academicJournals

Vol. 13(4), pp. 604-615, 22 January, 2014
DOI: 10.5897/AJB2013.13432
ISSN 1684-5315 ©2014 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Seasonal variation in phytochemicals and antioxidant activities in different tissues of various *Broccoli* cultivars

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Accepted 10 January, 2014

Florets, leaves, and stems of twelve commercial broccoli cultivars grown in the spring and fall seasons at the National Institute of Horticultural and Herbal Science (NIHHS), Rural Development Administration (RDA), Suwon, South Korea were evaluated for glucosinolates, vitamin C, total phenol, and total flavonoid contents and antioxidant activity. The levels of all phytochemicals and antioxidant activity were significantly influenced by cultivar (C), plant part (P), and growing season (S). Among the glucosinolates, glucoraphanin and glucobrassicin were the major constituents. The highest total glucosinolate content was found in the florets of plants grown in both seasons. Phenols and flavonoids were highest levels in leaves, while vitamin C was highest in stems, suggesting that broccoli leaves and stems may be good sources of such phytochemicals. The levels of all phytochemicals were generally higher in florets in the spring than in the fall, but were higher in leaves and stems during the fall than the spring. Furthermore, higher cultivar-dependent and tissue-dependent variation was observed in the spring than in the fall. Total phenol content exhibited a strong positive correlation (r = 0.674) with antioxidant activity, followed by total flavonoid content (r = 0.497**), indicating their significant contribution to total antioxidant activity.**

Key words: Antioxidant activity, broccoli, glucosinolate, seasonal variation, total phenol, vitamin C.

INTRODUCTION

Broccoli is one of the most commonly consumed green vegetables. Like other species of the *Brassica* family, broccoli is a source of health-promoting phytochemicals. Broccoli is known mainly for its wide range of bioactive compounds and is rich in both nutritional and non-nutritional antioxidants, including vitamin C, vitamin E, and phenolic compounds including flavonoids, carotenoids, and glucosinolates (Lin and Chang, 2005) which possess both antioxidant and anticancer activities (Williamson et al., 1998; Cohen et al., 2000; Chu et al., 2002; Gundgaard et al., 2003; Podsedek, 2007). Glucosinolates constitute a major group of natural plant compounds in the family Brassicaceae. They are responsible for the hot and pungent flavor of crucifers and exhibit anti-cancer activity (Fahey et al., 2001). Glucosinolates can be used as an alternative to synthetic pesticides for pest and disease control (Kirkegaard and Sarwar, 1998). Vitamin C is a health-promoting antioxidant compound that protects against cell death, directly scavenges superoxide radicals, hydrogen peroxide singlet oxygen, and hydroxyl radicals

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Abbreviations: DEAE, Diethyl aminoethyl; **DPPH,** 2,2-diphenyl-1-picrylhydrazyl; **HPLC,** high performance liquid chromatography; **UPLC,** ultra-performance liquid chromatography; **PDA,** photodiode array; **C,** cultivar; **P,** plant part; **S,** season.

(Gliszczynska-Swiglo et al., 2006) and cooperates with vitamin E to regenerate membrane-bound oxidized α-tocopherol, creating an 'antioxidant network' (Valko et al., 2006). Phenolic compounds are secondary metabolites that can neutralize or quench free radicals (Picchi et al., 2012). Flavonoids and their derivatives are the largest group of plant polyphenols (Hounsome et al., 2009). They possess strong antioxidant activity due to their ability to scavenge reactive oxygen species and inhibit oxidative stress (Rice-Evans et al., 1995; Pourcel et al., 2007). Variation in the amounts of these phytochemicals depends upon factors such as cultivar genotype, developmental stage, growing conditions, season, soil properties, and post-harvest storage conditions (Kurilich et al., 1999; Vallejo et al., 2002; Jeffery et al., 2003; Singh et al., 2007; Nath et al., 2011; Samec et al., 2011; Samec et al., 2013).

Although genotypic differences in the contents of glucosinolates, vitamin C, phenolics, and total flavonoids and antioxidant activity in broccoli florets have been reported (Zhang and Hamauzu, 2004; Singh et al., 2007; Koh et al., 2009; Balouchi et al., 2011; Naguib et al., 2012), information regarding the content of such phytochemicals specifically in leaves and stems is limited. Characterization of such phytochemicals to establish their distribution patterns in leaves and stems would also be useful. Seasonal variation in glucosinolate content in broccoli cultivars has been reported (Rosa and Rodrigues, 2001; Vallejo et al., 2003); however, most of the research focused only on variations in glucosinolate profiles. This study was conducted to evaluate glucosinolate, vitamin C, total phenol, and total flavonoid contents and to measure antioxidant activity in commercially cultivated broccoli cultivars in South Korea and to evaluate the cultivar- and season-dependent variation in such compounds in florets, leaves, and stems of different broccoli cultivars.

MATERIALS AND METHODS

Authentic standards and chemicals

Nine glucosinolate standards, glucoiberin, progoitrin, glucoraphanin, sinigrin, gluconapin, glucobrassicanapin, glucoerucin, glucobrassicin, and gluconasturtiin, were purchased from Cfm Oskar Co. (Germany). Authentic standards for diethyl aminoethyl (DEAE) Sephadex-A25, aryl sulfatase from *Helix pomatia*, vitamin C, glucose, sucrose, fructose, gallic acid, and catechin hydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chemicals, including sodium hydroxide, sodium carbonate, sodium nitrite, aluminum chloride, Folin-Ciocalteu reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals including acetonitrile (high performance liquid chromatography (HPLC)) grade, methanol (HPLC grade), and formic acid (ACS reagent) were purchased from J.T. Baker (Phillipsburg, NJ, USA).

Plant materials and growing conditions

Twelve commercial broccoli cultivars, 05-C3, AMaGi, BaeRiDom, CheongJae, Diamond, Grace, Grandeur, JikNok No. 28, NokJae, NokYeom No. 1, TS-2319, and YuDoRI No. 1 were used in this study study. The cultivars were grown in the field at the National Institute of Horticultural and Herbal Science (NIHHS), Rural Development Administration (RDA), Suwon, South Korea in the spring and fall growing seasons of 2011. Sowing dates were 5 March 2011 for the spring season and 25 July 2011 for the fall season. For both seasons, seedlings were transplanted to the cultivation field 33 days after sowing. Seedlings were planted in rows with 50 cm between plants and 60 cm between rows. The plants were harvested 35 and 30 days after planting in the spring and fall seasons, respectively. During the field experiments, water, fertilizers, and pesticides were applied according to standard cultural practices at the NIHHS, RDA. After harvest, the plants were separated into different parts (florets, stems, and leaves), cut into small pieces, and freeze-dried. The samples were ground into a fine powder and stored at -80°C for subsequent analyses of glucosinolates, vitamin C, total phenol, and total flavonoid content and antioxidant activity.

