similar to those of whole Shiraz red grapes in Thailand (Butkhup *et al.*, 2010) but lower than the Shiraz red grape when KWGs were pressed and turned to pomace.

Whole grape contained 32% more water than grape pomace (Table 1), had higher amounts of epigallocatechin, epicatechin, galocatechin, quercetin 3-D-galactoside, rutin (trihydrate), isorhamnetin, and myricetin compared to grape pomace (Table 4). Most flavonoids found in whole grape were 1.48-11.88 times higher than in grape pomace, which is not in agreement with

Ratnasooriya *et al.* (2010), showing higher flavan-3-ols in grape pomace. As mentioned above, both epigallocatechin and quercetin 3-D-galactoside seem to be lost in grape juice (Meng *et al.*, 2004) so neither epigallocatechin nor quercetin 3-D-galactoside was detectable in grape pomace. On the other hand, both naringenin and quercetin seemed to concentrate after grape juice and were 1-2 times greater in grape pomace. As quercetin is an antioxidant against both water and lipid soluble free radicals (Faustino *et al.*, 2004), quercetin may not fully be soluble in water. Therefore, quercetin as well as naringenin is not easily eluted grape juice.

In conclusion, this study was carried out for spectral

Table 1. Spectral analysis for total polyphenol, flavonoid and anthocyanin of whole Korean wild grape, grape skin and grape pomace and proximate water and pH of whole Korean wild grape and grape pomace

	Korean Wild Grape skin (<i>Vitis coignetiae</i>)					Korean wild Grape (<i>Vitis coignetiae</i>)			
	Ethanol		SEM ^z	p-value		100% Ethanol		SEM ^z	p-value
	100%	75%				Whole grape	Grape pomace		
Polyphenol (mg/g)	1.13 ^b	1.15 ^a	0.05	0.01	Moisture (%)	79.46 ^a	54.41 ^b	4.18	0.01
Flavonoid (mg/g)	0.84 ^b	1.08 ^a	0.03	0.01	pH	4.00 ^b	4.41 ^a	0.10	0.01
					Phytochemicals				
Kaempferol (ng/g)	2.59	2.07	0.55	0.37	Polyphenol (mg/g)	2.94 ^b	6.75 ^a	0.58	0.01
					Flavonoid (mg/g)	2.86 ^b	6.54 ^a	0.48	0.01
Anthocyanin (ug/g)	8.62		0.05	-	Anthocyanin (ug/g)	4.32 ^b	12.42 ^a	1.23	0.01

^zSEM = standard error of the mean.^{a,b}Mean values within a row followed by different letters indicate significant difference at $p < 0.05$.

Table 2. Experimental parameters of LC/MS/MS for the detection of flavonoid in whole Korean wild grape and grape pomace

Analyte	MW ^z	RT ^y	ESI ^x mode	MS ₁	MS ₂
Apigenin	270.24	11.94	[M+H] ⁺	270.9	152.9
Benzoic acid	122.12	11.84	[M-H] ⁺	121	77
Caffeic acid	180.16	9.91	[M-H] ⁺	179	135.1
Catechin (hydrate)	290.27	5.26	[M+H] ⁺	290.9	138.9
Cyanin chloride	611.52	8.52	[M-H] ⁺	610.9	287
Epicatechin	290.27	9.72	[M+H] ⁺	290.9	138.9
Epigallocatechin	306.27	4.89	[M+H] ⁺	306.9	138.9
Epigallocatechingallate	458.37	9.82	[M+H] ⁺	458.9	138.9
Gallic acid	170.12	3.91	[M-H] ⁺	169	125
Gallocatechin	306.27	8.09	[M+H] ⁺	306.9	139
Isorhamnetin	316.26	12.27	[M+H] ⁺	316.9	152.9
Isovanillic acid	168.16	10.08	[M-H] ⁺	167	107.9
Kaempferol	268.23	12.16	[M+H ₂ O] ⁺	286.9	164.9
Luteorin	286.24	11.26	[M+H] ⁺	286.8	134.9
Myricetin	318.24	10.58	[M+H] ⁺	318.9	152.9
Naringenin	272.26	11.89	[M+H] ⁺	272.9	153
Pelargonidin chloride	271.24	10.02	[M-H] ⁺	270.9	121
Quercetin	302.24	11.52	[M+H] ⁺	302.9	152.9
Quercetin 3-D-galactoside	464.38	10.08	[M+H] ⁺	464.9	302.9
Rosmarinic acid	360.31	10.07	[M-H] ⁺	359.1	161.1
Rutin (trihydrate)	610.52	9.86	[M+H] ⁺	611	302.8

^zMW = Molecular weight.^yRT = Retention time.^xESI = Electrospray ionization.

