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Glucoraphanin and 4-Hydroxyglucobrassicin Contents in Seeds of 59 Cultivars of Broccoli, Raab, Kohlrabi, Radish, Cauliflower, Brussels Sprouts, Kale, and Cabbage

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The importance of dietary sulforaphane in helping maintain good health continues to gain support within the health-care community and awareness among U.S. consumers. In addition to the traditional avenue for obtaining sulforaphane, namely, the consumption of appropriate cruciferous vegetables, other consumer products containing added glucoraphanin, the natural precursor to sulforaphane, are now appearing in the United States. Crucifer seeds are a likely source for obtaining glucoraphanin, owing to a higher concentration of glucoraphanin and the relative ease of processing seeds as compared to vegetative parts. Seeds of several commonly consumed crucifers were analyzed not only for glucoraphanin but also for components that might have negative health implications, such as certain indole-containing glucosinolates and erucic acid-containing lipids. Glucoraphanin, 4-hydroxyglucobrassicin, other glucosinolates, and lipid erucic acid were quantified in seeds of 33 commercially available cultivars of broccoli, 4 cultivars each of kohlrabi, radish, cauliflower, Brussels sprouts, kale, and cabbage, and 2 cultivars of raab.

KEYWORDS: Cruciferous vegetables; brassicaceae; glucosinolates; glucoraphanin; 4-hydroxyglucobrassicin; erucic acid

INTRODUCTION

Health-care professionals have long regarded several members of the plant family Cruciferae (more properly Brassicaceae) as important dietary contributors to good health. Among their many healthy attributes, cruciferous vegetables provide important nutrients such as vitamins C and A, folic acid, calcium, potassium, and dietary fiber and are low in calories and fat (*1*). Increasingly, consumers are also receiving the message that crucifers are the best dietary source for obtaining health-promoting glucosinolate conversion products, the most important of which may be sulforaphane (4-methylsulfinylbutyl isothiocyanate).

Although sulforaphane has several reported beneficial properties, its greatest contribution to the maintenance of human health is likely a potent capacity for inducing phase 2 enzymes, one of our body's important natural defense mechanisms. Phase 2 enzymes, such as glutathione transferases and NAD(P)H: quinone reductases, detoxify electrophilic carcinogens, which if left unchecked can lead to mutations in DNA and cancer (*2*−*4*). In fact, it is this property of sulforaphane, and to a lesser extent other crucifer isothiocyanates, of inducing phase 2 enzymes that is one of the leading biochemical hypotheses for explaining the epidemiological link between the consumption of cruciferous vegetables and a reduced risk of cancer (*5*−*9*). Other important, but less widely documented, health benefits of sulforaphane include the inhibition of *Helicobacter pylori*, a pathogen linked to the development of peptic ulcers and gastric cancer (*10*), and the protection of human retinal cells against severe oxidative challenges (*11*).

Despite these promising advances in identifying and substantiating the medical benefits of sulforaphane and their communication, not only in technical journals but also by the popular press and television, convincing U.S. consumers to increase their consumption of cruciferous vegetables containing sulforaphane continues to present a substantial challenge. Although U.S. consumption of cruciferous vegetables, especially broccoli, has steadily increased over the past several years (*12*), the contribution of crucifers to the total recommended five or more servings of fruits and vegetables remains very low, being on the order of 0.2−0.5 serving/day (*12, 13*). A recent two-day food intake behavior study involving 4806 adult Americans revealed that fewer than one in five consumed any kind of cruciferous vegetable and fewer than 3% ate broccoli over the reporting period (*13*). To focus more attention on this situation, some nutritionists are suggesting a high-profile campaign specifically promoting the consumption of crucifers be initiated (*13*). The success of such an undertaking may be limited,

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however, by the simple fact that many people do not like the taste of these vegetables (*14*) and are likely to resist changing their eating behavior irrespective of the soundness of the health argument.

To help overcome this hurdle, a step worthy of serious consideration is the addition of sulforaphane to popular-tasting processed food products, such as beverages and snacks. In this way, even those individuals who prefer not to eat cruciferous vegetables might still gain the health benefits of sulforaphane. However, direct fortification of foods with sulforaphane is likely to be problematic owing to its relatively high reactivity and pungent flavor. It is expected that products containing added sulforaphane would have a very short shelf life and low consumer acceptance. A more attractive option is the use of the parent compound, glucoraphanin (4-methylsulfinylbutyl glucosinolate). It is heat stable and water soluble and has few to no adverse flavor properties (*2*, *15*, *16*). Fairly recently, Brassica teas containing added glucoraphanin have appeared in several regional U.S. markets (*17*).

As a sulforaphane delivery system, however, the use of glucoraphanin in foods is not a perfect solution. Without myrosinase, an enzyme naturally present in crucifers and responsible for sulforaphane generation when the plant material is macerated, the production of sulforaphane must rely on conversion by bowel microflora, a less efficient process (*18*−*20*). To offset this loss, larger amounts of glucoraphanin will be required to generate the same amount of sulforaphane that would have been available were myrosinase present. Inclusion of myrosinase to foods containing added glucoraphanin is not a viable option unless the enzyme can be prevented from interacting with glucoraphanin and forming sulforaphane in the product.

The seeds of glucoraphanin-containing cruciferous vegetables, as opposed to the vegetative parts, appear to contain the highest concentration of the compound, suggesting their use as a convenient source for its isolation (*21*−*23*). However, unless the glucoraphanin is highly purified, which might increase the cost prohibitively, significant differences in the phytochemical content between partially purified seed isolates and the usually consumed vegetative parts could have important safety implications. For example, we recently found substantially higher amounts of erucic acid in the lipids of broccoli seeds as compared to florets (*24*). The negative health issues associated with erucic acid include myocardial lipidosis, myocardial necrosis, and impaired oxidative phosphorylation (*25*−*27*). Also, 4-hydroxyglucobrassicin has been reported to be the major crucifer seed indole-containing glucosinolate, whereas the major indole in mature vegetative parts is glucobrassicin (*28*). Compared to glucobrassicin, little has been documented regarding the safety of the conversion products of 4-hydroxyglucobrassicin, but it is known that 4-hydroxyglucobrassicin forms oligomeric compounds under acidic conditions in vitro (*29*). Analogous oligomeric compounds generated from glucobrassicin conversion in the stomach are thought by some investigators to be potentially very dangerous to human health (*30*, *31*).

Recently we reported the development of improved chromatographic methodology for the analysis of glucoraphanin in broccoli seeds and sprouts, which provided a solution to the problem of inadequate separation of glucoraphanin from another structurally similar alkyl glucosinolate while, at the same time, improving the resolution of those containing indole moieties (*32*). For this study, our goals were to (1) determine the glucoraphanin content of a fairly large sampling of commercial cultivars of broccoli seeds and other species of commonly consumed crucifers reported to contain glucoraphanin, (2) determine the 4-hydroxyglucobrassicin content of all seed accessions, (3) identify seeds from species of commonly consumed crucifers highest in glucoraphanin content, but with the lowest number and concentration of other glucosinolates, and (4) determine the hexane-soluble lipid content of all seeds and the percentage of erucic acid present in these lipids.

MATERIALS AND METHODS

Plant Materials and Chemicals. Seeds were obtained from New England Seed Co. (Hartford, CT), Johnny's Selected Seeds (Winslow, ME), Seedway Inc. (Elizabethtown, PA), and Thompson & Morgan Seedsmen Inc. (Jackson, NJ). Seeds used in the production of BroccoSpouts (variety not disclosed) were from Caudill Seed Co. (Louisville, KY). All chemicals were of ACS or HPLC grade. Sinigrin (allyl glucosinolate) was obtained from Sigma-Aldrich (Milwaukee, WI).

