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Highly pigmented vegetables: Anthocyanin compositions and their role in antioxidant activities

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ABSTRACT

Twelve highly pigmented (red or purple) vegetables (carrots, cabbage, cauliflower, potatoes, onions, asparagus and eggplant) were investigated for their total anthocyanin contents (TAC) and compositions of the individual anthocyanins and anthocyanidins by UPLC and LC–DAD–ESI–MS, and their antioxidant activities by DPPH, FRAP (ferric reducing antioxidant power) and ORAC (oxygen radical absorption capacity) assays. While a total of 26 anthocyanins were identified, the main aglycones were only found to be limited to 4 anthocyanidins (cyanidin, petunidin, pelargonidin and delphinidin). The TAC ranged from 0.08 to 2.01 mg Cyanidin-3-glucoside (C3G)/g DW and the total phenolic contents (TPC) was from 1.30 to 2.19 mg GAE/g DW suggesting that anthocyanins were the main phenolics in certain vegetables but not others. DPPH radical scavenging activities were 54.91–81.94%, FRAP values 10.00–70.07 μmol AAE/g DW and ORAC values were 3.74–189.32 μmol TE/g DW. The two cruciferous vegetables (purple cauliflower and cabbage) showed the highest TPC, TAC, DPPH and FRAP values, and the onions the lowest. The antioxidant activities in the DPPH and FRAP assays correlated well with the TPC and TAC, but ORAC did not. Results of this study are comparable and provide a rapid and effective method for the identification and quantification of all major anthocyanidins and their glycosides (anthocyanins), and how they might contribute to the antioxidant activity, therefore important information in developing anthocyanin-rich nutraceuticals and functional foods.

1. Introduction

In recent years, increasing attention has been paid to plants and foods rich in natural antioxidants, particularly fruits and vegetables, which have been associated with reduced risks of a number of chronic diseases in human (Hassan & Abdel-Aziz, 2010; Olsson, Gustavsson, Andersson, Nilsson, & Duan, 2004; Steinberg, 1995; Yi, Akoh, Fischer, & Krewer, 2006). The major antioxidants of vegetables are vitamins C and E, carotenoids and phenolic compounds, especially the subgroup of flavonoids. Pigmented flavonoids, mainly the anthocyanins are considered the most important subcategory of flavonoids in plant foods due to their strong antioxidant activity and other beneficial physicochemical and biological properties (De Pascual-Teresa & Sanchez-Ballesta, 2008; Jackman, Yada, Tung, & Speers, 1987).

Anthocyanins are water-soluble compounds and could impart color in plants (leaves, stems, roots, flowers and fruits) to appear red, purple or blue according to the pH and their structural features (Fossen, Andersen, ØVstedal, Pedersen, & Raknes, 1996). pH and the glycosylation patterns can affect the color of the anthocyanins. Anthocyanins play important roles in plant–animal interactions and act as antioxidants, phytoalexins or plant's chemical defense mechanism infections (De Pascual-Teresa & Sanchez-Ballesta, 2008; Petersson, Puerta, Bergquist, & Turner, 2008). High intake of foods rich in anthocyanins has been shown to have potential health beneficial effects on various disorders like cancer, aging, neurological diseases, inflammation, diabetes as well as bacterial infections (De Pascual-Teresa & Sanchez-Ballesta, 2008; Hassan & Abdel-Aziz, 2010; Hudson, Dinh, Kokubun, Simmonds, & Gescher, 2000; Olsson et al., 2004; Yi et al., 2006).

Anthocyanins are glycosides of anthocyanidins (Fig. 1), and they are the more likely form of pigments found in plants. While hundreds of anthocyanins have been reported due to the complex glycosylation patterns, only less than a dozen aglycones (anthocyanidins) have been identified, and the number of anthocyanidins found in commonly consumed plant foods is even more limited to a few aglycones containing the same flavylium ion backbone (Fig. 1). In fact, 90% of anthocyanins are based on cyanidin, delphinidin and pelargonidin

Abbreviations: AAE, Ascorbic acid equivalent; FRAP, Ferric reducing antioxidant power; C3G, Cyanidin-3-glucoside; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, Gallic acid equivalent; HPV, Highly pigmented vegetables; LC–MS, Liquid chromatography–mass spectrometry; ORAC, Oxygen radical absorption capacity; TAC, Total anthocyanin contents; TAI, Total anthocyanin index; TE, Trolox equivalent; TPC, Total phenolic contents; UPLC, Ultra Performance Liquid Chromatography.

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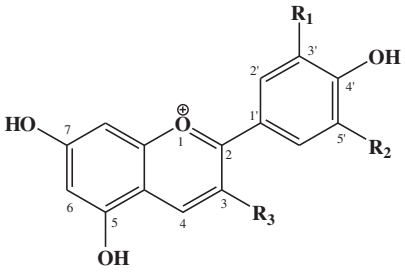

	R_1	R_2	R_3
Delphinidin	-OH	-OH	-OH
Luteolinidin	-OH	-H	-H
Cyanidin	-OH	-H	-OH
Petunidin	-OH	$-OCH_3$	-OH
Pelargonidin	-H	-H	-OH
Peonidin	$-OCH_3$	-H	-OH
Malvidin	$-OCH_3$	$-OCH_3$	-OH

Fig. 1. Structures of major anthocyandins found in highly pigmented vegetables.

and their methylated derivatives. Like other flavonoids, the aglycones and glycosides may have different biological activities, thus knowing the anthocyanin composition of plant foods may help further understanding the stability, bioavailability and actual health benefits of these phytochemicals (De Pascual-Teresa & Sanchez-Ballesta, 2008).

Highly pigmented fruits, particularly small berries such as blueberry, blackberry, cherry, raspberry and strawberry fruits, have been heavily studied, and anthocyanins have been shown to contribute significantly to the antioxidant activity *in vitro* (Juroszek, Lumpkin, Yang, Ledesma, & Ma, 2009; Yang, Zheng, & Cao, 2008), and to play important roles in oxidative stress related chronic diseases *in vivo* (Hassan & Abdel-Aziz, 2010; Morimitsu et al., 2002). Specifically, Hassan and Abdel-Aziz (2010) reported that black berry juice rich in anthocyanins helped preventing fluoride-induced oxidative stress and bolstering the cellular antioxidant defense system as shown by increased levels of thiobarbituric acid reactive substances in the examined organs of rats. Certain dark varieties of grains, such as black rice, blue wheat and purple barley, have also been shown to possess higher potential in maintaining health as compared to their respective common varieties (Hudson et al., 2000; Laokuldilok, Shoemaker, Jongkaewwattana, & Tulyathan, 2010; Siebenhandl et al., 2007). On the other hand, the chemistry, biochemistry and the potential health benefits of highly pigmented vegetables (HPV), particularly those rich in anthocyanins, have only been under close examination in very recent years (Gonzali, Mazzucato, & Perata, 2009; Hwang, Choi, Choi, Chung, & Jeong, 2011; Jackman et al., 1987; Kolodziejczyk, Saluk-Juszczak, Posmyk, Janas, & Wachowicz, 2011). These vegetables generally possess stronger antioxidant activities than their respective non-pigmented cultivars, and like in fruits and the dark grains, anthocyanins have been found to be the major contributors.

Anthocyanin-rich vegetables may have several advantages over small fruits and grains. For example, the tuber and root types of vegetables such as potatoes, carrots and onions, are less expensive and can be stored longer, and are consumed in large quantity (potato is the fourth largest staple food in the world). Breeding and production of HPV for enhanced health benefits is a new research direction, therefore as part of our current effort in finding antioxidant-rich foods, several HPV have been selected and examined in this study. These cultivars were found to contain high antioxidant activities due to the higher total phenolic and anthocyanin contents in our recent studies (data not shown). The main objective of this study is therefore to identify and evaluate the total and individual anthocyanin compositions and their contribution to the antioxidant activities of highly pigmented (red and purple) cultivars of several commonly consumed vegetables grown or available in Ontario.

2. Materials and methods

2.1. Plant materials

Purple carrots (*Rain* and *Haze*), red cabbage (*Gario*), purple cauliflower (*Graffitti*), purple potatoes (*Majesty* and *Mackintosh*), red potatoes (*Y38* and *Thumb*) were grown at the Elora Research Station, University of Guelph (Elora, Ontario, Canada −43°41′N, 80°26′W) on a Conestoga silt loam soil in 2009. Red onions (*Pier-c* and *Pearl*) and purple asparagus (*Albenga*) were Ontario products, and eggplant (*Black Beauty*) with unknown source of origin, all purchased from a local grocery store (July 2011). Four sub-samples each having at least 5 whole vegetables were washed with tap water, cut into pieces and ground with a commercial blender (7011, Waving® Laboratory Science, USA) in order to obtain a homogeneous paste, except for eggplant samples which had 2 fruits. A known amount of about 30 g of this paste was freeze-dried (Bulk tray dryer, Labconco, USA) and ground into fine powder. These materials were stored in polyethylene tubes at −80 °C prior to analysis.

