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Carotene, Tocopherol, and Ascorbate Contents in Subspecies of *Brassica oleracea*

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Cruciferous vegetables contain high levels of vitamins that can act as antioxidants, compounds that may protect against several degenerative diseases. The edible portions of 50 broccoli and 13 cabbage, kale, cauliflower, and Brussels sprouts accessions were assayed to determine variation in α-carotene, β-carotene, α-tocopherol, γ-tocopherol, and ascorbate contents within and between subspecies of *Brassica oleracea*. Ascorbate content was estimated in fresh samples using HPLC. Tissues for carotene and tocopherol analysis were lyophilized prior to extraction. Carotene and tocopherol concentrations were simultaneously measured using a reverse phase HPLC system. Results indicate that there is substantial variation both within and between subspecies. Kale had the highest levels of vitamins, followed by broccoli and Brussels sprouts with intermediate levels and then by cabbage and cauliflower, with comparatively low concentrations. Variability in vitamin content among the broccoli accessions suggests that potential health benefits that accrue with consumption are genotype dependent.

Keywords: *Brassica oleracea; carotenes; tocopherols; ascorbate; antioxidants*

INTRODUCTION

Fruits and vegetables contain compounds that contribute to health and wellness both by their traditional nutritive value and through enhancing the body's defense against chronic disease. Several carotenes, tocopherols, and ascorbate act both as traditional vitamins and as antioxidants. Carotenes have the potential for inhibition of neoplastic transformation (Bertram and Bortkiewicz, 1995; Bushway, 1985), photoprotection (Mathews-Roth, 1986), immunoprotection (Bendich and Shipiro, 1986), and activation of gene expression (Bendich, 1993). Both α- and β-carotenes are precursors to vitamin A, which is important for healthy skin, bone, gastrointestinal, and respiratory systems. Tocopherols provide antiproliferative effects (Azzi et al., 1995), anticlotting activity (Dowd and Zheng, 1995), and immunoprotection (Meydani, 1995) as well as reduction of platelet adhesion and protection against platelet-induced thrombosis formation (Opie, 1995; Steiner, 1993). Vitamin E activity of the tocopherols is important for maintaining stable cell membranes and preventing oxidative damage to tissues (Combs, 1992). Ascorbate is involved in synthesis of collagen tissue, metal ion metabolism, anti-histamine reactions, and enhancement of the immune system (Combs, 1992). Throughout the rest of this paper the carotenes, tocopherols, and ascorbate will be referred to as vitamins.

Several epidemiological studies have indicated an inverse association between consumption of vegetables from *Brassica oleracea* (*B. oleracea*) and a reduced risk of cancer (Stoewsand et al., 1988; Wattenberg et al., 1989; Peto et al., 1981). This biological action is considered to be due in part to a group of phytochemicals termed glucosinolates (Verhoeven et al., 1997). These secondary metabolites have been shown to break down into bioactive components that when added to experimental diets protect animals from cancer (Verhoeven et al., 1997). However, there are other components in cruciferous vegetables, such as the vitamin antioxidants, that may also play a role in offering protection against degenerative disease. As antioxidants, the carotenes, tocopherols, and ascorbate have the potential to prevent and treat malignant diseases (Byers et al., 1992; Evangelou et al., 1997). Epidemiological studies have associated these vitamins with a reduced risk of several types of cancer (Mirvish, 1986). However, under the right conditions, these compounds may also act as prooxidants as seen for β-carotene in several recent studies (Rautalahti et al., 1997; Potter, 1997).

Although carotene, tocopherol, and ascorbate concentrations in subspecies of *B. oleracea* have been quantified in some cases (Khachik et al., 1986; Granando et al., 1992; Hart and Scott, 1995; Piironen et al., 1996; Booth et al., 1963; Vanderslice et al., 1991), no study has been conducted that includes data on all three of these primary antioxidants, nor have surveys been conducted on a broad range of germplasm. Many previous studies have been limited by the use of bulked samples with no knowledge of genotype or postharvest conditions. In this study, we assayed the edible portions of 63 genotypes from 5 of the *B. oleracea* groups including var. Italica (broccoli), var. Capitata (cabbage), var. Acephala (kale), var. Botrytis (cauliflower), and var.

