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Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris* subspecies *cycla*) extracts

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Abstract

The antioxidant activity (radical scavenging and thiocyanate method), total phenolic contents (gallic acid equivalents) and phenolic composition (coulometric array detection) in methanol extracts of different parts and tissue types of hydroponically grown Swiss chard (*Beta vulgaris* subspecies *cycla*) were determined. Significant differences ($P < 0.01$) in the antioxidant activity were found between leaves and stems. Phenolic content and composition of the leaves and the stems also were found to be different. A positive linear correlation ($R = 0.943$) was demonstrated between radical scavenging activity and total phenolic content of each extract. The major phenolic acid and flavonoid in leaves of Swiss chard were syringic acid and kaempferol. Coulometric array detection showed good reproducibility (CV, 0.06–1.05%) and sensitivity (1 ng/ml, detection limit) for simultaneous detection of 13 phenolics. Our data indicates that Swiss chard has potential as a good dietary source of phenolic antioxidants.

Keywords: Swiss chard (*Beta vulgaris* subspecies *cycla*); Antioxidant activity; Phenolics; Coulometric array detection

1. Introduction

Plants are potential sources of natural antioxidants. Fruits and vegetables in the diet have been found in epidemiological studies to be protective against several chronic diseases associated with aging such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction (Ames, Shigenaga, & Hagen, 1993; Block, Patterson, & Subar, 1992; Vinson, Jang, Dabbagh, Serry, & Cai, 1995). These natural protective effects have been attributed to various components, such as carotenoids, vitamin C, E, and phenolic and thiol (SH) compounds (Paganga, Miller, & Rice-Evans, 1999).

Many studies have focused on the biological activities of phenolics which are potent antioxidants and free-radical scavengers (Marja et al., 1999; Rice-Evans, Miller, Bolwell, Bramly, & Pridham, 1995; Sugihara, Arakawa, Ohnishi, & Furuno, 1999). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Catherine, Rice-Evans, Nicholas, & George, 1996; Ramarathnam, Ochi, & Takeuchi, 1997; Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). In addition, they have a metal chelation potential (Kuo, Leavitt, & Lin, 1998; Yoshino & Murakami, 1998).

Recent studies have shown that many dietary polyphenolic constituents derived from plants are more effective antioxidants in vitro than vitamin E or C, and thus might contribute significantly to the protective effects in vivo (Catherine, Rice-Evans, Nicholas, & George, 1997; Rene, Yolanda, & Zeno, 2001; Vinson, Hao, Su, & Zubik, 1998). An investigation of such antioxidative phenolic compounds in edible plants has been conducted to improve our understanding of their dietary value and potential benefits.

Swiss chard (*Beta vulgaris* subspecies *cycla*), sometimes called stem chard because of its broad and flat stalks, is a herbaceous biennial related to the beet root (*Beta vulgaris* Subspecies *maritima*) but cultivated for its

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edible leaves (Anthony, Mark, & Margot, 1992). It has been grown in Europe since classical antiquity and is now widely cultivated in temperate regions including parts of Northern India and South America (Tindall, 1983). The distinctive color of chard is due to the petioles and most varieties are categorized as being either white or red (Ib Libner Nonnecke, 1989). The red color is largely from betacyanin, a compound closely related to anthocyanin which accounts for most of the red colors in plants (Anthony et al., 1992; Donald & George, 1997). The leaves can be used in salads or cooked like spinach, and the stems are usually chopped and cooked like celery. Yet, the plant is more robust and easier to grow than spinach and celery (Donald & George, 1997). The leaves and the stalks of chard contain nutritionally significant concentrations of vitamin A, C, B, calcium, iron, and phosphorus (Anthony et al., 1992; Donald & George, 1997; Tindall, 1983). Recently, Maria, Federico, Francisco, and Tomas (1998) reported that the flavonoid content of chard leaves was in the range 2.4–3.0 mg/g fresh weight and a new flavonoid, 6″-Malonyl-2″-xylosyl vitexin, was isolated. But no literature was found reporting antioxidant activity and phenolic composition in Swiss chard.

