

Original Article

Content of ascorbic acid, quercetin, kaempferol and total phenolics in commercial broccoli

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ABSTRACT

A total of 80 commercial broccoli samples were collected over a two-year period to determine seasonal variation in the levels of ascorbic acid (AA), vitamin C (the sum of AA and dehydroascorbic acid [DHAA]), the predominant flavonoids quercetin and kaempferol, and total phenolics at the consumer level. Levels of AA and vitamin C varied between 13.37–110.30 and 57.35–131.35 mg/100 g fresh weight (FW), respectively. The levels of quercetin and kaempferol ranged from 0.03 to 10.85 and 0.24 to 13.20 mg/100 g FW, respectively. Total phenolic activity ranged from 48.15 to 157.77 mg/100 g FW. Significant seasonal variation and year-to-year variability were observed in the content of all phytochemicals examined. Total phenolic levels correlated positively with levels of vitamin C, quercetin, and kaempferol. Based upon the USDA/ERS 2007 database for consumption based upon sales, and the data reported herein, the daily availability of vitamin C from broccoli was estimated at 8.99 mg; accounting for 15% of Reference Daily Intake (RDI). The average daily availability of quercetin, kaempferol and total phenolics from broccoli was estimated as 0.23, 0.32 and 5.50 mg, respectively.

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1. Introduction

Broccoli is one of the most commonly consumed green vegetables in the United States with per capita consumption of 8.3 pounds in 2005 (USDA/ERS, 2007). Like other species of the Brassica family, broccoli is a rich source of health promoting phytochemicals (Bahorun et al., 2004; Chun et al., 2005). Epidemiological studies have shown an inverse association between the consumption of Brassica vegetables and the risk of cancer (Day et al., 1994). Of the case-controlled studies, 56% demonstrate a strong association between increased broccoli consumption and the protection against cancer (Verhoeven et al., 1996). This protective effect has largely been attributed to the complement of phytochemicals, in broccoli which include the vitamins C and E, the flavonols quercetin and kaempferol, the carotenoids β -carotene and lutein, and the glucosinolates (Podsedek, 2007).

Flavonoids are particularly interesting as they are potent in vitro antioxidants (Duthie and Crozier, 2000; Pietta, 2000), display free radical scavenging activity (Van Acker et al., 1995), induce protective enzymes (Nijveldt et al., 2001) and are thought to play

key roles in many of the processes underlying vascular dysfunction and the development of atherosclerosis (Schroeter et al., 2006). The levels of quercetin and kaempferol, the predominant flavonoids in broccoli, are reported as less than 13.70 mg/100 g FW and 0.70–9.15 mg/100 g FW, respectively (USDA/ARS, 2007). In general, the levels of flavonoids in commercially available broccoli will depend on many factors including: cultivar (Vallejo et al., 2002), environmental pressures (Dixon and Paiva, 1995), agricultural production methods (Chassy et al., 2006; Esteban et al., 2001; Mitchell et al., 2007; Tovar et al., 2002) and post-harvest transport and handling (Vallejo et al., 2003a). Genotype is regarded as the most influential factor determining the levels of flavonoids in foods (Kurilich et al., 1999; Vallejo et al., 2002).

Agricultural commodities are rarely consumed at the farm gate and therefore post-harvest transport and storage conditions will influence the levels of labile and other key phytochemicals in commercial foods. Storage, transport and processing have been determined to significantly influence the levels of AA, glucosinolates and flavonoids in broccoli (Leja et al., 2001; Vallejo et al., 2003a; Van der Sluis et al., 2001; Wills et al., 1984). For example, Vallejo et al. (2003a) demonstrated major losses of total glucosinolates (71–80%) and total flavonoids (62–59%) but not vitamin C in broccoli inflorescences stored for 7 days at 1 °C to mimic cold storage followed by a 3-day period at 15 °C to mimic retail period. Conversely, Winkler et al. (2007) showed that

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extended storage (1 or 4 °C for up to 28 days) followed by a 3-day period did not influence glucosinolates or flavonoids in broccoli inflorescences. Vitamin C levels were not evaluated in this study. Although market-basket studies are not controlled studies, they provide valuable information about the actual concentrations of nutrients and phytochemicals that a consumer would normally encounter under typical post-harvest handling and storage conditions. However, market-basket studies require multiple sampling over extended periods in order to compensate for seasonal, annual and varietal variations. In a recent market-basket study, Harnly et al. (2006) evaluated the flavonoid content of more than 60 fresh fruits, vegetables and nuts commonly consumed in the United States. This study included broccoli, however, only a limited number of samples were evaluated and only twice in a one-year period.