2.2. Chemicals and reagents

Delphinidin, luteolinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin standards were purchased from Indofine (Belle Mead, NJ, USA). *L*-Ascorbic acid, fluorescein, gallic acid, 1,3,5-tri(2-pyridyl)-2,4,6-triazine (TPTZ), 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AAPH), Folin-Ciocalteu's phenol reagent, Trolox and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, MO, USA). Sodium acetate, ferric chloride hexahydrate, sodium phosphate monobasic, sodium phosphate dibasic and HPLC grade solvents, including methanol, glacial acetic acid, formic acid and hydrochloric acid were purchased from Caledon Laboratories (Georgetown, ON, Canada). All chemical reagents used were of analytical grade.

2.3. Colorimetric study

Instrumental measurements of color were conducted at room temperature in a Minolta Chromometer (Chroma Meter CR-200; Minolta Camera Co., Ltd., Osaka, Japan) by placing the vegetable powder in a 15 mm thick and transparent plastic cell without cover and by using a black plate as the background to standardize the measurements (Heredia, Peinado, Barrera, & Grau, 2009). The chromometer consisted of an 8 mm diameter measuring area and diffuse illumination/viewing was utilized. The tristimulus values of CIE L^*, a^*, b^* readings were calibrated against a standard white plate. CIE 1976 uniform color space was taken into account for the colorimetric analysis. Within the CIELAB uniform space a psychometric index of lightness, L^* (ranging from 0, black, to 100, white), and two color coordinates, a^* (which takes positive values for the direction of redness and negative values for the direction of the complement green) and b^* (positive for yellowness and negative for blueness), are defined (Arslan & Musa Özcan, 2010). The values a^* and b^* were used to calculate the hue angle ($H = \arctan(b^*/a^*)$) and metric chroma value ($C = (a^{*2} + b^{*2})^{1/2}$). The data of each measurement are the average of triplicate measures on equidistant points of the sample.

2.4. Sample extraction

Anthocyanins were extracted from the dried powder of the HPV by acidified aqueous methanol. In brief, the freeze-dried vegetable powder (2 g) was accurately weighed and transferred into a 50 mL tube containing 30 mL of 0.1% HCl (v/v) in 80% methanol (Longo,

Scardino, & Vasapollo, 2007; Tsao, 2010). The extraction was carried out on a rotary shaker (Scientific Industries Inc., USA) overnight (ca. 15 h; 400 rpm) at room temperature (Benakmoum, Abbeddou, Ammouche, Kefalas, & Gerasopoulos, 2008; Toor, Savage, & Lister, 2006). The mixture was centrifuged at 4000 rpm for 5 min (Eppendorf centrifuge 5810R, Brinkman Instruments Inc., Westbury, NY). The extraction was repeated three times and the supernatants were combined, topped up to 90 mL, filtered through a 0.2-μm PTFE membrane filter (VWR International, ON, Canada), and used as crude extract for further purification of anthocyanins and analyses of TPC, TAC, TAI (total anthocyanin index), anthocyanin composition and antioxidant activities. Acidified extraction solvent (0.1% HCl in 80% methanol) was necessary to prevent degradation of the anthocyanins (Longo & Vasapollo, 2006). Samples were extracted in triplicate.

2.5. Hydrolysis

Twenty milliliter crude extract and 5 mL of 6 M HCl were mixed in a 40 mL tube tightly sealed with a screw cap, flushed with nitrogen, and then incubated in a shaking water bath at 90 °C for 2 h to hydrolyze the anthocyanins (You et al., 2011). The samples were allowed to cool down, and then centrifuged at 2000 rpm for 5 min. The supernatant was filtered through a 0.2-μm PTFE membrane filter and subjected to UPLC analysis.

2.6. UPLC analyses of anthocyanins

Seven anthocyanidin standards were separated by UPLC in 9 min using a newly developed method. The UPLC system Accela (Thermal Technologies Co., Ltd., USA) was equipped with a diode array detector (DAD) and an Ezchrom workstation for data processing. Separation was done in a Phenomenex® Kinetex XB-C_{18} 1.7 μm column (100 × 2.1 mm, Phenomenex, Torrance, CA, USA) with a Phenomenex® C_{18} guard column (4 × 3 mm). The column was thermostatically controlled at 30 °C and the flow rate was set to 450 μL/min. The mobile phase consisted of two solvents: methanol–water-formic acid (A, 95:2:3, v/v/v) and water–methanol-formic acid (B, 95:2:3, v/v/v). The solvent gradient was as follows: 0–3 min, 10–30% A; 3–6 min, 30–40% A; 6–8 min, 50–100% A, and held at 100% A for 1 additional min. There was a 2-min post-run which brings back to the starting conditions. The UV–visible absorbance of the peaks was collected between 200 and 620 nm using DAD and monitored at 520 nm. Stock solutions of the standards were prepared separately by dissolving 10 mg of each compound in 5 mL DMSO and then topped up to 100 mL in a volumetric flask with methanol (final concentration 100 μg/mL). All 7 anthocyanidins in the HPV samples were quantified with external standards by using respective standard curves generated from serial dilutions of 10, 20, 40, 60, 80 and 100 μg/mL.

2.7. LC–MS analysis

LC–MS experiments were carried out using a Finnigan LCQ DECA ion trap mass spectrometer (ThermoFnnigan, San Jose, CA, USA) equipped with electrospray ionization (ESI) source. Separation was done using the same binary solvent system as in the UPLC method, but a different column (Luna 3 μm PFP (2) (4.6 mm × 100 mm; Phenomenex, Torrance, CA, USA)) and gradient program: 0–26 min, 10–100% B; 26–28.5 min, 100–10% B, then the gradient was held at 10% A for an additional 5 min. The flow rate was set at 1.0 mL/min. The UV–visible absorbance of the peaks was collected between 190 and 800 nm. Positive mode was selected for data collection. Before sample analysis, the instrument was tuned by using cyanidin standard to reach its optimum performance. As a result, the shear gas and auxiliary flow rates were set at 96 and 3 (arbitrary unit), respectively. The capillary voltage was 32.5 kV and its temperature was controlled at 350 °C. The entrance lens voltage was fixed at −58.0 V and the multipole RF amplitude was at 770 V. The ESI needle voltage was 5 kV. The tube lens offset was 55.0 V, the multipole lens 1 offset was −4.40 V and the multipole lens 2 offset was −8.00 V. The electron multiplier voltage was set at −1030 V for ion detection.

2.8. Quantification of total phenolics and anthocyanins

The TPC of the extracts was estimated using a method by Wang, Meckling, Marcone, Kakuda, and Tsao (2011). Briefly, 25 μL gallic acid standard or vegetable extract was mixed with 125 μL Folin–Ciocalteu reagent in 96-well microplates and allowed to react for 10 min at room temperature. Then 125 μL saturated sodium carbonate (Na_2CO_3) solution was added and allowed to stand for 30 min at room temperature before the absorbance of the reaction mixture was read at 765 nm using a visible–UV microplate kinetic reader (EL 340, Bio-Tek Instruments Inc., Winooski, VT, USA) (Wang et al., 2011). The results were expressed as milligram gallic acid equivalent/g dry weight (mg GAE/g DW). All samples were tested in triplicate.

The TAC was estimated by a modified pH differential method (Cheng & Breen, 1991). Extract of a vegetable sample (10 μL) was mixed separately with 272 μL of buffer at pH 1.0 (0.1 M HCl/4.9 mM KCl) and another at pH 4.5 (24.8 mM Sodium Acetate NaAC). The buffer was adjusted to 1.0 or 4.5 by hydrochloric acid if necessary. Absorbance was measured in a UV–visible microplate kinetics reader (EL 340, Bio-Tek Instruments, Inc., Winooski, VT, USA) at 510 nm and at 700 nm in buffers of pH 1.0 and pH 4.5, respectively. The total absorbance (A) was calculated as follows:

$$A = (A_{510} - A_{700})pH_{1.0} - (A_{510} - A_{700})pH_{4.5}.$$

The TAC was derived using cyanidin-3-glucoside whose molar extinction coefficient was 26900 $L \cdot cm^{-1} \cdot mol^{-1}$ and molecular weight was 449.2 g/mol. Results were expressed as milligrams of cyanidin-3-glucoside equivalent per gram of dry weight sample (mg C3G/g DW).