Glucosinolate analysis

For glucosinolates analysis, freeze-dried broccoli powder (0.1 g) was extracted with 1 mL of boiling methanol (70%) for 20 min and centrifuged at 12,000 rpm for 10 min at 4°C. The pellet was re-extracted once following the same procedure, and the supernatants were combined. Desulfoglucosinolates were then prepared and quantitatively determined by ultra-performance liquid chromatography (UPLC) using purified sulphatase isolated from *H. pomatia* according to Lee et al. (2013). Briefly, the extract was loaded onto a Mini Biospin chromatography column (Bio-Rad) containing 0.5 mL of DEAE-Sephadex A 25 which was preactivated with 0.1 M sodium acetate (pH 4.0). Then, desulfation was carried out by the addition of 200 µL of purified aryl sulphatase (EC 3.1.6.1, type H-1 from *H. pomatia).* The column was capped and left for 24 h at room temperature. The desulphoglucosinolates were eluted with 1.5 mL distilled water, filtered through a 0.2 µm syringe filter, and 10 µL samples were injected into a UPLC system (H-Class, Waters Co., USA) equipped with a sample manager flow-through-needle auto injector, quaternary solvent manager, and a photodiode array (PDA) eλ detector set at 229 nm. Separation was performed using a BEH-C18 column (2.1 × 100 mm, 1.7 µm, Waters Co., USA) at 25°C with a gradient elution of solvent A (100% distilled water) and solvent B (20% acetonitrile) with a flow rate of 0.2 mL min^{-1}. The gradient program used was as follows: a linear step from 1 to 99% of solvent B within 6 min, a constant step for 10 min, followed by a rapid dropdown to 1% solvent B at 12 min, and isocratic conditions with 1% solvent B for 3 min. Authentic glucosinolate standards were used for the identification and quantification of the peaks.

Vitamin C analysis

Vitamin C content was determined according to a method described by Spinola et al. (2012) with modifications. Dried and powdered broccoli samples (0.5 g) were extracted in 5% metaphosphoric acid solution. After centrifugation and filtration (with a 0.20 µm syringe filter), the sample was analyzed using a UPLC system (Waters, USA), an Acquity UPLC® HSS T3 (2.1 x 100 mm, 1.8 µm) column, and a PDA detector (Waters, USA) set at a wavelength of 254 nm. The mobile phase used was 99% methanol and 1% distilled water with 0.1% formic acid solution at a flow rate of 0.3 mL min^{-1}. An authentic ascorbic acid standard was used for identification and quantification of the peak.

Determination of total phenol content

Total phenolic content was estimated using the Folin-Ciocalteu colorimetric method based on the procedure of Singleton and Rossi (1965) using gallic acid as a standard phenolic compound. Freeze-dried powdered samples (1 g) were extracted in 80% methanol for

15 h at room temperature on an orbital shaker. The extracts were centrifuged and filtered through 0.45 µm syringe filters and 1 mL of each supernatant was mixed with 3.0 mL distilled water in 15 mL Falcon tubes. After adding 1 mL Folin reagent, the solutions were incubated in a water bath at 27°C for 5 min followed by addition of 1 mL of saturated sodium carbonate. After 1 h, absorbance of the extracts at 640 nm was measured using a micro plate reader (EON-C) (BioTek, USA) using 80% methanol as a blank. Gallic acid standards of various concentrations (5.0, 10.0, 25.0, 50.0, 75.0, and 100.0 ppm) were used for calibration and total phenol content was expressed as milligrams of gallic acid equivalent per gram (mg GAE g^{-1}) dry weight.

Determination of total flavonoid content

The broccoli extracts obtained for total phenol analysis were also subjected to total flavonoid analysis using a colorimetric method described by Zhishen et al. (1999). One milliliter of methanol extract (80%) was added to a 15 mL Falcon tube, mixed with 4 mL distilled water, and 0.3 mL 5 % sodium nitrite added. After 5 min, 10% $AlCl_3$ was added to the solution. At the sixth minute, 2 mL 1 M NaOH was added and the solution was brought to a final volume of 10 mL with distilled water. The solution was mixed thoroughly and absorbance was measured at 510 nm in a micro plate reader (EON-C) (BioTek, USA) using 80% methanol as a blank. Catechin hydrates of different concentrations (5.0, 10.0, 25.0, 50.0, 75.0, and 100.0 ppm) were used as standards and total flavonoid was expressed as milligrams of catechin hydrate equivalent per gram (mg CE g^{-1}) dry weight.

Determination of antioxidant activity

Antioxidant activity in extracts of different broccoli tissues was determined using the DPPH radical-scavenging method according to Koleva et al. (2002) with modifications. The extracts obtained for total phenol analysis were also used for the measurement of antioxidant activity. A 400 µM DPPH solution in 80% methanol was prepared. Then, 100 µL of the DPPH solution were mixed with 100 µL of various concentrations (0.5, 1.0, 2.5, 5.0, 7.5, and 10.0 mg mL^{-1}) of the extracts in 96-well plates. After 30 min in darkness at room temperature, absorbance at 517 nm was measured in a micro plate reader (EON-C) (BioTek, USA) using 80% methanol without DPPH as a blank. Similarly, absorbance of samples was also measured after mixing 100-µL samples with 100 µL of 80% methanol. Free-radical-scavenging activity (%) was calculated using the following equation:

$$\% \text{ DPPH radical-scavenging activity} = (B - A) \times 100/B$$

Where, A is the absorbance of [(Sample + DPPH) – (Sample + Methanol)] and B is the absorbance of [(Methanol + DPPH) – (Methanol)]. The IC_{50} value, which is the concentration required to obtain 50% antioxidant capacity, was calculated and was used to compare the antioxidant activities of sample extracts.

Statistical analysis

Means of at least two independent sample replications were used for all statistical analyses. Data were analyzed using the SAS (version 9.2) software. The statistical significance of differences among cultivars, growing seasons, and plant tissues was assessed using a fixed-factor analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Glucosinolates content