To date, there is no systematic study representing seasonal variation throughout the year for the complement of flavonoids, AA and vitamin C in commercial broccoli representing a range of different cultivars, agricultural and post-harvest handling and storage condition histories. Nonetheless, there is a growing interest and need for quantitative data on the levels and variability of key phytochemicals in commercial produce. These values are critical for estimating availability and for developing accurate databases. Accordingly, the aims of this study were to determine the content of AA, vitamin C, the main flavonoids quercetin and kaempferol and total phenolics in commercial broccoli collected in even-numbered months over a two-year period (August 2005 to February 2007) and to estimate the daily availability of vitamin C and total phenolics from broccoli in the US population using these values and the USDA/ARS database for broccoli consumption.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu phenol reagent (2N), quercetin dihydrate (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one dehydrate, 98%), gallic acid (3,4,5-trihydroxybenzoic acid, 98%), and dehydroascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, 95%) and luteolin (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one, 98%) were obtained from Indofine (Hillsborough, NJ, USA). Ascorbic acid (99.5%), o-phosphoric acid (85%), m-phosphoric acid glacial (35.7%), sodium carbonate (99.8%), and the HPLC solvents were from Fisher Scientific (Fair Lawn, NJ, USA). DL-1,4-Dithiothreitol (DTT, 99%) was purchased from ACROS ORGANICS (Geel, Belgium).

2.2. Sampling and sample preparation

Fresh broccoli was purchased from 5 independent grocery stores located in Davis and Woodland California in the United States. Samples were collected on the same day of every other month over two years. Sampling dates include: August, October, and December (2005), February, April, October, and December (2006), and February (2007). A total of thirty independent bunches of broccoli were purchased each month; fifteen organic and fifteen conventional. These broccoli were pooled into three independent replicate samples within each category by randomly selecting three bunches of broccoli and combining them together. This process was repeated at each of the five locations sampled. In total, six samples for each month/location were collected. Commercial samples were taken, regardless of broccoli cultivar identification, in order to reflect real consumer purchases over different seasons as cultivars and growing locations change with seasons. To preserve freshness, broccoli was transported to the laboratory

on ice. Upon arrival, stalks were cut at lowest deviation from the florets and three florets per broccoli crown were randomly selected and apportioned into one of four vacuum pouches (Prime Source Packaging Ltd., Spring, TX, USA) for the analysis of moisture and flavonoids, vitamin C, total phenolics and long-term –80 °C storage. Samples were vacuum packed and immediately frozen using a blast freezer. Frozen samples were stored at –80 °C until chemical analysis were performed. All analyses were performed around 6 weeks post storage.

2.3. Determination of moisture

The moisture content of the samples was determined by freeze-drying. Approximately 25.0 g of each pooled sample was weighed into plastic weighing dishes (VWR, West Chester, PA, USA) and lyophilized in a VirTis freeze-drier (SP Industries Inc., Warminster, PA, USA) until the weight of the sample reached a constant value.

