

Variation of Glucosinolates in Vegetable Crops of *Brassica oleracea*

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Glucosinolates were evaluated in 5 groups and 65 accessions of *Brassica oleracea* (50 broccoli, 4 Brussels sprouts, 6 cabbage, 3 cauliflower, and 2 kale) grown under uniform cultural conditions. Glucosinolates and their concentrations varied among the different groups and within each group. The predominant glucosinolates in broccoli were 4-methylsulfinylbutyl glucosinolate (glucoraphanin), 3-butenyl glucosinolate (gluconapin), and 3-indolylmethyl glucosinolate (glucobrassicin). Glucoraphanin concentration in broccoli ranged from 0.8 $\mu\text{mol g}^{-1}$ DW in EV6-1 to 21.7 $\mu\text{mol g}^{-1}$ DW in Brigadier. Concentrations of the other glucosinolates in broccoli varied similarly over a wide range. In Brussels sprouts, cabbage, cauliflower, and kale, the predominant glucosinolates were sinigrin (8.9, 7.8, 9.3, and 10.4 $\mu\text{mol g}^{-1}$ DW, respectively) and glucobrassicin (3.2, 0.9, 1.3, and 1.2 $\mu\text{mol g}^{-1}$ DW, respectively). Brussels sprouts also had significant amounts of gluconapin (6.9 $\mu\text{mol g}^{-1}$ DW). Wide variations in glucosinolate content among genotypes suggest differences in their health-promoting properties and the opportunity for enhancement of their levels through genetic manipulation.

Keywords: *Glucosinolates; broccoli; Brassica oleracea; health; HPLC*

INTRODUCTION

Epidemiological data show that a diet rich in cruciferous vegetables, such as broccoli, cabbage, Brussels sprouts, cauliflower, and kale, can reduce the risk from a number of cancers and that the risk can be significantly reduced by an intake of as little as 10 g per day (Graham, 1983; Wattenberg, 1993; Kohlmeier and Su, 1997). The underlying mechanism(s) for the reduction of cancer by cruciferous vegetables is not clear. However, these vegetables are rich in sulfur-containing glucosides called glucosinolates. Glucosinolates are a class of more than 100 plant secondary metabolites present primarily in the crucifer family (Fenwick and Heaney, 1983). Glucosinolates exhibit minimal anticancer activity; however, upon cell damage they undergo hydrolysis by myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) to yield glucose, sulfate, and aglucones that can undergo fragmentation and/or molecular rearrangement yielding isothiocyanates, thiocyanates, oxazolindine-2-thiones, and nitriles, depending on the specific glucosinolate substrate, myrosinase isozyme, reaction pH, and presence of certain ions (Bones and Rossiter, 1996). A number of aglucone breakdown products have been reported to protect cells against cancer (Faulkner et al., 1998; Kohlmeier and Su, 1997; Tawfiq et al., 1995).

Several mechanisms have been proposed for cancer prevention by breakdown products of glucosinolates. There is substantial evidence linking ingestion of a glucosinolate rich diet with an increase in activity of phase II detoxification enzymes. Nijhoff et al. (1995)

reported that consumption of cooked Brussels sprouts reduced colorectal cancer and enhanced the synthesis of the detoxification enzyme glutathione *S*-transferase. In murine hepa1c1c7 cells, glucosinolate breakdown products from cruciferous vegetables have been proposed to act as blocking agents against carcinogenesis by inducing quinone reductase activity (Tawfiq et al., 1995). The isothiocyanates, indole-3-carbinol (a product of glucobrassicin), phenylethylisothiocyanate (a product of gluconasturtiin), and sulforaphane (a product of glucoraphanin) have been shown to upregulate the synthesis of hepatic detoxification enzymes (Bradfield and Bjeldanes, 1984; Guo et al., 1992; Zhang et al., 1992) and protect against carcinogenesis (Morse et al., 1989; Wattenberg and Loub, 1978; Zhang et al., 1994). While most of the nitrile breakdown products have not been evaluated for their anticancer activity, a recent study demonstrated that the nitrile crambene (a product of progoitrin or epiprogoitrin) also upregulated the detoxification enzymes glutathione *S*-transferase and quinone reductase (Staack et al., 1998) and protects against aflatoxin-induced hepatocarcinogenesis in rats (Jeffery et al., 1996).

Comparative studies of glucosinolate distribution and variability between and within groups of the most widely consumed cruciferous vegetables such as broccoli, cabbage, cauliflower, Brussels sprouts, and kale are limited. To our knowledge, there are only two studies that have compared glucosinolate levels in the edible portions of more than three groups of these vegetables (Carlson et al., 1987b; Mullin and Sahasrabudhe, 1977). Even though one of these studies (Mullin and Sahasrabudhe, 1997) reports only total glucosinolates, the data suggest considerable differences in glucosinolate levels between and within the *Brassica* groups. In the more detailed study (Carlson et al., 1987a), 13 different glucosinolates have been reported in broccoli, Brussels sprouts, cauliflower, and kale. The authors reported that

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certain cultivars were highly correlated in their glucosinolate pattern and could be grouped together. Thus, broccoli with higher levels of glucoraphanin formed one group, Brussels sprouts and cauliflower with higher levels of glucobrassicin formed a second group, collard with higher levels of progoitrin formed a third group, and mustard green with higher levels of sinigrin formed a fourth group (Carlson et al., 1978a). However cabbage, an important *Brassica* vegetable, was not included in this study, and the glucosinolate comparisons were based on data collected over several years. This may confound the data, since in turnip, cabbage, and *Brassica napus* significant variations in glucosinolates are reported from year to year due to differences in climatic conditions (Carlson et al., 1987b; Fieldsend and Milford, 1994; Rosa et al., 1996).