Variations in glucosinolate profiles in florets, leaves, and stems of different broccoli cultivars during different growing seasons are presented in Tables 1, 2, and 3, respectively. Glucoraphanin was the most abundant glucosinolate in all three tissue types in both cultivars in both growing seasons. Glucobrassicin was the second most abundant glucosinolate in florets and leaves. Glucoerucin was present only in stems and was the second most abundant glucosinolate after glucoraphanin. Progoitrin, sinigrin, and gluconapin were found in only some cultivars, which showed different cultivar-dependent distribution patterns according to tissue type and growing season. Similar variations in glucosinolate distributions in different growing seasons were reported by Vallejo et al. (2003). In florets, the highest content of total glucosinolates was measured in the TS-2319 cultivar (17.81 µmol g^{-1}) in the spring season, while the JikNok No. 28 cultivar (13.80 µmol g^{-1}) had the highest glucosinolate levels in the fall season (Table 1). In both seasons, we observed lower glucosinolate levels than reported by Lee et al. (2012), who found total glucosinolate levels of 15.90–59.30 µmol g^{-1} from analysis of 95 broccoli accessions. This difference in values might be due to differences in genotypes and other environmental factors. Florets showed higher total glucosinolate levels (7.45 µmol g^{-1}) in the spring than in the fall (6.96 µmol g^{-1}); however, no specific trends were found among the cultivars. This result was somewhat similar to those of Charron and Sams (2004) and Justen et al. (2012) who also reported higher total glucosinolate amounts in spring growing conditions. These changes in glucosinolate concentration might be due to the interactions of several factors (Charron and Sams, 2004; Fabek et al., 2012). Two major glucosinolates, glucoraphanin and glucobrassicin, were present in all of the cultivars in both seasons, but other glucosinolates showed cultivar-dependent distribution patterns. The average total glucosinolate content was lower (4.20 µmol g^{-1}) in the spring than in the fall (5.35 µmol g^{-1}). Among the 12 cultivars, the highest total glucosinolate contents in the spring and fall seasons were found in NokYeom No. 1 (5.76 µmol g^{-1}) and AMaGi (6.73 µmol g^{-1}), respectively, suggesting their superiority in terms of leaf glucosinolate content.

Stems contained one additional glucosinolate, glucoerucin, not found in florets or leaves. Glucoerucin was one of the three major glucosinolates in stems in all cultivars and constituted approximately 25 and 10% of total glucosinolates in the spring and fall seasons, respectively (Table 3). Other major glucosinolates included glucoraphanin (spring, 2.19 µmol g^{-1}; fall, 3.60 µmol g^{-1}) and glucobrassicin (spring, 0.27 µmol g^{-1}; fall, 0.29 µmol g^{-1}). The average total glucosinolate content in stems showed a pattern similar to that in leaves with higher total glucosinolate levels in fall (4.96 µmol g^{-1}) than in spring (4.18 µmol g^{-1}). Among the 12 cultivars, only 2, BaeRiDom and TS-2319, contained all 6 glucosinolates analyzed in this study in their florets, leaves, and stems in both seasons. Among the three tissue types, florets exhibited the highest total glucosinolates, followed by leaves and stems, in both seasons, sug-

Table. 1. Seasonal variation in glucosinolate contents (μmol g^{-1}, dry weight) in florets of broccoli cultivars.

Cultivar	Progoitrin		Glucoraphanin		Sinigrin		Gluconapin		Glucobrassicin		Total glucosinolates	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
05-C3	0.00±0.00[z]	0.17±0.00	2.30±0.15	4.03±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.58±0.10	4.31±0.10	3.88±0.25	8.51±0.07
AMaGi	2.25±0.08	1.83±0.01	2.61±0.11	1.41±0.01	0.00±0.00	0.00±0.00	0.57±0.02	0.60±0.01	1.54±0.09	2.57±0.11	6.95±0.28	6.40±0.12
BaeRiDom	1.28±0.00	1.15±0.02	1.81±0.05	1.37±0.06	0.21±0.00	0.19±0.00	0.32±0.01	0.34±0.02	0.68±0.03	1.56±0.13	4.29±0.07	4.60±0.24
CheongJae	0.19±0.01	0.56±0.04	1.67±0.00	2.62±0.20	0.00±0.00	0.10±0.00	0.06±0.00	0.18±0.02	0.34±0.00	2.35±0.13	2.26±0.01	5.79±0.01
Diamond	0.00±0.00	0.00±0.00	4.34±0.02	2.05±0.14	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	3.51±0.06	1.63±0.06	7.85±0.08	3.68±0.20
Grace	0.00±0.00	0.00±0.00	5.83±0.33	1.75±0.10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.46±0.10	1.42±0.08	7.29±0.23	3.17±0.18
Grandeur	0.00±0.00	0.00±0.00	4.28±0.22	2.37±0.07	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	4.21±0.03	4.09±0.04	8.48±0.01	6.46±0.02
JikNok No. 28	2.58±0.25	3.72±0.14	3.38±0.26	5.12±0.52	0.00±0.00	0.00±0.00	0.83±0.01	0.97±0.02	1.98±0.21	4.00±0.04	8.76±0.73	13.8±0.32
NokJae	0.00±0.00	0.00±0.00	3.74±0.40	3.54±0.31	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.88±0.07	2.49±0.07	5.62±0.33	6.03±0.38
NokYeom No. 1	2.64±0.27	2.55±0.01	2.16±0.06	3.32±0.12	0.00±0.00	0.00±0.00	0.56±0.06	0.82±0.01	2.94±0.18	2.80±0.10	8.30±0.20	9.49±0.21
TS-2319	4.48±0.11	2.11±0.19	7.64±0.07	1.92±0.09	0.54±0.00	0.26±0.01	0.49±0.01	0.69±0.05	4.68±0.28	1.27±0.11	17.81±0.31	6.24±0.46
YuDoRi No. 1	1.86±0.07	2.36±0.19	3.31±0.09	3.49±0.09	0.30±0.03	0.00±0.00	0.44±0.01	0.61±0.04	2.09±0.18	3.00±0.20	7.99±0.18	9.45±0.53
Average	2.18	1.80	3.59	2.75	0.35	0.18	0.47	0.48	2.24	2.62	7.46	6.96

[z] Each value is the mean ± SD of two independent replications.

Table 2. Seasonal variation in glucosinolate contents (μmol g^{-1}, dry weight) in leaves of broccoli cultivars.