2.9. Antioxidant assays

2.9.1. DPPH assay

The antiradical activity of the extracts was determined spectrophotometrically in a UV–vis plate reader (EL 340, Bio-Tek Instruments Inc., Winooski, VT, USA) based on a previously described method (Wu et al., 2011). Briefly, 100 μL of a methanolic solution of DPPH (0.065 mM) was mixed with 20 μL of vegetable extract or a standard solution in a 96 well plate and let stand for 30 min at room temperature before the absorbance was recorded at 517 nm using the aforementioned microplate kinetic reader. The reaction was conducted at room temperature for 30 min at which time the absorbance was stabilized. The radical scavenging activity of the extracts was calculated as follows:

$$\text{Percent scavenging } (\%) = \frac{A_0 - (A_1 - A_s)}{A_0} \times 100$$

where A_0 is the absorbance of DPPH alone, A_1 is the absorbance of DPPH and extract and A_S is the absorbance of the extract only. All samples were tested in triplicate.

2.9.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined according to the method of Wu et al. (2011). The FRAP assay measures the ability of the antioxidants in the vegetable extracts to reduce ferric-tripyridyl-triazine (Fe^{3+}-TPTZ) complex to the blue colored ferrous form (Fe^{2+}) which absorbs light at 593 nm. Briefly, a standard or sample extract (10 μL) was mixed with 300 μL of ferric-TPTZ reagent (prepared by mixing

300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ at a ratio of 10:1:1 (v/v/v)) and added to the wells. The plate was incubated at 37 °C for the duration of the reaction. The absorbance readings were taken at 593 nm at 30 min using the aforementioned microplate kinetic reader. Six concentrations of 25, 50, 100, 200, 400 and 800 μmol/L were used to prepare the standard curve of *L*-ascorbic acid. The antioxidant activities are expresses as micromole ascorbic acid equivalent (AAE) per gram dry weight tomato (μmol AAE/g DW). Stronger absorption therefore indicates higher reducing power of the phytochemical, thus, higher antioxidant activity.

2.9.3. Oxygen radical absorption capacity (ORAC) assay

The assay for the oxygen radical scavenging capacity was conducted according to Wang et al. (2006). AAPH, a water-soluble azo compound, was used as a peroxyl radical generator. Trolox, a water-soluble tocopherol analog, was used as standard and fluorescein as fluorescent probe. Briefly, 25 μL of blank, Trolox standard or a sample extract (in triplicate) were mixed with 200 μL fluorescein solution (0.0868 nM) and incubated for 30 min at 37 °C before injection of 25 μL 2,20-azobis-(2-methylpropionamidine) dihydrochloride (AAPH, 153 mM). The fluorescence was measured every minute for about 120 min until it reached zero (excitation wavelength 485 nm, emission wavelength 528 nm) in a Bio-Tek Fluorescence Spectrophotometer equipped with an automatic thermostatic holder (PL_X 800, Bio-Tek Instruments, Inc., Winooski, VT, USA). A calibration curve was constructed daily by plotting the calculated differences of area under the fluorescein decay curve between the blank and the sample for a series of standards of Trolox solutions (6.25, 12.5, 25, 50 and 100 μmol/L). The results were expressed as micromole Trolox equivalent (TE) per gram dry weight vegetable sample (μmol TE/g DW) (André et al., 2009).

2.10. Statistical analysis

Results were expressed as mean ± SD of three independent extractions. One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered significant at $p<0.05$. All statistical analyses were performed with Statistix for Windows version 9.0 (Analytical Software, Tallahassee, FL, USA).

3. Results and discussion

3.1. Colorimetric study

To systematically study the color quality of the anthocyanin-rich vegetables, the CIE $L^*a^*b^*$ system (International Commission on Illumination, Vienna) which has been favored by the USA food industry for measuring color of food products, was used. The a^*/b^* values of all pigmented vegetables ranged from −3.42 to 0.64, and the saturation *C* was from 4.45 to 24.27 (Table 1). Different vegetables and the same vegetable with different colors might have a similar distinctive *Hue* angle as found in purple carrots, red onions, and purple and red potatoes (Table 1). In purple asparagus, due possibly to the greater green element (*e.g.* chlorophylls), although it appeared to be purple, the powder actually showed green rather than red in this study (a^* showed negative value, others were positive). The color activity concept and the CIE $L^*a^*b^*$ color system are useful tools for studying key colorants in complex mixtures and help to understand the contribution of individual pigments to the overall color of vegetables, thus this information is important for the development as functional foods with HPV.

3.2. UPLC determination of anthocyanidins and method validation

A UPLC method was successfully established for 7 anthocyanidins in 9 min (Fig. 3), which is shorter than a conventional HPLC method. All anthocyanins in the crude extracts were hydrolyzed to their respective anthocyanidins (aglycones) before being separated and identified by matching the retention times, UV–visible spectral and mass spectrometric data with those of the standards, *i.e.* delphinidin, luteolinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin.

The above method was validated vigorously for sensitivity, linearity, precision and accuracy in the analysis of the aforementioned 7 anthocyanidins. Correlation between the concentration and the peak area (520 nm) of all anthocyanidins was highly linear ($R^2>0.99$) between 0.20 and 100 μg/mL. The LOD (limit of detection) values were less than 0.20 μg/mL; the LOQ (limit of quantification) values were less than 0.65 μg/mL; the precision was evaluated by intra- and inter-day assays, and the relative standard deviation (RSD) values were lower than 1.48% and 4.26%, respectively. The recovery studies for all quantified compounds were 93.4–101.2%. These data validates the UPLC method as suitable for analysis of all anthocyanidins found in the HPV of this study.

3.3. Identification of anthocyanins

Anthocyanins exist primarily as glycosidic conjugates of anthocyanidins in vegetables. While the most prevalent glycosylation in anthocyanins is with glucose; other sugar units including rhamnose, galactose, xylose and arabinose have also been found to be present in anthocyanins. In addition, the glycosides are frequently acylated by aromatic and aliphatic acids such as *p*-coumaric, caffeic, ferulic, gallic, acetic, malonic and malic acids (Petersson et al., 2008). Acylated anthocyanins are chemically more stable, thus considered to be

Table 1
Colorimetric parameters of highly pigmented vegetables[a].

Cultivar	L^*	a^*	b^*	a^*/b^*	C^b	*Hue* (°)[c]
Purple carrot *Rain*	43.22 ± 0.22	20.15 ± 0.13	−9.67 ± 0.03	−2.08 ± 0.01	22.35 ± 0.13	334.37 ± 0.10
Purple carrot *Haze*	46.47 ± 0.08	22.30 ± 0.06	−9.04 ± 0.03	−2.47 ± 0.08	24.07 ± 0.06	337.93 ± 0.08
Red cabbage *Gario*	47.34 ± 0.35	18.98 ± 0.10	−16.23 ± 0.03	−1.17 ± 0.00	24.97 ± 0.10	319.48 ± 0.10
Purple cauliflower *Graffitti*	64.63 ± 0.02	12.52 ± 0.04	−9.22 ± 0.02	−1.36 ± 0.00	15.55 ± 0.04	323.61 ± 0.05
Purple potato *Majesty*	51.94 ± 0.05	13.63 ± 0.07	−12.32 ± 0.03	−1.11 ± 0.00	18.37 ± 0.07	317.90 ± 0.07
Purple potato *Mackintosh*	58.47 ± 0.03	14.76 ± 0.02	−13.76 ± 0.01	−1.07 ± 0.00	20.17 ± 0.01	317.01 ± 0.02
Red potato *Y38*	65.40 ± 0.01	22.82 ± 0.04	−6.68 ± 0.01	−3.42 ± 0.00	23.78 ± 0.04	343.68 ± 0.01
Red potato *Thumb*	71.63 ± 0.03	17.99 ± 0.05	−5.57 ± 0.01	−3.23 ± 0.01	18.83 ± 0.05	342.81 ± 0.06
Red onion *Pier-c*	84.24 ± 0.03	4.18 ± 0.02	−1.53 ± 0.01	−2.74 ± 0.00	4.45 ± 0.02	339.92 ± 0.02
Red onion *Pearl*	71.02 ± 0.05	9.51 ± 0.03	−3.35 ± 0.01	−2.84 ± 0.01	10.08 ± 0.03	340.58 ± 0.06
Purple asparagus *Albenga*	51.21 ± 0.10	−5.85 ± 0.04	20.11 ± 0.17	−0.29 ± 0.00	20.94 ± 0.18	286.23 ± 0.06
Eggplant *Black Beauty*	49.33 ± 0.21	13.13 ± 0.29	20.39 ± 0.33	0.64 ± 0.03	24.27 ± 0.12	57.21 ± 1.01

[a] Data are expressed as the mean ± SD, $n=3$.
[b] C = chroma value.
[c] Hue (°) = hue angle expressed in degree.

better candidates than non-acylated anthocyanins as food colorants (Tsao, 2010), and they are bioavailable to animals and humans in intact glycoside forms (Charron, Clevidence, Britz, & Novotny, 2007). However, the complex glycosylation patterns make the identification of individual anthocyanins highly difficult even when aided with LC–MS. As Fig. 2 shows, depending on the type of vegetables, the anthocyanin profiles can be completely different. Peaks found in different vegetables that appeared to have very close retention time such as peaks 3, 18, 26 and 11, 16, 23, respectively (Fig. 2 and Table 2) were found to be totally different glycosides of different aglycones. Also, standard reference materials are not commercially available for most of the anthocyanins. For these reasons, anthocyanins are often analyzed by studying their hydrolysis products, the aglycones (anthocyanidins).

