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DETERMINATION OF ANTHOCYANIN CONTENT AND ANTIOXIDANT CAPACITY OF DIFFERENT GRAPE VARIETIES

DETERMINAÇÃO DA COMPOSIÇÃO ANTOCIÂNICA E DA ACTIVIDADE ANTIOXIDANTE DA UVA DE DIFERENTES VARIEDADES DE VIDEIRA

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SUMMARY

The aim of this study was to determine anthocyanin content and antioxidant activity of 20 different grape varieties, using HPLC and DPPH, FRAP and ABTS assays, respectively. The identified anthocyanins were malvidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside, cyanidin-3 glucoside, and peonidin-3-glucoside on the basis of their retention times. Comparatively the total anthocyanin content varied from 181.2 mg/ 100 g FW ('Vidal Black') to 716.4 mg/ 100 g FW ('Catawba'). High anthocyanin content was found in 'Catawba' (716.4 mg/100 g), 'Ruby Seedless' (634.5 mg/100 g) and 'Campbell Early' (611.1 mg/100 g) extracts. The antioxidant activities of grape extracts varied from 32.8% ('Campbell Early') to 87.6% ('Hongiseul') by DPPH, 79.1% ('Campbell Early') to 197% ('Hongiseul') in case of FRAP and 11.1% ('Chasselas Rouge') to 74.5% ('Flouxa') by ABTS antioxidant assay. The results suggested that the anthocyanin content in studied grape varieties showed statistically significant correlation with free radical scavenging activity. Thus depending on results we say that these grape varieties may serve as a potential source of nutraceuticals and functional food development.

RESUMO

O objectivo do presente estudo foi determinar a composição antociânica e a actividade antioxidante da uva de 20 variedades de videira, por HPLC e através dos métodos DPPH, FRAP e ABTS, respectivamente. As antocianinas identificadas foram a malvidina-3-glucósido, delfinidina-3-glucósido, petunidina-3-glucósido, cianidina-3-glucósido e peonidina-3-glucósido, de acordo com os seus tempos de retenção. O teor total de antocianinas variou entre 181,2 mg/ 100 g FW ('Vidal Black') e 716,4 mg/ 100 g FW ('Catawba'). Os teores mais elevados foram detectados nos extractos de uvas das variedades 'Catawba' (716,4 mg/100 g), 'Ruby Seedless' (634,5 mg/100 g) e 'Campbell Early' (611,1 mg/100 g). A actividade antioxidante dos extractos de uvas variou entre 32,8% ('Campbell Early') e 87,6% ('Hongiseul') pelo método DPPH, entre 79,1% ('Campbell Early') e 197% ('Hongiseul') pelo método FRAP, e entre 11,1% ('Chasselas Rouge') e 74,5% ('Flouxa') pelo método ABTS. Os resultados sugerem que a composição antociânica nas variedades estudadas apresenta uma correlação significativa com a actividade antiradicalar. Assim, considera-se que as uvas destas variedades de videira podem constituir uma fonte potencial de compostos nutracêuticos e com interesse no desenvolvimento de alimentos funcionais.

Key words: grape, HPLC, anthocyanin, antioxidant.
Palavras-chave: uva, HPLC, antocianinas, antioxidante.

INTRODUCTION

Anthocyanins constitute a large family of polyphenols in plants and are responsible for many of the fruit and floral colours observed in nature (Nile and Park, 2014a). They are pigments dissolved in the vacuolar sap of the epidermal tissues of flowers and fruit which impart a pink, red, blue, or purple colour (Mazza and Maniati, 1993). Accumulating data show that anthocyanins and anthocyanin-rich plant extracts could provide various health benefits, including protection from DNA cleavage (Lazze *et al.*, 2003), anti- inflammatory activity (Rossi *et al.*, 2003), anticancer activity (Hou, 2003; Hou *et al.*, 2004), antioxidant activity (Matsumoto *et al.*, 2002; Oh *et al.*, 2008), anti-diabetic activity (Jankowski *et al.*, 2000; Tsuda *et al.*, 2003), and prevention of cardiovascular and neurodegenerative disease (Youdim *et al.*, 2000). Many of the grape varieties like 'Catawba', 'Concord', 'Niagara', 'Ontario',