Analyses. *Moisture.* Seed moisture was determined using the low constant temperature oven method described in the International Rules for Seed Testing Sections 9.1.5.3 and 9.1.5.8 (*33*). The moisture content ranged from 3.9 to 8.2% (mean value of 5.3%), values consistent with those reported by others (*34*).

Hexane−*Extractable Lipid (HL) and Fatty Acid Distribution (FAD).* Before extraction, seeds were rapidly pulverized under argon in a high-speed blender. Except when limited by sample amount, accurately weighed (nearest 0.01 g) triplicate ∼40 g samples were Soxhlet extracted with hexane overnight immediately after grinding. The solvent was removed under water aspirator rotary vacuum evaporation with bath temperatures not exceeding 40 °C, and extracts were subjected to additional evaporation until consecutive weighings were within 0.1%. The relative standard deviation (RSD) for repeat HL analyses was routinely <1.0%. HL samples were kept frozen (−15 °C) until FAD analysis using procedures previously reported (*24*). The hexane-defatted seed powders were dried under high vacuum (<27 Pa) at room temperature (∼21 °C) overnight and refrigerated (5 °C) until analyzed for glucosinolates.

Glucosinolates. Duplicate 1.00 ± 0.01 g samples of seed powder were rapidly dispersed with stirring into ∼30 mL of boiling water contained in 100 mL beakers. Samples were continuously stirred with glass rods for 5 min with occasional removal from heat as required to allow settling of foam. After hot water extraction, samples were allowed to cool for several minutes before quantitative transfer into tubes and centrifugation at 18000*g* for 20 min. The supernates were poured into 100 mL volumetric flasks and the pellets homogenized using a 10 mm diameter coarse dispersing tool [Ultra-Turrax T25 homogenizer (IKA Works Inc., Wilmington, NC)] in 20 mL of room temperature water for 30 s at the maximum speed setting. After centrifugation as previously described, each supernate was transferred to the appropriate volumetric flask and the process was again repeated. Once brought to volume, a portion of each of the combined supernates was filtered (0.45 μm nylon Acrodisc, Pall Gelman Laboratory, Ann Arbor, MI) and analyzed by HPLC-UV and HPLC-MS using methodology recently described (*32*), with Prevail C_{18} columns (Alltech Associates, Deerfield, IL) used for all chromatography (5 μm, 250 × 4.6 mm column used for quantitation; 3 μm, 150 × 2.1 mm column used for mass spectrometry). MS-MS was used to confirm all identified peaks as homogeneous and consistent with their assigned chemical structure (*32*, *35*). Quantitation was based on commercially available high-purity (99.3%) sinigrin, a technique used previously by us as well as others (*32*, *36*). Over the course of the investigation, the RSD for repeat standard injections was 1.4%. Glucosinolate concentrations were expressed in units of micromoles per gram of extracted weight (EW; hexane−extracted and vacuum-dried seed powder) and recalculated by taking into account moisture and hexane lipid to provide a value for fresh weight (FW). Although the major focus of this study was on glucoraphanin and 4-hydroxyglucobrassicin, other glucosinolates were identified and quantified, especially those present in broccoli seed. However, because the chromatography was optimized for the title compounds, the resolution of glucosinolates with relatively short elution

times [<4 min; glucoiberin (3-methylsulfinylbutyl glucosinolate), progoitrin (2(*R*)-hydroxy-3-butenyl glucosinolate), and sinigrin] was not optimum for quantitative purposes when most or all are present in the same extract. The quality of the results in these few instances should be viewed in this light (refer to **Figure 3**, for example). Contrary to our previous report (*32*), progoitrin and epiprogoitrin (2(*S*)-hydroxy-3-butenyl glucosinolate) are resolved by this system and can be quantified separately.

On the basis of glucoraphanin content, the first extraction step was found to remove >85% of the glucosinolates and the subsequent two extractions removed an extra 12 and 2%, respectively. Additional extractions were therefore deemed to be of limited value and not performed. Recovery of sinigrin added to samples at 2.2 mg/g seeds was >99%. Throughout the study, the RSDs of replicate determinations were routinely less than 0.5 and 1.0% for glucoraphanin and 4-hydroxyglucobrassicin, respectively. Although lower limits of detection are possible (*32*), we chose 1.50 μmol of glucosinolate/g of EW and 1.00 μmol of glucosinolate/g of FW as the lower limits for reporting results. The only exceptions made to these limits were for documenting the low glucoraphanin content of several cultivars presented in **Table 2**.

When the described procedure was used, seed myrosinase was likely not inactivated until the glucosinolate extraction step. To determine if significant amounts of glucosinolates were converted, and therefore lost before their analysis, the concentrations of glucoraphanin and 4-hydroxyglucobrassicin were measured using a procedure in which seeds were initially boiled to inactivate myrosinase before grinding and defatting. For these analyses, seeds of two broccoli cultivars containing widely differing amounts of glucoraphanin (Gypsy and BroccoSprouts) were separately added to boiling water (0.07 g of seeds/mL of water) and boiled for 5 min with constant stirring (*18*, *20*). The hot mixtures were then poured into precooled stainless steel trays, frozen, and freeze-dried. When dry, the material in the trays (seeds and matter leached from seeds) was ground, defatted, and analyzed in duplicate for glucosinolates. The differences in glucoraphanin and 4-hydroxyglucobrassicin content of the "boiled-first" samples for either cultivar were not statistically significant from controls analyzed using our usual procedure. Therefore, our initial sample preparatory steps of grinding and defatting before treatment with boiling water to ensure myrosinase inactivation (and extract glucosinolates) did not result in measurable losses of glucosinolates. Additionally, defatted and ground samples stored under refrigeration for up to 6 months showed no statistically significant losses of glucoraphanin as has been reported for unprocessed seed (*34*).

Statistics. Statistical analyses were performed using ProStat version 3 (Poly Software International, Pearl River, NY), JMP version 5 (SAS Institute Inc., Cary, NC), and Control Chart version 3 (WindowChem Software Inc., Fairfield, CA) software. Means were separated using the Tukey Kramer HSD test, $p = 0.05$ (analysis of variance), and two independent sample t test, $p = 0.05$ (comparison of two sample experiments).

RESULTS AND DISCUSSION

From a "consumer-friendly ingredients" perspective, it is our contention that seeds of cruciferous plants with familiar names and, more preferably those with vegetative parts recognized as edible by U.S. consumers, are to be preferred over less recognized or inedible varieties when a source for obtaining glucosinolates for food fortification purposes is considered. Therefore, in addition to broccoli (*Brassica oleracea* var. *italica*), we also chose to analyze seeds of kohlrabi (*Brassica oleracea* var. *gongylodes*), cauliflower (*Brassica oleracea* var. *botrytis*), Brussels sprouts (*Brassica oleracea* var. *gemmifera*), cabbage (*Brassica oleracea* var. *capitata*), kale (*Brassica oleracea* var. *acephala* or *viridis* and *Brassica napus* var. *pubularia*), raab (*Brassica ruvo*), and radish (*Raphanus sativa*). All selections have been previously reported to contain at least some glucoraphanin in the vegetative portions of the plant, if not the seeds (*21*, *28*, *37*−*39*).

