Variation in Glucosinolates in Oriental Brassica Vegetables

Curtis B. Hill and Paul H. Williams
Department of Plant Pathology, University of Wisconsin, Madison, WI, 53706
Diana G. Carlson and H.L. Tookey
Agricultural Research Service, U.S. Department of Agriculture, Northern Regional Research Center, Peoria, IL 61604

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Abstract. The glucosinolates (GSs) were estimated in the normally eaten portions of 72 cultivars of Oriental brassica vegetables including mustard greens (Brassica juncea L.), Chinese kale (B. oleracea L. Alboglabra Group Bail.), Chinese cabbage (B. rapa L. Pekinski Group Bail.), pak choy (B. rapa Chinensis Group Bail.), tendergreen (B. rapa Perviridis Group Bail.), turnip (B. rapa L. Rapifera Group Bail., B. narinos. Bail., and B. nipposinica Bail.). Variation in GS profiles was complex. There was variation in percentages of major GSs and total GS among B. juncea, B. oleracea, and the combination B. rapa plus naringosa and nipposinica and among four subspecific groups of rapa plus the two species closely related to rapa: naringosa and nipposinica. B. juncea had distinctively high proportions of alyl-GS, ranging from 81% to 94%, whereas B. oleracea had distinctively high proportions of 4-methylsulfanylbutil-GS, ranging from 9% to 68%. Differences in GS profiles among the rapa groups, naringosa and nipposinica, were less distinctive. Cultivars of pak choy from China differed in percentages of three minor GSs from cultivars from Japan and elsewhere. There was also variation among cultivars of Chinese kale and between turnip foliage and roots.

Vegetable brassicas including Chinese cabbage, pak choy, turnip, tendergreen, B. narinos. B. nipposinica, mustard greens, and Chinese flowering kale long have been important in the diet of East Asians (10, 25, 27), and they are increasing in popularity in the United States and Europe (8). Important in crucifers are the biologically active thioglucosides known as glucosinolates (GSs), the chemistry of which has been extensively reviewed (18, 20). The hydrolytic products of GSs have been implicated in mustard flavor (11), insect attraction (6), disease susceptibility (1), toxicity to animals (13), and anticarcinogenesis (26).

As part of a comprehensive study to provide information on levels and variation of GSs in cruciferous vegetables (2, 3, 5, 19), we have analyzed the GS contents in the edible parts of 72 cultivars or lines of mustard greens, Chinese flowering kale, B. narinos. B. nipposinica, and vegetables in four subspecific groups of B. rapa.

Materials and Methods

Seeds of 72 cultivars or breeding lines of oriental brassica vegetables were provided by seed companies, institutions, or individuals (Table 1). Seeds were sown in plastic pots containing 1 coarse sand; 1 compost; 1 peatmoss (by volume), and plants were grown in the greenhouse at 24°C and fertilized weekly with a 10N–10P–10K fertilizer. Twenty-one days after seeding, plants were transplanted to the field, which consisted of a Colwood silt-loam (fine, loamy, mixed, mesic, typic, haplaquolls) at the Univ. of Wisconsin agricultural experiment station, Madison. The Chinese flowering kale was grown in Plainfield loamy-sand (mixed, mesic, typic, udipsamments) at the Univ. of Wisconsin agricultural experiment station, Hancock. Chinese cabbage cultivars from China were sown in August and harvested in Nov. 1980; Chinese flowering kale was sown in June and harvested in Aug. 1981; the remainder of the vegetables were sown in June and harvested in July 1980. Two or three plants of each cultivar were chilled, harvested, and shipped to the USDA Northern Regional Research Center in Peoria, Ill., where they were stored at 4°C for up to 3 weeks before extraction. Storage of plants in the refrigerator had no effect on GS levels. The plant parts sampled were those normally eaten (Table 1). For Chinese kale, samples consisted of 100 g of fresh tissue.
from individual plants. For the other vegetables, nearly equal amounts of three plants were bulked to make 100-g composite samples. Wedges were cut from individual heads of Chinese cabbage and unpeeled roots of turnip.

To assess the interplant variation within a cultivar, seven cultivars of *B. rapa*, *B. nipposinica*, and *B. narinosa* were grown at Hancock, Wis. in 1981. Four plants of each cultivar were sampled and analyzed individually.