'Delaware' and 'Thomson Seedless' has been originated and developed from the early 20th century (Paul and Sanjun, 2003; Chiou *et al.*, 2007). Grapes are among the fruits containing the highest content of phenolic substances, which are partially extracted during wine making process and brewing (Revilla and Ryan, 2002). Many grape berries having significant amount of bioactive phenolics with antioxidant properties (including flavan-3-ols, anthocyanins, cinnamic acid derivatives, flavonol derivatives and trans-resveratrol) that may be separated in a single run by direct injection of red wine (Burns *et al.*, 2000). The phenolic compounds in fresh and commercial grape juices may also be beneficial in the prevention of coronary heart diseases as they also have strong antioxidant activity towards human LDL oxidation in vitro (Meyer *et al.*, 1998). South Korea is bestowed with diverse climatic conditions and conducive for the growth of different grapes known for their nutritional and wine producing values (Nile *et al.*, 2013). So far, there has not been any study on antioxidant activity of grape cultivars grown and consumed South Korea. The main objective of this study was to screen a large number of grapes cultivars grown and consumed in the Korean diet and wine industry with respect to their total anthocyanin content and antioxidant activity. Based on the above facts, the objectives of our work was to quantify anthocyanins and antioxidant activity from grape fractions of twenty different cultivars that are available in South Korea. Such studies are of particular importance because anthocyanins have been shown to differ considerably in their bioavailability and to exert different biological activities in vitro and in vivo. Thus, this data may contribute to the selection of suitable grape material for the extraction of phytochemicals as ingredients for nutraceuticals and functional food development.

MATERIAL AND METHODS

Materials

The 20 grape cultivars were obtained from vineyards from Suwon, Gyeonggi, Jeju and Muan, different geographic regions of Korea in 2012 (Table I). 5 Kg each fruits were harvested in summer after fully maturation, stored for about 5 months at -40 0C, homogenized with food processor, and lyophilized to concentrate each sample.

Table I

Classification of grape cultivars according to skin colour

Classificação das variedades de videira de acordo com a cor da película

Skin colour	Species		
	V. vinifera	*V. labrusca*	*V. hybrida*
White	Vidal Black	Niagara	Thomson Green (seedless)
	Italia	Catawba	Ontario
Red	Chasselas Rouge	Delawere	Hongiseul
	Red globe	Ruby Seedless	Honey Red
		Koho	
Black	Alphonse Lavallee	Concord	Flouxa
		Campbell Early	Tamnara
		Sherpher	Black Pegaru

Chemicals

Anthocyanin standards: HPLC grade malvidin-3-O-glucoside chloride, delphinidin 3-O-β-D-glucoside chloride, petunidin-3-O-glucoside chloride, cyanidin-3-O-glucoside chloride, and peonidin-3-O-glucoside chloride, Sigma Chemical Co. (St. Louis, MO), DPPH were obtained from Fluka Chemicals AG (Buchs, Switzerland). All other chemicals were procured from Daejung Chemicals, Seoul, South Korea (Kallithraka *et al.*, 2005).

Extraction of grape sample

A lot of 1 kg grape berries was carefully collected in the vineyard, cut from the clusters with the pedicel, and transferred quickly to the laboratory. Pedicels were removed and berries were weighted, manually skinned, and the skins were freeze-dried. The freeze-dried tissues were then extracted with 200 mL of 1% HCl in methanol. Extraction was carried out under stirring for 48 h, and repeated in triplicate. Extracts were pooled, and this mixture was used for further procedures after deep-freezing (-70 ºC) for no longer than 3 days.

HPLC analysis

The HPLC/DAD analysis was performed on a Shimadzu HPLC equipped with a diode array detector (SPD-M10A, Shimadzu, Japan) at 520 nm for quantification. The column used was a C¬18 HPLC column (Zorbax 300SB-C18, Agilent Technologies, Ris¬ing Sun, Md., USA). A temperature programmable column oven (Younglin Instrument, Seoul, Korea) was used to maintain the column temperature at 35 ºC during the HPLC analysis. The injection volume of the prepared sample was 10 µL. Solvent A was formic acid/water (10:90), and solvent B was formic acid/acetonitrile (10:90). The solvent gradient for all grape samples and reference standards (malvidin-3-O-glucoside chloride, delphinidin 3-O-β-D-glucoside chloride, petunidin-3-O-glucoside chloride, cyanidin-3-O-glucoside chloride, and peonidin-3-O-glucoside chloride) was 0 to 10 min, 10% B; 10 to 20 min, 10% to 20% B; 20 to 30 min, 15 % to 25 % B; and 35 to 50 min, 30 % B (Oh *et al.*, 2008).