With the recent advances in electrochemical detection, coulometric electrode array detection is becoming a powerful tool for detecting phenolic acids and flavonoids in a wide range of samples (Achilli, Cellerino, & Hamache, 1993; Guo, Cao, Sofic, & Prior, 1997; Mattila, Astola, & Kumpulainen, 2000; Peyrat-Maillard, Bonnely, & Berset, 2000). Electrochemical detection is already well-known for its capacity to detect trace amounts of compounds in complex matrices because of its excellent sensitivity and selectivity. It should also be of particular interest for the analysis of primary antioxidants, which are all electroactive (Gamache, Ryan, & Acworth, 1993; Guo et al., 1997). Thus, the purpose of this study was to (i) evaluate each methanol extract of the different parts (stems and leaves) and the tissue types (red and white) of Swiss chard that were grown under a flood hydroponic system with respect to their antioxidant activity and concentration of phenolic compounds (ii) develop an optimized single method for the determination of phenolic acids and flavonoids in Swiss chard utilizing reversed-phase chromatography coupled with a coulometric array detection system.

2. Materials and methods

2.1. Plant material

Fresh Swiss chard (white tissue cultivar ; *Large white ribbed* with creamy white stems, red tissue cultivar; *CXS 2550* with reddish stems) were cultivated in a green house located at Cook College, Rutgers University, under a flood hydroponic system and samples were collected for analysis. The leaves and stems were gathered separatetly from the two cultivars and dried to constant weight in a vacuum at 25 °C. This was powdered in a grinder to pass through a 40-mesh sieve and kept at −18 °C until used.

2.2. Extraction

Each sample of Swiss chard powder (25 g) was extracted by mixing using a magnetic stirrer with 150 ml of methanol at 30 °C for 5 h. Each extract was filtered through Whatman No. 4 filter paper and re-extracted with the same solvent for the extraction of antioxidant fractions. All extracts were pooled respectively and concentrated under vacuum at 40 °C.

2.3. Antioxidant activity using the thiocyanate method

The antioxidant activity of Swiss chard extracts was determined using the thiocyanate method (Singh, Chidambara, & Jajaprakasha, 2001; Yen & Hsieh, 1998). The linoleic acid emulsion was prepared by homogenizing 0.28 g linoleic acid, 0.28 g Tween-40 and 50 ml phosphate buffer (0.2 M, pH 7.0). Test samples were prepared in a MeOH:water mixture (6:4, v/v). Different test samples (0.5 ml) were mixed with 2.5 ml linoleic acid emulsion and 2.5 ml phosphate buffer (0.2 M, pH 7.0), and incubated at 37 °C for 150 h. The mixture prepared as above without the test sample served as the control. Aliquots (0.1 ml) were drawn from the incubation mixture at 24 h intervals and mixed with 5.0 ml 75% ethanol, 0.1 ml 30% ammonium thiocyanate and 0.1 ml 20 mM ferrous chloride in 3.5% HCl and allowed to stand at room temperature for 3 min. The color developed was measured at 500 nm in a spectrophotometer (Hitachi U-3110, Tokyo, Japan). The degree of linoleic acid peroxidation was calculated at 120 h using the following formula. Antioxidant activity (AA, %) = 100−(Increase in the absorbance of sample/Increase in the absorbance of control) × 100. Butylated hydroxytoluene (BHT) and α-tocopherol (100–1000 μg/ml) were included as standard antioxidants for comparison.

2.4. Radical scavenging activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method

The stable DPPH radical scavenging effect of Swiss chard extracts was measured according to Brand-Williams, Cuvelier, and Berset (1995) and Robards, Sanchez-Moreno, Larrauri, and Saura-calixto (1998). An aliquot of methanol solution containing different sample concentrations was added to 3.9 ml DPPH 0.025 g/l in methanol prepared daily. The absorbance at 515 nm was measured at different time intervals until the reaction reached the steady state. The inhibitory percentage of

DPPH was calculated according to the following equation. % Inhibition = absorbance control − absorbance sample/absorbance control × 100%. BHT and α-tocopherol dissolved in methanol (100–1000 μg/ml) were also analyzed for comparison.