Tissues were extracted in boiling methanol (19), and the extracts treated with thioglycosidase (EC 3.2.3.1.) to release glucose from GSs by hydrolysis (5, 21). Released glucose measured with Glucose Auto/Stat (Pierce) was used as a measure of total GS. Amounts of isothiocyanates and oxazolidine-2-thiones, formed during hydrolysis and measured by gas chromatography (4), were used to estimate the amounts of their respective GS precursors. The 3-indolylmethyl- and 3-(N-methoxylindolylmethyl)-GS (and other 3-indolylmethyl-GSs present) were estimated jointly by measuring the thiocyanate ion that formed during hydrolysis (2).

Comparisons of the data were made using one-way analysis of variance (ANOVA) and Fisher's least significant difference (i.e., LSDs were calculated only if the ANOVA was significant for \( P = 0.05 \) or less). Prior to statistical analysis, percentage data were transformed to the trigonometric angle of the percentage by calculating arcsin \( \frac{\text{percentage}}{100} \) \(^{1/2} \). Each cultivar was used as a sample in the ANOVA.


### Results

Among the *Brassica* species, *oleracea*, *juneae*, and *rapa* (including *narinosa* and *nipposinica*), there were significant differences in the percentages of allyl-, 3-butenyl-, and 4-methylsulfinylbutyl-GS and in the total amount of GS present in the edible portions (Table 2). The percentage differences indicate relative differences in GS contents among species. Allyl-GS was the predominant GS in *B. juneae* and was distinctive in that its range in percentage did not overlap with the ranges in the other two species. Similarly, the percentage of 4-methylsulfinylbutyl-GS differentiates *B. oleracea* from the other two species. That GS and 3-butenyl-GS were the major GSs in *B. oleracea*. 3-butenyl-GS was the predominant GS in *B. rapa* (including *narinosa* and *nipposinica*). Great variation was found among the cultivars in the percentages of these major GSs (>10% in proportion) as indicated by the large ranges.

Analysis of the experiment estimating interplant variation within a cultivar indicated that there was very large variation among four replicate plants (CV = 0.4 to 2.4) for the absolute levels of individual GS. Their percentages varied similarly. Because of this variation and sampling (two or three plants) of *B. rapa*, *B. narinosa*, *B. nipposinica*, and *B. juneae*, we believe that comparisons among cultivars within a vegetable group could not be meaningful.

In addition to the major GSs listed in Table 2, significant differences were found among the species in some minor GSs (<10% proportion). *B. juneae* cultivars had no 2-hydroxy-3-butenyl-GS, whereas proportions in *B. rapa* cultivars ranged.

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**Table 1. Scientific names, common names or description, sources, and parts of the vegetables analyzed.**

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name or description</th>
<th>Sources</th>
<th>Part analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. juncea</em> L.</td>
<td>Mustard greens</td>
<td>A.B.</td>
<td>Foliage</td>
</tr>
<tr>
<td><em>B. narinosa</em> Bail.</td>
<td>Dark green.</td>
<td>C.G.</td>
<td>Foliage</td>
</tr>
<tr>
<td><em>B. oleracea</em> L. Group Albuglabra Bail.</td>
<td>Chinese kale or broccoli</td>
<td>H</td>
<td>Flowering shoot</td>
</tr>
<tr>
<td><em>B. rapa</em> L. Group Chinensis Bail.</td>
<td>Pak choy</td>
<td>A.B.C.D.E.F</td>
<td>Foliage</td>
</tr>
<tr>
<td><em>B. rapa</em> L. Group Pekinensis Bail.</td>
<td>Chinese cabbage, petsai</td>
<td>E</td>
<td>Head</td>
</tr>
<tr>
<td><em>B. rapa</em> L. Group Perviridis Bail.</td>
<td>Tendergreen, mustard spinach</td>
<td>A.B.</td>
<td>Foliage</td>
</tr>
<tr>
<td><em>B. rapa</em> L. Group Raphifera Bail.</td>
<td>Turnip</td>
<td>A.B.</td>
<td>Foliage and root</td>
</tr>
</tbody>
</table>

'\( A = \) Takii and Co., Kyoto, Japan.
'B = Mikado Seed Growers Co., Chiba City, Japan.
'C = Asian Vegetable Research and Development Center, Shanhua, Taiwan.
'D = C. Miller, Nickerson International Plant Breeders, Gilroy, Calif.
'E = Li Chia Wen, People's Republic of China.
'F = Chen Hang, Beijing Vegetable Research Institute.
'G = Watanabe Seed Co., Kogota, Japan.
'H = F. Heyn, Feldstrasse 36, D-2222 Mame, Federal Republic of Germany.'
Table 2. *Brassica* *rapa* (including *B. narinos* and *B. nipposinica*), *B. juncea* and *B. oleracea*: Major glucosinolates (GSs) in fresh tissue that were significantly different (*P* = 0.05 or less in analysis of variance) in percentages of all GSs individually measured, and total amounts among the species.