Table 1. Glucosinolate Content of Broccoli Seed Cultivars

	μmol/g of FW[a] (μmol/g of EW)					
broccoli seed cv.	GR[b]	4OHGB[b]	GE[b]	GI[b]	GV[b]	total[c]
Premium Crop	104.9 a (153.1)	10.19 (14.88)	26.76 (39.07)	NR[d] (NR)[e]	NR (NR)	141.9 (207.1)
San Miguel (1)[c]	104.8 a (161.4)	11.90 (18.33)	18.92 (29.13)	NR (NR)	NR (NR)	138.1 (212.7)
Arcadia	95.00 b (153.9)	10.88 (17.63)	26.77 (43.37)	NR (NR)	NR (NR)	132.7 (214.9)
Gypsy	93.13 b (141.6)	10.91 (16.58)	32.16 (48.88)	NR (NR)	NR (NR)	136.2 (207.1)
Everest	92.84 bc (155.1)	9.97 (16.64)	34.10 (56.95)	NR (NR)	NR (NR)	136.9 (228.7)
Patron	90.72 c (145.2)	11.79 (18.86)	22.81 (36.50)	NR (NR)	NR (NR)	125.3 (200.6)
Southern Comet (2)[c]	87.82 d (137.0)	14.66 (22.87)	25.49 (39.76)	NR (NR)	NR (NR)	129.5 (202.1)
Destiny	86.46 d (118.5)	13.09 (17.94)	37.79 (51.76)	45.82 (62.77)	7.99 (10.95)	191.2 (261.9)
Green Comet	82.23 e (120.1)	10.54 (15.39)	35.40 (51.68)	NR (NR)	NR (NR)	128.2 (187.2)
Climax (3)[c]	81.23 e (135.7)	11.98 (20.01)	19.18 (32.02)	9.86 (16.47)	1.08 (1.81)	125.2 (209.2)
Pirate	80.75 ef (113.9)	16.42 (23.16)	28.36 (39.98)	NR (NR)	NR (NR)	125.5 (177.0)
Monterey	78.70 f (121.2)	14.32 (22.05)	21.11 (32.51)	NR (NR)	NR (NR)	114.1 (175.8)
Major	71.60 g (116.7)	7.24 (11.80)	17.33 (28.25)	36.29 (59.16)	3.55 (5.78)	136.0 (221.7)
Tierra (4)[c]	69.07 h (107.8)	15.23 (23.76)	13.05 (20.35)	18.37 (28.66)	1.76 (2.74)	118.6 (185.1)
Green Valiant	68.98 h (104.9)	12.41 (18.87)	27.98 (42.52)	NR (NR)	NR (NR)	109.4 (166.3)
Triathlon	67.69 h (111.7)	13.73 (22.24)	18.14 (29.39)	NR (NR)	NR (NR)	99.56 (163.3)
Early Dividend	62.00 i (98.28)	7.27 (11.33)	15.82 (26.17)	33.60 (52.42)	3.77 (5.88)	123.5 (194.1)
Green Goliath	62.77 i (98.55)	15.75 (24.72)	39.72 (62.36)	NR (NR)	NR (NR)	118.2 (185.6)
Super Dome	62.68 i (94.02)	8.76 (13.14)	25.24 (37.86)	40.55 (60.82)	5.25 (7.88)	142.5 (213.7)
Captain	62.14 i (98.18)	7.02 (11.09)	15.98 (25.24)	34.63 (54.72)	3.52 (5.56)	123.3 (194.8)
Marathon	59.81 j (97.48)	15.10 (24.61)	21.31 (34.73)	26.40 (43.04)	4.84 (7.89)	127.5 (207.8)
Centauro	56.92 k (83.68)	10.81 (15.88)	19.77 (29.06)	NR (NR)	NR (NR)	87.50 (128.6)
Packman acc. 2	55.67 k (95.74)	9.83 (16.90)	17.28 (29.72)	NR (NR)	NR (NR)	82.78 (142.4)
Zeus	54.69 k (85.31)	8.78 (13.70)	11.23 (17.52)	NR (NR)	NR (NR)	74.70 (116.5)
Legacy	54.68 k (79.28)	16.17 (23.45)	21.30 (30.88)	26.44 (38.33)	4.91 (7.11)	123.5 (179.1)
Small Miracle	46.92 l (75.54)	11.66 (18.77)	39.95 (64.32)	NR (NR)	NR (NR)	98.53 (158.6)
Headline	46.06 lm (70.47)	10.92 (16.71)	17.10 (26.16)	NR (NR)	NR (NR)	74.08 (113.3)
BroccoSprouts (5)[c]	44.27 mn (70.39)	11.75 (18.69)	12.87 (20.47)	21.82 (34.70)	2.76 (4.39)	96.77 (153.9)
Southern Star	42.03 no (70.19)	6.20 (10.35)	12.23 (20.41)	NR (NR)	NR (NR)	60.46 (101.0)
Packman acc. 1	41.46 o (71.31)	9.70 (16.68)	12.67 (21.79)	NR (NR)	NR (NR)	63.83 (109.8)
DeCicco (6)[c]	34.49 p (57.60)	12.08 (20.17)	10.75 (17.96)	14.63 (24.43)	2.44 (4.06)	77.80 (129.9)
Waltham 29 (7)[c]	32.70 p (54.93)	11.19 (18.80)	9.89 (16.62)	13.85 (23.26)	2.12 (3.56)	73.59 (123.6)
Montecristo	29.45 q (45.64)	9.60 (14.89)	19.16 (29.70)	7.34 (11.38)	1.32 (2.05)	66.87 (103.7)
Endeavor	5.38 r (8.93)	11.64 (19.32)	46.04 (76.43)	30.62 (50.66)	NR (NR)	93.68 (155.3)

[a] Cultivars not connected by the same letter are significantly different. [b] Glucosinolates: GR, glucoraphanin; 4OHGB, 4-hydroxyglucobrassicin; GE, glucoerucin; GI, glucoiberin; GV, glucoibervirin; GA, glucoalyssin; P, progoitrin; S, sinigrin. [c] Includes GA, P, and S for 1–7: (1) GA 2.52, (3.88); (2) GA 1.56, (2.43); (3) GA 1.90, (3.17); (4) GA 1.12, (1.74); (5) P 2.05 (3.27), S 1.25 (1.99); (6) P 2.35 (3.92), S 1.06 (1.77); (7) P 2.75 (4.61), S 1.09 (1.82). [d] NR means <1.00 μmol/g of FW. [e] (NR) means <1.50 μmol/g of EW.

The glucosinolates quantified in broccoli cultivars with concentrations above our reporting limits are presented in **Table**

Table 2. Glucoraphanin (GR) and 4-Hydroxyglucobrassicin (4OHGB) Contents of Other Crucifer Seeds

	μmol/g of FW (μmol/g of EW)	
crucifer seed, cv.	GR	4OHGB
raab, Spring Raab	0.13 (0.24)	10.28 (18.60)
raab, Sessantina Grossa	0.12 (0.21)	11.95 (21.75)
kohlrabi, Grand Duke	40.25 (64.80)	10.68 (17.19)
kohlrabi, Purple Vienna	36.81 (62.20)	14.76 (24.95)
kohlrabi, White Vienna	33.16 (58.02)	12.76 (22.32)
kohlrabi, Winner	0.78 (1.34)	13.00 (22.35)
radish, Diakon	1.79 (3.02)	7.91 (13.37)
radish, White Icicle	1.73 (2.91)	11.11 (18.66)
radish, Nero Tonda	1.42 (2.72)	11.56 (22.07)
radish, Altaglobe	1.09 (1.83)	12.28 (20.63)
cauliflower, Brocoverde	40.70 (62.67)	13.00 (20.03)
cauliflower, Shasta	1.90 (3.06)	15.91 (25.61)
cauliflower, Early Dawn	1.84 (3.03)	19.58 (32.30)
cauliflower, Snow March	0.32 (0.53)	14.99 (24.58)
Brussels sprouts, Long Island	6.24 (10.92)	15.43 (27.01)
Brussels sprouts, Oliver	4.37 (6.86)	17.99 (28.24)
Brussels sprouts, Dutch Treat	3.47 (5.58)	16.37 (26.35)
Brussels sprouts, Bubbles	0.82 (1.30)	20.66 (32.84)
kale, Red Russian	3.02 (5.27)	13.19 (23.09)
kale, Siberian	2.89 (5.32)	12.25 (22.54)
kale, Dwarf Blue Curled	2.74 (4.66)	12.34 (20.97)
kale, Blue Ridge	0.84 (1.41)	19.60 (32.92)
cabbage, Danish Ballhead	6.95 (11.67)	17.30 (29.07)
cabbage, Golden Acre	5.80 (9.80)	18.77 (31.72)
cabbage, Emblem	4.02 (6.39)	13.23 (21.03)
cabbage, Blue Vantage	1.53 (2.55)	26.21 (43.52)

1. Results for the glucoraphanin and 4-hydroxyglucobrassicin content of all other crucifer seed accessions are listed in **Table 2**. Representative chromatograms (using 3 μm, 150 × 2.1 mm column) of the extracts obtained from one variety or cultivar of each type of plant seed are documented in **Figures 1−8**. The chemical structures of all identified glucosinolates may be found in **Table 3**.