2.5. Determination of total phenolics

Total phenolics were determined using Folin-Ciocalteu reagent (Singleton & Slinkard, 1977). Samples (200 μl) were introduced into test tubes, and then 1.0 ml Folin-Ciocalteu's reagent and 0.8 ml sodium carbonate (7.5%) were added. The absorbance of all samples was measured at 765 nm after incubating at 30 °C for 1.5 h. Results were expressed as milligram of gallic acid equivalent (GAE) per gram of fresh weight (FW).

2.6. Extraction and hydrolysis of phenolics

Flavonols and hydroxybenzoic acids occur in plants mainly as glycosides, whereas hydroxycinnamic acids are bound to cell wall polymers or they occur as simple esters (Herrmann & Schuster, 1989). Hydrolysis can release the combined flavonoids and phenolic acids. The optimal extraction and hydrolysis of phenolics were carried out as follows according to Hertog, Hollman, and Venema (1992) and Häkkinen, Karenlampi, Heinonen, Mykkanen, and Torronen (1998). The sample (mixture of unconjugated phenolic standards or 0.5 g sample of Swiss chard powder) was weighed and rinsed with 25 ml methanol. To this extract, 10 ml 6 M HCl was added by careful mixing (final HCl concentration 1.2 M) and the solution was sonicated for 5 min. The remaining air in the bottle was replaced by nitrogen gas. The extract was shaken at 35 °C in the dark. After 20 h, the extract was allowed to cool and was then filtered. A 15 ml portion of the filtrate was evaporated to dryness at 35 °C. The residue was dissolved in 5 ml of methanol. The solution was passed through a C18 solid-phase extraction cartridge which had been preconditioned with methanol and 0.1% HCl respectively (Schieber, Keller, & Carle, 2001). After washing with water, the analytes were eluted with methanol (3 ml) and used for chromatographic analyses.

2.7. HPLC analysis of phenolics using coulometric array detection

The HPLC system consisted of an ESA model 582 pump and an ESA analytical coulochem II electrochemical detector (model 5600 A, CoulArray, ESA, MA, USA). The coulometric array (electrochemical) detector was controlled using the ESA Coularray for Windows Data Processing Module Version 1.00 computer program. The analytical cell pack contained two porous graphite working electrodes with associated palladium reference electrodes and platinum counter electrodes. The detector was operated using −50 mV to 825 mV potentials (−50, 0, 70, 250, 375, 500, 675, 825 mV). After multiple preliminary assays, a Zorbax column (C18, 250 mm × 4.6 mm, i.d.; particle size, 5 μm) and a gradient elution program using methanol–water–acetonitrile as solvent was chosen. Mobile phase A was 25 mM sodium acetate and 25 mM citric acid in methanol/water (5/95, v/v), while mobile phase B was composed of 25 mM sodium acetate and 25 mM citric acid in water/methanol/acetonitrile (20/40/40, v/v/v). The following elution profile was used: isocratic 6% B from 0 to 10 min, linear increase of phase B from 6 to 30% over 35 min, linear increase of phase B from 30 to 100% from 36 to 45 min, isocratic at 6% phase B until 50 min. The flow rate was 1 ml/min, and the injection volumes were 10 μl of the standards and the sample extracts. All phenolics were quantified using the external standard method. Calibration curves of the standards were made by diluting stock standards in 50% acquous methanol to yield 0.1–500 μg/ml. The samples were prepared and analyzed in triplicate.

2.8. Standards

All authentic standards (13 compounds) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The stock standard solutions were prepared by dissolving the standard phenolic compounds (nine phenolic acids; gallic acid, *p*-OH-benzoic acid, protocatechuic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid and ferulic acid, and four flavonoids; (+) catechin, myricetin, quercetin and kaempferol) in the appropriate volume of 50% aqueous methanol to produce a final concentration of 1 mg/ml. Stock/working solutions of the standards were stored in the dark at −18 °C.