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Gemmifera (Brussels sprouts). Fifty of the 63 accessions surveyed were broccoli. Emphasis was placed on this subspecies due to its economic importance and consumer preference. An accompanying paper (Kushad et al., 1999) surveys these same genotypes for variability in glucosinolate content.

This investigation was conducted to provide consumers and dieticians with a more complete survey of the variation in carotene, tocopherol, and ascorbate concentrations found in *B. oleracea* vegetables. The genetic variation uncovered in this study will also provide plant breeders with information relevant to the development of cultivars with increased content of compounds with potential health benefits.

EXPERIMENTAL PROCEDURES

Plant Material. Seeds of broccoli, cauliflower, Brussels sprouts, cabbage, and kale were kindly provided by Peto Seeds, Asgrow Seed Co., the USDA Plant Genetic Resource Unit (Cornell University), and Dr. Mark Farnham at the USDA Vegetable Research Center (Charleston, SC). Lines included commercial hybrids, open-pollinated varieties, land races, inbreds, and doubled haploids generated from commercial hybrids (Farnham, 1997). During the 1996 season, seeds were germinated in the greenhouse and grown for 4 weeks before transplantation into plots at the University of Illinois, South Farm, in Champaign, IL. The field design was a randomized complete block with three replications containing 8−10 plants per replication. Irrigation, fertilization, and pest control were applied according to standard commercial practices.

Broccoli and cabbage heads, cauliflower curds, kale leaves, and Brussels sprout buttons were harvested at commercial maturity. Approximately equal amounts of tissue were collected from three to six plants in each replicate and bulked. All samples were placed on ice for transport to the laboratory. A 100 g sample of each was weighed and frozen in liquid nitrogen. Due to the large number of samples, the tissue was lyophilized and ground into a powder to preserve the lipid-soluble vitamin content. Freeze-dried tissue was stored at −20 °C until further analysis. A second 100 g subsample of fresh tissue was weighed and immediately analyzed for ascorbate content. Moisture content was determined by comparing the fresh weight of the tissue to the lyophilized weight.

Carotenoid and Tocopherol Extraction and Analysis. Carotenes and tocopherols were extracted according to a modification from a procedure published by Weber (1987). Lyophilized tissues of each genotype were extracted from the three field replicates under yellow lights, using 300 mg each of broccoli, Brussels sprouts, and kale and 600 mg each of cabbage and cauliflower in 25 mL glass test tubes. Ten milliliters of ethanol containing 0.1 g of butylated hydroxytoluene was added to each sample before it was placed in a 70 °C water bath for 15 min. Upon removal from the water bath, 180 μL of 80% potassium hydroxide was added to each tube, and samples were vortexed and then saponified in a water bath at 70 °C for 30 min. Saponification was deemed necessary for maximal extraction of carotenes and their esters (Khachik et al., 1986; Hart and Scott, 1995). Samples were placed directly into an ice bath where 2.5 mL of double-distilled, deionized water and 2.5 mL of hexane/toluene (10:8) were added. Tubes were vortexed for 20 s before centrifugation at 1000*g* (2100 rpm) for 5 min. The upper layer (hexane/toluene fraction) was then transferred to a separate test tube. Hexane/toluene extractions were repeated two more times. The combined hexane/toluene fractions were dried using a Speed Vac Plus SC 110A (Savant, Holbrook, NY). Residue was reconstituted in 400 μL (kale), 300 μL (broccoli and Brussels sprouts), or 200 μL (cabbage and cauliflower) of tetrahydrofuran (THF) prior to injection (20 μL) onto the liquid chromatograph.