Cultivars	Progoitrin		Glucoraphanin		Sinigrin		Gluconapin		Glucobrassicin		Total glucosinolates	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
05-C3	0.00±0.00[z]	0.00±0.00	3.18±0.23	4.70±0.29	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.10±0.01	1.55±0.08	5.28±0.24	6.24±0.21
AMaGi	0.34±0.02	0.60±0.02	2.05±0.16	3.38±0.06	0.00±0.00	0.00±0.00	0.20±0.00	0.25±0.01	2.31±0.01	2.51±0.01	4.90±0.13	6.73±0.03
BaeRiDom	0.26±0.02	0.22±0.01	2.00±0.09	1.36±0.11	0.07±0.00	0.06±0.01	0.18±0.01	0.10±0.01	1.16±0.01	0.68±0.01	3.66±0.05	2.41±0.12
CheongJae	0.00±0.00	0.19±0.01	1.66±0.11	5.05±0.06	0.00±0.00	0.06±0.00	0.00±0.00	0.08±0.00	0.21±0.00	1.74±0.11	1.87±0.11	7.11±0.18
Diamond	0.00±0.00	0.00±0.00	1.27±0.09	4.13±0.31	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.02±0.02	2.16±0.02	3.29±0.10	6.28±0.33
Grace	0.00±0.00	0.00±0.00	3.56±0.03	4.11±0.05	0.00±0.00	0.00±0.00	0.12±0.01	0.00±0.00	0.75±0.03	1.02±0.02	4.42±0.05	5.13±0.07
Grandeur	0.00±0.00	0.00±0.00	2.40±0.07	3.70±0.31	0.00±0.00	0.00±0.00	0.10±0.00	0.00±0.00	2.22±0.06	3.14±0.17	4.72±0.01	6.84±0.14
JikNok No. 28	0.48±0.00	0.34±0.02	2.66±0.13	1.61±0.06	0.00±0.00	0.00±0.00	0.24±0.00	0.12±0.00	1.55±0.10	1.34±0.08	4.91±0.03	3.40±0.04
NokJae	0.00±0.00	0.00±0.00	3.39±0.11	7.45±0.33	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.84±0.08	1.27±0.06	5.23±0.03	8.72±0.39
NokYeom No. 1	0.54±0.00	0.45±0.04	1.92±0.00	2.07±0.11	0.00±0.00	0.00±0.00	0.20±0.00	0.12±0.00	3.10±0.00	1.68±0.08	5.76±0.00	4.31±0.23
TS-2319	0.10±0.01	0.38±0.01	1.56±0.04	2.82±0.07	0.05±0.00	0.08±0.00	0.11±0.00	0.15±0.01	2.01±0.03	0.77±0.03	3.82±0.01	4.19±0.05
YuDoRi No. 1	0.21±0.01	0.27±0.01	1.09±0.06	1.41±0.10	0.00±0.00	0.06±0.00	0.17±0.00	0.11±0.00	1.08±0.00	0.96±0.01	2.54±0.09	2.80±0.12
Average	0.32	0.35	2.23	3.48	0.06	0.06	0.16	0.13	1.70	1.57	4.20	5.35

[z] Each value is the mean ± SD of two independent replications.

Table 3. Seasonal variation in glucosinolate contents (μmol g^{-1}, dry weight) in stems of broccoli cultivars.

Cultivar	Progoitrin		Glucoraphanin		Sinigrin		Gluconapin		Glucoerucin		Glucobrassicin		Total glucosinolate	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
05-C3	0.00±0.00[z]	0.00±0.00	2.95±0.10	6.42±0.40	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.89±0.01	1.17±0.06	0.52±0.00	0.33±0.02	4.35±0.11	7.92±0.35
AMaGi	1.16±0.04	1.01±0.00	2.39±0.14	3.22±0.06	0.00±0.00	0.00±0.00	0.33±0.00	0.32±0.03	1.56±0.01	0.28±0.01	0.30±0.01	0.54±0.00	5.74±0.07	5.37±0.09
BaeRiDom	0.88±0.01	0.84±0.05	1.7±0.06	2.05±0.19	0.13±0.01	0.12±0.02	0.15±0.01	0.13±0.00	1.59±0.10	0.27±0.02	0.21±0.01	0.15±0.00	4.64±0.08	3.55±0.12
CheongJae	0.00±0.00	0.31±0.00	1.00±0.04	3.07±0.24	0.00±0.00	0.05±0.00	0.00±0.00	0.11±0.01	0.00±0.00	0.15±0.00	0.04±0.00	0.40±0.00	1.04±0.04	4.08±0.25
Diamond	0.00±0.00	0.00±0.00	2.65±0.12	3.98±0.12	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.36±0.01	0.48±0.03	0.39±0.00	0.24±0.01	3.39±0.13	4.70±0.09
Grace	0.00±0.00	0.00±0.00	2.98±0.25	3.79±0.06	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.41±0.07	0.54±0.00	0.29±0.03	0.29±0.01	5.68±0.21	4.61±0.04
Grandeur	0.00±0.00	0.00±0.00	2.77±0.24	5.82±0.18	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.48±0.00	0.49±0.01	0.53±0.04	0.56±0.01	3.77±0.20	6.86±0.18
JikNok No. 28	1.22±0.01	1.29±0.06	1.55±0.00	1.40±0.03	0.00±0.00	0.00±0.00	0.29±0.01	0.32±0.00	1.20±0.02	0.18±0.01	0.17±0.00	0.23±0.00	4.41±0.02	3.41±0.10
NokJae	0.00±0.00	0.00±0.00	4.18±0.33	7.22±0.08	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.09±0.07	0.37±0.01	0.26±0.01	0.22±0.00	5.53±0.27	7.81±0.07
NokYeom No. 1	1.11±0.02	1.33±0.10	1.16±0.07	2.46±0.13	0.00±0.00	0.00±0.00	0.97±0.04	0.20±0.00	1.29±0.04	0.30±0.01	0.23±0.03	0.24±0.00	4.76±0.13	4.52±0.02
TS-2319	0.62±0.00	0.82±0.01	1.65±0.07	2.15±0.20	0.11±0.00	0.09±0.01	0.16±0.00	0.14±0.00	0.78±0.03	0.53±0.00	0.15±0.00	0.11±0.00	3.46±0.04	3.84±0.36
YuDoRi No. 1	0.78±0.06	0.74±0.06	1.31±0.12	1.59±0.02	0.10±0.00	0.08±0.01	0.12±0.01	0.13±0.01	0.95±0.08	0.23±0.02	0.23±0.01	0.15±0.00	3.47±0.29	2.91±0.08
Average	0.96	0.90	2.19	3.60	0.11	0.09	0.33	0.19	1.14	0.42	0.27	0.29	4.19	4.96

[z] Each value is the mean ± SD of two independent replications.

gesting that florets are a good source of glucosinolates. Variation in glucosinolate amounts, measured as the coefficient of variation (CV %), was highest in florets in both the spring (CV 51.9%) and fall (CV 42.4%) seasons. Two major glucosinolates (glucobrassicin and glucoraphanin) as well as total glucosinolates exhibited higher variation in florets in the spring than in the fall, but the opposite was the case in leaves and stems. In our study, amounts of total as well as individual glucosinolates were significantly dependent on cultivar (C), plant part (P), and season (S) (Table 4) in most cases. Most of the interactions were significant, except for C x S and C x S x P for gluconapin and S x P for glucoerucin (Table 4). This result is in agreement with Rosa and Rodrigues (2001) and Vallejo et al. (2003) who also reported cultivar- and season-dependent variation in total and individual glucosinolate amounts. Such variation in total as well as individual glucosinolate contents in different plant tissues may be due to differences in the control mechanisms of the glucosinolate biosynthetic pathway, alteration of substrate availability, and degradation and mobilization of glucosinolates (Sang et al., 1984; Chen and Andreasson, 2001).