Glucosinolate levels in *Brassica* are also affected by the growing location. Shelp and McLellan (1993) evaluated glucosinolate distribution in two broccoli cultivars grown at three different locations. They reported that differences in glucosinolate content among growing sites were greater than differences between cultivars. Factors that may have contributed to these differences included soil type, sulfate and nitrate fertilizer application, plant spacing, and date of harvest (Josefsson, 1970; Heany and Fenwick, 1980b; Griffiths et al., 1991; Rosa et al., 1996). Higher levels of glucosinolates were found in plants grown on clay soil than on sandy soil and in plants treated with sulfate than in plants treated with nitrate (Josefsson, 1970; Heaney and Fenwick, 1980b). In a study of *Brassica napus*, it was reported that higher nitrogen application increased the proportion of progoitrin at the expense of sinigrin (Zhao et al., 1994).

While previous studies on glucosinolate distribution in *Brassica* vegetables have indicated differences between and within groups, a significant portion of the variation may have resulted from variations in environmental and cultural factors. Therefore, the objective of this study was to compare glucosinolate levels in the edible portions of 66 genotypes from five *Brassica oleracea* groups including 50 var. *italica* (broccoli), 4 var. *gemmifera* (Brussels sprouts), 6 var. *capitata* (cabbage), 3 var. *butrytis* (cauliflower), and 2 *acephala* (kale) grown under uniform cultural practices in an attempt to identify differences due to genetic factors. Emphasis was placed on broccoli because of its economic importance and consumer preference.

MATERIALS AND METHODS

Materials. Broccoli, Brussels sprouts, cabbage, cauliflower, and kale seeds were obtained from the Asgrow Seed Co. and Peto Seeds (Seminis vegetable seeds), the USDA Plant Genetics Resource Unit (Geneva, NY), and Dr. Mark Farnham of the USDA Vegetable Research Center (Charleston, SC). Lines included commercial hybrids, open-pollinated varieties, land races, inbreds, and doubled haploids developed by Farnham (1997) from commercial hybrids. In July 1996, seeds were germinated in Fafard growing mix no. 2 (Conrad Fafard, New Brunswick, Canada) in a greenhouse under natural day light. After 4 weeks in the greenhouse, seedlings were transplanted into a field plot of silty clay loam (Drumer) soil (pH 6.7) at the University of Illinois vegetable farm, in Champaign, IL. The field design was a randomized complete block (RCB) with three replicates of 15 plants each. Transplants of each entry were planted in single rows 75 cm apart and 40 cm in the row for a population equivalent of 33 333 plants per hectare. Water, fertilizer, and pesticides were applied according to standard cultural practices developed for cruciferous vegetables in Illinois (Foster and Maynard, 1998).

At optimum maturity, three uniform size plants free of insect and/or mechanical damage were selected from each replicate and the edible portions were cut, placed on ice, and immediately transported to the laboratory. Subsamples of 100 g from each plant per replicate were combined, weighed, frozen in liquid nitrogen, and lyophilized. Freeze-dried tissue was ground into a fine powder using a coffee mill and stored at -20°C until further analysis.

Glucosinolates Analysis. Intact glucosinolates were analyzed according to Wathelet et al. (1991), with slight modification. A 0.2 g sample of freeze-dried powder from each replicate was placed in a capped 15 mL glass tube and heated on a heating block (Reacti-Therm III, Pierce, Rockford, IL) set at 95°C for 15 min. To each tube, 2 mL of boiling deionized water and 500 μL of 1 μM benzylglucosinolate (internal standard, Canola Council of Canada, Manitoba, Canada) were added. The tubes were heated for an additional 5 min, immediately cooled on ice, and centrifuged at 12000g for 10 min at 4°C , and the supernatant was saved on ice. The pellet was re-extracted with 1 mL of boiling water and centrifuged at 12000g for 10 min at 4°C , and the supernatant was collected, combined with the previously saved supernatant, and mixed. A 1 mL fraction of the supernatant was combined with 150 μL of 0.5 M barium acetate, vortexed for 5 s, and layered on a DEAE Sephadex A-25 column. Glucosinolates were desulfated with arylsulfatase while on the column by adding 10 units of sulfatase suspended in 500 μL of glass-distilled water to each column and capping it for 18 h. The desulfated glucosinolates were eluted from the column with 2 mL of water and separated on a Hitachi HPLC system (Hitachi Ltd., Tokyo, Japan) consisting of a variable UV detector set at 229 nm wavelength, a refrigerated autosampler, a column heater set at 32°C , and a Lichosphere RP-18 column (Merck, Darmstadt, Germany). Desulfoglucosinolates were eluted off the column in 46 min with a linear gradient of 0–20% acetonitrile in water at a flow rate of 0.8 mL/min. The type and amount of glucosinolates in each *Brassica* vegetable were calculated in comparison to certified glucosinolate levels in a standard rapeseed reference material (BCR 367, Commission of the European Community Bureau of References, Brussels, Belgium). Using benzylglucosinolate as an internal standard, the recovery of glucosinolates from the *Brassica* vegetables using this procedure was estimated at 95–97%. A representative chromatogram of the HPLC elution profile of glucosinolates from a broccoli sample is shown in Figure 1.