Carotene and tocopherol concentrations were simultaneously quantified using reverse phase, high-performance liquid chromatography (HPLC). The system consisted of a Waters model 680 automated gradient controller, a model 510 pump, a model 710B autosampler, and a model 490E programmable multiwavelength detector (Millipore Corp, St. Louis, MO). A C18 reverse phase 250 × 4.6 mm 5 μ Prodigy ODS (3) column protected by a 30 × 4.6 5μ Prodigy ODS (3) guard column from Phenomenex (Torrance, CA) was used. The mobile phase was a modification of one published by Bushway (1985) for carotene separation with a combination of acetonitrile/methanol/THF at 52:40:8 (v/v/v). It was degassed using an ERC 3510 degasser from ERMA Optima Ltd. (Anspec Co., Ann Arbor, MI). Flow rate was 2 mL/min. Absorbance was measured at 290 and 450 nm simultaneously for tocopherols and carotenes, respectively.

For identification and quantification of carotenes and tocopherols, α-carotene, β-carotene, α-tocopherol, and γ-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO) and used as standards. Carotene standards were prepared in HPLC grade hexane, and tocopherol standards were prepared in absolute ethanol. Compound identification was based on retention time of known standards. Individual standards were also added to samples to confirm retention time of spiked peaks. Concentrations of standards were determined spectrophotometrically using spectral absorption coefficients: α-carotene = 2800 at 444 nm, β-carotene = 2592 at 452 nm, α-tocopherol = 75.8 at 292 nm, and γ-tocopherol = 91.4 at 298 nm (NIST, 1994).

Recoveries were 95, 114, 81, and 80% for α-carotene, β-carotene, γ-tocopherol, and α-tocopherol, respectively. These values were based on average concentrations from one sample replicated three times and compared to three replicates of the same sample spiked with known concentrations of compounds. Within-day and between-day variation measurements were based on the average standard deviations of three samples run three times in 1 day and the same three samples run three times over the following 2 days. Within-day variabilities for total carotenes and tocopherols were ±0.14 and ±0.43 μg/g, respectively. Between-day variations for total carotenes were ±0.97 and ±2.41 μg/g for total tocopherols, respectively.

Broccoli samples of Wintergarden and Majestic were sent to the USDA in Beltsville, MD, for independent analysis of carotene content. The following values were reported for α- and β-carotene, respectively: Wintergarden, 1.8 and 40.0 μg/g of dry weight; Majestic, 1.90 and 41.5 μg/g of dry weight. In addition, samples of Majestic were sent to the University of Illinois—Chicago campus for independent analysis of tocopherol content. They reported 79.6 μg/g of dry weight for γ-tocopherol, but α-tocopherol was below the level of detection. These concentrations are comparable to data reported in this investigation; we found α-carotene and β-carotene contents for Wintergarden were 1.81 and 51.5 μg/g, respectively; α-carotene, β-carotene, and γ-tocopherol contents for Majestic were 1.55, 44.3, and 77.3 μg/g of dry weight, respectively. We were also able to detect levels of α-tocopherol in Majestic using our protocol.

Ascorbate Extraction and Analysis. Extracts for ascorbate analysis were prepared by blending 100 mL of 1% *m*-phosphoric acid and 25 g of fresh frozen tissue in a Waring blender for 2 min. The sides of the blender jar were washed with 1% *m*-HPO_3 (50 mL) and blended for an additional 2 min. The slurry was adjusted to 250 mL with 1% *m*-HPO_3 and then filtered using Whatman 2V fluted filter paper. One milliliter of filtrate and 1 mL of 5% dithiothreitol were mixed. Sample extracts were diluted to 10 mL with 1% *m*-HPO_3. The sample was filtered through a 0.20 μm filter, and 10 μL was injected onto the liquid chromatogragh.

Ascorbate concentrations were measured using an isocratic HPLC system consisting of a Beckman model 421 controller, a Beckman model 100A pump, and a Beckman Altex C-R1A integrator. The detector was a Waters M-490 programmable multiwavelength (Millipore, St. Louis, MO.). The stationary phase was a Rainin Dynamax −60 Å amine, 4.6 × 250 mm column protected by a Rainin Dynamax amine 8 μm, 1.5 cm guard column (Varion, Walnut Creek, CA). The mobile phase consisted of acetonitrile/0.05 M KH_2PO_4 (pH 5.95), 75:25.