2.9. Method validation

Linearity of the detector responses and the detection limits were tested for the phenolics. The within-day and the between-day repeatability of the methods were tested by analyzing the reference sample. Identity and purity of the phenolic peaks was monitored using voltammetric data (in addition to monitoring retention times and the symmetry of the peak). The purity match of the sample peaks was obtained by comparing channel area or height ratios (dominant vs. post- and predominant channel areas or heights) (Guo et al., 1997).

2.10. Statistical analysis

All analysis were run in triplicate and averaged. Data were analyzed by an analysis of variance ($P < 0.05$) and the means separated by Duncan's multiple range test.

3. Results and discussion

3.1. Antioxidant activity and total phenolic content

Comparison of antioxidant activity between each methanol extract of Swiss chard, and the total phenolic (TP) content are shown in Fig. 1. In the DPPH radical scavenging assay the leaf extracts of red tissue cultivars showed significantly ($P<0.01$) higher radical scavenging activity while the stem extracts of white tissue cultivars showed the lowest activity. The radical scavenging capacity of the leaves and red tissue compared with the stems and white tissue was stronger at 300–700 μg/ml concentration respectively (data not shown). Among each extract the red leaf extracts showed a particularly high activity at 500 μg/ml (87.0±1.2%). This activity was significantly ($P<0.01$) stronger than those of BHT (65.3±0.1%) and α-tocopherol (80.4±0.1%) at the same concentration. The reason for the high activity of red tissue remains unclear; possibly the extract contained additional phenolic anthocyanins such as betacyanin. This finding on the differences in the color of tissue agreed with the results reported by others (Furuta, Suda, Nishiba, & Yamakawa, 1998; Joe & Barbara, 1995; José, Concepcion, & Fulgencio, 1998). Recently, Oki et al. (2002) reported that anthocyanins and polyphenols play an important role in the DPPH radical scavenging activity of purple-fleshed sweet potatoes.

There was a good linear correlation ($R=0.943$) between the TP content and the scavenging of DPPH radical in each extract (Fig. 1). These results indicated that the radical scavenging capacity of each extract might be mostly related to their concentration of phenolic hydroxyl group. The antiradical activity of phenolic compounds depends on their molecular structure, that is, on the availability of phenolic hydrogens and on the possibility for stabilization of the resulting phenoxyl radicals formed by hydrogen donation (Catherine et al., 1996; Ramarathnam et al., 1997). A linear correlation between radical scavenging activity and polyphenolic concentration has been reported in an extensive range of vegetables, fruits and beverages (Gil, Tomas-Barberan, Hess Pierce, Holcroft, & Kader, 2000; Guo et al., 1997; Marja et al., 1999; Velioglu, Mazza, Gao, & Oomah, 1998).

The antioxidant activity (AA, %) of each extract was further evaluated using linoleic acid peroxidation by the thiocyanate method, which is used to measure the peroxide level during the initial stage of lipid oxidation (Fig. 2). Similar trends of antioxidant activity were observed using either the DPPH or the thiocyanate method. The leaf extracts exibited significantly ($P<0.01$) higher antioxidant activity (%) than the stem extracts at 500–1000 μg/ml concentration (data not shown). However, no significant difference ($P>0.05$) was found between red and white tissue. The red leaf extracts (AA = 72.5%) exibited the highest activity at 1000 μg/ml but less than that of BHT (AA = 88.2%) and nearly equal to or stronger than that of α-tocopherol (AA = 71.8%) at the same concentration. These results suggested that the leaf extracts might react with free radicals, particularly peroxy radicals, which are the major propagators of the autooxidation of fat, thereby terminating the chain reaction (Frankel, 1991; Shahidi & Wanasundara, 1992).

Using the DPPH radical scavenging method and the thiocyanate method, the antioxidant activity of each extract from Swiss chard were in the order red leaf > white leaf > red stem > white stem. This result was consistent with the order of total phenolic content of each extract.