Statistical Analysis. Analysis of variance was performed on data between and within the groups. Mean separation was determined by least significant differences (LSD) at $P = 0.05$ and standard error of the mean (SE). Mean separation was also determined for the effect of harvest date on glucosinolates in broccoli. Pearson's correlations of glucosinolates and vitamins evaluated in a companion study by Kurilich et al. (1998) were also determined.

RESULTS AND DISCUSSION

Variation in Glucosinolates among *Brassica oleracea* Groups. Intact glucosinolates in broccoli, Brussels sprouts, cabbage, cauliflower, and kale were evaluated in order to determine variation in amounts and types across groups grown under similar conditions and using the same analytical procedure. A profile of 12 glucosinolates in *Brassica oleracea* groups is shown in Table 1. Data show significant differences in content of individual glucosinolate levels among the groups. In broccoli, the predominant glucosinolates were glucoraphanin (7.1 $\mu\text{mol g}^{-1}$ DW), glucobrassicin (1.1 $\mu\text{mol g}^{-1}$ DW), gluconapin (1.0 $\mu\text{mol g}^{-1}$ DW), and progoitrin (1.0 $\mu\text{mol g}^{-1}$ DW). Glucoraphanin and glucobrassicin concentrations in broccoli in this study were similar to those previously reported by Shelp et al. (1993) and Goodrich et al. (1989), but nearly 4-fold less than those reported by Hansen et al. (1995). Using gas chromatography,

Table 1. Mean Glucosinolate Content ($\mu\text{mol g}^{-1}$ Dry Mass) in the Edible Tissues of *Brassica oleracea*^a

glucosinolates		broccoli	Brussels sprouts	cabbage	cauliflower	kale
group	form					
aliphatics	sinigrin	0.1 ± 0.4	8.9 ± 0.2	7.8 ± 0.1	9.3 ± 0.1	10.4 ± 0.0
	gluconapin	1.0 ± 1.5	6.9 ± 0.7	0.7 ± 0.4	0.3 ± 0.2	1.0 ± 0.1
	glucobrassicinapin	0.3 ± 0.1	0.5 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
	progoitrin	1.0 ± 0.8	2.4 ± 0.4	0.2 ± 0.2	0.3 ± 0.1	0.6 ± 0.1
	epiprogoitrin	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	napoleiferin	0.7 ± 0.6	0.4 ± 0.3	0.0 ± 0.1	0.2 ± 0.1	0.0 ± 0.0
	glucoiberin	0.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	glucoraphanin	7.1 ± 2.5	1.0 ± 1.3	0.1 ± 0.6	0.5 ± 0.3	1.0 ± 0.2
	glucoalysin	0.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	gluconasturtiin	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
	glucobrassicin	1.1 ± 0.4	3.2 ± 0.2	0.9 ± 0.1	1.3 ± 0.1	1.2 ± 0.0
phenyl indoles	4-OH glucobrassicin	0.2 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	1.6 ± 0.0	0.1 ± 0.0
	4-CH ₃ OH glucobrassicin	0.4 ± 0.4	0.4 ± 0.2	0.3 ± 0.1	1.0 ± 0.1	0.2 ± 0.0
	neoglucobrassicin	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0
	total	12.8	25.1	10.9	15.1	15.0

^a Values represent the mean ± SEM. Means and SEM were calculated from the following accessions: broccoli, 50; Brussels sprouts, 4; cabbage, 6; cauliflower, 3; kale, 2.

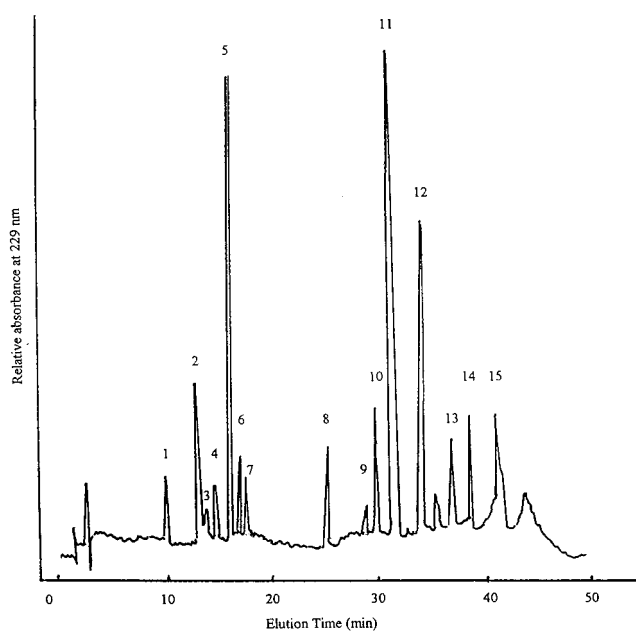


Figure 1. Typical HPLC chromatogram of desulfoglucosinolates in broccoli. Peaks: 1, glucoiberin; 2, progoitrin; 3, epiprogoitrin; 4, sinigrin; 5, glucoraphanin; 6, napoleiferin; 7, glucoalysin; 8, gluconapin; 9, 4-hydroxy-glucobrassicin; 10, glucobrassicinapin; 11, benzylglucosinolate (internal standard); 12, glucobrassicin; 13, gluconasturtiin; 14, 4-methoxyglucobrassicin; 15, neoglucobrassicin.