Table 1. Carotene, Tocopherol, and Ascorbate Means and Ranges in Edible Tissues of *B. oleracea*[a]

	α-carotene	β-carotene	α-tocopherol	γ-tocopherol	ascorbate
broccoli					
mean ($n = 51$)	0.03 b	0.89 b	1.62 a	0.13 b	74.71 a
range	0−0.073	0.37−2.42	0.46−4.29	0.02−0.64	54.0−119.8
cauliflower					
mean ($n = 2$)	ND	0.07 c	0.17 c	0.06 c	41.98 b
range		0.07−0.08	0.16−1.20	0.05−0.08	39.6−44.3
Brussels sprouts					
mean ($n = 4$)	0.01 c	0.90 b	0.83 b	0.04 cd	NA
range	0.00−0.011	0.77−1.02	0.49−1.20	0.02−0.05	
cabbage					
mean ($n = 5$)	0.002* c	0.08 c	0.17 c	0.005** d	27.32 c
range	0.00−0.002	0.01−0.13	0.06−0.27	0.00−0.006	22.6−32.9
kale					
mean ($n = 2$)	0.06 a	4.86 a	1.92 a	0.23 a	NA
range	0.05−0.07	3.65−6.08	1.03−2.80	0.15−0.31	
LSD	0.017	0.536	0.330	0.049	6.168

[a] All values are reported in mg/100 g of fresh weight. *, measurable in one accession only; **, measurable in two accessions only. Means with different letters are significantly different at $p < 0.05$. ND, below level of detection; NA, not available due to tissue limitations.

Detection was at 268 nm with a sensitivity of 0.02 AUFS. Flow rate was 1.5 mL/min.

Ascorbic acid standards (USPC, Inc., Rockville, MD) were prepared by diluting 0.01 ± 0.002 g of USP grade L-ascorbic acid to 100 mL with 1% *m*-HPO_3. This solution (1 mL) was mixed with 5% dithiothreitol (1 mL) and diluted to 10 mL with 1% *m*-HPO_3 to produce a 10 ppm ascorbic acid standard.

Statistical Analysis. Analysis of variance was performed on data between and within subspecies. Least significant differences (LSD) were determined among genotypes at $p \leq 0.05$%. Analysis of variance (SAS Institute, 1988) was performed for early- and late-harvested broccoli accessions. Associations among individual vitamin concentrations in broccoli were determined using Pearson's correlation analysis (SAS Institute, 1988).

RESULTS AND DISCUSSION

Cruciferous vegetables, including subspecies of *B. oleracea*, are relatively abundant sources of antioxidants with potential anticarcinogenic activity. Among the subspecies shown to be high in antioxidants are Brussels sprouts, cabbage, kale, cauliflower, and broccoli (Cao et al., 1996). Among the 22 vegetables assayed by Cao and co-workers, kale was the most potent vegetable source of antioxidants, with Brussels sprouts and broccoli ranked third and fifth, respectively.

Sixty-three accessions of broccoli, cauliflower, cabbage, kale, and Brussels sprouts were surveyed for α- and β-carotene, α- and γ-tocopherol, and ascorbate contents to obtain a profile of variability between (Table 1) and within (Table 2) subspecies of *B. oleracea*. Concentrations of β-carotene reported in this study were generally in accordance with previously published results (Khachik et al., 1986; Granando et al., 1992; Mangels et al., 1993; Souci et al., 1994; Hart and Scott, 1995). In contrast, α-carotene levels reported here are generally greater than those previously published. Tocopherol content was similar to that reported by Piironen et al. (1986), Bauernfeind (1980), and Souci et al. (1994) for broccoli, cauliflower, Brussels sprouts, and cabbage. Kale was not surveyed in either of the first two studies, and our values were lower than those reported by Souci et al. (1994). However our study, like that of Souci, shows kale as having the highest tocopherol content of all the subspecies evaluated. From a comparison of these data with previously published work, freeze-drying of the tissue does not appear to result in changes in lipid-soluble vitamin concentration. The variations between these values and other published values are not dramatic and may be due to differences in genotype assayed and extraction procedure used. Ascorbate content of *B. oleracea* subspecies has been reported (Van Duyne et al., 1945; Vanderslice et al., 1991; Souci et al., 1994). These earlier studies reported slightly higher amounts of ascorbate in cabbage compared to our survey. However, the ascorbate levels in broccoli that we report are within the same range as previously reported, although our range is substantially broader.