In the present study, identification of the anthocyanidins in the hydrolysate of the vegetable extracts was relatively straightforward. The retention times and UV/Vis spectra of all peaks were matched with those of the respective standards, and similarly the molecular ion and the fragmentation pattern of the mass spectrum of the peaks were confirmed by LC–ESI–MS. As a result, peaks 1–7 in Fig. 3, with molecular ions at *m/z* 303 $[M]^+$, 271 $[M]^+$, 287 $[M]^+$, 317 $[M]^+$, 301 $[M]^+$, 271 $[M]^+$ and 331 $[M]^+$, were identified as delphinidin, luteolinidin, cyanidin, petunidin, peonidin, pelargonidin and malvidin, respectively (Fig. 3). Compared to the anthocyanin profiles of the crude extract (Fig. 2), the anthocyanidin profiles of the respective samples were much simpler; in fact, all vegetables of this study showed only one predominant anthocyanidin (Fig. 3). More specifically, it was found that cyanidin was the main aglycone in carrots, cabbage, cauliflower, onions and purple asparagus, whereas petunidin, pelargonidin and delphinidin were the main aglycones in purple potatoes, red potatoes and eggplant, respectively (Table 3). This suggests that different plants may synthesize anthocyanins based on the same anthocyanidin, but more interestingly, plants of the same family, or even the same species but different cultivar may produce different anthocyanidins as were found in the potatoes and eggplant which belong to the same Solanaceae family (Fig. 3). A clear understanding of the aglycone profile of a particular HPV sample can help significantly the identification of the glycosides. As mentioned above, even though carrots, cabbage, cauliflower, onions and purple asparagus had the same aglycone, the glycoside profiles, particularly those of the different vegetables were completely different (Fig. 2). The anthocyanin compositions of the vegetables as monitored at 520 nm revealed different major peaks in carrots (peaks 1–4), red cabbage (peaks 5, 6, 8 and 9), cauliflower (peaks 5, 7, 8 and 10), purple potatoes (peaks 11–13), red potatoes (peaks 14–17), red onions (peaks 18–21), purple asparagus (peaks 22–24) and eggplant (peaks 25 and 26) (Fig. 2). These 26 major anthocyanins were tentatively identified by comparing the aglycone information after hydrolysis and the LC–MS data obtained in this study with those reported in the literature, and the result is summarized in Table 2.

The two purple carrot cultivars had nearly the same anthocyanin profile with four distinctive peaks (Fig. 2, Peaks 1–4) which showed $[M]^+$ ions at *m/z* 743, 949, 919 and 889, respectively (Table 2). The MS data showed that in addition to the molecular ion, all these 4 peaks had only the aglycone fragment ion at *m/z* 287, suggesting that the glycosidic units of these anthocyanins are attached to the aglycone at one single position. This observation was in agreement with the identification of the 4 cyanidin-3-glycosides by others for the purple carrot *Haze* (Montilla, Arzaba, Hillebrand, & Winterhalter, 2011; Schwarz, Wray, & Winterhalter, 2004). The MS data combined with the UV–vis spectra, the hydrolysis products (overwhelmingly cyanidin) and the elusion order of the peaks in LC, has led to the tentative identification of peaks 1–4 in Fig. 2 to be cyanidin-3-(xylosyl) glucoside-galactoside, cyanidin-3-(xylosyl)(sinapoyl)glucoside-galactoside, cyanidin-3-(xylosyl)(feruloyl)glucoside-galactoside and cyanidin-3-(xylosyl)(coumaroyl)glucoside-galactoside, respectively.

Anthocyanins in the red cabbage *Gario* would be mainly glycosides of cyanidin as well according to Fig. 3. Peak 5 gave major fragment

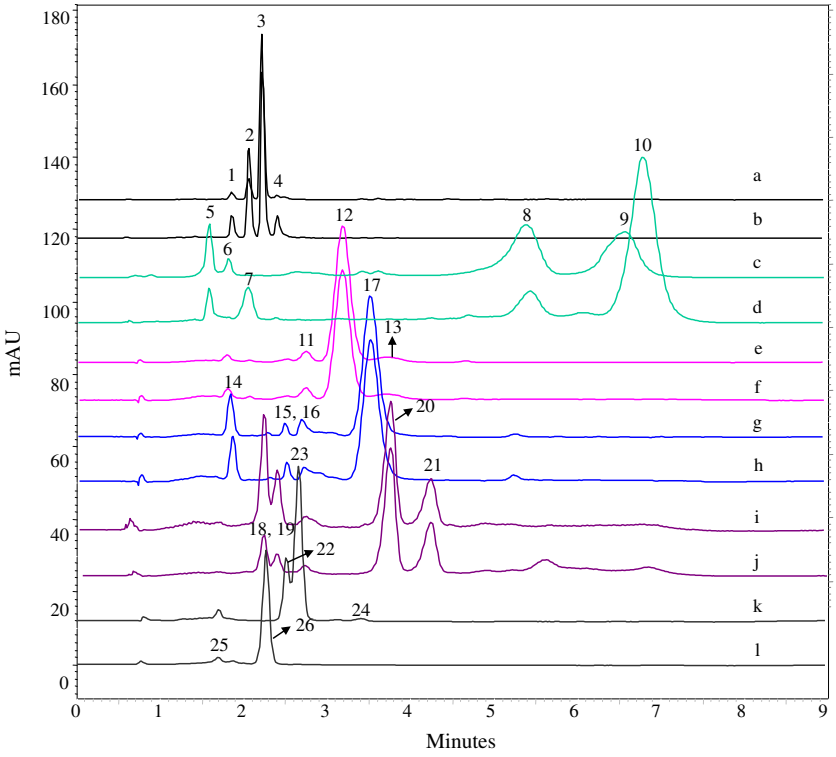

Fig. 2. UPLC chromatograms of anthocyanins in highly pigmented vegetables (before hydrolysis) at 520 nm.a = Purple carrot *Rain*; b = purple carrot *Haze*; c = red cabbage *Gario*; d = purple cauliflower *Graffitti*; e = purple potato *Majesty*; f = Purple potato *Mackintosh*; g = red potato *Y38*; h = red potato *Thumb*; i = red onion *Pier-c*; j = red onion *Pearl*; k = purple asparagus *Albenga*; l = eggplant *Black Beauty*.

Table 2
MS data of the major anthocyanin peaks in the crude extract of highly pigmented vegetables.