Carlson et al. (1987b) found that in broccoli, the concentrations of glucoraphanin and glucobrassicin were similar. However, in the present study we found glucoraphanin concentration to be about 7-fold higher than that of glucobrassicin (Table 1). It is interesting to note that while estimation of glucoraphanin and glucobrassicin levels in the previous studies were made in mature flower heads, a more recent study (Fahey et al., 1997) reported a complete absence of glucobrassicin in broccoli seedlings. Similarly, we found 50% higher glucobrassicin in fully developed Packman broccoli heads (15–20 cm diameter) than in immature heads (5–10 cm diameter, data not shown). These results suggest that glucobrassicin synthesis is active during later stages of broccoli development. In Brussels sprouts, the predominant glucosinolates are sinigrin ($8.9 \mu\text{mol g}^{-1}$ DW), gluconapin ($6.9 \mu\text{mol g}^{-1}$ DW), glucobrassicin ($3.2 \mu\text{mol g}^{-1}$

DW), and progoitrin ($2.4 \mu\text{mol g}^{-1}$ DW). Our results are similar to those previously reported by Heaney and Fenwick (1980b) and Goodrich et al. (1989). In contrast, Carlson et al. (1987a) reported a higher level of glucobrassicin in Brussels sprouts than that reported in the present study and that the glucobrassicin concentration is nearly 40-fold higher than the sinigrin concentration. However, Carlson et al. (1987a) quantified glucobrassicin indirectly by measuring the amount of thiocyanate ion released by the action of myrosinase. This procedure appears to overestimate glucobrassicin concentration and does not differentiate between glucobrassicin and neoglucobrassicin (Heaney and Fenwick, 1980a). In a more recent study, Heaney and Fenwick (1993) compared several glucosinolate analysis procedures and suggested that high-pressure liquid chromatography is more reliable and less time-consuming than other methods.

In cabbage, the predominant glucosinolates were sinigrin ($7.8 \mu\text{mol g}^{-1}$ DW), glucobrassicin ($0.9 \mu\text{mol g}^{-1}$ DW), and gluconapin ($0.7 \mu\text{mol g}^{-1}$ DW). In agreement with our data, Pocock et al. (1987) found high levels of sinigrin and glucobrassicin compared to the other glucosinolates in English white cabbage. In addition, they found significant amounts of glucoiberin in the three cultivars tested. In a survey of 22 cabbage cultivars, VanEtten and Daxenbichler (1976) found higher levels (10–50%) of glucoiberin than either sinigrin or glucobrassicin. Rosa (1997) reported that glucoiberin concentration in cabbage is about 2-fold higher in the roots than in the leaves and it decreased in both tissues with an increase in plant age. In the present study, glucoiberin concentration was very low ($\leq 0.06 \mu\text{mol g}^{-1}$ DW) in the six accessions tested. Both Pocock et al. (1987) and VanEtten and Daxenbichler (1976) used GC to detect glucoiberin, which suggests that GC may be more sensitive than HPLC for detecting this compound.

In cauliflower, the most abundant glucosinolates were sinigrin ($9.3 \mu\text{mol g}^{-1}$ DW), 4-hydroxy glucobrassicin ($1.6 \mu\text{mol g}^{-1}$ DW), and glucobrassicin ($1.3 \mu\text{mol g}^{-1}$ DW). Carlson et al. (1987b) and Sones et al. (1984) also found higher levels of sinigrin and glucobrassicin compared to other glucosinolates in cauliflower. However, the amounts that they reported are significantly less than those we report in this study. Both Carlson et al. (1987b) and Sones et al. (1984) used GC to estimate the level of glucosinolates. According to Sones et al. (1984), the GC

procedure underestimates the level of glucosinolates in cauliflower.

In kale, the most abundant glucosinolates were sinigrin ($10.4 \mu\text{mol g}^{-1}$ DW), glucobrassicin ($1.2 \mu\text{mol g}^{-1}$ DW), gluconapin ($1.0 \mu\text{mol g}^{-1}$ DW), and glucoraphanin ($1.0 \mu\text{mol g}^{-1}$ DW, Table 1). Similarly, Carlson et al. (1987a) and Rosa et al. (1996) reported higher sinigrin and glucobrassicin but only a trace amount of glucoraphanin in kale leaves. However, in flowering Chinese kale, up to 68% of the total glucosinolates were reported to be glucoraphanin (Hill et al., 1987). In this study, gluconasturtiin concentration in kale was similar to that in the other four vegetable groups (Table 2).