Vitamin C content

Vitamin C contents in different tissue types of broccoli cultivars cultivated in the spring and fall seasons are presented in Figure 1. Vitamin C amounts varied according to cultivar, tissue type, and season. In florets, vitamin C amounts ranged from 2.42 (JikNok No. 28) to 5.53 mg g^{-1} (Grandeur) with an average of 3.94 mg g^{-1} in the spring, and from 1.22 (NokJae) to 5.27 mg g^{-1} (BaeRiDom) with a lower average value of 3.05 mg g^{-1} in the fall. Variation in vitamin C amounts was higher in the fall (CV 41.7%) than in the spring (CV 23.0%). In leaves, the average vitamin C content (4.28 mg g^{-1}) was similar in both seasons, but variation was higher in the fall (CV 33.1%) than in the spring (CV 15.4%). In contrast, stems showed higher vitamin C levels (5.54 mg g^{-1}) in most of the cultivars and higher cultivar-dependent variation (CV 18.0%) in the spring than in the fall (CV 15.0%). The presence of higher vitamin C levels in the spring growing season might be due to higher temperature and radiation, as was reported previously for broccoli and other plants species (Merzlyak and Solovchenko, 2002; Hakala et al., 2003; Yao et al., 2005; Nilsson et al., 2006; Aires et al., 2011). However, no cultivar has

Table 4. Results of analyses of variance for antioxidant amounts and activityies in broccoli cultivars.

Source of variance	Progoitrin	Glucoraphanin	Sinigrin	Gluconapin	Glucoerucinin	Glucobrassicin	Total glucosinolate	Vitamin C	Total phenol	Total flavonoid	Antioxidant activity
Cultivar (C)	***	***	***	***	***	***	***	***	***	***	***
Season (S)	NS	***	***	NS	***	*	***	***	**	***	***
Plant Parts (P)	***	**	***	***	***	***	***	***	***	***	***
C X S	***	***	***	NS	***	***	***	***	***	***	***
C X P	***	***	***	**	***	***	***	***	***	***	***
S X P	*	***	***	*	NS	***	***	***	***	***	***
C X S X P	***	***	***	NS	***	***	***	***	***	***	***

NS, *, **, ***: Non-significant or significant at $p < 0.05$, 0.01, and 0.001, respectively.

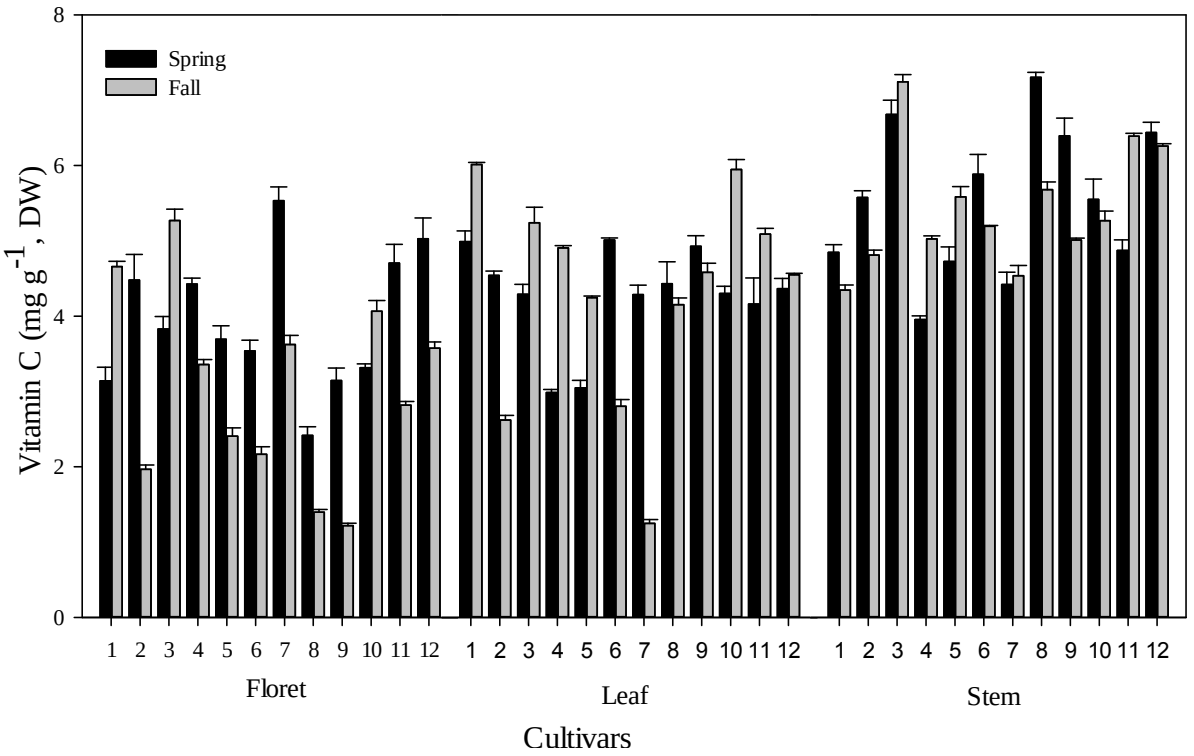

Figure 1. Seasonal variation in the vitamin C contents of florets, leaves, and stems of broccoli cultivars. Each bar represents the mean ± SD of three independent replications. 1, 05-C3; 2, AMaGi; 3, BaeRiDom; 4, CheongJae; 5, Diamond; 6, Grace; 7, Grandeur; 8, JikNok No. 28; 9, NokJae; 10, NokYeom No. 1; 11, TS-2319; and 12, YuDoRi No. 1.