Among the accessions analyzed by us, there can be little doubt that broccoli seeds are usually the best source for obtaining glucoraphanin. The one exception was cv. Endeavor, for which not only was the glucoraphanin content considerably lower compared to that of other broccoli cultivars but glucoraphanin was also the minor glucosinolate quantified. In every other broccoli seed cultivar analyzed, glucoraphanin was always the major glucosinolate present. Of the other 26 crucifers tested, only the seeds of 3 kohlrabi cultivars (Grand Duke, Purple Vienna, and White Vienna) and 1 of the cauliflower accessions (cv. Brocoverde) contained levels of glucoraphanin similar to those of some of the broccoli cultivars. However, two of the kohlrabi cultivars (White Vienna and Purple Vienna) contained measurable amounts of progoitrin (4.50 and 9.09 μmol/g of FW, respectively), and all three contained glucoiberin (22.84−26.25 μmol/g of FW). Cauliflower var. Brocoverde also contained substantial amounts of glucoiberin (72.50 μmol/g of FW). Owing to reported goitrogenic and other so-called "antinutritional" effects of their conversion products, we believe it is best to exclude seeds from consideration that contain progoitrin and/or epiprogoitrin (*37*, *40*). Although concerns over the safety of glucoiberin appear to be unwarranted (*41*, *42*), the conversion product of this glucosinolate, 3-methylsulfinylpropyl isothiocyanate, has low (∼10−20%) phase 2 enzyme inducer activity as compared to sulforaphane (*42*−*44*). In fact, some previous reports of the phase 2 enzyme induction capacity of broccoli were erroneously overstated due to the coelution of glucoraphanin and glucoiberin (*39*), a problem corrected with our methodology (*32*).

As seen in **Table 1**, ∼40% (14 of 33) of the broccoli cultivars also contained glucoiberin and all accessions contained glucoerucin (3-methylthiobutyl glucosinolate). Similar to glucoiberin, glucoerucin presents no reported safety issue, but the activity of the corresponding isothiocyanate to induce phase 2 enzymes is again much less than that of sulforaphane (*43*, *44*). Three cultivars (BroccoSprouts, DeCicco, and Waltham 29) were found to contain progoitrin. However, the presence of this goitrogenic precursor in these seeds is of no practical significance, because all three are in the lower 20% of broccoli accessions, on the basis of glucoraphanin content, and would therefore not be considered to be good candidates for glucoraphanin isolation. Other glucosinolates identified and quantified in broccoli seed accessions included glucoibervirin (3-methylthiopropyl glucosinolate), glucoalyssin (5-methylsulfinyl glucosinolate), and sinigrin. Glucobrassicin was also present in several cultivars, but at levels below our reporting limit. The conversion products of these glucosinolates are thought to be relatively weak phase 2 enzyme inducers (*43*−*45*).

Our crucifer seed accessions all contained 4-hydroxyglucobrassicin as the major indole-containing glucosinolate in concentrations ranging from 6.2 to 26.21 μmol/g of FW (mean of 12.87 μmol/g of FW). Truscott and co-workers first reported the presence of 4-hydroxyglucobrassicin in crucifer seeds including cabbage and rapeseed (*Brassica napus*) (*46*). The concentration of 4-hydroxyglucobrassicin in one rapeseed cultivar was ∼14 μmol/g of defatted meal, an amount consistent with our range, and mean EW values of 10.35−43.52 and 20.90 μmol/g, respectively, for all crucifer seeds. The 4-hydroxyglucobrassicin concentration of cabbage seeds was not reported by Truscott. A subsequent study by Sang et al. corroborated the presence of 4-hydroxyglucobrassicin in cabbage seed and rapeseed and also recorded its presence in the seeds of three other crucifers, including mustard (*Brassica juncea*), swede (*B. napobrassica*), and radish, but no concentrations were given (*28*). These same investigators were first to note that while leaf tissue contained mainly glucobrassicin and little 4-hydroxyglucobrassicin, the reverse was true for seeds. Although the presence of 4-hydroxyglucobrassicin in broccoli seed appears to have been alluded to previously (*36*), Troyer and co-workers may be the first to have actually documented its presence (cv. Marathon); however, no quantitative data were reported (*39*). Matthäus and Luftman identified 4-hydroxyglucobrassicin in 8

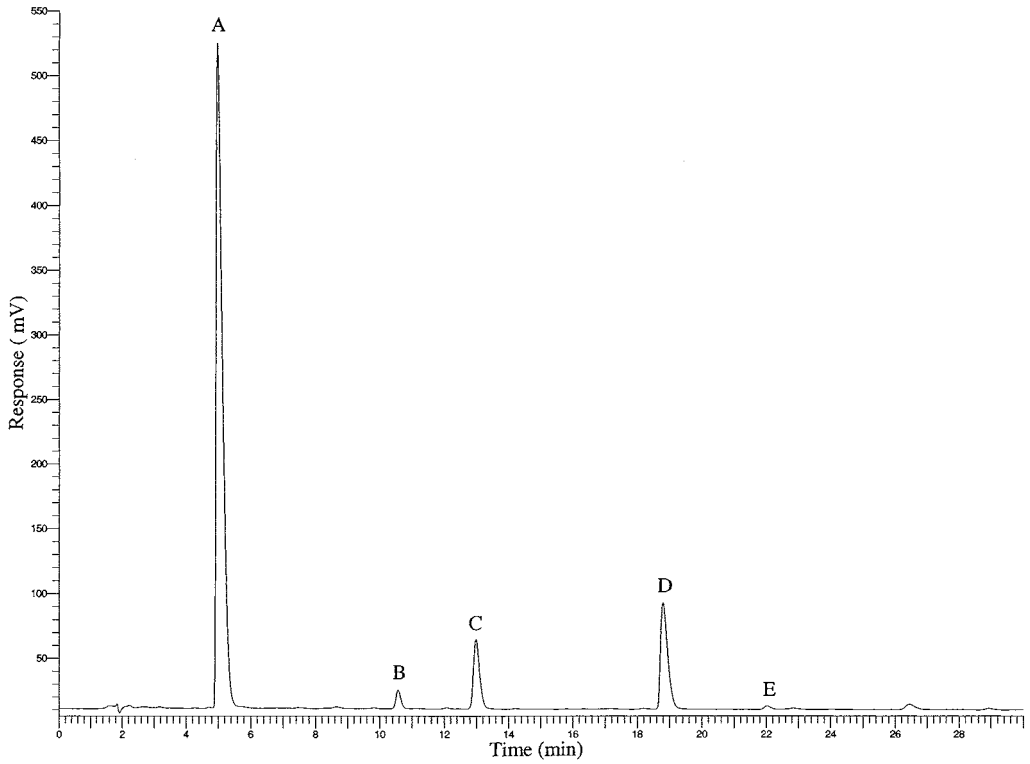


Figure 1. HPLC-UV chromatogram of broccoli seed cv. San Miguel glucosinolates: (A) glucoraphanin, (B) glucoalyssin, (C) 4-hydroxyglucobrassicin, (D) glucoerucin, and (E) glucobrassicin.