Total anthocyanin content

Total anthocyanin content was determined using a previously described method (Connor *et al.*, 2002), in which each grape extract was diluted (5:95, v/v) in 1% HCl in methanol to obtain an absorbance between 0.500 and 1.000 at 530 nm. The values were expressed as mg cyanidin-3-glucoside (c3g) equivalents per 100 g fresh weight using a molar extinction coefficient of 27.900. All determinations were performed in triplicates.

DPPH free radical scavenging activity

The bleaching rate of a stable free radical DPPH was monitored at a characteristic wavelength in the presence of the grape sample. In this, radical form of DPPH absorbs at 517 nm upon reduction by an antioxidant. This activity was measured according the previously described method, briefly as: 100 µM solution of DPPH was prepared in 10 mL of methanol and 2.7 mL of this solution was added to 0.5 mL of grape extract in methanol at the same concentration (0.1 mg/mL). After 10 min, the absorbance was measured at 517 nm. The percentage of remaining DPPH was calculated as, DPPH scavenging effect (%) = [(AControl - ASample/AControl) × 100], Where AControl is the absorbance of the DPPH reaction and ASample is the absorbance in the presence of grape extracts. All determinations were performed in triplicate (Nile *et al.*, 2013).

Ferric reducing antioxidant power (FRAP) assay

The assay was based on the reducing power of a compound (antioxidant) as previously described (Nile and Park, 2014b). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 µM, pH 3.6), a solution of 10 µM TPTZ in 20 µM HCl, and 10 µM $FeCl_3$ at 10:1:1 (v/v/v). The reagent (300 µL) and sample solutions (10 µL) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 10 min. Standard curve was prepared using different concentrations of Trolox. The results were corrected for dilution and expressed in micromolar Trolox per 100 g of dry weight (DW). All determinations were performed in triplicate.

Free radical scavenging ability by ABTS

The free-radical-scavenging activity was determined by ABTS radical cation decolorization assay (Nile and Park, 2014c). ABTS was dissolved in water to a 5 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.50 mM potassium persulfate (final concentration) and kept in the dark at room temperature for 12-16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the study of infusion, the samples containing the $ABTS^{\bullet+}$ solution were diluted with redistilled water to an absorbance of 0.700 (0.02) at 734 nm and equilibrated at 30 ºC. After the addition of 3.0 mL of diluted $ABTS^{\bullet+}$ solution (A734 nm) 0.700 (0.02) to 10 µL of polyphenolic extracts of grape, the absorbance reading was exactly 6 min after initial mixing. The results were corrected for dilution and expressed in micromolar Trolox per 100 g of dry weight (DW). All determinations were performed in triplicate.

Statistical analysis

The experiments were performed in triplicate. The results were expressed as mean ± SD. Also, linear regressions between the content of total anthocyanins with the results of the antioxidant assays were assessed.

RESULTS AND DISCUSSION

All grape samples were analyzed by HPLC to quantify their anthocyanins. The obtained results revealed differences in the concentrations of total anthocyanins in grapes according to the variety (Table II). Baseline separation of all anthocyanins was achieved within 30 min, 4 of them being identified as the malvidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside, cyanidin-3 glucoside, and peonidin-3-glucoside on the basis of their HPLC retention times by comparison with those of reference compounds (peak 1-5, Figure 1). The total anthocyanin content varied from 181.2 mg/ 100 g FW ('Vidal Black') to 716.4 mg/ 100 g FW ('Catawba'). High anthocyanin content was found in 'Catawba' (716.4 mg/100 g), 'Ruby Seedless' (634.5 mg/100 g) and 'Campbell Early' (611.1 mg/100 g) extracts. Most of black skin colour grape cultivars were rich in cyanidin-3 glucoside, this cyanidin-3 glucoside anthocyanin varying from 32.5 ('Vidal Black') to 89.5 ('Hongiseul') mg/100 g fresh weight. Malvidin-3-glucoside varied from 32.8 ('Campbell Early') to 87.6 ('Hongiseul') mg/100 g fresh weight. Delphinidin-3-glucoside varied from 80.9 ('Honey Red') to 456.8 ('Catawba') mg/100 g fresh weight. Peonidin-3-glucoside varied from 11.1 ('Chasselas Rouge') to 74.5 ('Flouxa') mg/100g fresh weight. Petunidin-3-glucoside varied from 12.6 ('Vidal Black') to 65.9 ('Flouxa') mg/100g fresh weight. The other anthocyanin profile was distinctive for each cultivar, but the degree of ripeness may be critical in this respect, because anthocyanin distribution can be considerably affected during different maturation stages of grape fruits.