On the basis of concentration, the most important vitamins are β-carotene, α-tocopherol, and ascorbate. Carotenes and tocopherols are found in lipid-soluble fractions of biological systems, such as membranes and low-density lipoproteins. They protect cellular membranes by scavenging or physically quenching free radicals. Ascorbate is a water-soluble free radical scavenger found in cytosol, plasma, and other bodily fluids. It is found in relatively higher concentrations (30−150 μmol/L) in the plasma than either carotenes (0.3−0.6 μmol/L) or tocopherols (15−40 μmol/L) (Sies and Stahl, 1995). In relation to their ability to react with peroxy radicals, β-carotene has the highest rate constant at 1.5×10^9 mol L^{-1} s^{-1} and, therefore, the greatest antioxidant activity, followed by α-tocopherol at 5×10^8 mol L^{-1} s^{-1} and ascorbate at 2×10^8 mol L^{-1} s^{-1} (Sies and Stahl, 1995). With respect to quenching of oxygen free radicals, again, β-carotene has the highest rate constant followed by α-tocopherol and ascorbate (5×10^9, 8×10^7, and 1×10^7 mol L^{-1} s^{-1}, respectively) (Sies and Stahl, 1995).

Synergistic activity between these antioxidants has also been observed. Evangelou et al. (1997) reported that high doses of ascorbate and tocopherol together could be associated with prevention and treatment of malignant tumors. Palozza and Krinsky (1992a) observed inhibition of lipid peroxidation by a combination of β-carotene and α-tocopherol in rat liver. These authors have also provided evidence that α-tocopherol protects β-carotene from oxidation (Palozza and Krinsky, 1992b).

Compound Variation among *B. oleracea* Subspecies. The variability of each compound between or within a subspecies is relevant because it can be used to estimate the potential maximal concentration of each compound that can be achieved through genetic manipulation. The greater the variability for a specific

Table 2. Means and Coefficients of Variation (%CV) of Each Antioxidant for Individual Accessions Surveyed within Each Subspecies of *B. oleracea*[a]