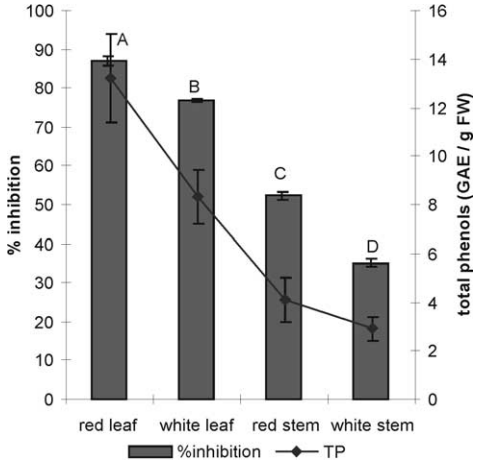

Fig. 1. The relation ($R=0.943$) of DPPH radical scavenging (% inhibition at 500 μg/ml) and total phenols (TP, gallic acid equivalents/g fresh weight) in Swiss chard extracts. Different letters indicate significant difference ($P<0.01$). Each value is the mean±standard deviation of three replicate analysis.

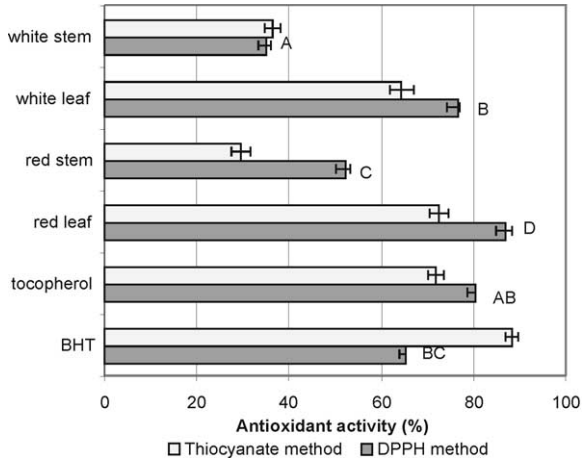

Fig. 2. Antioxidant activity of each extract from Swiss chard. The activity was determined by the DPPH and the thiocyanate method, and the concentration of each method was 500 and 1000 μg/ml, respectively. Different letters indicate significant difference ($P<0.01$). Each value is the mean±standard deviation of three replicate analysis.

Therefore, total phenols in each extract are likely to be responsible for the antioxidant properties studied.

3.2. Analysis of phenolic compounds

Selected phenolics in each extract were separated and identified by using reversed-phase HPLC with coulometric array detection system. Most of the external standards used in this study were phenolic acids and flavonoids that commonly occur in vegetables. Anthocyanins—although important phenolic compounds in red tissue—are not included because the extraction and the hydrolysis procedure developed in this study is not sensitive enough for these compounds. It is not an easy task to find a single method which is adequate for an analysis of a diverse group of phenolics because of the differing chemical structures and the varying sensitivity of the compounds to the conditions of hydrolysis and extraction (Howard & Gary, 2000).

The present methods showed good reproducibility (coefficient of variation; CV, 0.06–1.05%) and sensitivity (1 ng/ml, detection limit) for the simultaneous detection of these external standards. The within-day (CV, 0.06–1.05%) and the between-day (CV, 0.09–4.2%) voltammetric response (peak area, μC) variabilities were tested by repeated injection of the standard mixture (50 μg/ml). Chromatographic and electrochemical characteristics for some studied flavonoids and phenolic acids are listed in Table 1. In regard to voltammetric behavior, the phenolic acids responded dominantly at low potentials (70–375 mV), except *p*-hydroxybenzoic acids, which has a dominant potential at 825 mV, while the flavonoids had two oxidation waves (70–250 mV and 675–825 mV). However, the methoxylation of the phenolic groups increased the dominant potential; vanillic, syringic, and ferulic acids respond at 675 mV. These results agree with previous work (Peyrat-Maillard et al., 2000). In general, the higher phenolic compound's ability to act as primary antioxidants, the lower is their oxidization potential, since this value provides an estimate of the energy required to donate an electron (Brenna, Buratti, Cosio, & Mannino, 1998).