Table II

Individual anthocyanin content in grape varieties (*Vitis vinifera, Vitis labrusca and Vitis hybrida*) extracts[a]

Teores de antocianinas nos extractos de uvas de diversas variedades (Vitis vinifera, Vitis labrusca and Vitis hybrida)[a]

Sr. No	Varieties	Anthocyanin content (mg 100 g^{-1} FW)					
		malvidin-3-glucoside	delphinidin-3-glucoside	peonidin-3-glucoside	cyanidin-3 glucoside	petunidin-3-glucoside	Total anthocyanin
1	Thomson Green (seedless)	42.2 ± 2.1	134.0 ± 1.4	28.0 ± 2.2	40.2 ± 1.2	24.1 ± 1.0	268.5 ± 7.9
2	Vidal Black	34.5 ± 1.3	86.7 ± 2.0	14.9 ± 0.4	32.5 ± 1.1	12.6 ± 0.5	181.2 ± 5.3
3	Italia	48.5 ± 4.1	283.2 ± 3.3	28.0 ± 1.5	38.5 ± 1.4	30.1 ± 1.2	428.3 ± 11.5
4	Niagara	60.1 ± 3.1	88.2 ± 1.6	20.7 ± 1.2	58.4 ± 2.3	22.1 ± 1.6	249.5 ± 9.8
5	Catawba	56.1 ± 1.5	456.8 ± 2.4	52.5 ± 2.8	50.4 ± 1.7	48.6 ± 1.4	716.4 ± 9.8
6	Ontario	71.5 ± 2.4	196.3 ± 2.6	17.4 ± 1.7	72.4 ± 1.8	15.6 ± 1.0	373.2 ± 9.5
7	Hongiseul	87.6 ± 2.4	217.0 ± 2.2	18.2 ± 1.4	89.5 ± 2.7	20.2 ± 1.8	432.5 ± 10.5
8	Delaware	67.5 ± 2.3	218.9 ± 2.7	25.9 ± 1.9	70.2 ± 1.5	20.1 ± 2.4	402.6 ± 10.8
9	Ruby Seedless	72.5 ± 2.2	432.5 ± 3.6	36.0 ± 2.4	75.2 ± 1.2	18.3 ± 1.6	634.5 ± 11
10	Chasselas Rouge	80.1 ± 2.1	102.9 ± 1.8	11.1 ± 3.6	84.1 ± 2.1	15.3 ± 1.7	293.5 ± 11.3
11	Honey Red	63.6 ± 1.4	80.9 ± 3.2	13.6 ± 1.7	65.2 ± 1.5	14.8 ± 1.2	238.1 ± 9
12	Red Globe	48.2 ± 1.8	165.0 ± 1.3	25.3 ± 0.9	50.1 ± 1.1	22.6 ± 1.4	311.2 ± 6.5
13	Koho	59.0± 3.1	364.2 ± 1.9	39.0± 2.6	62.2± 1.4	35.4± 2.1	559.8 ± 11.1
14	Flouxa	78.2± 3.2	186.0 ± 2.5	74.5± 1.8	80.5± 2.1	65.9± 1.8	485.1 ± 9.6
15	Black Pegaru	40.2 ± 1.9	85.9 ± 2.8	15.4 ± 2.4	45.3 ± 1.3	18.5 ± 1.0	205.3 ± 9.4
16	Sherpher	56.3 ± 1.4	223.2 ± 2.8	20.4 ±1.6	58.6 ± 1.4	25.4 ± 2.1	383.9 ± 9.3
17	Concord	37.7 ± 3.1	199.1 ± 1.8	17.7 ± 0.9	41.2 ± 1.6	15.6 ± 1.2	311.3 ± 8.6
18	Campbell Early	32.8 ± 1.4	449.1 ± 2.9	48.2 ± 2.0	35.8 ± 2.2	45.2 ± 2.0	611.1 ± 10.5
19	Alphonse Lavallee	48.6 ± 2.7	277.8 ± 4.7	38.2 ± 1.2	50.6 ± 1.7	35.1 ± 2.1	450.3 ± 12.4
20	Tamnara	38.2 ± 2.6	85.6 ± 2.1	13.7 ± 1.7	42.5 ± 1.1	20.1 ± 1.2	200.1 ± 8.7

[a]Values are expressed as mean ± SD (*n*=3). All values area expressed as cyanidin-3-rutinoside equivalents.