accession	*N*	% moisture	α-carotene		*β*-carotene		α-tocopherol		*γ*-tocopherol		ascorbate		seed source[b]
			mean	%CV	mean	%CV	mean	%CV	mean	%CV	mean	%CV	
broccoli													
De Cicco	3	84.5	0.029	21	0.87	33	1.70	37	0.066	22	88.98	27	C
Green Comet	3	85.3	0.017	9	0.50	13	0.46	83	0.040	21	119.80	40	C
Pinnacle	2	87.3	0.021	4	0.64	3	1.04	13	0.108	33	77.97	10	C
Premium Crop	3	83.6	0.021	66	0.53	49	0.71	45	0.033	27	98.85	9	C
Zeus	3	82.1	0.019	13	0.57	5	0.67	20	0.137	15	75.57	10	C
Shogun	3	81.6	0.030	20	0.81	15	1.37	24	0.088	21	56.04	27	C
Packman	3	87.4	0.015	0	0.49	35	0.60	37	0.020	61	67.24	21	C
Atlantic	3	80.8	0.032	34	1.47	7	2.61	9	0.138	23	65.45	31	C
G31824	3	84.6	0.013	31	0.49	11	1.26	7	0.221	38	82.17	24	C
G31825	3	85.0	0.018	26	0.60	11	0.81	4	0.062	14	79.29	39	C
Brigadier	3	83.8	0.021	23	0.83	33	1.41	36	0.064	29	86.62	15	C
Pirate F1	2	81.9	0.027	6	0.80	7	2.20	1	0.118	10	71.89	14	C
Greenbelt	3	83.2	ND		0.80	14	1.49	3	0.137	6	57.59	27	C
Persius	2	79.8	0.038	1	1.11	1	1.64	7	0.117	10	57.96	24	C
Wintergarden	3	81.6	0.033	17	0.95	18	1.45	30	0.162	31	68.33	8	C
High Sierra	3	82.7	0.037	9	0.96	30	3.31	30	0.136	36	80.57	4	C
Cavolo Broccolo RC	2	74.1	0.073	49	2.42	20	3.49	26	0.293	0	97.99	17	C
Peto 3	3	83.3	ND		0.75	8	1.62	0	0.068	6	81.90	8	B
Peto 5	3	83.1	0.023	42	0.75	30	1.53	35	0.058	36	103.78	25	B
Peto 6	3	83.0	0.033	15	0.84	7	1.16	36	0.090	8	67.94	20	B
Peto 7	3	76.1	0.043	14	1.21	2	4.29	6	0.166	2	NA		B
Peto 8	3	83.1	0.024	17	0.95	5	0.68	45	0.078	29	96.68	10	B
Peto 10	2	78.4	0.032	5	0.74	15	0.53	66	0.111	23	NA		B
Peto 11	3	82.1	0.029	25	0.67	21	1.12	32	0.061	73	75.69	11	B
Peto 12	2	83.7	0.016	5	0.94	9	0.75	0	0.053	6	69.04	6	B
Peto 13	3	81.7	0.034	29	1.55	11	4.07	6	0.176	12	70.57	9	B
Peto 14	3	82.3	0.026	8	1.06	25	2.22	8	0.102	49	68.24	21	B
Peto 15	3	82.4	0.028	5	1.19	7	2.70	21	0.118	13	59.55	20	B
Peto 16	3	84.0	0.035	30	1.15	15	1.93	31	0.097	29	60.84	23	B
Eu 4-1	2	82.9	0.021	26	0.94	16	0.78	79	0.092	57	55.76	5	D
Eu 8-1	2	83.7	0.035	15	1.20	2	3.31	6	0.178	25	70.54	13	D
GV 8-1	3	81.2	0.019	14	0.78	14	0.54	58	0.068	15	58.67	8	D
GC 3-2	3	83.5	0.037	12	1.52	25	1.05	18	0.072	22	67.22	26	D
GC 1-2	3	85.7	0.042	2	0.93	26	1.14	15	0.047	29	63.86	67	D
EV 6-1	3	84.8	0.022	31	0.58	14	0.69	15	0.206	24	69.63	6	D
EV 2-1	3	82.6	0.032	13	1.12	18	1.00	21	0.185	31	81.23	19	D
Fu 549 DH IS	2	83.6	0.018	47	0.42	47	1.10	47	0.185	47	68.74	2	D
Su 006 DH IS	3	82.9	0.024	19	1.13	20	3.78	16	0.145	16	55.29	6	D
VI 158 DH IS	2	84.0	0.023	7	0.37	70	1.51	20	0.148	9	75.83	18	D
HS 061 DH IS	3	78.2	0.026	39	0.98	28	2.54	18	0.161	8	67.80	29	D
HS 067 DH IS	3	79.9	0.023	16	0.74	5	2.06	20	0.137	8	NA		D
MA 065 DH IS	3	84.5	0.020	11	0.65	15	2.15	26	0.241	27	82.33	15	D
MA 191 DH X6	2	84.0	0.024	13	0.37	65	1.74	26	0.543	87	62.63	4	D
Legacy	3	85.3	0.018	15	0.52	9	0.90	21	0.115	21	60.54	13	A
Majestic	3	83.6	0.025	19	0.73	10	1.27	25	0.097	85	71.97	16	A
Gem	2	84.4	ND		0.93	11	1.34	14	0.094	2	100.39	19	A
Galaxy	3	84.6	0.033	20	0.71	14	0.57	20	0.057	62	85.99	19	A
Baccus	3	87.1	0.022	12	0.90	21	1.06	15	0.049	23	65.78	25	A
Florette	2	81.8	ND		1.15	1	1.09	61	0.641	87	NA		A
Big Sur	3	81.1	0.045	29	1.01	22	2.33	41	0.098	27	86.00	1	A
cauliflower													
Peto 17	3	91.1	ND		0.08	1	0.18	18	0.077	31	44.33	13	B
Snow Crown	3	92.9	ND		0.07	2	0.16	2	0.048	53	39.63	22	C
Brussels sprouts													
Long Island	2	83.6	0.004	16	0.77	14	1.20	17	0.054	17	NA		C
Jersey	2	83.9	0.009	12	1.02	19	0.66	3	0.047	7	NA		C
Cambridge No. 5	2	84.0	0.008	53	0.78	0	0.97	22	0.036	18	NA		C
Yates Darkcrop	3	83.8	0.011	35	1.00	28	0.49	28	0.020	24	NA		C
cabbage													
Jersey Wakefield	3	94.0	ND		0.01	25	0.06	63	0.005	100	22.61	24	C
PI 214148	3	92.0	ND		0.04	7	0.06	39	ND		31.85	19	C
Peto 22	3	92.2	0.002	4	0.10	10	0.21	3	ND		22.84	17	B
Peto 23	3	90.0	ND		0.12	2	0.27	0	0.006	48	26.47	17	B
Peto 24	3	90.8	ND		0.13	9	0.24	2	ND		32.82	18	B
kale													
Winterborne	2	80.8	0.071	1	6.08	7	2.80	5	0.305	0	NA		C
Vates	3	85.9	0.048	75	3.65	32	1.03	80	0.153	72	NA		C
LSD ($p = 0.05$)			0.015		0.37		0.62		0.164		24.62		