Ratio accuracy (%) values in Table 1 greater than 77.1% excluding *p*-OH-benzoic acid (36.0%), chlorogenic acid (41.4%), and caffeic acid (50.1%) were considered to be good matches, while values less than 70% probably indicated a different compound that co-eluted with the compound of interest. The 70% ratio accuracy point was the default setting recommended by the ESA coularray software (model 5600A, CoulArray). Although the precision of the method is not high for all of the phenolics, a reasonable amount of information is obtained within a single analysis. As shown in Table 2 the peak number (peak heights >10 nC) of each leaf sample (red; 241±12, white; 174±10) was larger than those of kale (117±4), spinach (67±3), and brussel

Table 1
Chromatographic and electrochemical characteristics and chemical structures of phenolic compounds found in Swiss chard extracts

Name	Substituent at								[a]tR (min)	[b]DP (mV)	[c]RA (%)
	C-2	C-3	C-4	C-5	C-2′	C-3′	C-4′	C-5′			
Benzoic acids											
Gallic	H	OH	OH	OH					4.9	250	88.1
Protocatechuic	H	OH	OH	H					10.4	250	83.2
p-OH-benzoic	H	H	OH	H					19.6	825	36.0
vanillic	H	OCH_3	OH	H					24.1	675	90.1
Cinnamic acids											
Syringic	H	OCH_3	OH	OCH_3					26.6	675	90.5
Chlorogenic[d]	H	OH	OH	H					18.6	70	41.4
Caffeic	H	OH	OH	H					25.6	250	50.1
p-Coumaric	H	H	OH	H					33.1	675	81.7
Ferulic	H	OCH_3	OH	H					36.5	375	80.9
Flavan-3-ols											
(+)Catechin					H	OH	OH	H	22.5	70,675	77.1
Flavonols											
Myricetin					H	OH	OH	OH	39.9	70,825	77.6
Quercetin					H	OH	OH	H	40.9	250	82.6
Kaempferol					H	H	OH	H	41.7	250	85.8

[a] tR, retention time; min. All data expressed as mean ($n=3$, CV = 0.7%).
[b] DP, dominant oxidation potential; millivolts.
[c] RA, ratio accuracy; analyte peak area ratio (lower/dominant, upper/dominant) accuracy (%) between Swiss chard extracts and standard solutions are expressed as mean ($n=3$, CV = 9.2%)
[d] COOH of chlorogenic acid esterified by quinic acid.

Table 2
Voltammetric response (peak number and peak area) and phenolic compounds in Swiss chard extracts

	Red tissue (*CXS 2550*)		White tissue (*Large white ribbed*)	
	Leaf	Stem	Leaf	Stem
Peak number[a]	241±12	78.9±9	174±10	64±5
Peak area (μC/μl)[b]	230±2.2	85.7±2.5	195.2±4.3	67.4±0.6
Flavonoids				
(+)Catechin	6.7[c]	0.3	1.5	0.1
Myricetin	2.2	0.1	3.1	nd[d]
Quercetin	7.5	0.5	2.2	0.3
Kaempferol	9.2	1.7	5.8	0.5
Phenolic acids				
Gallic	1.4	1.2	3.7	1.5
p-OH-benzoic	4.7	3.9	1.9	2.4
Protocatechuic	5.4	0.3	4.5	1.7
Syringic	44.9	7.5	45.1	1.5
Vanillic	5.1	0.9	3.2	5.4
Chlorogenic	7.7	3.1	3.9	0.8
Caffeic	14.8	0.2	7.8	1.9
p-Coumaric	10.4	4.4	8.0	3.7
Ferulic	8.1	5.6	10.8	3.4

[a] Peak heights greater than 10.0 nanocoulomb.
[b] All data are from the diluted aqueous extracts of Swiss chard and expressed as mean±SD ($n=3$).
[c] All data are expressed as mean ($n=3$, CV = 9.7%) of mg/100 g fresh weight of Swiss chard.
[d] nd, not detect.