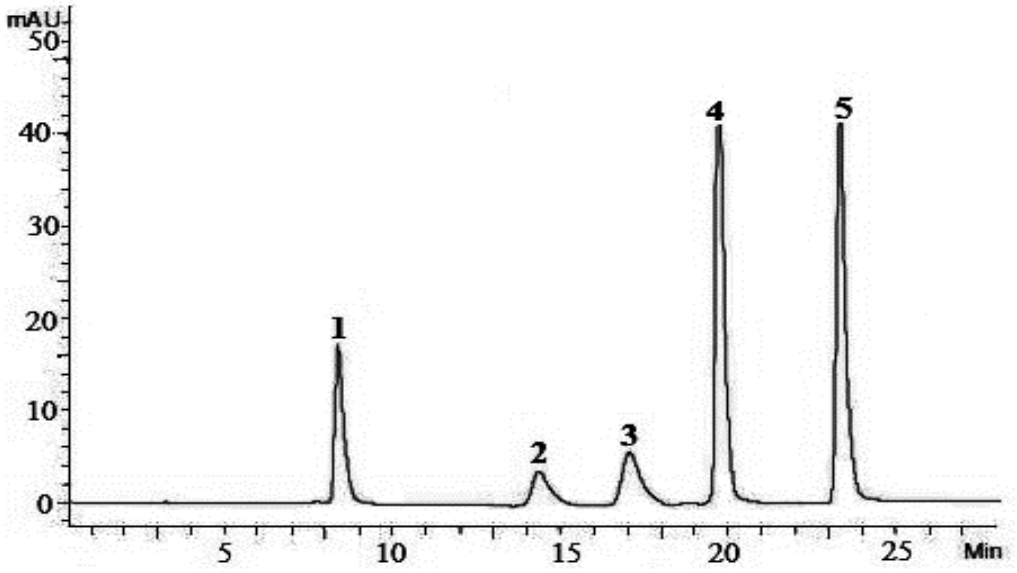

Figure 1. HPLC separation of reference anthocyanins (520 nm). 1: Delphinidin-3-*O*-β-D-glucoside; 2: Cyanidin-3-*O*-glucoside; 3: Petunidin-3-*O*-glucoside; 4: Peonidin-3-*O*-glucoside; 5: Malvidin-3-*O*-glucoside.

Separação de antocianinas de referência por HPLC (520 nm). 1: Delfinidina-3-O-*β-D*-glucósido; *2: Cianidina-3-O-glucósido; 3: Petunidina-3-O-glucósido; 4: Peonidina-3-O-glucósido; 5: Malvidina-3-O-glucósido.*