2.4. Determination of AA

The AA determination was based on the method of Sánchez-Mata et al. (2000) with a slight modification. To increase both extraction efficiency and sample homogeneity, frozen broccoli was rapidly pulverized into a homogenous powder using a wooden mallet. The powdered samples were then weighed into a 50-mL tube, mixed with 25 mL of 2.5% m-phosphoric acid on a vortex mixer for 1 min and centrifuged for 20 min at 4 °C at 3000 × g. The supernatant was filtered through a cheese cloth into a 50-mL volumetric flask, residue re-extracted with 20 mL of 2.5% m-phosphoric acid, centrifuged, filtered into the same volumetric flask, brought to volume and filtered through a 0.45 µm PTFE membrane with glass microfiber prefilter (Whatman, Florham Park, NJ). This extract was used to measure AA. For total vitamin C determination, 1.00 mL of the filtrate was treated with 0.20 mL of DTT (40 mg/mL) at 40 °C for 2 h for reduction of DHAA into AA. Extracts were analyzed using a Hewlett Packard series 1090 liquid chromatograph (Agilent, Palo Alto, CA, USA) equipped with a diode array detector monitoring at 245 nm. Separations were achieved on an Agilent Zorbax XDB C₁₈ column (4.6 × 250 mm, 5 µm) with a guard column (4.6 × 12.5 mm, 5 µm) of the same material (Santa Rosa, CA). An isocratic flow rate of 1.0 mL/min of 0.05 M KH₂PO₄ (pH 2.6) was used for analysis. The linear range of quantification of AA was 0.0125–0.1000 mg/mL.

2.5. Determination of total phenolic content

Total phenolic content was measured by the Folin-Ciocalteu method (Singleton and Rossi, 1965). Approximately 10 g of chopped, frozen broccoli was weighed into a 250 mL centrifuge bottle and was vortexed with 95 mL acetone/water (80/20, v/v) for 1 min. The samples were incubated for 1 h in the refrigerator, centrifuged at 10,444 × g for 20 min, filtered into a 100 mL volumetric flask through a cheese cloth and brought to volume. A 125 µL of the sample solution was placed in a glass tube, 125 µL of Folin-Ciocalteu reagent was added, vortexed and allowed to stand for 5 min at ambient temperature. Next, a 1.75 mL of 5% sodium carbonate in water was added, mixed, and then allowed to react for 1.5 h. The absorbance was measured at 765 nm on a Shimadzu UV-1700 UV-visible spectrophotometer (Jiangsu, China). Gallic acid was used as an external standard and the linear range of quantification was 0.05–0.20 mg/mL. AA and DHAA are known to contribute to the response measured in the Folin-Ciocalteu assay. Therefore, a correction factor is applied to prevent overestimation of total phenolics. Correction factors are determined by measuring the relative response of increasing concentrations of AA and DHAA in Folin-Ciocalteu assay over a range of

concentration typical to the samples being tested. In this study, the relative response of AA was measured over the range of 0.0125–0.100 mg/mL and DHAA was measured over a range of 0.010–0.100 mg/mL. A plot of absorbance against concentration resulted in a linear equation for the relative response of AA and DHAA. These equations are then used to correct for AA and DHAA in the samples as follows: (1) the content of AA and DHAA are determined in the sample using independently derived standard curves, (2) the absorbance at 765 nm corresponding to the measured amount of AA and DHAA is then determined using the respective linear equation derived from the relative response curves for AA and DHAA, (3) the total phenolic content is measured in the Folin-Ciocalteu assay measuring absorbance at 765 nm, (4) this value is then corrected by subtracting the absorbance contribution calculated using the relative response curves for AA and DHAA, and (5) the concentration of total phenolics is then determined using the corrected absorbance value and an independently generated standard curve.

2.6. Determination of flavonoids

Flavonoid analysis was based on the method of Merken and Beecher (2000). Frozen broccoli samples (2.0 g) were mixed with 80 mL of methanol/water (62.5:27.5, v/v) in a 500-mL round bottomed flask and spiked with an internal standard of luteolin to a final concentration of 0.005 mg/mL. Samples were refluxed for 4 h at 100 °C. A 1.5 h reflux time in 1.2N hydrochloric acid was used as the optimal condition for quercetin, kaempferol, and luteolin. The mixture was refluxed at 100 °C for 1.5 h with 20 mL of 6N hydrochloric acid to hydrolyze flavonoid glycosides. A 2.0-mL aliquot of the mixture was taken from the flask, cooled on ice and then centrifuged. A sub-aliquot of 200 µL of hydrosylate was mixed with 200 µL methanol. The mixture was sonicated for 10 min and filtered using 0.45 µm Millex-FH (PTFE) SLFH 013 NL (Millipore, Bedford, MA, USA) filters and analyzed on an Agilent HPLC equipped with a photodiode array detector. The column was a Zorbax Eclipse XDB C₁₈ column (4.6 × 250 mm, Agilent, Palo Alto, CA, USA). The mobile phase consisted of 0.05% trifluoroacetic acid (TFA) in water (A), 0.05% TFA in methanol (B), and 0.05% TFA in acetonitrile (C). The linear gradients used at a flow rate of 1.0 mL/min were as follows: 90–85% A, 6–9% B, 4–6% C (0–5 min), 85–71% A, 9–17.4% B, 6–11.6% C (5–30 min), 71–0% A, 17.4–85% B, 11.6–15% C (30–60 min) and 0–90% A, 85–6% B, 15–4% C (60–71 min). The linear range of quantification of quercetin, kaempferol, luteolin were 0.5–8.0, 0.5–10.00, and 0.25–10.00 µg/mL, respectively. Internal standard recovery was 92–93% for luteolin. We used standard curves to calculate the concentrations of flavonoids in broccoli. The results were expressed as mg per 100 g FW with consideration for luteolin recovery.