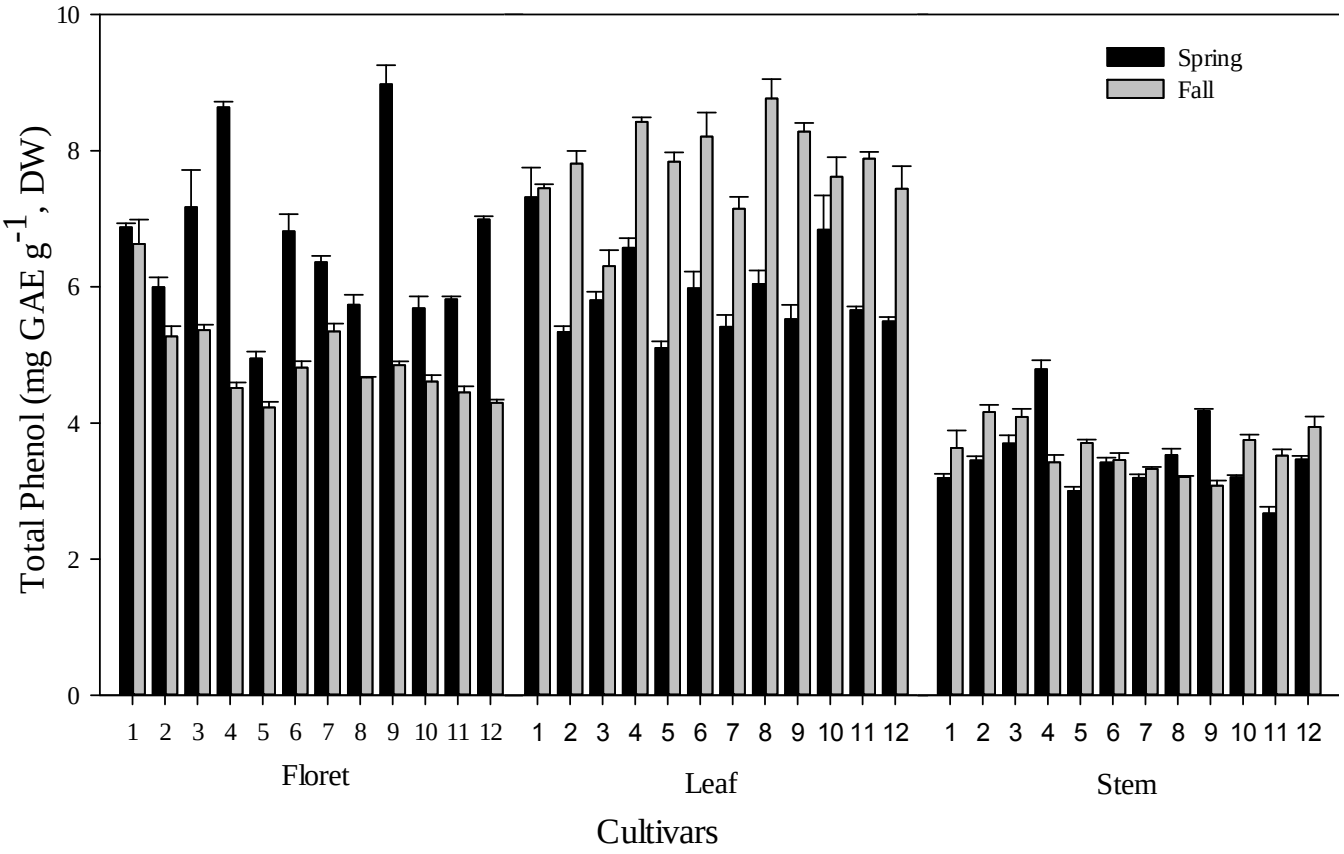

Figure 2. Seasonal variation in the total phenol contents of florets, leaves, and stems of broccoli cultivars. Each bar represents the mean ± SD of three independent replications. 1, 05-C3; 2, AMaGi; 3, BaeRiDom; 4, CheongJae; 5, Diamond; 6, Grace; 7, Grandeur; 8, JikNok No. 28; 9, NokJae; 10, NokYeom No. 1; 11, TS-2319; and 12, YuDoRi No. 1.

higher vitamin C contents in both seasons as well as their respective parts (florets, leaves and stems), which suggests that genotype is one of the most important factors and that the effects of tissue types and environmental factors associated with growing seasons on vitamin C content are dependent on the genotype.

The average vitamin C content was highest in stems (spring, 5.54 mg g^{-1}; fall, 5.44 mg g^{-1}), followed by leaves, and was lowest in florets, indicating that stems are a good source of vitamin C in broccoli. In contrast, cultivar-dependent variation was highest in florets in both growing seasons. The data revealed that vitamin C levels were significantly ($p < 0.001$) affected by C, P and S (Table 4). Similarly, all interactions (C x S, C x P, S x P, and C x S x P) were statistically significant. Kurilich et al. (1999) also reported significant differences in vitamin C content among various cultivars in different growing seasons. Our study is the first report, to our knowledge, of changes in vitamin C content in different plant tissues and in different growing seasons in broccoli. Although our results suggest that the vitamin C content in broccoli plants is higher in stems than in florets or leaves and higher in the spring than in the fall, further careful investigation of vitamin C content using fresh samples is required.

Total phenol content

Phenolic compounds, which are important secondary metabolites, possess various biological activities, the most important of which is antioxidant activity associated with reduced cancer risk (Manach et al., 2005; Picchi et al., 2012). The seasonal variation in total phenol content in different tissues of various broccoli cultivars is presented in Figure 2. In florets, the total phenol content in the spring growing season ranged from 4.95 mg GAE g^{-1} (Diamond) to 8.98 mg GAE g^{-1} (NokJae), with an average of 6.67 mg GAE g^{-1}; this was higher than the average phenol content in the fall season (4.92 mg GAE g^{-1}). The variation in total phenol content in florets was higher in the spring (CV 17.9%) than in the fall (CV 13.5%). In contrast, leaves and stems exhibited higher total phenol contents in the fall (7.76 mg GAE g^{-1} and 3.61 mg GAE g^{-1}, respectively) than in the spring (5.92 mg GAE g^{-1} and 3.48 mg GAE g^{-1}, respectively). Similar to florets, variation was higher in leaves (CV 11.3%) and stems (CV 15.9%) in the spring than in the fall. Similar to the results of Howard et al. (2002), we found higher total phenol content in florets (6.67 mg GAE g^{-1}) than in leaves or stems in the spring growing season, possibly due to the