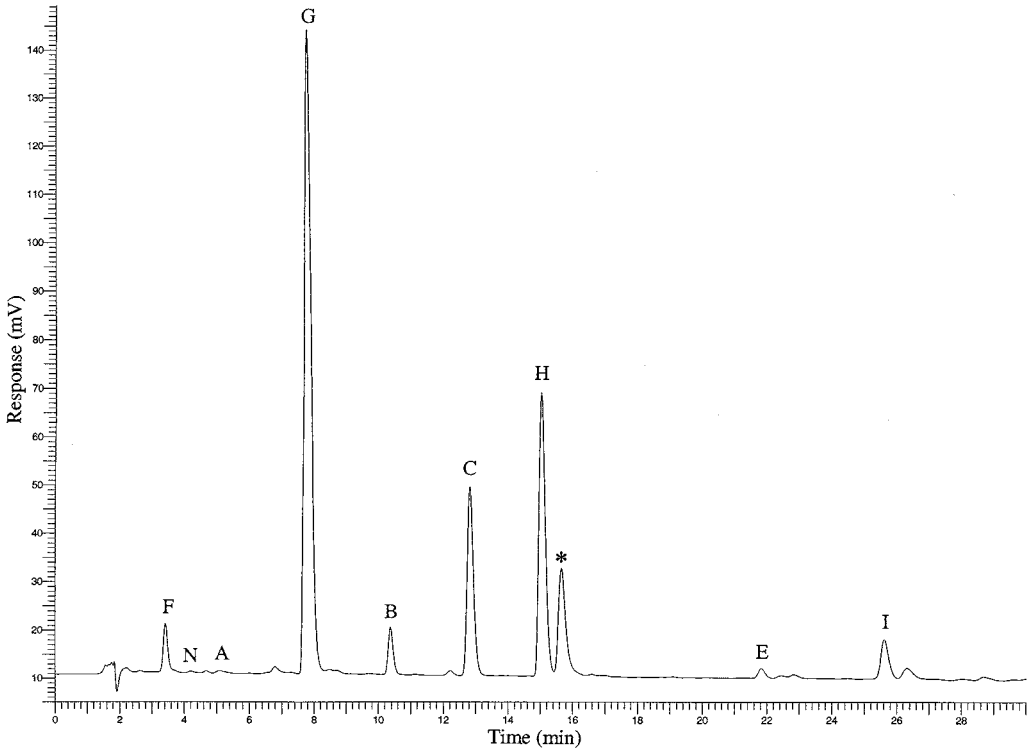


Figure 2. HPLC-UV chromatogram of raab seed cv. Sessantina Grossa glucosinolates: (A) glucoraphanin, (B) glucoalyssin, (C) 4-hydroxyglucobrassicin, (E) glucobrassicin, (F) progoitrin, (G) gluconapin, (H) glucobrassicanapin, (I) gluconasturtiin, (N) epiprogoitrin, and (∗) unidentified, but not likely a glucosinolate (missing *m*/*z* 97 ion [HSO_4^-], which is appreciably detected using MS/MS and MS/MS/MS for typical glucosinolates).

of 14 crucifer seed acquisitions, including radish (*R. sativus* var. *nigher*; 1.4 μmol/g, *R. sativus* var. *oleiformis*; 1.9 μmol/g, and *R. sativus* var. *sativus*; 1.3 μmol/g) and Brussels sprouts (1.4 μmol/g) (*47*). Although not specifically stated, these results were likely based on fresh weight and are almost 10-fold lower than ours.

Substantial differences in the glucosinolate content of crucifer seeds reported by other investigators are not limited to only 4-hydroxyglucobrassicin content, however. Comparison of our broccoli seed glucoraphanin results with previous reports reveals considerable variation. For example, Carlson and co-workers reported the glucoraphanin content for four broccoli seed

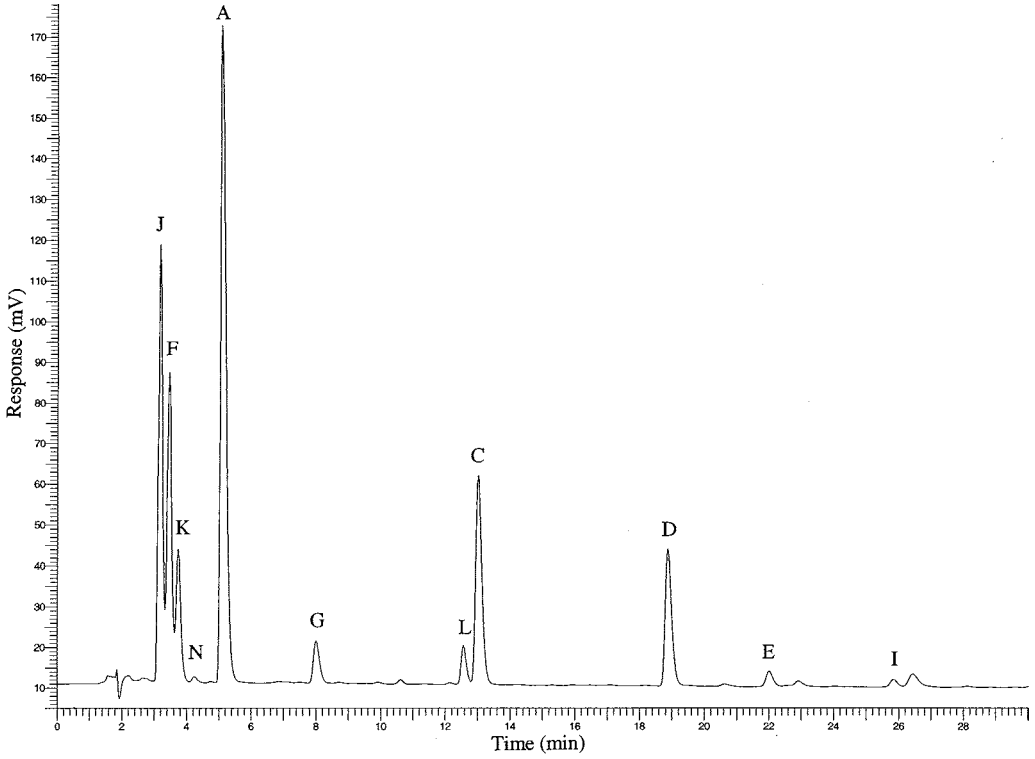


Figure 3. HPLC-UV chromatogram of kohlrabi seed cv. Purple Vienna glucosinolates: (A) glucoraphanin, (C) 4-hydroxyglucobrassicin, (D) glucoerucin, (E) glucobrassicin, (F) progoitrin, (G) gluconapin, (I) gluconasturtiin, (J) glucoiberin, (K) sinigrin, (L) glucoibervirin, and (N) epiprogoitrin.