2.7. Estimation of daily availability

The average daily availability of phytochemicals from broccoli in the US population were calculated using the average experimentally determined values of vitamin C, total phenolics, quercetin and kaempferol (this study) and using the daily broccoli availability derived from US broccoli consumption per capita in 2005 (USDA/ERS, 2007).

2.8. Statistical analysis

Statistical analysis was performed with SAS software (SAS Institute, Cary, NC, USA). Data were from three different composites per sample. Seasonal variation was evaluated using one-way analysis of variance (ANOVA, Tukey method at $P < 0.05$) to determine the presence of significant differences in the

collection times. Associations among phytochemicals levels in broccoli were determined by using Pearson's correlation analysis. Year-to-year variability was generated by univariate analysis of variance (t -test) with the levels of phytochemicals in broccoli in the two consecutive years.

3. Results and discussion

A total of 80 fresh broccoli samples were analyzed for AA, vitamin C, total phenolics and flavonoids in order to establish actual levels of these key phytochemicals in typical broccoli that consumers would purchase at the market. Samples were collected from five local stores at approximately two-month intervals in Northern California during 2005–2007. A combination of organic and conventional broccoli was purchased at every market to reflect consumer trends of increased consumption of organic produce. As this was a market-basket study, no information for cultivar, growing conditions, or post-harvest storage conditions was available and therefore no distinction was made between cultivation practices.

3.1. Ascorbic acid and vitamin C

The levels of AA and vitamin C in broccoli varied between 13.37–110.30 and 57.35–131.35 mg/100 g FW, respectively (Table 1). The variation observed for AA was much larger than the range reported for Hawaiian broccoli (41.2–63.8 mg/100 g FW) by Franke et al. (2004). The range of vitamin C levels was similar to other reported values (Favell, 1998; Kurilich et al., 1999; Singh et al., 2007; USDA/ARS, 2006; Vallejo et al., 2002, 2003b), although our data are generally distributed in the upper range of these reported values. The variation of AA (8-fold) and vitamin C (2-fold) likely arises from various factors including seasonal cultivar selection and variations in post-harvest handling conditions. Kurilich et al. (1999) reported that the vitamin C levels in 50 subspecies of broccoli were dependent on the cultivar, and ranged from 54.0 to 119.8 mg/100 g FW. Carvalho and Clemente (2004) found that vitamin C levels in broccoli stored at 1 °C decreased over 15 days, with the most significant loss between 12 and 15 days. Similarly, vitamin C levels decreased over 3 days (13.2% loss) when stored at 15 °C simulating retail market conditions (Vallejo et al., 2003a).

Seasonal fluctuations in vitamin C and AA levels in commercial broccoli are given in Fig. 1. These results are in accordance with the result of Vallejo et al. (2003c) who demonstrated that vitamin C levels in broccoli were significantly influenced by the growing season. Levels of AA were lower, and demonstrated a wider range of variation, as compared to levels of vitamin C; suggesting that AA is rapidly oxidized to DHAA in fresh broccoli. Not surprisingly, an inverse association between AA and DHAA ($r = -0.71$) was found (Fig. 2). Shigenaga et al. (2005) found that the contents of reduced

Table 1

Content of moisture, ascorbic acid, vitamin C, total phenolics and flavonoids in commercial broccoli^a.