An important factor that may have contributed to the difference in glucosinolate content among the groups is the difference in tissue types tested. In this study, immature flowers were analyzed in broccoli and cauliflower, leaves in cabbage and kale, and axillary buds in Brussels sprouts. Variation in glucosinolate types and concentrations among plant parts was previously reported in turnip, cabbage, and *Brassica napus* (Booth and Walker, 1991; Carlson et al., 1987a; Clossais-Besnard and Larher, 1991; Rosa, 1997). In turnip, gluconapin and glucobrassicin were higher in the tops than in the roots, while glucoraphanin, progoitrin, glucoalysin, glucobrassicin, and total glucosinolates were higher in the roots than in the tops (Carlson et al., 1987a; Rosa et al., 1996). In cabbage, Rosa (1997) found higher indole glucosinolates in the roots and higher aliphatic glucosinolates in the leaves. Differences in glucosinolate levels between plant parts are not linked to mobilization between organs; instead they are believed to be synthesized and stored within each organ (Rosa et al., 1996; Clossais-Besnard and Larher, 1991).

Variation in Glucosinolates within *Brassica oleracea* Groups. Evaluation of glucosinolate levels showed significant differences between accessions within each group (Table 2). Among the broccoli accessions tested, the highest amount of glucoraphanin was found in Brigadier ($21.7 \mu\text{mol g}^{-1}$ DW) followed by Wintergarden ($17.5 \mu\text{mol g}^{-1}$ DW), and Majestic ($16.0 \mu\text{mol g}^{-1}$ DW). The lowest amount of glucoraphanin ($0.8 \mu\text{mol g}^{-1}$ DW) was found in EV6-1. Carlson et al. (1987b) also found differences in glucoraphanin among six broccoli accessions; however, the differences were relatively small (less than 3-fold) compared to differences reported in this study (more than 27-fold). In *Brassica napus* and *Brassica oleracea*, Rosa (1996) reported a significant effect of accessions on glucosinolate levels.

In some broccoli accessions (example Peto no. 3), the glucoraphanin level represents up to 90% of the aliphatic glucosinolates. The action of the hydrolyzing enzyme myrosinase on glucoraphanin causes the release of glucose and an unstable intermediate that rearranges irreversibly to form sulforaphane and/or sulforaphane nitrile. Basic pH and high temperature enhance sulforaphane formation, while acidic pH and ferrous ions enhance sulforaphane nitrile formation (Fenwick et al., 1983). Sulforaphane is the most potent naturally occurring inducer of phase II enzymes, including quinone reductase and glutathione *S*-transferases (Prochaska et al., 1992). Induction of phase II enzymes has been shown to protect cells against toxic electrophiles, including carcinogens such as 9,10-dimethyl-1,2-benzanthracene (Zhang et al., 1992, 1994). Sulforaphane nitrile, however, is inactive against hepatic glutathione concentration and glutathione-*S*-transferase activity

(Ringenberg and Wallig, 1996). In broccoli, the concentration of sulforaphane was reported to be between 1.9 and 3.7 mg g^{-1} DW and sulforaphane nitrile between 0.2 and 0.3 mg g^{-1} DW (Chiang et al., 1998).

Other aliphatic glucosinolates found in varying amounts in broccoli include epiprogoitrin, napoleiferin, glucoalysin, and gluconapin. The sum of these compounds is listed as residual aliphatics (Table 1). Differences in concentrations of these compounds among the broccoli accessions were relatively small, except in DeCicco, which showed significantly higher concentration of glucoalysin ($16.0 \mu\text{mol g}^{-1}$ DW), and Peto no. 7, which showed significantly higher concentrations of progoitrin ($7.9 \mu\text{mol g}^{-1}$ DW) and gluconapin ($17.1 \mu\text{mol g}^{-1}$ DW). Differences in the profile of aliphatic glucosinolates among broccoli lines have been attributed to genetic variation (Giamoustaris and Mithen, 1996). Several loci have been identified that are involved in the partitioning of aliphatic glucosinolates. The *Gsl-elong* locus is responsible for the elongation of the carbon side chain (Mithen et al., 1995), while several other loci are affiliated with side chain rearrangements (Giamoustaris and Mithen, 1996; Magrath et al., 1994). Partitioning into the various forms has been proposed to be regulated by the presence of nonfunctional alleles at these loci (Giamoustaris and Mithen, 1996).

Broccoli accessions also showed significant differences in indole glucosinolates content. Glucobrassicin is the most abundant indole glucosinolate in broccoli, representing nearly 60% of the indoles and about 10% of the total glucosinolates (Table 2). The highest concentration of glucobrassicin in broccoli was found in the accessions Pirate ($2.8 \mu\text{mol g}^{-1}$ DW) and Shogun ($2.4 \mu\text{mol g}^{-1}$ DW), while the lowest concentration was found in Galaxy, Majestic, and MA 191 DH X6 ($0.3 \mu\text{mol g}^{-1}$ DW). The sums of residual indole glucosinolates (4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin) were small and relatively similar among the broccoli accessions (Table 2), except in Peto no. 10 which contained a significant amount of 4-hydroxy glucobrassicin ($3.4 \mu\text{mol g}^{-1}$ DW). In contrast to aliphatic glucosinolates, which are predominately under genetic control (Magrath et al., 1994), the concentration of the indole forms has been proposed to be regulated primarily by environmental and/or physiological factors (Mithen et al., 1995).