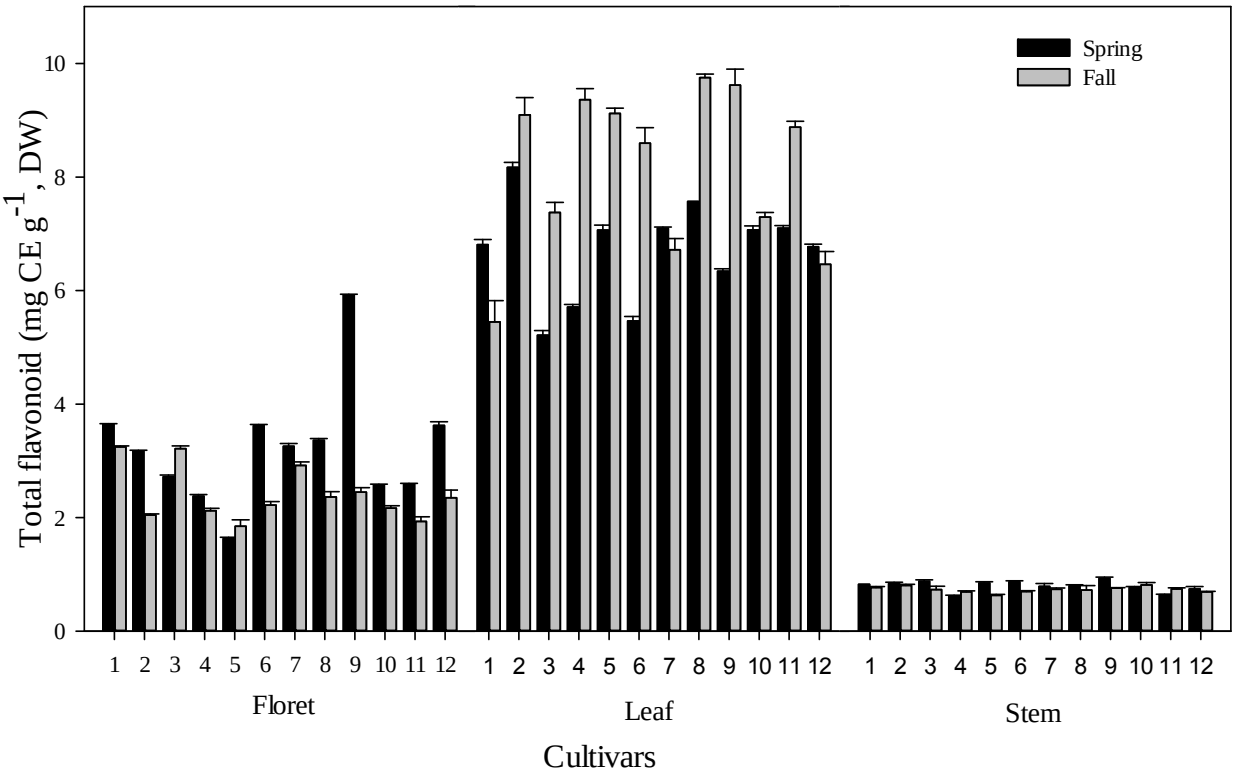

Figure 3. Seasonal variation in the total flavonoid contents of florets, leaves, and stems of broccoli cultivars. Each bar represents the mean ± SD of three independent replications. 1, 05-C3; 2, AMaGi; 3, BaeRiDom; 4, CheongJae; 5, Diamond; 6, Grace; 7, Grandeur; 8, JikNok No. 28; 9, NokJae; 10, NokYeom No. 1; 11, TS-2319; and 12, YuDoRi No. 1.

influence of biosynthesis of phenolic com-pounds; however, average total phenol contents were higher in leaves (7.76 mg GAE g^{-1}) and stems (3.60 mg GAE g^{-1}) in the fall season. Among the three tissue types, cultivar-dependent variation was highest in florets in both seasons (spring season, 17.9%; fall season, 13.5%). We found that the total phenol contents of broccoli were significantly affected by C, P, S and interactions between these factors (Table 4). However, seasonal variation was lower than cultivar-dependent and tissue-dependent variation in this case. Similar cultivar- and season-dependent variation was also reported by Eberhardt, et al. (2005), Singh et al. (2007) and Balouchi et al. (2011). However, this study describes the variation in total phenol among various tissue types of several broccoli cultivars.

Total flavonoid content

Flavonoids are important secondary plant metabolites (Koh et al., 2009) that possess strong antioxidant activity due to their ability to scavenge reactive oxygen species and inhibit oxidative stress (Hounsome et al., 2009). The seasonal variation in total flavonoid content in florets, leaves, and stems of broccoli cultivars is presented in Figure 3. Total flavonoid content in florets ranged in the spring from 1.63 mg CE g^{-1} in Diamond to 5.92 mg CE g^{-1} in NokJae, with an average of 3.21 mg CE g^{-1}; this was higher than the average flavonoid content in the fall season (2.41 mg CE g^{-1}). Total flavonoid content was also higher in stems in the spring (0.80 mg CE g^{-1}) than in the fall (0.74 mg CE g^{-1}). However, leaves had higher average total flavonoid contents in the fall (8.14 mg CE g^{-1}) than in the spring (6.70 mg CE g^{-1}). Flavonoid contents ranged from 5.45 to 9.75 mg CE g^{-1} in the 05-03 and JikNok No. 28 cultivars, respectively. Among the three tissue types, leaves had a significantly higher average total flavonoid content ca. threefold higher than in florets and eightfold higher than in stems in both seasons, indicating that leaves are a good source of flavonoids. Florets showed the highest cultivar-dependent variation in both the spring (CV 32.7%) and fall (CV 19.7%), while the variation was lowest in stems (spring, CV 11.8%; fall, CV 7.0%). No cultivar exhibited higher flavonoid contents in all tissue types and in both growing seasons. We found that total flavonoid content in broccoli was significantly ($p > 0.001$) affected by C, P, S and their interactions (Table 4), suggesting that total flavonoid content in broccoli cultivars is markedly influenced by genotype, growing season, and tissue type. Similar cultivar- and season-dependent variation was also reported by Koh et al. (2009) and Balouchi et al. (2011). However, this study addresses the variation in the total flavonoid contents in various tissue types of several broccoli cultivars.