	Range	Mean ± SD
Moisture (%)	83.87–90.27	87.03 ± 1.29
Ascorbic acid (mg/100 g)	13.37–110.30	47.57 ± 18.84
Vitamin C (mg/100 g)	57.35–131.35	87.19 ± 12.75
Total phenolics (mg/100 g)	48.15–157.77	87.32 ± 25.60
Corrected total phenolics (mg/100 g) ^b	15.18–121.38	53.28 ± 23.62
Quercetin (mg/100 g)	0.03–10.85	2.27 ± 2.07
Kaempferol (mg/100 g)	0.24–13.20	3.14 ± 2.30

^a Represents the values in 80 commercial broccoli based on the fresh weight (FW).

^b Represents total phenolics contents after the deduction of the contribution of AA and DHAA.

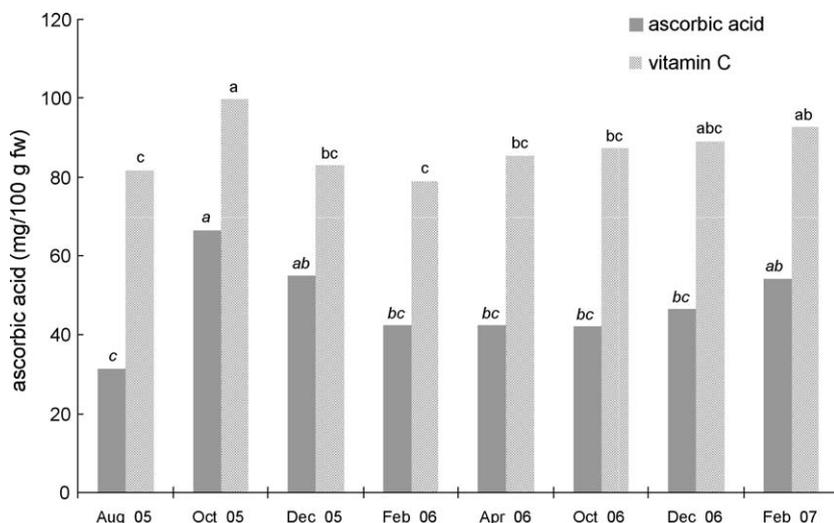


Fig. 1. Seasonal variation of the levels of ascorbic acid and vitamin C in commercial broccoli (2005–2007). The different letters on the top of bars represent a significant difference in the levels of ascorbic acid (italic) and vitamin C at $P < 0.05$.

glutathione and AA, which constitute ascorbate-glutathione cycle to reduce DHAA to AA in plant, decreased gradually after 6 days storage of broccoli. In fresh broccoli, AA is the predominant form of vitamin C, but the ratio of DHAA/vitamin C increased from 5% to 26% after 4 days storage at 20 °C (Vanderslice et al., 1990; Wills et al., 1984). Our results indicate that approximately 33–62% of the vitamin C is present as DHAA in commercial broccoli. These findings suggest that the rate of oxidation of AA is faster than the reduction of DHAA probably due to decreased capacity of ascorbate-glutathione cycle during the post-harvest storage of broccoli.

3.2. Total phenolics

Total phenolics are presented in Table 1 and expressed as mg of gallic acid equivalents/100 g FW. Total phenolics ranged from 15.18 to 121.38 mg/100 g FW over the two years. This is a larger variation as compared to the combined range (80.76–109.9 mg/100 g) reported for other studies (Baharun et al., 2004; Chu et al., 2002; Gliszczynska-Świgło et al., 2006). However, we would like to point out that these studies did not correct for AA in the total phenolic assay. AA can react with the Folin-Ciocalteu reagent used in the determination of total phenolic compounds (Singleton and Rossi, 1965). Interestingly, we found that DHAA is also reactive

with Folin-Ciocalteu reagent with a contribution factor of 0.146, which was four times lower than the factor determined for AA (0.599). As these studies did not correct for AA or DHAA, they likely resulted in the overestimation of total phenolics in broccoli and possibly mask subtle changes in phenolic levels.