Culture	Peak[a]	Retention time (min)	Proposed anthocyanins[b]	Molecular ion $[M^+]$ (m/z)	Fragment ions[c] (m/z)
Purple carrot *Rain*	1	1.800	Cy 3-(xyl)-glc-gala	743	**287** (Schwarz et al., 2004; Montilla et al., 2011)
Purple carrot *Haze*	2	1.968	Cy 3-(xyl)(sin)-glc-gala	949	**287** (Schwarz et al., 2004; Montilla et al., 2011)
	3	2.083	Cy 3-(xyl)(fer)-glc-gala	919	**287** (Schwarz et al., 2004; Montilla et al., 2011)
	4	2.250	Cy 3-(xyl)(coum)-glc-gala	889	**287** (Schwarz et al., 2004; Montilla et al., 2011)
Red cabbage *Gario*	5	1.550	Cy 3-glc-glc-5-glc	773	**287**, 449, 611 (McDougall et al., 2007)
	6	1.750	Cy 3-(sin)-glc-glc-5-glc	979	**287**, 449, 817 (McDougall et al., 2007)
	8-1	5.385	Cy 3-(*p*-coum)-glc-glc-5-glc	919	**287**, 449, 757 (McDougall et al., 2007)
	8-2	5.385	Cy 3-(fer)-glc-glc-5-glc	949	**287**, 449, 787 (McDougall et al., 2007)
	8-3	5.385	Isomer of Cy 3-(sin)-glc-glc-5-glc	979	**287**, 449, 817 (McDougall et al., 2007)
	9-1	6.533	Cy 3-(fer)(fer)-glc-glc-5-glc	1125	**287**, 449, 963 (McDougall et al., 2007)
	9-2	6.533	Cy 3-(sin)(fer)-glc-glc-5-glc	1155	**287**, 449, 993 (McDougall et al., 2007)
Purple cauliflower *Graffitti*	5	1.550	Cy 3-glc-glc-5-glc	773	**287**, 449, 611 (Scalzo et al., 2008)
	7	1.967	Isomer of Cy 3-(sin)-glc-glc-5-glc	979	**287**, 449, 817 (Scalzo et al., 2008)
	8-1	5.385	Cy 3-(*p*-coum)-glc-glc-5-glc	919	**287**, 449, 757 (Scalzo et al., 2008)
	8-2	5.385	Cy 3-(fer)-glc-glc-5-glc	949	**287**, 449, 787 (Scalzo et al., 2008)
	10	6.953	Cy 3-(sin)(sin) -glc-glc-5-glc	1185	**287**, 449, 1023 (Scalzo et al., 2008)
Purple potato *Majesty*	11	2.883	Pet 3-(caf)-rut-5-glc	949	**317**, 479, 787 (Ieri et al., 2011; Mulinacci et al., 2008)
Purple potato *Mackintosh*	12	3.367	Pet 3-(*p*-coum)-rut-5-glc	933	**317**, 479, 771, 787 (Ieri et al., 2011; Mulinacci et al., 2008)
	13	3.817	Mal 3-(*p*-coum)-rut-5-glc	947	**331**, 493, 785, 801 (Ieri et al., 2011; Mulinacci et al., 2008)
Red potato *Y38*	14	1.833	Pel 3-rut-5-glc	741	**271,** 433, 579 (Ieri et al., 2011; Mulinacci et al., 2008)
Red potato *Thumb*	15	2.517	Pel 3-rut	579	**271** (Ieri et al., 2011; Mulinacci et al., 2008)
	16	2.700	Pel 3-(*p*-coum)-rut-5-glc	887	**271,** 443, 725, 741 (Ieri et al., 2011; Mulinacci et al., 2008)
	17-1	3.567	Peo 3-(*p*-coum)-rut-5-glc	917	**301,** 463, 755 (Ieri et al., 2011; Mulinacci et al., 2008)
	17-2	3.567	Pel 3-(fer)-rut-5-glc	917	**271,** 433, 755 (Ieri et al., 2011; Mulinacci et al., 2008)
Red onion *Pier-c*	18	2.183	Cy 3-glc	449	**287** (Fossen et al., 1996; Petersson et al., 2008)
Red onion *Pearl*	19	2.378	Cy 3-(malo)-glc	535	**287** (Fossen et al., 1996; Petersson et al., 2008)
	20	3.733	Isomer of Cy 3-(malo)-glc	535	**287** (Fossen et al., 1996; Petersson et al., 2008)
	21	4.217	Cy 3-(malo)-glc-glc	697	**287,** 449 (Fossen et al., 1996; Petersson et al., 2008)
Purple asparagus *Albenga*	22	2.516	Cy 3-glc-rha-5-glc	757	**287,** 449, 595[39]
	23	2.667	Cy 3-rut	595	**287** (Sakaguchi et al., 2008)
	24	3.493	Peo 3-rut	609	**301** (Sakaguchi et al., 2008)
Eggplant *Black Beauty*	25	1.700	Delp 3-glc	465	**303** (Azuma et al., 2008)
	26	2.283	Delp 3-rut	611	**303** (Azuma et al., 2008)

[a] Peak's numbers refers to Fig. 2.
[b] Abbreviations used: Cy, cyaniding; Pel, pelargonidin; Peo, peonidin; Pet, petunidin; Mal, malvidin; Delp, delphinidin; caf, caffeoyl; rut, rutinoside; *p*-coum, para-coumaroyl; xyl, xylosyl; fer, feruloyl; sin, sinapoyl; malo, malonyl; glc, glucoside; gala, galactoside; rha, rhamnopyranosyl.
[c] Aglycone ions are in bold.

ions *m/z* at 773 $[M]^+$, 611 $[M\text{-}Glc]^+$, 449 $[M\text{-}2Glc]^+$ and 287 $[M\text{-}3Glc]^+$ (Table 2), which were consistent with cyanidin-3-diglucoside-5-glucoside. Peak 6 gave *m/z* 979 $[M]^+$, 817 $[M\text{-}Glc]^+$, 449 $[M\text{-}2Glc\text{-}sinapoyl]^+$ and 287 $[M\text{-}3Glc\text{-}sinapoyl]^+$, which can be assigned to cyanidin-3-(sinapoyl)diglucoside-5-glucoside. Peak 8 was one of the two major anthocyanin peaks in the crude extract of the red cabbage. This broad peak was found to be made up of three partially over-lapping compounds of *m/z* 919, 949 and 979. The earlier eluting portion of the peak 8-1 showed *m/z* 919 $[M]^+$, 757 $[M\text{-}Glc]^+$, 449 $[M\text{-}2Glc\text{-}p\text{-}coumaroyl]^+$ and 287 $[M\text{-}3Glc\text{-}p\text{-}coumaroyl]^+$; the middle portion peak 8-2 gave *m/z* 949 $[M]^+$, 787 $[M\text{-}Glc]^+$, 449 $[M\text{-}2Glc\text{-}feruloyl]^+$ and 287 $[M\text{-}3Glc\text{-}feruloyl]^+$, and the MS data of the latter portion of peak 8-3 gave *m/z* 979 $[M]^+$, 817 $[M\text{-}Glc]^+$, 449 $[M\text{-}2Glc\text{-}sinapoyl]^+$ and 287 $[M\text{-}3Glc\text{-}sinapoyl]^+$. Overlapping anthocyanidin glycosides have also been found in cabbage extract by others (McDougall, Fyffe, Dobson, & Stewart, 2007), therefore the three compounds contained in peak 8 in Fig. 2 were putatively identified as cyanidin-3-(*p*-coumaroyl)diglucoside-5-glucoside, cyanidin-3-(sinapoyl)diglucoside-5-glucoside and cyanidin-3-(feruloyl)diglucoside-5-glucoside, respectively according to the eluting order and MS data (McDougall et al., 2007). Similarly, peak 9 was found to contain two partially overlapping compounds whose molecular ions were *m/z* 1125 $[M]^+$ and 1155 $[M]^+$ (Table 2). The early eluting portion of peak 9-1 gave *m/z* 1125 $[M]^+$, 963 $[M\text{-}Glc]^+$, 449 $[M\text{-}2Glc\text{-}2feruloyl]^+$ and 287 $[M\text{-}3Glc\text{-}2feruloyl]^+$, and the latter portion peak 9-2 showed *m/z* 1155 $[M]^+$, 993 $[M\text{-}Glc]^+$, 449 $[M\text{-}2Glc\text{-}feruloyl\text{-}sinapoyl]^+$ and 287 $[M\text{-}3Glc\text{-}feruloyl\text{-}sinapoyl]^+$. These major components were tentatively identified as cyanidin-3-(feruloyl)(feruloyl)diglucoside-5-glucoside and cyanidin-3-(feruloyl) (sinapoyl)diglucoside-5-glucoside, respectively by comparing their MS data and elution order with those in a previous report (McDougall et al., 2007).