In Brussels sprouts, the accession Jersey had nearly 2–3-fold higher aliphatic glucosinolates than the other four accessions (Table 2). The predominant aliphatic glucosinolates in Jersey were gluconapin, sinigrin, and progoitrin at 12.5 , 9.1 , and $7.3 \mu\text{mol g}^{-1}$ DW, respectively. Significant differences were also detected in indole glucosinolates content among the five Brussels sprouts accessions (Table 2). The accessions Cambridge no. 5 and Yates Darkcrop have 56 and 69% higher glucobrassicin concentration than the accession Long Island, respectively. Other indole glucosinolates (summed as residual) were relatively similar among the Brussels sprouts accessions (Table 2).

Among cabbage accessions, PI 1214148 had the highest concentration of total aliphatic glucosinolates ($39.0 \mu\text{mol g}^{-1}$ DW), while Peto no. 27 had the lowest ($5.6 \mu\text{mol g}^{-1}$ DW) concentration. The predominant aliphatic glucosinolates in PI 1214148 were sinigrin ($21.1 \mu\text{mol g}^{-1}$ DW), gluconapin ($13.4 \mu\text{mol g}^{-1}$ DW, included in the residual aliphatic), and progoitrin ($2.3 \mu\text{mol g}^{-1}$ DW).

Table 2. Glucosinolates Content ($\mu\text{mol g}^{-1}$ DW) of Individual Accessions within Five *Brassica oleracea* Subspecies