<table>
<thead>
<tr>
<th><em>Brassica</em> sp.</th>
<th>3-butenyl</th>
<th>Allyl</th>
<th>4-methyl-</th>
<th>Total</th>
<th>4-methylsulfinylbutyl</th>
<th>Total individual GS*</th>
<th>Total glucose released</th>
<th>4-methylsulfinylbutyl</th>
<th>Total glucose released</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. rapa</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
<td>µmol/100g</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
</tr>
<tr>
<td>0%</td>
<td>---</td>
<td>29 ± 1 a</td>
<td>0-86</td>
<td>1 b</td>
<td>0-11</td>
<td>139 ± 15 b</td>
<td>5-458</td>
<td>79 ± 3</td>
<td>13-123</td>
</tr>
<tr>
<td><em>B. oleracea</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
<td>µmol/100g</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
</tr>
<tr>
<td>4 ± 1 b</td>
<td>0-13</td>
<td>46 ± 1 a</td>
<td>7-77</td>
<td>29 ± 1 a</td>
<td>9-68</td>
<td>230 ± 39 b</td>
<td>139-103</td>
<td>89 ± 3</td>
<td>82-103</td>
</tr>
<tr>
<td><em>B. juncea</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
<td>µmol/100g</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
</tr>
<tr>
<td>90 ± 1 a</td>
<td>81-94</td>
<td>4 ± 1 b</td>
<td>2-9</td>
<td>0 b</td>
<td>---</td>
<td>391 ± 45 a</td>
<td>192-556</td>
<td>80 ± 4</td>
<td>62-95</td>
</tr>
</tbody>
</table>

*The percentages were transformed to the trigonometric angle by arcsin (%/100)^1/2.*

*Combined data of subspecific groups Chinensis, Pekinensis, Perviridis, and Rapifera and also including *B. narinos* and *B. nipposinica,* excluding *Rapifera* foliage. Number of cultivars tested: 59 (*rapa*), 6 (*oleracea*), 7 (*juncea*).

*Mean ± se.*

*Mean total amount of GS in fresh tissue as measured by the glucose released after enzymatic hydrolysis of the extracts. Use 457 as the average molecular weight of GS."

Means across a row followed by a common letter were not significantly different at *P* = 0.05 using Fisher’s least significant difference.

from 0% to 24%, and, in *B. oleracea*, 1% to 11%. The percentages of 4-pentenyl-GS were significantly higher in *B. rapa*, but there were exceptions because some cultivars had none. 4-pentenyl-GS, not found in *B. oleracea*, was present in <1% proportion in only one *B. juncea* cultivar. *B. rapa* and *B. oleracea* had small amounts of 5-methylsulfinylpentyl-GS, whereas it was absent in *B. juncea*. *B. rapa* had a significantly higher percentage of 2-phenylethyl-GS than *B. oleracea* and *B. juncea*, but some *B. rapa* cultivars had none. The percentages of 3-indolylnethyl-GS did not differ significantly among the three species.

Significant differences in the percentages of major GSs and in total amounts of GS were found also among four subspecific groups of *B. rapa*, *B. narinos*, and *B. nipposinica*. There was great variation, as indicated by the large and overlapping ranges (Table 3). The predominant GS in Group Chinensis (pak choy), Group Perviridis (tendergreen), and *B. nipposinica* was 3-butenyl-GS, whereas in Group Pekinensis (Chinese cabbage), it was 3-indolylnethyl-GS. Both of those GSs were major constituents in *Rapifera* foliage and *B. narinos*. The GS profile of Group *Rapifera* foliage (not listed in Table 3) resembled that of Group Perviridis.