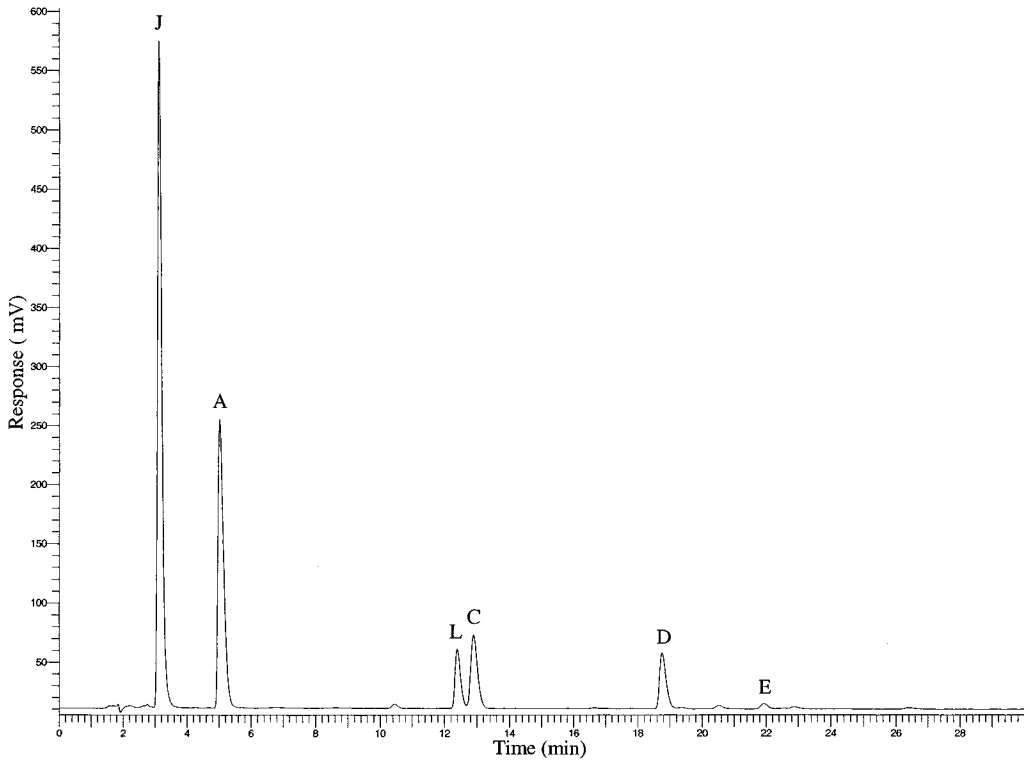


Figure 4. HPLC-UV chromatogram of cauliflower seed cv. Brocoverde glucosinolates: (A) glucoraphanin, (C) 4-hydroxyglucobrassicin, (D) glucoerucin, (E) glucobrassicin, (J) glucoiberin, and (L) glucoibervirin.

cultivars with results ranging from 31.5 to 86.1 μmol/g of defatted weight (mean value of 56.3 μmol/g of defatted weight; converted from units of millimoles/100 g by us) (*38*). Our comparable EW values ranging from 8.93 to 161.4 μmol/g (mean of 101.6 μmol/g) are essentially double. Green Comet was the only cultivar analyzed in both studies, and our finding was almost 4 times greater (31.5 μmol/g compared to 120.1 μmol/g). Our glucoraphanin result for cv. Marathon was also higher than that reported by Troyer et al. (59.81 μmol/g of FW compared to 36 μmol/g of FW) (*39*). In contrast, our glucoraphanin results are almost half the value of those recently reported by Rangkadilok and co-workers (*34*). Results for their

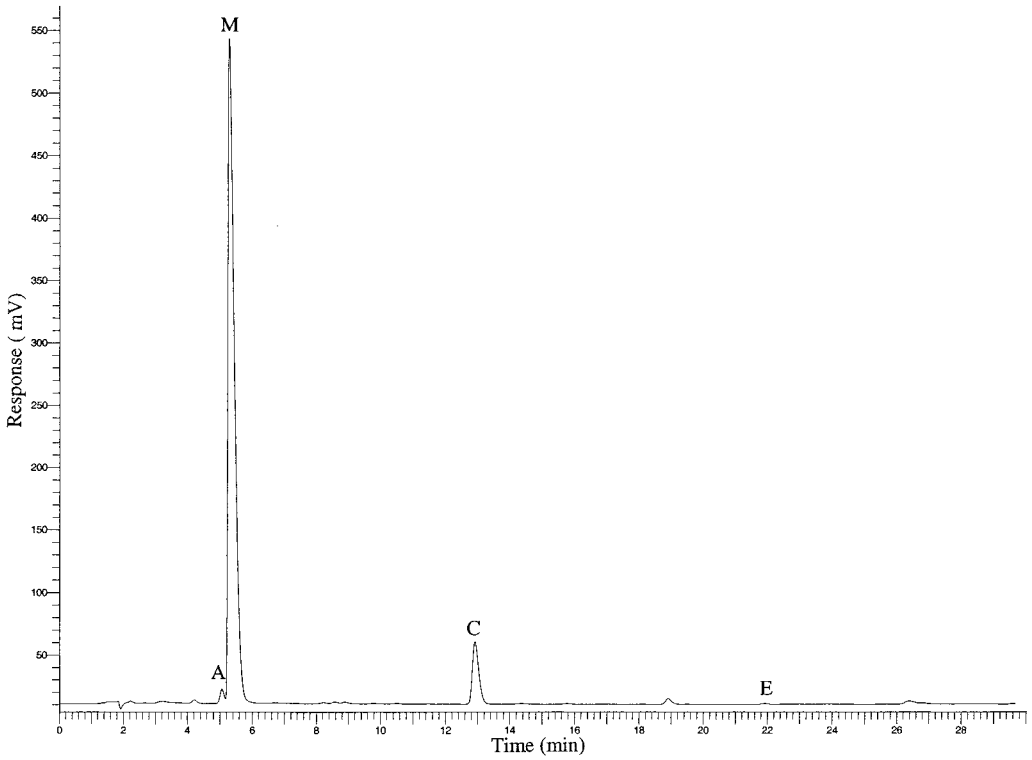


Figure 5. HPLC-UV chromatogram of radish seed cv. Nero Tonda glucosinolates: (A) glucoraphanin, (C) 4-hydroxyglucobrassicin, (E) glucobrassicin, and (M) glucoraphenin.

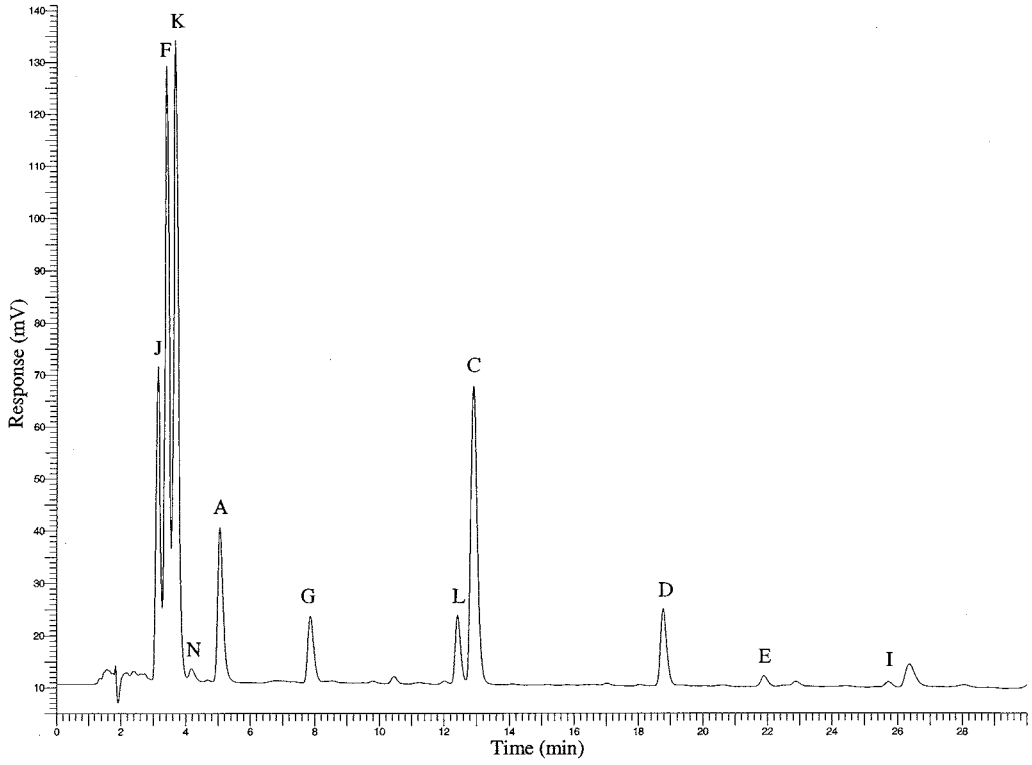


Figure 6. HPLC-UV chromatogram of Brussels sprouts seed cv. Long Island glucosinolates: (A) glucoraphanin, (C) 4-hydroxyglucobrassicin, (D) glucoerucin, (E) glucobrassicin, (F) progoitrin, (G) gluconapin, (I) gluconasturtiin, (J) glucoiberin, (K) sinigrin, (L) glucoibervirin, and (N) epiprogoitrin.