The phenolics, flavonoids and anthocyanins are the main class of natural compounds with significant antioxidant activities which have been identified and quantified in several fruits, vegetables and berries (Rockenbach *et al.*, 2011; Nile and Park, 2014a). Grapes and wine contains high amounts of phenolics, flavonoids and anthocyanins and acts as antioxidants (Yildirim *et al.*, 2005). Also it was found that the grape extracts consist of high amount of anthocyanins from the skin and procyanidins from the seeds (Shrikhande, 2000). Phenolics, flavonoids and anthocyanins are primary antioxidants which can donate hydrogen or electron, and radical intermediates can be stabilized by these types of compounds (Yilmaz and Toledo, 2006). Ricardo-da-Silva *et al.* (1991) studied and found that the FRAP values of grape extracts were reported to be highly correlated with total anthocyanin content and ABTS radical scavenging activity values. In the present study, we also found that total anthocyanin content were highly correlated with antioxidant activity (FRAP, DPPH and ABTS) of grape extracts (Figure 2). Thaipong *et al.* (2006) also reported a positive correlation coefficient between the total phenolic content and antioxidant activity of methanolic extracts of guava fruit by comparing ABTS, DPPH, FRAP, and ORAC assays. Concerning the study of antioxidant effectiveness, the use of different in vitro models has recently been recommended, due to the differences between the various free radical scavenging assay systems (Ruberto *et al.*, 2007). Thus, the determination of the antioxidant activity of the extracts was carried out using the ABTS and DPPH methods and reducing power through the FRAP method (Table III). Therefore, the ripening stage may be a major factor for anthocyanin levels in grape together with the cultivars. The effects of free radical scavenging by DPPH, FRAP and ABTS radical for all samples are shown in Table III. The antioxidant activities of grape extracts varied from 32.8% ('Campbell Early') to 87.6% ('Hongiseul') by DPPH. The radical scavenging activities of grape extracts varied from 79.1% ('Campbell Early') to 197% ('Hongiseul') by FRAP assay and in case of ABTS assay the scavenging activities of grape extracts varied from 11.1% ('Chasselas Rouge') 74.5% ('Flouxa'). The contents of anthocyanin in grape demonstrated lower correlations with the effects of free radical scavenging by ABTS radical. These results were similar to those of a previous study (Kallithraka *et al.*, 2005). The results presented herein provided valuable data with regard to anthocyanin composition of several technologically important wine grape varieties from Korea. The vinification of grapes with high anthocyanin potential has been regarded as a principal criterion for grapes,

which are enriched with these biologically active phenolic compounds like anthocyanins. In some small fruits like raspberries, sweet potatoes and cranberries the antioxidant capacity has been correlated to a significant degree with anthocyanin content, indicating that anthocyanins may govern the antioxidant capacity of several plant tissues to a certain extent. In grapes, however, fractions containing cyanidin-3 glucoside has efficient activity as inhibiting the LDL oxidation whereas; the highest activity was seen with fractions containing flavonols in grapes (Oki *et al.*, 2002; Wada and Ou, 2002). According to many studies antioxidant activity of fruits, results mainly from phenolics, particularly flavonoids and found a strong correlation among antioxidant capacity, total phenols and anthocyanins (Kalt *et al.*, 1999; Wang and Lin, 2000). On the other hand, some investigations also indicated that anthocyanins may be less significantly correlated with the antioxidant properties (Burns *et al.*, 2000; Arnous *et al.*, 2002). These results presented herein provided valuable data for the total anthocyanin content and antioxidant activity of several commercially important grape varieties from South Korea. Furthermore, the correlation of the antioxidant activity and total anthocyanins was found to be significant (Figure 2) and which was similar and comparative with previous studies.

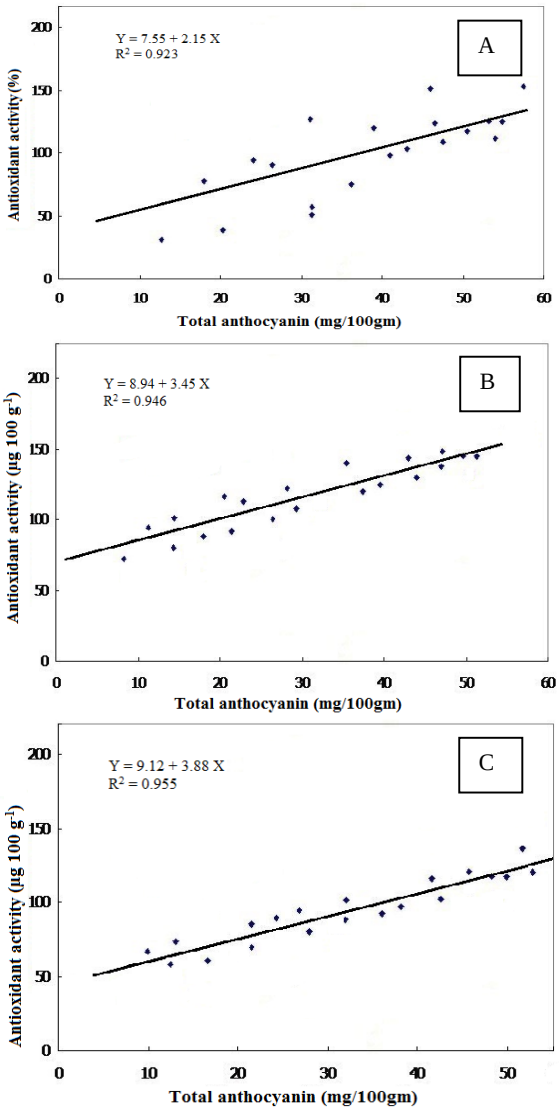

Figure 2. Correlation of total anthocyanin content and antioxidant activity (%) in grapes (A: DPPH assay, B: FRAP assay, C: ABTS assay).