Similar to vitamin C, the levels of total phenolics in fresh produce are influenced by several factors including cultivar selection, growing season and storage conditions. The levels of total phenolics can vary by up to 200% between different cultivars of broccoli (Eberhardt et al., 2005; Singh et al., 2007). Total phenolics are also higher in over-wintered spinach harvested in the spring as compared to spinach, which was planted and harvested in the fall, indicating that growing conditions influenced the biosynthesis of phenolic compounds (Howard et al., 2002). In the present study, there was greater seasonal variability in the levels of total phenolics as compared with levels of vitamin C (Fig. 3). This is not surprising as total phenolics represent a range of secondary plant metabolites including vitamin C.

3.3. Flavonoids

Flavonoids are important secondary plant metabolites which increase with plant stress (Dixon and Paiva, 1995; Harborne and Williams, 2000). Herein we demonstrate that the ranges of

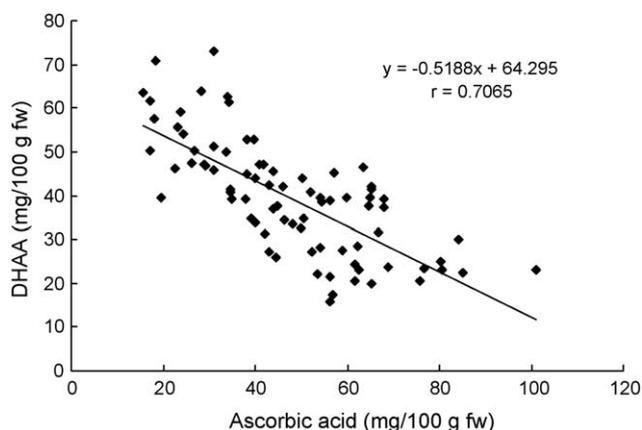


Fig. 2. Correlation between the content of ascorbic acid and dehydroascorbic acid in commercial broccoli (2005–2007).

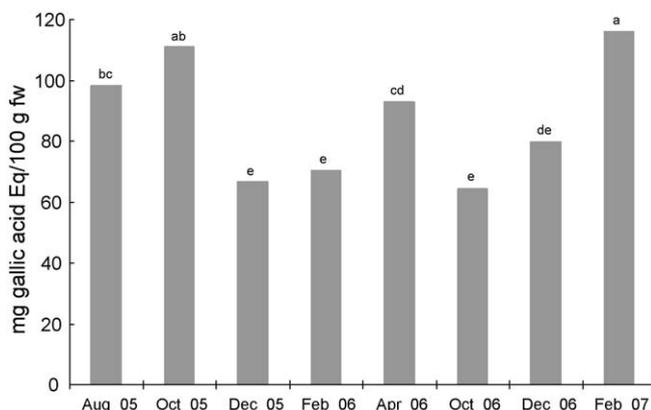


Fig. 3. Seasonal variation of total phenolics content in commercial broccoli (2005–2007).

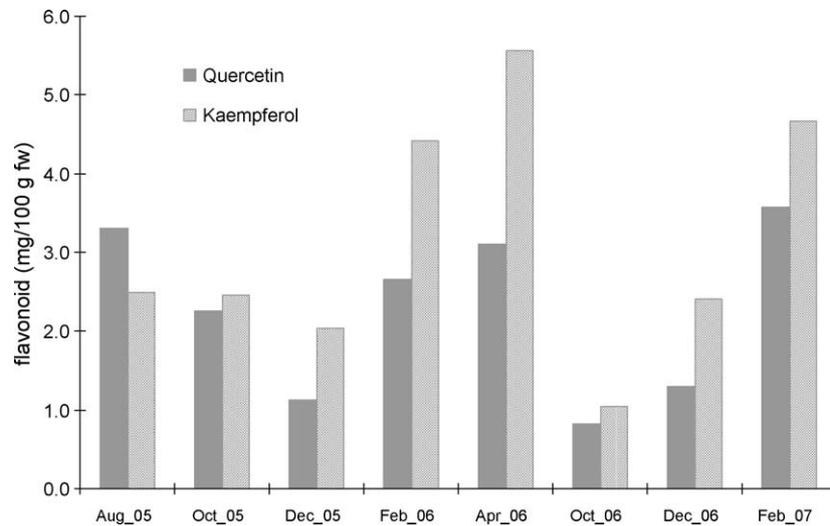


Fig. 4. Seasonal variation of the levels of quercetin and kaempferol levels in commercial broccoli (2005–2007).