[a] All values are in mg/100 g of fresh weight. ND, below level of detection. NA, not available. LSD, least significant difference. [b] A, Asgrow Seed Co.; B, Peto Seeds; C, USDA Plant Genetic Resource Unit (Cornell University); D, USDA Vegetable Research Center.

Table 3. Correlations between α-Carotene, β-Carotene, α-Tocopherol, γ-Tocopherol, and Ascorbate in Broccoli Accessions[a]

	α-carotene	β-carotene	α-tocopherol	γ-tocopherol
β-carotene	0.7907			
	0.0001			
α-tocopherol	0.5276	0.5936		
	0.0002	**0.0001**		
γ-tocopherol	0.1921	0.1383	0.2220	
	0.2009	0.3383	0.1213	
ascorbate	0.0577	−0.0457	−0.1312	−0.1446
	0.7133	0.7632	0.3848	0.3376

[a] Numbers below each correlation coefficient are probability values. Values in bold are significantly different at $p < 0.05$.

trait, the greater the opportunity for genetic improvement by plant breeding.

Variability among subspecies is shown in Table 1. The variation observed here may be due to several causes, including genetic and environmental influences as well as differences in plant tissues used for analysis. Immature flower buds were analyzed in broccoli and cauliflower, whereas leaf tissue was used in cabbage and kale, and axillary buds were analyzed in Brussels sprouts. Another source of variation may be associated with the number of days from planting to harvest, which varied from 55 to 89 days among broccoli accessions (data not shown). To test this hypothesis, the first 10 accessions harvested were compared to the last 10. No significant differences were found based on early versus late harvest for any of the antioxidants surveyed here.