Purple cauliflower *Graffitti* belongs to the same crucifer family as the red cabbage, and the hydrolysis product was also nearly exclusively cyanidin (Fig. 3). Peaks with the same retention time, *i.e.* peaks 5, 8-1 and 8-2 in cauliflower were therefore readily identified. Peak 7 gave *m/z* 979 $[M]^+$, 817 $[M\text{-}Glc]^+$, 449 $[M\text{-}2Glc\text{-}sinapoyl]^+$ and 287 $[M\text{-}3Glc\text{-}sinapoyl]^+$, which was identical to cyanidin-3-(sinapoyl)diglucoside-5-glucoside, which is the same as peak 6 in red cabbage (Scalzo, Genna, Branca, Chedin, & Chassaigne, 2008). These 2 peaks may only differ at the position of the sinapoyl moiety, however, their absolute structures need to be confirmed in further studies. Peak 7 was only found in purple cauliflower and peak 6 was only in red cabbage. Peak 10 gave *m/z* 1185 $[M]^+$, 1023 $[M\text{-}Glc]^+$, 449 $[M\text{-}2Glc\text{-}2sinapoyl]^+$ and 287 $[M\text{-}3Glc\text{-}2sinapoyl]^+$, which was tentatively identified as cyanidin-3-(sinapoyl)(sinapoyl)diglucoside-5-glucoside (Scalzo et al., 2008).

Hydrolyzing the purple potato extracts produced mainly petunidin (Fig. 3), suggesting that the majority of the anthocyanins would be petunidin glycosides. The minor peak 11 in the crude extract was characterized by ions at m/z 949 $[M]^+$, 787 $[M\text{-}Glc]^+$, 479 $[M\text{-}Glc\text{-}caffeoyl]^+$, 317 $[M\text{-}Glc\text{-}caffeoyl\text{-}rutinoside]^+$, which can be assigned to petunidin-3-(caffeoyl)-rutinoside-5-glucoside. The molecular and fragment ions of the major peak 12 were *m/z* 933 $[M]^+$, 771 $[M\text{-}Glc]^+$, 479 $[M\text{-}Glc\text{-}p\text{-}coumaroyl]^+$, 317 $[M\text{-}Glc\text{-}p\text{-}coumaroyl\text{-}rutinoside]^+$. These are characteristic of petunidin-3-(*p*-coumaroyl)-rutinoside-5-glucoside. However, peak 13 showed *m/z* 947 $[M]^+$, 785 $[M\text{-}Glc]^+$, 493 $[M\text{-}Glc\text{-}p\text{-}coumaroyl]^+$, 331 $[M\text{-}Glc\text{-}p\text{-}coumaroyl\text{-}$

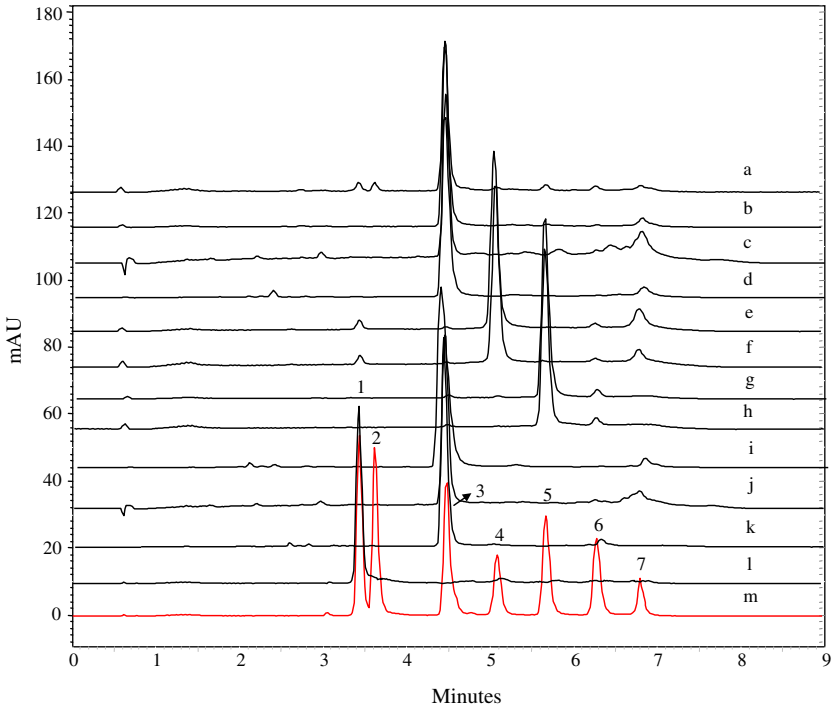

Fig. 3. UPLC chromatograms of anthocyanidins of highly pigmented vegetables after hydrolysis (a–l) and mixed standards (m) at 520 nm.a = Purple carrot *Rain*; b = purple carrot *Haze*; c = red cabbage *Gario*; d = purple cauliflower *Graffitti*; e = purple potato *Majesty*; f = purple potato *Mackintosh*; g = red potato *Y38*; h = red potato *Thumb*; i = red onion *Pier-c*; j = red onion *Pearl*; k = purple asparagus *Albenga*; l = eggplant *Black Beauty*; m = mix standards.Peaks: 1 = delphinidin; 2 = luteolinidin; 3 = cyanidin; 4 = petunidin; 5 = pelargonidin; 6 = peonidin; 7 = malvidin.

rutinoside]$^+$, which suggests malvidin-3-(*p*-coumaroyl)-rutinoside-5-glucoside, this explains the identification of malvidin in the hydrolysate of the purple potatoes (Fig. 3). It was interesting to note that although they belong to the same species, the red potato cultivars had different anthocyanidin profile than the purple ones; the dominant aglycone was pelargonidin in the red instead of petunidin in the purple cultivars (Fig. 3). Four anthocyanins peaks were identified (Fig. 2, Peaks 14–17). The MS spectrum of peak 14 gave *m/z* 741 [M]$^+$, 579 [M-Glc]$^+$, 433 [M-rutinoside]$^+$ and 271 [M-Glc-rutinoside]$^+$, which is in agreement with typical fragments of pelargonidin-3-rutinoside-5-glucoside. Peak 15 had *m/z* 579 [M]$^+$ and 271 [M-rutinoside]$^+$, which is identified as pelargonidin-3-rutinoside. Similarly, peak 16 gave *m/z* 887 [M]$^+$, 741 [M-*p*-coumaroyl]$^+$, 725 [M-Glc]$^+$, 443 [M-*p*-coumaroyl-rutinoside]$^+$ and 271 [M-*p*-coumaroyl-rutinoside-Glc]$^+$, which matched with pelargonidin-3-(*p*-coumaroyl)-rutinoside-5-glucoside. Peak 17 was the predominant glycoside peak, but a close examination using SIM showed that there was one minor and one major partially overlapping compounds; the minor peak 17-1 produced *m/z* 917 [M]$^+$, 755 [M-Glc]$^+$, 463 [M-*p*-coumaroyl-rutinoside]$^+$ and 301 [M-*p*-coumaroyl-rutinoside-Glc]$^+$ and the major peak 17-2 also had the same molecular ion *m/z* 917 [M]$^+$ and the fragment ion *m/z* 755 [M-Glc]$^+$ as peak 17-1, but with two different fragment ions *m/z* 433 [M-feruloyl-rutinoside]$^+$ and 271 [M-feruloyl-rutinoside-Glc]$^+$. These two peaks were tentatively identified as peonidin-3-(*p*-coumaroyl)-rutinoside-5-glucoside and pelargonidin-3-(feruloyl)-rutinoside-5-glucoside (Ieri, Innocenti, Andrenelli, Vecchio, & Mulinacci, 2011; Mulinacci et al., 2008). Identification of the peonidin glycoside (Peak 17-1) is also confirmed by the positive identification of peonidin in the hydrolysate as a minor anthocyanidin (Fig. 3).

In red onions, like in the purple carrots and the crucifers, the only major aglycone was cyanidin (Fig. 3), however, the cyanidin

Table 3
Total anthocyanin contents and individual anthocyanidin concentrations in different highly pigmented vegetables (mg/100 g DW) (n = 3)[A].