accession	aliphatics					indoles			phenyl
	sinigrin	progoitrin	glucoraphanin	residual	total	glucobrassicin	residual	total	gluconasturtiin
Broccoli									
Atlantic	0.0	0.3	11.6	1.1	12.9	0.7	0.6	1.4	0.5
Baccus	0.0	0.7	1.5	1.2	3.4	0.4	0.7	1.0	0.1
Big Sur	0.0	0.2	2.2	0.6	3.0	0.4	1.0	1.4	0.4
Brigidier	0.0	0.9	21.7	3.8	26.3	1.2	1.0	2.2	0.7
Cavalo Broccolo Ramoso	0.2	1.4	10.2	6.2	18.0	1.8	3.9	5.7	0.8
De Cicco	0.0	0.4	10.7	2.7	13.8	1.7	1.0	2.8	0.6
Eu 4-1	0.0	0.2	7.6	0.4	8.2	0.9	0.6	1.5	0.3
Eu 8-1	0.1	7.9	9.6	3.3	20.9	1.3	1.0	2.3	0.6
EV 2-1	0.0	0.2	1.7	1.1	3.0	1.5	1.3	2.8	0.8
EV 6-1	0.0	0.1	0.8	0.4	1.3	0.6	0.5	1.1	0.2
Florette	0.0	0.3	8.7	1.5	10.4	1.0	0.5	1.6	0.6
Fu 549 DH I.S.	0.0	0.3	3.9	3.9	8.1	0.7	0.3	1.1	0.4
G31824	0.0	0.5	7.1	2.5	10.1	0.5	1.6	2.1	0.8
G31825	0.0	3.9	3.3	4.4	11.6	0.6	0.2	0.8	0.2
Galaxy	0.0	1.0	3.4	0.7	5.1	0.3	0.2	0.5	0.2
GC 1-2	0.0	0.1	5.1	0.5	5.7	0.7	0.8	1.4	0.2
GC 3-2	0.3	2.1	1.6	3.2	7.2	1.3	0.2	1.5	0.2
Gem	0.0	0.0	5.0	1.3	6.4	0.5	0.3	0.7	0.3
Green Comet	0.0	1.4	6.4	1.4	9.2	0.4	0.4	0.9	0.3
Greenbelt	0.0	0.4	8.5	0.9	9.8	1.2	0.7	1.8	0.3
GV 8-1	0.0	2.0	6.6	3.9	12.4	0.7	0.9	1.6	0.3
High Sierra	0.3	2.3	4.5	2.9	9.9	0.8	0.6	1.3	0.3
HS 061 DH I.S.	0.0	0.2	7.2	1.1	8.5	0.8	0.4	1.2	0.3
HS 067 DH I.S.	0.0	0.3	7.4	0.7	8.4	0.8	0.4	1.2	0.3
Legacy	0.0	0.1	5.5	1.9	7.5	0.4	0.4	0.8	0.4
MA 065 DH I.S.	0.0	0.2	6.2	0.6	7.0	1.6	0.7	2.4	0.4
MA 191 DH X6	0.1	0.0	13.8	3.8	17.7	0.3	0.7	1.1	0.4
Majestic	0.0	0.1	16.0	3.0	19.1	0.3	0.4	0.7	0.2
Packman	0.1	0.9	4.2	2.4	7.5	0.1	0.3	0.4	0.1
Persius	0.0	0.5	10.6	0.8	11.9	1.0	0.5	1.6	0.5
Peto no. 3	0.0	0.2	7.1	0.6	7.8	0.5	0.3	0.8	0.1
Peto no. 5	0.0	0.3	8.8	0.9	10.1	1.1	0.5	1.5	0.3
Peto no. 6	0.0	0.0	5.5	1.3	6.8	1.5	0.9	2.3	0.4
Peto no. 7	0.1	7.2	5.9	18.3	31.4	1.2	2.4	3.6	0.6
Peto no. 8	0.0	0.1	5.1	0.5	5.8	0.9	0.5	1.4	0.4
Peto no. 10	0.0	0.4	8.5	1.4	10.3	1.8	4.2	6.0	0.6
Peto no. 11	0.0	0.9	2.6	1.4	4.9	1.0	0.8	1.8	0.2
Peto no. 12	0.1	0.1	3.6	4.3	8.0	0.3	0.5	0.8	0.1
Peto no. 13	0.0	0.3	6.0	1.1	7.4	1.0	0.4	1.4	0.4
Peto no. 14	0.0	0.1	7.6	0.9	8.6	0.7	0.5	1.2	0.2
Peto no. 15	0.0	0.6	2.2	0.9	3.7	0.7	0.3	1.0	0.2
Peto no. 16	0.0	0.2	9.1	1.0	10.2	1.5	0.4	1.9	0.4
Pinnacle	0.1	0.3	8.8	2.2	11.4	1.8	1.9	3.7	0.7
Pirate F1	0.0	0.5	10.7	1.9	13.0	2.8	1.3	4.1	0.6
Premium Crop	0.0	1.1	4.7	2.4	8.2	1.3	0.8	2.1	0.4
Shogun	0.8	2.6	11.9	3.3	18.7	2.4	3.8	6.2	0.9
Su 006 DH I.S.	0.2	0.4	1.5	8.7	10.7	1.8	0.8	2.7	0.4
VI 158 DH I.S.	6.0	5.3	9.4	10.5	31.2	1.3	0.6	1.9	0.5
Wintergarden	0.0	0.5	17.5	0.9	18.9	1.6	0.9	2.5	0.5
Zeus	0.0	0.1	2.9	0.8	3.8	1.1	1.3	2.3	0.3
Brussels Sprouts									
Cambridge no. 5	3.2	1.9	0.6	6.2	11.9	5.6	1.6	7.1	0.6
Jersey	9.1	7.3	1.3	14.1	31.7	3.9	0.8	4.6	0.6
Long Island	4.6	1.1	0.4	5.5	11.5	3.6	1.3	4.9	0.2
Yates Darkcrop	5.1	2.2	1.4	6.1	14.8	6.1	1.8	7.9	0.6
Cabbage									
Jersey Wakfield	6.9	0.6	0.4	2.5	10.5	0.6	1.0	1.5	0.4
Peto no. 22	11.2	0.4	0.3	1.2	13.1	0.6	0.4	1.0	0.4
Peto no. 23	7.1	0.2	0.1	0.1	7.4	0.5	0.5	1.0	0.2
Peto no. 24	7.4	0.2	0.1	0.8	8.5	1.3	1.0	2.3	0.6
Peto no. 27	4.3	0.2	0.1	1.0	5.6	1.3	1.0	2.3	0.2
PI 1214148	21.1	2.3	1.8	13.8	39.0	0.9	0.6	1.5	0.7
Cauliflower									
Feng Shan	9.5	0.3	0.2	0.4	10.3	1.1	1.7	2.8	0.6
Peto no. 17	5.7	0.3	0.4	0.8	7.1	0.3	2.4	2.7	0.2
Snow Crown	12.9	0.3	0.9	0.6	14.6	2.3	3.8	6.1	0.4
Kale									
Vates	7.4	0.5	0.6	1.1	9.5	1.6	0.5	2.2	0.4
Winterborne	13.3	0.7	1.4	1.2	16.6	0.7	0.3	1.0	0.4
LSD 0.01 (all)	3.6	1.4	6.3	0.4	11.0	1.8	1.2	3.0	0.5
LSD 0.01 (broccoli only)	1.0	1.3	7.2	0.1	9.7	1.5	1.1	2.7	0.5

^a Values represent the mean of three replicates per accession.

Concentrations of indole glucosinolates were relatively similar among the cabbage accessions tested.

In cauliflower, sinigrin concentration varied among accessions (Table 2). The highest concentration of sinigrin was found in Snow Crown ($12.9 \mu\text{mol g}^{-1}$ DW) while the lowest concentration was found in Peto no. 17 ($5.7 \mu\text{mol g}^{-1}$ DW). Similarly, Snow Crown showed a significantly higher concentration of indole glucosinolates, especially of glucobrassicin ($2.3 \mu\text{mol g}^{-1}$ DW) and 4-hydroxy glucobrassicin ($3.4 \mu\text{mol g}^{-1}$ DW, included in residual indoles), than Peto no. 17 and Feng Shan. Peto no. 17 showed a significantly higher concentration of methoxy glucobrassicin ($1.8 \mu\text{mol g}^{-1}$ DW) compared to Snow Crown and Feng Shan (0.3 and $0.7 \mu\text{mol g}^{-1}$ DW, respectively). No major differences in the other indole glucosinolates were observed among the three cauliflower accessions.