On the basis of concentration, ascorbate was the most abundant vitamin antioxidant observed among the subspecies. Due to tissue limitations, ascorbate was not quantified in accessions of kale and Brussels sprouts. Food composition tables indicate that a substantial amount of ascorbate can be found within these subspecies (Souci et al., 1994). Broccoli had the highest levels of ascorbate relative to all other groups, followed by cauliflower. All subspecies contained measurable amounts of β-carotene and α-tocopherol. However, α-carotene and γ-tocopherol were generally found in lower concentrations and were below the level of detection (0.00001 and 0.0001 mg/100 g, respectively) in some accessions (Table 2). Kale contained comparatively high amounts of β-carotene and α-tocopherol, whereas broccoli and Brussels sprouts had moderate levels and cabbage and cauliflower relatively low amounts. In general, the best sources of all compounds are kale and broccoli. Broccoli, as the only subspecies with a substantial data set, was utilized to investigate compound variability within a subspecies.

Compound Variation within Var. Italica. The public perceives all broccoli as a good source of vitamins and anticarcinogens. However, Table 2 shows that there is substantial diversity in vitamin content within this subspecies. This diversity indicates that potential health benefits depend greatly on the genotype consumed.

If one is to improve vitamin content of vegetable varieties through plant breeding, it is important to know the extent to which variability is associated with genetic control and how these compounds interact in their biosynthesis. To address the first question, analysis of variance was conducted (SAS Institute, 1988) across broccoli accessions. The analysis of variance indicated that 79% of β-carotene, 82% of α-tocopherol, and 55% of ascorbate total experimental variation is associated with genetic differences among the broccoli accessions, with the remainder affiliated with variation among replicates. Although this information is limited to data from one year at one location, it does suggest that accessions with enhanced vitamin content can be produced through genetic manipulation and plant breeding efforts. Current research is underway to estimate genetic and environmental variability over several years.

To investigate the biosynthetic interaction among these vitamins, Pearson correlation coefficients were generated using means of individual compounds among the broccoli accessions (Table 3). All correlations were positive or nonsignificant between compounds. Discussion of correlations involving compounds with relatively low concentrations (α-carotene and γ-tocopherol) should be treated with some caution due to proportionally higher coefficients of variation associated with lower mean concentrations. The carotenes and tocopherols are synthesized via the mevalonic acid pathway, which may partially explain the significant positive correlation between β-carotene and α-tocopherol concentrations. The data indicate that β-carotene is positively and highly correlated with both α-carotene and α-tocopherol, suggesting that increasing the levels of one vitamin could result in increased levels of the other vitamins. Surprisingly, γ-tocopherol was not correlated with α-tocopherol. The proposed pathway for tocopherol biosynthesis suggests that γ-tocopherol is converted to α-tocopherol (Mann, 1995). Allelic variability among the accessions at loci encoding enzymes regulating rates of α-tocopherol conversion may be responsible for this lack of correlation. If this is the case, then tocopherol levels could be genetically manipulated so that the γ-form could be increased without a concomitant reduction in α-tocopherol concentration. Ascorbate was not correlated with any of the other antioxidants surveyed here. This is not surprising because it is found in the aqueous phase, whereas carotenoids and tocopherols are associated with the lipid phase in biological systems. These results do, however, indicate that carotenoid, tocopherol, and ascorbate levels could be enhanced simultaneously, without efforts to increase one negatively affecting another. In an accompanying paper (Kushad et al., 1999) in which glucosinolate concentrations were quantified in the same accessions, γ-tocopherol was observed to correlate with total aliphatic glucosinolates ($r = 0.28$, $p \leq 0.05$).

This study was the first phase of a program designed to enhance the concentration of several vitamins with potential health benefits in *B. oleracea* subspecies. On the basis of the above information, the next phase of the program will focus on developing new germplasm with increased levels of these vitamins. A subset of the accessions surveyed here will be grown in additional environments and over years to obtain a more precise understanding of genetic influence on these compounds. A breeding program has been instigated to enhance antioxidant levels in these vegetables and identify molecular markers associated with genes influencing individual compounds. Ultimately, bioassays using animals will be conducted to determine actual doses of tissue required for anticarcinogenic activity.

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