In addition to variation in major GSs, significant differences existed among the four *rapa* groups, *B. narinos*, and *B. nipposinica* for GSs that usually occurred in relatively minor proportions: 4-methylthiobutyl-, 5-methylthiopentyl-, and 5-methylsulfinylpentyl-GS. Some of these GSs were actually major constituents in a few cultivars; for example, in the Group Pekinensis 'Chi Bei Tsai', 5-methylthiopentyl-GS made up 52% of the GSs.

Group Chinensis (pak choy) cultivars from China differed significantly from those from elsewhere in percentages of the minor GSs: 2-hydroxy-3-butenyl-, 5-methylsulfinylpentyl-, and 2-phenylethyl-GS (Table 4).

Turnip (Group *Rapifera*) foliage and roots had significant differences in the percentages of seven GSs (Table 5). The total amount of GS was significantly less in the foliage than in the roots (Table 5).

In the Chinese kale (*B. oleracea* Group Alloglabra), where there were three replicate plants sampled and individually analyzed for each line, significant differences were found among the lines for the percentages of 3-butenyl- and 4-methylsulfnylbutyl-GS and for the total GS.

**Discussion**

The information reported in this study indicating considerable

Table 3. Glucosinolates (GSs) significantly different (*P* = 0.05 or less in analysis of variance) in percentages of all GSs individually measured and total amounts, in fresh tissue, among four subspecific groups of *B. rapa* (Chinensis, Pekinensis, Perviridis, Rapifera), *B. narinos*, and *B. nipposinica*.

<table>
<thead>
<tr>
<th><em>Brassica</em> <em>rapa</em> subspecific group</th>
<th>3-butenyl</th>
<th>4-penemyn</th>
<th>2-phenylethyl</th>
<th>3-indolylnethyln</th>
<th>Total</th>
<th>Total individual GS*</th>
<th>Total glucose released x 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
<td>Percentage</td>
<td>Range</td>
<td>µmol/100g</td>
<td>Range</td>
</tr>
<tr>
<td>Chinensis</td>
<td>44 ± 1 b</td>
<td>0-79</td>
<td>12 ± 1 a</td>
<td>0-34</td>
<td>3 ± 1 c</td>
<td>0-16</td>
<td>7 ± 1 c</td>
</tr>
<tr>
<td>Pekinensis</td>
<td>1 d</td>
<td>0-8</td>
<td>11 ± 1 a</td>
<td>3-25</td>
<td>12 ± 1 b</td>
<td>3-27</td>
<td>40 ± 1 a</td>
</tr>
<tr>
<td>Perviridis</td>
<td>70 ± 1 a</td>
<td>62-77</td>
<td>16 ± 1 a</td>
<td>4-25</td>
<td>2 ± 1 e</td>
<td>1-3</td>
<td>5 ± 1 e</td>
</tr>
<tr>
<td>Rapifera</td>
<td>28 ± 1 c</td>
<td>6-49</td>
<td>5 ± 1 b</td>
<td>2-10</td>
<td>19 ± 1 a</td>
<td>7-37</td>
<td>27 ± 1 b</td>
</tr>
<tr>
<td><em>B. narinos</em></td>
<td>37 ± 1 b</td>
<td>24-75</td>
<td>2 ± 1 b</td>
<td>0-7</td>
<td>10 ± 1 b</td>
<td>2-25</td>
<td>20 ± 6 b</td>
</tr>
<tr>
<td><em>B. nipposinica</em></td>
<td>70 ± 1 a</td>
<td>39-86</td>
<td>4 ± 1 b</td>
<td>1-2</td>
<td>1 ± 1 c</td>
<td>1-2</td>
<td>6 ± 1 c</td>
</tr>
</tbody>
</table>

*The percentages were transformed to the trigonometric angle by arcsin (%/100)^1/2.*

*Combined data of group Chinensis (pak choy) cultivars from China and from elsewhere. Number of cultivars tested: 17 (Chinensis), 14 (Pekinensis), 5 (Perviridis), 15 (Rapifera), 3 *B. narinos*, 5 *B. nipposinica*.

*Data of group Rapifera (turnip) roots only.*

*Mean ± se.*

*Mean total amount of GS in fresh tissue as measured by the glucose released after enzymatic hydrolysis of the extracts. Use 457 as the average molecular weight of GS.

Means across a row followed by a common letter were not significantly different at *P* = 0.05 using Fisher’s least significant difference.