In kale, the accession Winterborne had about 2-fold higher total aliphatic glucosinolates content (predominantly sinigrin) than the accession Vates. However, Vates had about 2-fold higher total indole glucosinolates (predominantly glucobrassicin) than Winterborne (Table 2).

In addition to genetic factors, variation in glucosinolate content within the groups of *Brassica* examined in this study may have resulted from differences in dates of harvest. In broccoli, we have found a positive and significant correlation ($r = 0.52$, $P = 0.004$) between glucoraphanin levels and date of harvest. For example, the late maturing accessions Peto no. 5 and Peto no. 10 (maturing 79 and 85 days after transplanting, respectively) contained 8.84 and $8.50 \mu\text{mol g}^{-1}$ DW glucoraphanin, while the earlier maturing accessions EV6-1 and Peto no. 11 (both maturing at 55 days after transplanting) contained 0.8 and $2.6 \mu\text{mol g}^{-1}$ DW glucoraphanin, respectively (Table 2). Glucoraphanin levels drop precipitously during the first 10 days of growth from seed (Fahey et al., 1997). Rosa et al. (1996) reported higher glucosinolates in spring than fall harvested cabbage. However, these differences were not linked to variation in growing temperatures (Rosa, 1997). In contrast, in a companion study we have found no correlation between broccoli harvest date and carotenoids, tocopherols, and ascorbate levels (Kurilich et al., 1998).

Interaction between Glucosinolates and Vitamins. In a companion study, using the same cruciferous accessions, we have evaluated the levels of the antioxidants α - and γ -tocopherols, α - and β -carotenes, and ascorbate (Kurilich et al., 1998). Pearson's correlations between glucosinolates and antioxidants in broccoli showed that there are significant and positive interactions between glucoraphanin and α -tocopherol ($r = 0.30$, $P = 0.02$). Significant and positive correlations were also found between total aliphatic glucosinolates and γ -tocopherol ($r = 0.38$, $P = 0.006$) and gluconasturtiin and γ -tocopherol ($r = 0.28$, $P = 0.05$). However, there were no significant correlations between indole glucosinolates and any of the antioxidants (Kurilich et al., 1998). Vang et al. (1997) reported that anticarcinogenic properties of dietary fruit and vegetables are likely to be due to a combination of substances acting simultaneously. The significant and positive correlations between glucosinolates and α - and γ -tocopherol reported in this study indicate added health benefit from consuming crucifer vegetables rich in these compounds.

Variation in glucosinolate levels between and within each group of *Brassica* vegetables suggests differences in their health-promoting properties. Recently, Faulkner et al. (1998) reported 10-fold higher glucoraphanin levels in hybrid lines developed from crosses between wild *Brassica oleracea* and broccoli accessions. When murine hepatoma Hepa 1c1c7 were treated with extracts from the high glucoraphanin lines, they exhibited more than a 100-fold increase in quinone reductase activity over broccoli accessions. Quinone reductase, glutathione *S*-reductase, and UDP-glucuronosyl transferase are phase II detoxification enzymes that have been shown to be induced by a diet rich in crucifer vegetables (Prochaska et al. 1992). These enzymes, through modification of the reactive products of cytochrome P450 oxidation, cause detoxification of carcinogens (Prochaska et al., 1992). Staack et al. (1998) reported that a mixture of glucosinolate breakdown products, similar to that found in a crucifer diet, greatly enhanced the synthesis of detoxification enzymes. They also found that glucosinolates interacted synergistically to induce higher levels of phase II detoxification enzymes and that the induction is dose dependent.

The diversity in glucosinolate levels reported in this and other studies suggests that the potential health benefits from crucifer vegetables are greatly dependent on the accession selected. Previous studies have suggested that enhancing the level of glucosinolates in cruciferous vegetables through conventional breeding or genetic engineering can be expected to enhance the chemopreventive properties of these vegetables. The wide range of variability in glucosinolates among and within the five *Brassica* groups that were evaluated in this study offer an important information base for developing accessions with enhanced health benefits.

NOMENCLATURE

Sinigrin, allyl-glucosinolate; gluconapin, butyl-3-enyl-glucosinolate; glucobrassicinapin, pent-4-enyl-glucosinolate; progoitrin, (2*R*)-2-hydroxybut-3-enylglucosinolate; epiprogoitrin, (2*S*)-2-hydroxybut-3-enylglucosinolate; glucoiberin, 3-methylsulfinylpropyl-glucosinolate; glucoraphanin, 4-methylsulfinylbutyl-glucosinolate; glucoalysin, 5-methylsulfinylpentyl-glucosinolate; gluconasturtiin, phenethyl-glucosinolate; glucobrassicin, indol-3-ylmethyl-glucosinolate; neoglucobrassicin, *N*-methoxyindol-3-ylmethyl-glucosinolate; 4-OH glucobrassicin, 4-hydroxyindol-3-ylmethyl-glucosinolate; 4-CH₃O glucobrassicin, 4-methoxyindol-3-ylmethyl-glucosinolate; sulforaphane, 1-isothiocyanate-4-(methylsulfinyl)-butane.

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