quercetin and kaempferol aglycones were 0.03–10.85 mg and 0.24–13.20 mg/100 g FW, respectively (Table 1). These levels are similar to the ranges reported for quercetin (0–13.70 mg/100 g) and kaempferol (0.7–9.15 mg/100 g) in the USDA flavonoid database (USDA/ARS, 2007). The mean value of quercetin (2.27 mg/100 g FW) herein is much higher than the 0.4 mg/100 g found in broccoli reported by Harnly et al. (2006), yet similar to the USDA database value of 2.51 mg/100 g (USDA/ARS, 2007). Variation in the levels of flavonoids in vegetables can be attributed to numerous factors including: genotype, agronomic environment, developmental stage at harvest and post-harvest handling conditions (Gliszczynska-Swiglo et al., 2007; Vallejo et al., 2002, 2003b). Although flavonoids have been reported to be generally stable under refrigerated conditions after harvest (Van der Sluis et al., 2001; Winkler et al., 2007), Vallejo et al. (2003a) demonstrated that the total flavonoid content decreased up to 59% during transportation as compared to broccoli at harvest. In the current study, we demonstrate that the levels of flavonoids, especially kaempferol, varied considerably month-to-month over the two-year period (Fig. 4).

Most of earlier studies demonstrated that kaempferol was the most abundant flavonoid, followed by quercetin in broccoli (Franke et al., 2004; Gliszczynska-Swiglo et al., 2007; Price et al., 1998), whereas Bahorun et al. (2004) reported that quercetin levels were about five times higher than kaempferol levels in broccoli. As shown in Fig. 4, on average the most abundant flavonoid in this study was kaempferol over the two-year period, with an exception of August 2005 where levels of quercetin predominated. Seasonal variation in quercetin, possibly due to cultivar selection or environmental conditions might help to explain the discrepancy between these two findings.

3.4. Pearson's correlation between phytochemicals

Pearson's correlation coefficients were generated to determine the possible association between the phytochemicals of interest. All correlations were either positive or not significant (Table 2). AA correlated highly with vitamin C in broccoli. The levels of quercetin and kaempferol, which are produced via the same biosynthesis pathway in plants, were significantly correlated. Interestingly, total phenolics correlated positively and highly with AA, DHAA and both flavonoids, indicating that the level of total phenolics may be a good indicator for predicting, or comparing the levels of these phytochemicals in broccoli.

3.5. Year-to-year variability

In order to determine year-to-year variability, broccoli was collected twice on October, December, and February in the consecutive two years. As shown in Table 3, the levels of total phenolics differed significantly from year-to-year ($P < 0.01$ or $P < 0.001$). Of three time points observed, only October demonstrated annual variability for AA, DHAA, both flavonoids and total phenolics. These results demonstrate the importance of replicating analysis across multiple harvests when measuring secondary plant metabolites such as phenolic acids and flavonoids in fruits and

Table 2

Pearson's correlation coefficients between the contents of ascorbic acid, vitamin C, total phenolics, quercetin and kaempferol in broccoli in the fresh weight basis^a.

	Vitamin C	Total phenolics	Quercetin	Kaempferol
Total phenolics	0.6088 <.0001			
Quercetin	0.3836** 0.0025	0.6821 <.0001		
Kaempferol	0.1907 0.1445	0.5676 <.0001	0.9055 <.0001	
Ascorbic acid	0.7336 <.0001	0.4485 0.0003	0.3021* 0.0190	0.1661 0.2047

^a Numbers below each correlation coefficient are probability values. Values in bold are significantly different at $P < 0.001$ and asterisks (* and **) represent a significant difference at $P < 0.05$ and $P < 0.01$, respectively.

Table 3

The comparison of 2005 with 2006 in the levels of ascorbic acid, vitamin C, total phenolics, quercetin and kaempferol in broccoli grown on October, December, and February^a.