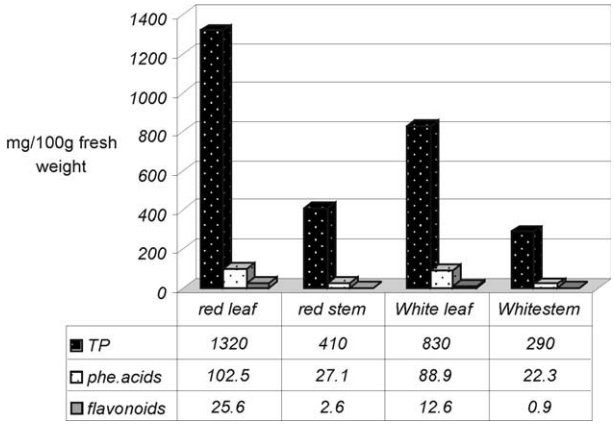

Fig. 3. The concentrations of total phenols (TP, gallic acid equivalents) and phenolic acids and flavonoids found in Swiss chard. Each value is the mean ($n=3$).

sprouts (74±2) reported by Guo et al. (1997). The electrochemical data expressed as total peak height or total peak area and peak number should be useful for roughly evaluating the overall antioxidant status of sample because all antioxidants are electroactive (Guo et al., 1997). The red leaf extract, which has been shown to exhibit the highest antioxidant activity, had the largest total peak area (230.3±2.2 μC/μl) summed across all channels, than other samples.

The concentrations of identified components were determined using the obtained calibration curves and listed in Table 2. Most of phenolic compounds selected were found or identified in each sample. The phenolic compositions of the leaves and the stems were found to be different (Fig. 3). In leaf extracts, total concentration of phenolics (red; 128.1 mg/100 g FW, white; 101.5 mg/100 g FW) was much higher than those of stem extracts (red; 29.7 mg/100 g FW, white; 23.2/100 g FW). In other foods, differences have been observed in the levels of flavonoids in the various parts of the plant (Hertog et al., 1992; Ulla, Pia, & Torben, 1998).

By comparing the results for the color of tissue types, it was observed that the total concentration of phenolics in red tissue (157.8 mg/100 g FW) was higher than in white tissue (124.7 mg/100 g FW). The high flavonoid content (28.2 mg/100 g FW) of the red tissue compared to the white tissue (13.5 mg/100 g FW) may well be a consequence of the proximity of the flavonol and anthocyanin biosynthesis pathways (Holton & Cornish, 1995) and warrants further study.

The information on the distribution of phenolic acids within plants is very limited. In the present study, syringic acid was the major phenolic compound in both red and white leaf extracts but in the stem extracts its concentration was quite low (Table 2). Selected flavonoids [myricetin, quercetin, kaempferol, (+)catechin] represented from 3.8 to 19.9% of the total phenolics extracted, that in the highest concentration being kaempferol. The red leaf extract, which was shown the highest antioxidant activity (Fig. 2) had the highest concentrations of flavonoid (25.6 mg/100 g FW). In general, flavonoids containing multiple hydroxyl groups have higher antioxidant activities against peroxyl radicals than do phenolic acids and possess antioxidant capacity towards a variety of easily oxidizable compounds (Robards et al., 1998). The antioxidant activities of flavonoids increased with the number of hydroxyl groups substituted on the B-ring, especially at C-3′, and a single hydroxy substituent generates little or no additional antioxidant capacity (Catherine et al., 1996; Ramarathnam et al., 1997). Moreover, a keto group at C-4 and a double bond between C-2 and C-3 in the pyrone ring seem to be of some importance (Cos et al., 1998). In this respect, it can be concluded that the difference of antioxidant activity from each extract of Swiss chard might be related to their phenolic content and composition.

In summary, the present study demonstrates for the first time the presence of both phenolic acids and flavonoids as antioxidant components in the methanol extract of Swiss chard. The present methods, a coulometric array detection chosen for peak identity and quantify analysis, were effective for the determination of phenolic acids and flavonoids in each extract.

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