15 broccoli seed cultivars ranged from 44.2 to 275.1 μmol/g of DW (dry weight; mean of 137.6 μmol/g), which even after taking into account an average value for moisture of 5% and converting to FW, are about double the values reported by us. Both studies have only the cultivar Marathon in common, and their result is again about twice ours [115.6 μmol/g of FW (value reported as 121.7 μmol/g of DW and converted to FW by us) compared to our value of 59.81 μmol/g of FW].

Differences in reported glucosinolate values between the various studies could be the result of multiple factors, including methodology (all reports used different approaches for extraction, chromatography, and quantification of glucosinolates) and

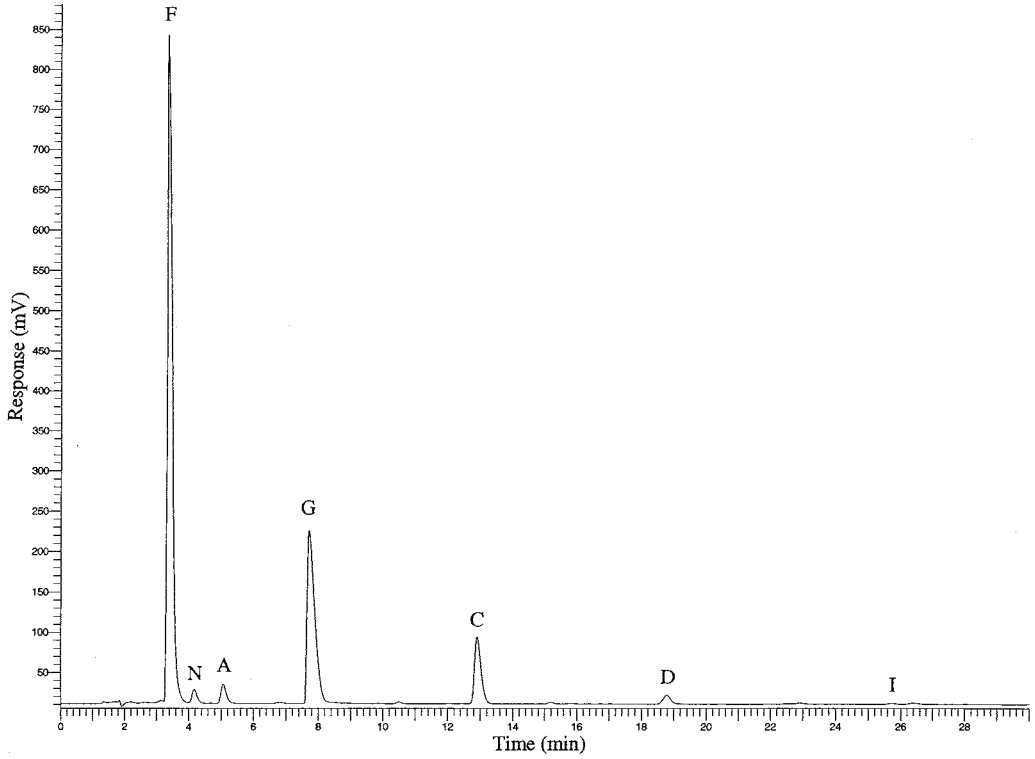


Figure 7. HPLC-UV chromatogram of kale seed cv. Red Russian glucosinolates: (A) glucoraphanin, (C) 4-hydroxyglucobrassicin, (D) glucoerucin, (F) progoitrin, (G) gluconapin, (I) gluconasturtiin, and (N) epiprogoitrin.

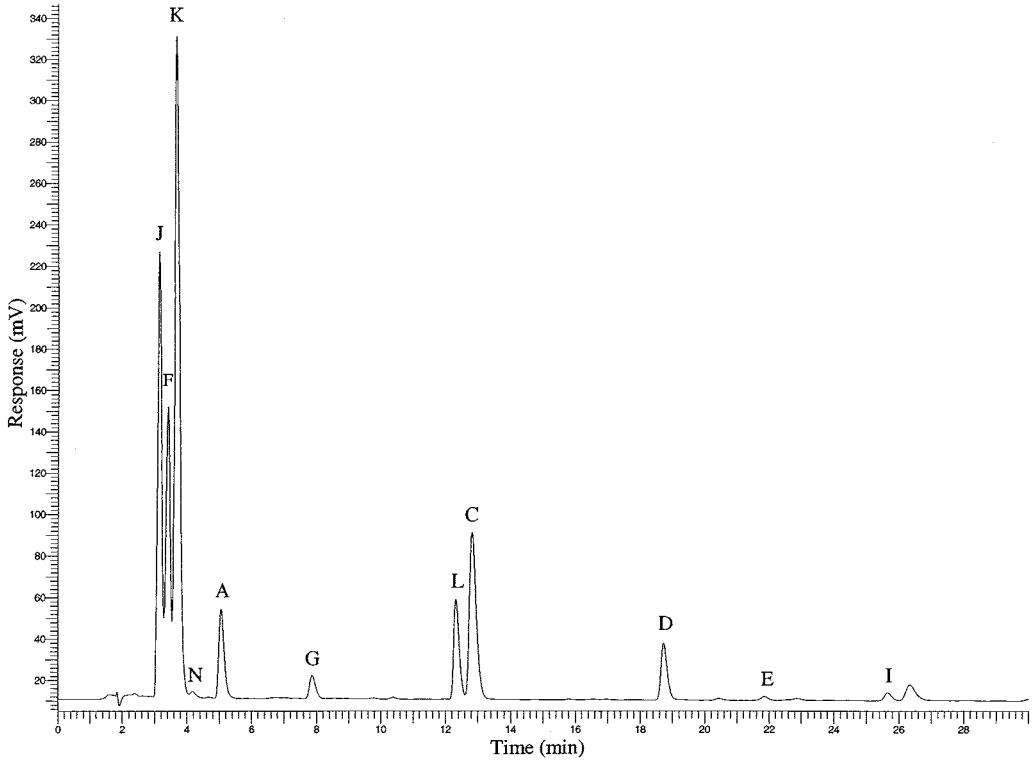
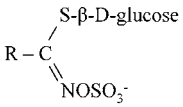


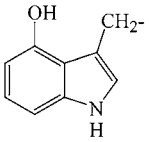
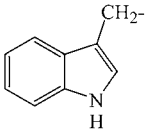
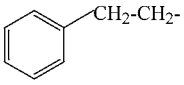
Figure 8. HPLC-UV chromatogram of cabbage seed cv. Danish Ballhead glucosinolates: (A) glucoraphanin, (C) 4-hydroxyglucobrassicin, (D) glucoerucin, (E) glucobrassicin, (F) progoitrin, (G) gluconapin, (I) gluconasturtiin, (J) glucoiberin, (K) sinigrin, (L) glucoibervirin, and (N) epiprogoitrin.

sample variation, including variables such as growing and storage environments (*34*, *48*). To this point of sample variation, we found statistically significant differences between two separate accessions of the cultivar Packman (55.67 and 41.46 μmol/g FW); differences we did not find between two accessions of Gypsy (93.13 and 91.22 μmol/g FW; data for only one accession presented in **Table 1**). Although further speculation regarding the root cause(s) of these differences without new data is of limited value, it is clear that work needs to be undertaken to identify and communicate to the various research groups the key parameters that better ensure the accuracy and consistency of seed glucosinolate measurements.