Component	October	December	February
Ascorbic acid	0.0045**	0.2414	0.1285
Vitamin C	0.0352*	0.1861	0.0107*
Total phenolics	0.0003***	0.0090**	0.0002***
Quercetin	0.0097*	0.7025	0.4595
Kaempferol	0.0044**	0.5243	0.8179

Asterisks (*, **, ***) represent significantly different at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

^a The statistics were generated by univariate analysis of variance to test if there are differences between the means of two different years on phytochemical levels in broccoli.

Table 4
Estimates of the average daily availability of phytochemicals from broccoli^a.

	Average ^b (mg/100g FW)	Availability (mg/day)	
		Fresh	Fresh + freezing
Vitamin C	87.19	6.07	8.99
Total phenolics ^c	53.28	3.71	5.50
Quercetin	2.27	0.16	0.23
Kaempferol	3.14	0.22	0.32
Total ^d		9.78	14.49

^a Calculated as follows: average daily availability (mg/day)=the average of vitamin C or total phenolics in broccoli (mg/100g FW)/100×daily broccoli availability of fresh (7g) or total (the sum of fresh and frozen, 10.3g). Daily availability of broccoli was calculated by using US per capita broccoli consumption in 2005 on the USDA/ERS updated February 15, 2007.

^b Obtained from this study.

^c Corrected for the contribution of ascorbic acid and dehydroascorbic acid.

^d Sum of the daily availability of vitamin C and total phenolics because total phenolics includes quercetin and kaempferol.

vegetables (Chassy et al., 2006; Gil et al., 2002; Hajslova et al., 2005; Howard et al., 2002).

3.6. Average daily availability

In the United States, annual fresh broccoli consumption has increased up to 5.6 pounds per capita in 2005 from 0.53 pounds in 1970, while annual frozen broccoli consumption per capita has grown at a slower rate from 1 pound in 1970 to 2.7 pounds in 2005 (USDA/ERS, 2007). Taking into consideration that California is the largest broccoli producer, accounting for 91% of total US production in 2002 (Brunke, 2003), the broccoli samples used in this study should reflect the broccoli available in most markets. To date, estimates of the daily availability of flavonoids and total phenols from broccoli are confounded from using data derived from a limited number of sample that are typically restricted to a single season or year (Chun et al., 2005; Vinson et al., 1998).

Therefore, we estimated the average daily availability of vitamin C, total phenolics and quercetin and kaempferol using the mean values reported herein, and by referencing the US database for average broccoli consumption based on sales per capita (USDA/ERS, 2007). These results are given in Table 4. The RDI for vitamin C (60 mg) is based on a caloric intake of 2000 calories per day (US FDA, 1998). Our data indicate that broccoli contributes about 8.99 mg vitamin C per day, accounting for 15% of RDI for vitamin C. The daily availability of total phenolics (5.50 mg) from broccoli in this study was slightly lower (6.7 mg) than intake values reported by of Vinson et al. (1998) and approximately 2-fold higher than the 2.4 mg value estimated by Chun et al. (2005). Interestingly, the average values of quercetin and kaempferol were lower than those reported in the USDA flavonoids database (2.51 mg/100 g for quercetin and 4.01 mg/100 g kaempferol) (USDA/ARS, 2007). Our estimates indicate that the average daily availability of quercetin and kaempferol from fresh broccoli are 0.23 and 0.32 mg, respectively (Table 4). These values are lower than the estimated daily flavonol intake (the sum of quercetin, kaempferol and myricetin; 1.4 mg) reported by Sampson et al. (2002). However, these are based upon flavonoids levels found in typical Dutch foods.

4. Conclusions

This study demonstrated the substantial seasonal variation in the levels of AA, vitamin C, total phenolics and flavonoids in commercial broccoli, possibly due to variability in cultivar

selection, growing conditions and/or post-harvest storage conditions. The range of AA, DHAA, quercetin, kaempferol and total phenolics in broccoli were generally broader than the range of values reported in the USDA databases for these compounds. Additionally, the average values for vitamin C and the flavonoids were lower than USDA database values. Total phenolics levels, in particular, were found to be significantly influenced by the season as well as cropping year.

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