Table 3. Regression equation and R-square of analytes for the detection of flavonoid in whole Korean wild grape and grape pomace

Analyte	Regression equation	R square
Apigenin	Y = 119.83x + 5020.7	0.9974
Benzoic acid	Y = 0.0533x + 1.6667	1
Caffeic acid	Y = 29.872x + 875.5	0.9949
Catechin (hydrate)	Y = 55.559x + 1090.7	0.9993
Cyanin chloride	Y = 104.16x + 931.4	0.9996
Epicatechin	Y = 77.954x + 948.56	0.9997
Epigallocatechin	Y = 66.903x + 1050.5	0.9988
Epigallocatechingallate	Y = 62.909x + 682.02	0.9992
Gallic acid	Y = 11.215x + 118.5	0.9997
Gallocatechin	Y = 35.732x + 16.444	1
Isorhamnetin	Y = 55.739x + 790.47	0.9989
Isovanillic acid	Y = 0.1794x + 11.333	1
Kaempferol	Y = 12.338x + 74.333	0.9991
Luteorin	Y = 28.215x + 1349.6	0.9965
Myricetin	Y = 56.393x + 938.33	0.9994
Naringenin	Y = 157.18x + 5840.3	0.9974
Pelargonidin chloride	Y = 87.617x + 5461	0.977
Quercetin	Y = 20.193x + 143.83	0.9998
Quercetin 3-D-galactoside	Y = 218.58x + 3785.8	0.99978
Rosmarinic acid	Y = 34.274x + 751.66	0.9967
Rutin (trihydrate)	Y = 73.935x + 1446.5	0.9989

analysis of total polyphenol, flavonoid and anthocyanin determination and to identify and quantify flavonoids of KWG pomace using a LC/MS/MS. Higher total polyphenol

Table 4. Flavonoid quantification of whole Korean wild grape and grape pomace by LC/MS/MS

	Korean Wild Grape (<i>Vitis coignetiae</i>)		SEM ^z	<i>p</i> -value
	Whole grape	Grape pomace		
Anthocyanin (ng/g)				
Cyanin chloride	11.38 ^a	0.75 ^b	3.08	0.01
Flavan-3-ols (ng/g)				
Epigallocatechin	1.48	Ta ^y	0.05	-
Epicatechin	521.69 ^a	147.58 ^b	86.85	0.01
Gallocatechin	4.16	0.35	1.21	0.12
Flavanone (ng/g)				
Naringenin	Ta	1.66	0.73	-
Flavone (ng/g)				
Quercetin 3-D-galacto	17.97	Ta	3.17	-
Rutin(trihydrate)	305.45	110.94	61.54	0.12
Flavonol (ng/g)				
Isorhamnetin	1.29 ^a	0.27 ^b	0.27	0.03
Myricetin	65.74 ^a	29.04 ^b	9.04	0.01
Quercetin	0.24	0.42	0.06	0.12

^zSEM = standard error of the mean.^yTa = traceable.^{a,b}Mean values within a row followed by different letters indicate significant difference at $p < 0.05$.

and flavonoid were determined when 75% ethanol was used as a grape skin extractant ($p < 0.05$). Additionally, grape pomace contained high total polyphenol, flavonoid and anthocyanin as compared to that of whole grape ($p < 0.05$). A total of 21 flavonoids and non-flavonoids were identified but only 10 flavonoids were quantified in whole grape and grape pomace. Both epicatechin and rutin are main flavonoids confirmed, and they were around 521 and 147 ng/g of epicatechin and 305 and 110 ng/g of rutin in both whole grape and grape pomace ($p < 0.05$). Therefore, it seems that KWG pomace is able to be used as a food material, and strategies to include KWG pomace in food products must be founded.

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