Additionally, further study into possible safety issues of the extraneous glucosinolates likely to be extracted along with

Table 3. Chemical Structures of Identified Glucosinolates



Compound	Common Name	R Group
A	glucoraphanin	CH_3-SO-CH_2-CH_2-CH_2-CH_2-
B	glucoalyssin	CH_3-SO-CH_2-CH_2-CH_2-CH_2-CH_2-
C	4-hydroxyglucobrassicin	
D	glucoerucin	CH_3-S-CH_2-CH_2-CH_2-CH_2-
E	glucobrassicin	
F	progoitrin (R)	CH_2=CH-C*HOH-CH_2-
G	gluconapin	CH_2=CH-CH_2-CH_2
H	glucobrassicanapin	CH_2=CH-CH_2-CH_2-CH_2-
I	gluconasturtiin	
J	glucoiberin	CH_3-SO-CH_2-CH_2-CH_2-
K	sinigrin	CH_2=CH-CH_2-
L	glucoibervirin	CH_3-S-CH_2-CH_2-CH_2-
M	glucoraphenin	CH_3-SO-CH=CH-CH_2-CH_2-
N	epiprogoitrin (S)	CH_2=CH-C*HOH-CH_2-

glucoraphanin, especially 4-hydroxyglucobrassicin, may be warranted to ensure that none possesses undesirable biological or nutritional effects at the levels likely to be encountered in semipurified glucoraphanin preparations. Although all of the alkyl- and indole-containing glucosinolates identified in broccoli seed have been reported in the edible parts of broccoli or other commonly consumed crucifers, the ratios of the differing glucosinolates vary greatly between seed and commonly consumed vegetative parts (*49*−*52*). Considering 4-hydroxyglucobrassicin as an example, Pereira et al. (*22*) as well as Fahey and co-workers (*36*) reported that indole-containing glucosinolates (4-hydroxyglucobrassicin and glucobrassicin) most often accounted for <10% of the total glucosinolates in 3-day-old broccoli sprouts (>70 cultivars evaluated). However, almost 65% of the broccoli seed cultivars we analyzed possessed 4-hydroxyglucobrassicin alone in concentrations >10%, with several as high as 15−16%. If any extraneous glucosinolate becomes suspect of causing potential harm, the development of procedures for minimizing its concentration will need to be considered. To this point, a method has been recently patented for the production of broccoli and cauliflower plants, which in effect decreases the level of indole-containing glucosinolates relative to those with alkyl moieties (*53*).

The development of procedures for the enrichment of glucoraphanin from crucifer seeds should also take into account the removal of high erucic acid-containing lipids. All seed accessions contained substantial amounts of hexane−extractable lipids ranging from 21.8 to 42.0% (mean of 32.8%; 21.8−37.0 and 30.9% range and mean, respectively, for broccoli cultivars only), which were composed of 27.0−56.7% (mean of 46.7%; 39.4−56.7 and 50.2% range and mean, respectively, for broccoli cultivars only) erucic acid. Because extraction with hexane does not remove polar lipids, the total amount of erucic acid-containing lipids might be expected to be even higher than presented. However, this is not the case for broccoli seeds as we previously reported that the percentage of erucic acid was essentially the same in extracts prepared using a total lipid

Table 4. Hexane-Extractable Lipid (HL) and Its Erucic Acid Content in Crucifer Seed Cultivars

crucifer seed, cv.	% HL	% erucic acid[a]
broccoli, Arcadia	33.0	46.6
broccoli, BroccoSprouts	31.4	51.6
broccoli, Captain	31.6	55.2
broccoli, Centauro	26.2	50.7
broccoli, Climax	34.8	56.7
broccoli, De Cicco	35.8	52.1
broccoli, Destiny	21.8	49.4
broccoli, Early Dividend	31.3	54.7
broccoli, Endeavor	35.0	50.0
broccoli, Everest	36.2	55.5
broccoli, Green Comet	26.3	50.7
broccoli, Green Goliath	31.0	49.7
broccoli, Green Valiant	28.8	53.3
broccoli, Gypsy	28.7	39.4
broccoli, Headline	29.3	51.8
broccoli, Legacy	25.9	47.9
broccoli, Major	30.4	53.5
broccoli, Marathon	33.6	49.8
broccoli, Montecristo	29.0	49.7
broccoli, Monterey	30.3	47.9
broccoli, Packman 1	36.6	43.3
broccoli, Packman 2	37.0	44.4
broccoli, Patron	33.1	46.8
broccoli, Pirate	23.5	47.5
broccoli, Premium Crop	25.6	47.5
broccoli, San Miguel	29.6	52.4
broccoli, Small Miracle	32.7	50.4
broccoli, Southern Comet	30.5	50.0
broccoli, Southern Star	35.1	51.9
broccoli, Super Dome	27.5	54.8
broccoli, Tierra	30.1	50.6
broccoli, Triathlon	33.6	46.6
broccoli, Waltham 29	35.4	51.9
broccoli, Zeus	31.2	52.7
raab, Sessantina Grossa	38.9	44.5
raab, Spring Raab	34.8	44.8
kohlrabi, Grand Duke	32.0	48.3
kohlrabi, Purple Vienna	35.1	43.8
kohlrabi, White Vienna	37.3	47.9
kohlrabi, Winner	36.7	50.3
radish, Altaglobe	35.3	34.0
radish, Diakon	36.3	32.5
radish, Nero Tonda	42.0	27.0
radish, White Icicle	35.7	28.8
cauliflower, Brocoverde	29.2	44.2
cauliflower, Early Dawn	34.0	55.2
cauliflower, Shasta	33.4	52.9
cauliflower, Snow March	31.2	40.3
Brussels sprouts, Bubbles	31.5	44.2
Brussels sprouts, Dutch Treat	32.7	36.8
Brussels sprouts, Long Island	37.8	33.6
Brussels sprouts, Oliver	31.8	35.5
kale, Blue Ridge	36.3	50.4
kale, Dwarf Blue Curled	36.8	38.9
kale, Red Russian	37.0	42.9
kale, Siberian	40.0	37.9
cabbage, Blue Vantage	34.8	40.7
cabbage, Danish Ballhead	36.0	44.7
cabbage, Emblem	32.4	48.9
cabbage, Golden Acre	36.5	43.1

[a] Expressed as methyl erucate, % of all methyl esters.

extraction method (Folch) or hexane alone (*24*). All lipid data are presented in **Table 4**.

This study represents the largest single report of the glucoraphanin and 4-hydroxyglucobrassicin contents of commercially available crucifer seed cultivars. Several cultivars of broccoli seed are clearly better sources for obtaining glucoraphanin than others, but even the best of these contain significant levels of potentially unwanted glucosinolates, namely, 4-hydroxyglucobrassicin and glucoerucin. Among the top six highest glucoraphanin-containing cultivars, Premium Crop and San Miguel are our preferred choices for the isolation of glucoraphanin. They contain a lower total percentage of extraneous glucosinolates relative to glucoraphanin than the others in this top group (~5% less) and are also among those lowest in hexane−extractable lipid content (which we currently view as a waste stream owing to the erucic acid content).

ABBREVIATIONS USED

FW, fresh weight; EW, extracted weight (hexane-extracted/dried); DW, dried weight; HL, hexane-extractable lipid; cv., cultivar; FAD, fatty acid distribution; RSD, relative standard deviation; acc, accessions; GR, glucoraphanin; 4OHGB, 4-hydroxyglucobrassicin; GE, glucoerucin; GI, glucoiberin; GV, glucoibervirin; GA, glucoalyssin; P, progoitrin; S, sinigrin.

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