Cultivar	Delphinidin	Luteolinidin	Cyanidin	Petunidin	Pelargonidin	Peonidin	Malvidin	TAI[B]	TAC[C]
Purple carrot *Rain*	0.34 ± 0.02 b	0.27 ± 0.01 b	13.68 ± 0.62 d	0.32 ± 0.01 c	0.47 ± 0.06 c	0.49 ± 0.03 e	0.88 ± 0.07 f	16.44 ± 0.81 e	44.27 ± 3.91 cd
Purple carrot *Haze*	ND[D]	ND	17.58 ± 1.09 c	ND	0.54 ± 0.01 c	0.26 ± 0.01 f	3.43 ± 0.02 d	21.82 ± 1.12 d	57.22 ± 5.46 c
Red cabbage *Gario*	ND	ND	54.06 ± 2.08 b	ND	ND	0.71 ± 0.01 d	8.20 ± 0.05 a	62.98 ± 2.14 b	198.61 ± 6.10 a
Purple cauliflower *Graffitti*	ND	ND	63.20 ± 1.71 a	ND	ND	ND	6.83 ± 0.15 b	70.03 ± 1.84 a	201.11 ± 5.87 a
Purple potato *Majesty*	0.55 ± 0.01 b	ND	0.14 ± 0.01 fg	29.72 ± 1.29 a	ND	0.83 ± 0.02 c	4.77 ± 0.05 c	36.00 ± 1.35 c	97.71 ± 3.71 b
Purple potato *Mackintosh*	0.34 ± 0.02 b	ND	0.02 ± 0.01 g	13.99 ± 0.82 b	ND	0.30 ± 0.01 f	2.70 ± 0.01 e	17.34 ± 0.83 e	48.74 ± 4.55 c
Red potato *Y38*	ND	ND	0.57 ± 0.03 fg	0.32 ± 0.02 c	31.31 ± 1.42 a	2.46 ± 0.06 a	ND	34.65 ± 1.49 c	85.23 ± 0.61 b
Red potato *Thumb*	ND	ND	0.52 ± 0.02 fg	ND	16.58 ± 0.58 b	1.38 ± 0.06 b	ND	18.49 ± 0.64 e	43.09 ± 1.59 cd
Red onion *Pier-c*	ND	ND	3.10 ± 0.12 f	ND	ND	ND	0.06 ± 0.10 g	3.16 ± 0.21 h	7.77 ± 0.38 f
Red onion *Pearl*	ND	ND	5.77 ± 0.14 e	ND	ND	ND	0.33 ± 0.05 g	6.11 ± 0.18 g	18.95 ± 3.63 ef
Purple asparagus *Albenga*	ND	0.37 ± 0.09 a	8.06 ± 0.13 e	ND	ND	ND	ND	8.43 ± 0.20 g	23.74 ± 1.85 e
Eggplant *Black Beauty*	11.53 ± 0.84 a	ND	ND[D]	0.55 ± 0.02 c	ND	ND	ND	12.08 ± 0.82 f	29.55 ± 2.35 de

[A] Values are mean ± SD, n = 3. Values followed by the same letter in the same row are not significantly different ($p < 0.05$).
[B] TAI: total anthocyanidin index (sum of individual anthocyanidin concentrations, mg/100 g dry weight).
[C] TAC: total anthocyanin content (mg Cyanidin-3-glucoside/100 g dry weight).
[D] ND: not detected.

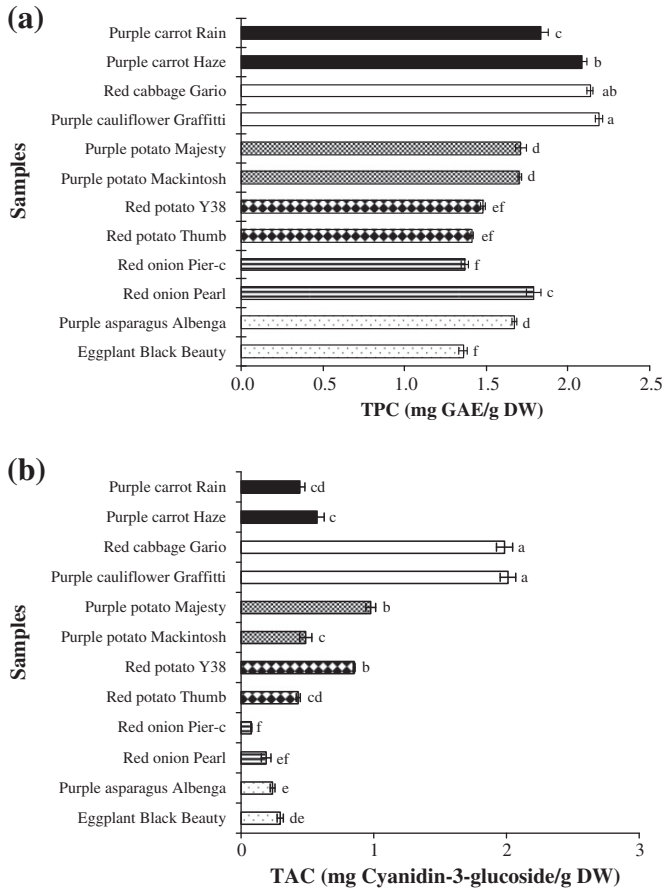

Fig. 4. The total phenolic and total anthocyanin contents of the hydrophilic extracts of highly pigmented vegetables.A: Total phenolic content, values are expressed as milligram gallic acid equivalent/g dry weight (mg GAE/g DW).B: Total anthocyanin content, values are expressed as milligram cyanidin-3-glucoside equivalent/g dry weight (mg cyanidin-3-glucoside/g DW).Values are means ± SD, n = 3. Values followed by the same letter in the same assay are not significantly different ($p<0.05$).

glycosides in red onions were found to be different. Peak 18 produced *m/z* 449 $[M]^+$ and 287 $[M\text{-}Glc]^+$; peaks 19 and 20 showed the same MS spectrum with *m/z* 535 $[M]^+$ and 287 $[M\text{-malonyl-}Glc]^+$, and peak 21 gave *m/z* 697 $[M]^+$, 449 $[M\text{-malonyl-}Glc]^+$ and 287 $[M\text{-malonyl-}2Glc]^+$. These compounds (peaks 18–21) have been assigned to cyanidin-3-glucoside, cyanidin-3-(malonyl)glucoside and cyanidin-3-(malonyl)diglcoside, respectively (Fossen et al., 1996; Petersson et al., 2008). Peaks 19 and 20 are highly likely cyanidin-3-(3″-malonyl)glucoside and cyanidin-3-(6″malonyl)glucoside, structural isomers in which the malonyl group is attached to different positions (Fossen et al., 1996; Petersson et al., 2008). The major difference between the anthocyanins in the onion and other vegetables are in the acylation of the sugar units; it was by the aliphatic acid (malonic acid) in the former, and aromatic acids (cinnamic acids) in the latter (McDougall et al., 2007; Petersson et al., 2008).

Three major anthocyanin peaks 22–24 were detected in purple asparagus extract (Fig. 2). Peak 22 was a minor peak, and its MS spectrum showed *m/z* 757 $[M]^+$, 595 $[M\text{-}Glc]^+$, 449 $[M\text{-}Glc\text{-}rhamnopyranose]^+$ and 287 $[M\text{-}2Glc\text{-}rhamnopyranose]^+$, which can be assigned to cyanidin-3-glucoside-(rhamnopyranosyl)-5-glucoside. Peak 23 was the dominant glycoside, its MS showed *m/z* 595 $[M]^+$ and 287 $[M\text{-}rutinose]^+$, which can be assigned to cyanidin-3-rutinoside. Peak 24 showed *m/z* 609 $[M]^+$ and 301 $[M\text{-}rutinoe]^+$, which are characteristic of peonidin-3-rutinoside (Sakaguchi et al., 2008).

The only hydrolysis product of the eggplant extract was delphinidin (Fig. 3), and the two glycoside peaks 25 and 26 in Fig. 2 were identified to be delphinidin-3-glucoside whose MS spectrum gave