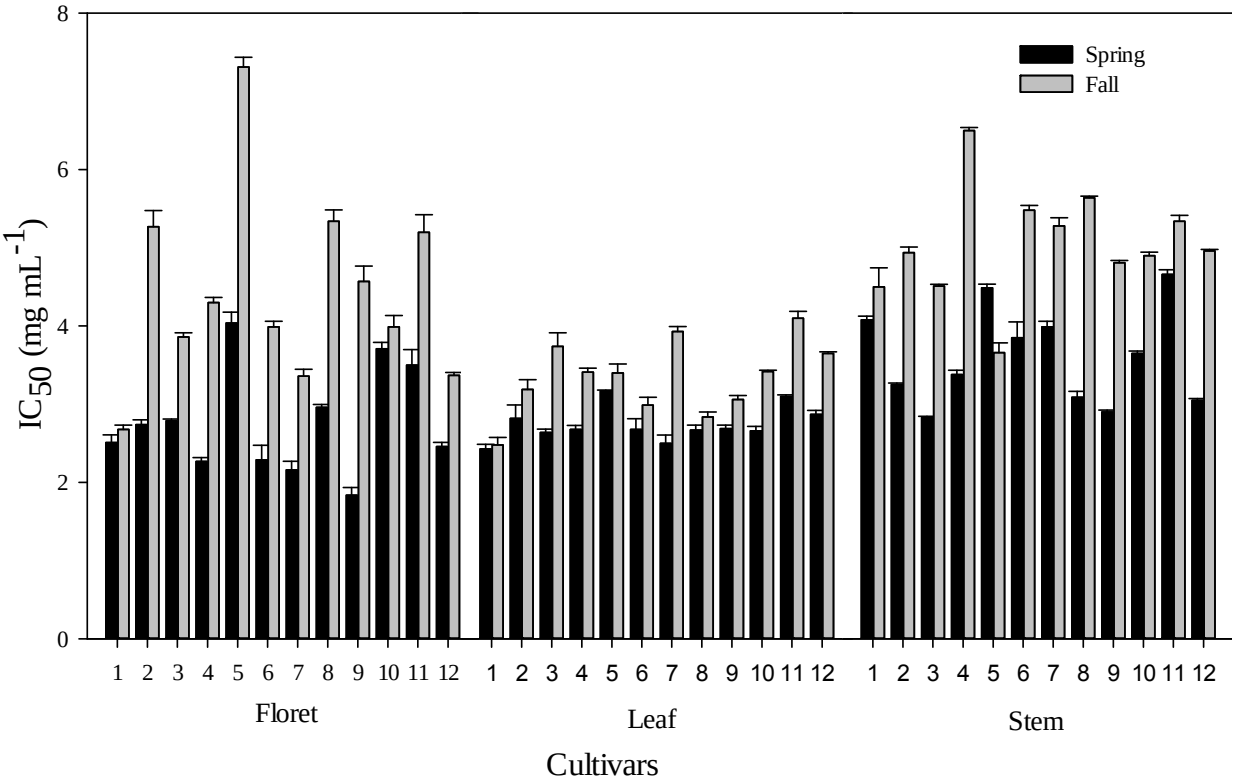

Figure 4. Seasonal variation in the antioxidant activities in florets, leaves, and stems of broccoli cultivars. Each bar represents the mean ± SD of three independent replications. 1, 05-C3; 2, AMaGi; 3, BaeRiDom; 4, CheongJae; 5, Diamond; 6, Grace; 7, Grandeur; 8, JikNok No. 28; 9, NokJae; 10, NokYeom No. 1; 11, TS-2319; and 12, YuDoRi No. 1.

Antioxidant activity

Antioxidant activities of broccoli samples were evaluated by measuring the DPPH radical scavenging activity of different concentrations of methanol extracts. IC_{50} (50% of inhibition) values were calculated after linear regression analysis of the observed inhibition percentages vs. concentration (Figure 4), where lower IC_{50} values indicate higher antioxidant activity. Measurement of DPPH radical scavenging activity is a technically simple and rapid method for the evaluation of antioxidant activity (Fukumoto and Mazza, 2000). In the spring season, the highest antioxidant activities were exhibited by NokJae (IC_{50} 1.84 mg mL^{-1}), 05-C3 (IC_{50} 2.43 mg mL^{-1}), and BaeRiDom (IC_{50} 2.83 mg mL^{-1}) in florets, leaves, and stems, respectively. In the fall, the antioxidant activities in florets, leaves, and stems were highest in 05-C3 (IC_{50} 2.68 mg mL^{-1}), 05-C3 (IC_{50} 2.48 mg mL^{-1}), and Diamond (IC_{50} 3.66 mg mL^{-1}), respectively (Figure 4). Almost all of the cultivars showed higher antioxidant activity in the spring than in the fall. The average antioxidant activities in the spring season in florets, leaves, and stems were 2.77, 2.74, and 3.60 mg mL^{-1}, respectively. This might be due to the higher temperature in the spring because temperature has an important influence on antioxidant activity (Aldrich et al., 2011). Among the three tissue types, leaves exhibited the highest antioxidant activity, followed by florets and stems. Cultivar-dependent variation was higher in florets in both the spring (CV 24.2%) and fall (CV 27.6%) than in leaves or stems. Similar to vitamin C, total phenol, and total flavonoid contents, antioxidant activity was also significantly influenced by C, P S and their interactions (Table 4).

Corelationships among phytonutrients

Several studies have evaluated the relationship between antioxidant activity and several antioxidants, such as vitamin C, phenolics, and flavonoids (Robards et al., 1999; Zhou and Yu, 2006; Sun et al., 2007; Aires et al., 2011; Naguib et al., 2012). To clarify the contribution of antioxidants to antioxidant activity and among phytonutrients (glucosinolates, vitamin C, total phenol and total flavonoid), we evaluated the correlations between vitamin C, phenolics, and flavonoids and antioxidant activity (Table 5). In this study, regardless of P or S, all of the glucosinolates showed significantly positive correlations with total glucosinolate contents, with the exception of glucoerucin, which was present only in stems and exhibited a non-significant negative correlation with total glucosinolates. Similarly, among the antioxidants, the total phenol content exhibited a significant positive correlation with total flavonoid content (r 0.808**), but a negative correlation with vitamin C content (r - 0.282**), and a non-

Table 5. Correlation coefficients among antioxidants and antioxidant activities in broccoli cultivars.

	Glucoraphanin	Sinigrin	Gluconapin	Glucoerucinin	Glucobrassicin	Total glucosinolate	Vitamin C	Total phenol	Total flavonoid	Antioxidant activity
Progoitrin	0.562**	0.983**	0.689**	-0.100	0.503**	0.837**	-0.367**	-0.205	-0.385**	-0.228*
Glucoraphanin		0.638**	0.234*	-0.098	0.269**	0.712**	-0.077	0.12	0.039	0.003
Sinigrin			0.799**	0.356	0.706**	0.881**	-0.188	0.091	-0.230	0.049
Gluconapin				-0.055	0.402**	0.594**	-0.439**	-0.204	-0.307**	-0.179
Glucoerucinin					-0.277**	-0.037	0.354**	-0.184	-0.169	0.247
Glucobrassicin						0.666**	-0.479**	0.378**	0.309**	0.286**
Total glucosinolate							-0.218**	0.086	-0.037	0.027
Vitamin C								-0.282**	-0.231**	-0.028
Total phenol									0.808**	0.674**
Total flavonoid										0.497**

*, **, Correlation is significant at $p < 0.05$ and 0.01, respectively.

significant positive correlation with total glucosenolate content (r 0.086 ^{NS}). Similarly, antioxidant activity exhibited a significantly positive correlation with total phenol content (r 0.674**) and total flavonoid content (r 0.497**). In contrast, non-significant positive and negative correlations were observed between antioxidant activity and total glucosinoate (r 0.027^{NS}) and vitamin C (r -0.028^{NS}) contents, respectively. The stronger correlations between antioxidant activity and total phenol content in this study are in agreement with Tavarini et al. (2008), Olajire and Azeez (2011), and Naguib et al. (2012), possibly due to the contribution of the high concentration of phenolics to antioxidant activity.

Conclusion

In this study, we identified changes in the amounts of various phytochemicals and in antioxidant activity in various broccoli tissues in different growing seasons. In most cases, the amounts of phytochemicals and antioxidant activities were significantly affected by C, P, S and their interactions. The average contents of vitamin C, glucosinolate, total phenol, and total flavonoid and antioxidant activities were significantly higher in florets in the spring than in the fall. Furthermore, florets exhibited the highest cultivar-dependent and season-dependent variation of all of the phytochemicals relative to leaves and stems. Similarly, leaves exhibited higher contents of phytochemicals in the fall. Stems exhibited higher levels of some of the phytochemicals in both seasons, indicating that seasonal variation in the phytochemical content of broccoli is dependent not only on genotype but is also affected by tissue type and the particular phytochemical compound. Similarly, leaves and stems had higher contents of vitamin C, total phenol, and total flavonoids than florets, indicating that leaves and stems are good sources of these phytochemicals.

ACKNOWLEDGEMENTS

This work was carried out with the support of the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ006722), Rural Development Administration, Republic of Korea.

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