Correlação entre o teor total de antocianinas e a actividade antioxidante (%) das uvas (A: método DPPH; B: método FRAP; C: método ABTS).

Table III

Antioxidant activity of grape varieties

Actividade antioxidante das uvas das diversas variedades

	Grape varieties	Antioxidant activity [a, b]		
		DPPH	FRAP	ABTS
1	Thomson Green (seedless)	42.2 ± 2.2	88.2 ± 1.4	28.0 ± 2.2
2	Vidal Black	34.5 ± 1.3	86.7 ± 2.0	14.9 ± 0.4
3	Italia	48.5 ± 4.1	113.2 ± 3.3	28.0 ± 2.2
4	Niagara	60.1 ± 3.1	134.0 ± 1.6	20.7 ± 1.7
5	Catawba	56.1 ± 1.5	156.8 ± 2.4	52.5 ± 2.8
6	Ontario	71.5 ± 2.4	190.3 ± 2.6	17.4 ± 1.7
7	Hongiseul	87.6 ± 2.4	197.0 ± 2.2	18.2 ± 2.4
8	Delaware	67.5 ± 2.3	148.9 ± 2.7	25.9 ± 1.9
9	Ruby Seedless	72.5 ± 2.2	162.5 ± 3.6	16.0 ± 2.4
10	Chasselas Rouge	80.1 ± 2.1	182.9 ± 1.8	11.1 ± 3.6
11	Honey Red	63.6 ± 1.4	179 ± 3.2	13.6 ± 1.7
12	Red globe	48.2 ± 1.8	115.0 ± 1.8	25.3 ± 0.9
13	Koho	59. 0± 3.1	134.2 ± 1.9	39. 0± 2.6
14	Flouxa	78.12± 3.2	186.0 ± 2.5	74.5± 1.8
15	Black Pegaru	40.2 ± 1.9	85.9 ± 2.8	15.4 ± 2.4
16	Sherpher	56.3 ± 1.4	123.2 ± 2.8	20.4 ±1.6
17	Concord	37.7 ± 3.1	87.2± 3.1	17.7 ± 0.9
18	Campbell Early	32.8 ± 1.4	79.1 ± 2.9	48.2 ± 2.0
19	Alphonse Lavallee	48.6 ± 2.7	177.8 ± 4.7	38.2 ± 1.2
20	Tamnara	38.2 ± 2.6	85.6 ± 6.7	13.7 ± 1.7

[a]DPPH antioxidant value expressed in percent inhibition and [b]FRAP and ABTS expressed in (μg 100 g^{-1}).Values are expressed as means of three determinations ± standard deviation.

CONCLUSIONS

The results obtained in this study showed significant differences among the varieties in relation to the anthocyanin content. The 'Catawba', 'Ruby Seedless' and 'Campbell Early' varieties were richest in total anthocyanins, respectively. Malvidin-3-glucoside, delphinidin-3-glucoside, petunidin-3-glucoside, cyanidin-3 glucoside, and peonidin-3-glucoside was the most abundant anthocyanin compounds identified in grape pomace. The antioxidant activity of extracts obtained from grape pomace showed that of the varieties studied the 'Hongiseul', 'Campbell Early' and 'Flouxa' varieties has the greatest potential as a source of compounds to be applied as natural antioxidants in food. This study showed that the grape varieties rich in anthocyanins might be utilised as functional food and natural remedy against various diseases and disorders related to oxidative stress and free radical effects. Thus, the grape varieties rich in anthocyanins may serve as a new potential source of nutraceuticals and functional food development. From this study we also concludes that the antioxidant assay methods differ from each other in terms of assay principles and reaction conditions, one single method is not enough to show all the antioxidants. Therefore this study compared three most widely used

spectrophotometric methods. A good correlation between the antioxidant activities determined by DPPH, ABTS, and FRAP methods *versus* total anthocyanin content was observed for the grapes.

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