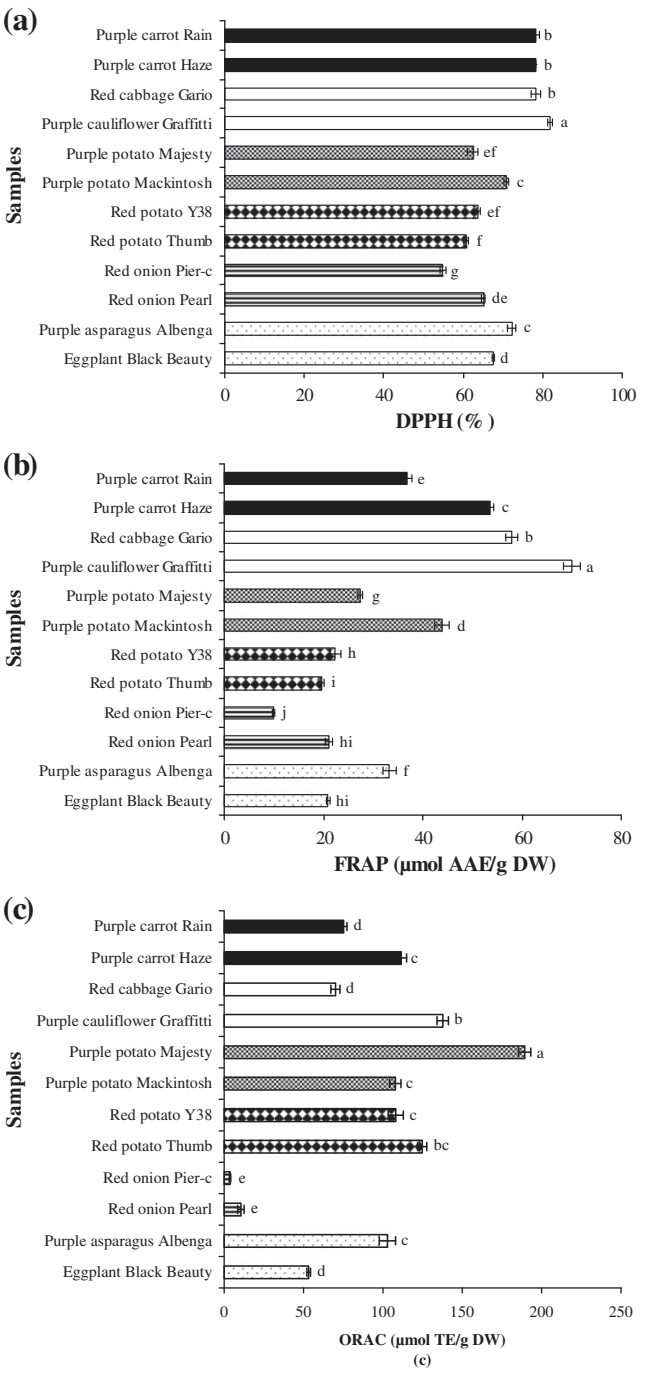

Fig. 5. Antioxidant activities of the hydrophilic extracts of highly pigmented vegetables.A: DPPH assay, values are expressed as percent scavenging (%);B: FRAP assay, values are expressed as micromole ascorbic acid equivalent (AAE)/g dry weight (μmol AAE/g DW).C: ORAC assay, values are expressed as micromole Trolox equivalent/g dry weight (μmol TE/g DW);Values are means ± SD, n = 3. Values followed by the same letter in the same assay are not significantly different ($p<0.05$).

m/z 465 $[M]^+$ and 303 $[M\text{-}Glc]^+$ and delphinidin-3-rutinoside whose MS spectrum showed *m/z* 611 $[M]^+$ and 303 $[M\text{-}rutinoside]^+$, respectively, which is in agreement with previous reports (Azuma et al., 2008).

3.4. Total phenolics and total anthocyanins

Concentration of the individual anthocyanidins was analyzed using the above UPLC method (Table 3). The total anthocyanin

index (TAI) which is the sum of concentrations of all anthocyanidins detected was also obtained for each individual vegetable. The TPC, TAC and TAI are shown in Fig. 4 and Table 3. The TPC varied significantly among the 12 HPV, ranging from 1.36 to 2.19 mg GAE/g DW. Purple cauliflower, red cabbage and purple carrot *Haze* were the top three vegetables significantly higher in TPC than others, having 2.19, 2.13 and 2.09 mg GAE/g DW, respectively (Fig. 4a). Purple cauliflower and red cabbage also topped the list in TAC and TAI, containing 2.01, 1.99 mg C3G/g DW and 0.70, 0.63 mg/g DW (70.03, 62.98 mg/100 g DW), respectively (Fig. 4b and Table 3), indicating anthocyanins may be the predominant phenolic compounds in these two cruciferous vegetables. The greater TAC values as compared with the TAI can be caused by the incomplete quantification of all peaks in the UPLC method, and the potential interferences by other components in the TAC method (Tsao & Yang, 2003). The TAC and TIC in all other vegetables were significantly lower, and considering the relatively narrower range of the TPC, it is safe to say that other vegetables may contain other phenolics in addition to anthocyanins. Such phenolic compounds may include phenolic acid and flavonoids as reported by others (Huang, Wang, Eaves, Shikany, & Pace, 2007). TAC and TAI in the two cultivars of red onions, purple asparagus and eggplant were very low due to the fact that only the surface of the scale leaves or the edible parts are pigmented. According to the Pearson's correlation coefficient, TAI was positively correlated with the TAC ($r=0.9596$) and moderate correlations were found for the pair of TAC and TPC ($r=0.6454$) and for TAI and TPC ($r=0.5899$).

3.5. Antioxidant activities

It should be noted that because the main focus of this study was on the anthocyanins extracted by aqueous methanol, the antioxidant activities of the vegetable extracts are therefore from the hydrophilic phytochemicals including anthocyanins and other phenolic compounds. Three commonly used *in vitro* assays were used to evaluate the antioxidant activities, and the results showed that different vegetables had significantly different degrees of antioxidant activity ($p<0.05$) (Fig. 5). In the DPPH assay, all extracts showed good radical scavenging activity with the percent scavenging ranging from 54.91 to 81.94%. The two cruciferous vegetables (purple cauliflower and red cabbage) and the two purple carrots *Rain* and *Haze* showed the highest antiradical activity in the DPPH assay (Fig. 5a). The antioxidant activities as measured by the FRAP assay ranged from 10.00 to 70.07 μmol AAE/g DW, a wider range of difference than what was found in the DPPH assay among the vegetables analyzed. However, the vegetables with the highest antioxidant activity were the same, *i.e.* the two crucifers and the purple carrot *Haze* (Fig. 5b). The antioxidant activity of the HPV varied even more significantly in the ORAC assay, ranging from 3.74 to 189.32 μmol TE/g DW (Fig. 5c). The highest ORAC value was found in the purple potato *Majesty*, followed by those in purple cauliflower and red potato *Thumb* (Fig. 5c).

Attempts were made to correlate the TPC, TAC, TAI and the antioxidant activities using the Pearson's correlation coefficients (r). In general, vegetables with higher TPC also had higher antioxidant activity, especially with the FRAP and DPPH assays ($r=0.8436$ and 0.7397, respectively). TAC and TAI correlated with the FRAP assay ($r=0.7554$ and 0.7227, respectively). The strong positive correlation between the TPC and FRAP, and the low or lack of correlation between the TPC and ORAC have been observed in other studies (Samec et al., 2010; Szydłowska-Czerniak, Trokowski, Karlovits, & Szłyk, 2010; Tsao & Yang, 2003). Red onions had the lowest TPC, TAC and TAI, and they also had the lowest antioxidant activity in all three assays, again suggesting that phenolics, particularly anthocyanins play an important role in the antioxidant potential of vegetables. In the mean time, the antioxidant activities of the vegetables as measured by the three assay methods were not always consistent. While those of the FRAP and DPPH were highly correlated ($r=0.8892$), the other pairs, *i.e.* between the FRAP and ORAC, and the DPPH and ORAC, were only weakly correlated ($r=0.4685$ and 0.3253, respectively), indicating the different mechanisms of these assay methods, as well as the different radical scavenging capabilities arising from the different phenolic or anthocyanin compositions under different assay conditions. Ou, Huang, Hampsch-Woodill, Flanagan, and Deemer (2002) also reported no correlation of antioxidant activity between the FRAP and ORAC assays among most of the 927 freeze-fried vegetable samples.

4. Conclusion

The HPV of this study showed distinctive $L^*a^*b^*$ values due to the different anthocyanin compositions. The color characteristics and stability are important factors in developing functional foods. A total of 26 anthocyanins were separated by UPLC in 9 min and identified in these vegetables using a combination of LC–DAD–ESI–MS, acid hydrolysis and congruent retention time of the hydrolysis products with standard anthocyanidins. These glycosides were composed of four main anthocyanidins cyanidin, petunidin, pelargonidin and delphinidin and different sugar units. Depending on the vegetables, anthocyanins may be the majority of the TPC such as those in the cruciferous cabbage and cauliflower, but may also be a small portion of the TPC in others such as the red onions. Higher TPC resulted in higher antioxidant activities in the FRAP and DPPH assays, whereas TAC and TAI only positively correlated with the FRAP value. The results indicate that vegetable crops higher in TPC, TAC and TAI are the greatest source of antioxidants, but those lack of anthocyanins, have relatively low antioxidant activities. While the purple or red cultivars of some vegetables have been individually studied elsewhere, results from different studies are often incomparable due to the different methods used. By investigating different HPV in one study using the same sets of analytical methods, the present study provides a more comprehensive approach in studying the potential health benefits of HPV, and avoids the incomparability of results found in individual studies. Obtaining a complete picture of the anthocyanin compositions and their roles in the antioxidant activities of the existing and new cultivars of these HPV may also help better understand the anthocyanin bioavailability and the mechanisms of antioxidant action in further studies, and provide fundamental information for the development of new vegetable cultivars with elevated health benefits, and anthocyanin-rich nutraceuticals and functional foods.

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