Table 4. *Brassica rapa* Group Chinensis (pak choy): Three minor glucosinolates (GSs) (<10% of total GSs) significantly different (P = 0.05 or less in analysis of variance) in percentages of all GSs individually measured\(^1\) in cultivars from different locations.

<table>
<thead>
<tr>
<th>Origin</th>
<th>No.</th>
<th>2-hydroxy-3-butenyl</th>
<th>5-methylsulfinylpentyl</th>
<th>2-phenylethyl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cultivars</td>
<td>Percentage</td>
<td>Range</td>
<td>Percentage</td>
</tr>
<tr>
<td>China</td>
<td>8</td>
<td>10 ± 1(^2)</td>
<td>0-24</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Elsewhere</td>
<td>9</td>
<td>1 ± 1</td>
<td>0-10</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

\(^1\)The percentages were transformed to the trigonometric angle by arcsin (\(^\%\)/100)\(^{1/2}\).

\(^2\)Mean ± SE.

Table 5. *Brassica rapa* Group Rapifera (turnip) roots vs. foliage: Glucosinolates (GSs) in fresh tissue significantly different (P = 0.05 or less in analysis of variance) in percentages of all GSs individually measured\(^1\) and total amounts in those plant parts in 15 cultivars.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>3-butenyl</th>
<th>2-hydroxy-3-butenyl</th>
<th>4-methylthiobutyl</th>
<th>4-pentenyl</th>
<th>5-methylthiophenyl</th>
<th>2-phenylethyl</th>
<th>3-indolylmethyl</th>
<th>Total</th>
<th>% released \times 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foliage</td>
<td>71 ± 1(^3)</td>
<td>26-89</td>
<td>0-13</td>
<td>1</td>
<td>19 ± 6</td>
<td>6-43</td>
<td>1 ± 1</td>
<td>0-1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Roots</td>
<td>28 ± 1(^3)</td>
<td>6-49</td>
<td>5 ± 1</td>
<td>10-17</td>
<td>5 ± 4</td>
<td>1-16</td>
<td>1 ± 1</td>
<td>2-10</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

\(^3\)Mean ± SE.

\(^1\)Mean total amounts of GS in fresh tissue as measured by the glucose released after hydrolysis of the extracts. Use 457 as the average molecular weight of GS.

variation in GS profiles among *Brassica* species and among the 10-chromosome Brassica vegetables is of potential interest to evolutionary biologists and has practical value for plant breeders. The distinctive interspecific variation found may be a reflection of the different genomes in each species. As noted previously, diploid *B. rapa*, *B. narifino*, and *B. nippoticana* contain the “a” genome of n = 10 chromosomes and *B. oleracea* has the “c” genome of n = 9 chromosomes (17), whereas disomic polypligl *B. juncea* contains the “b” genome n = 8 from *B. nigra* L. and the “a” genome, giving it a haploid ab = 18 genome (12). Roebben and Thies (15) compared the patterns of major GSs in the seedmeal of six cultivated *Brassica* species and concluded that the “b” genome contains the factor(s) controlling synthesis of allyl-GS and that the “c” genome has the factor(s) controlling 2-hydroxy-3-butenyl-GS. For *B. rapa*, they noted that 3-butenyl-GS is the predominant GS but that 4-pentenyl-GS was higher in some reports. *B. juncea* seedmeal from some countries had mainly allyl-GS, while *B. juncea* from India and Pakistan had 3-butenyl-GS alone or in combination with allyl-GS suggesting geographic variation (22, 23). Our data on the GS contents in edible vegetative parts tend to support some of these reported associations of particular GSs with particular genomes. An exception found in our data suggests that *B. oleracea* Group Albuglabra has factor(s) for low 2-hydroxy-3-butenyl GS synthesis, but has factor(s) for high 3-butenyl- and 4-methylsulfinylbutyl-GS syntheses. The evolutionary bases for the associations is unknown, but the associations may have chemotaxonomic value (24).

The practical value of the information on GS levels may be realized by plant breeders attempting interspecific hybridization among *Brassica* species. Presence or absence of certain GSs may help identify interspecific and intergeneric hybrid plants, and their GS contents may be predicted qualitatively. An outcome of interspecific and interspecific breeding strategies may be the creation of altered flavor characteristics. Pearson (14) reported on the changed GS contents and flavor of *B. oleracea* substituted in *B. nigra* cytoplasm. Glucosinolates have long been known to be associated with the pungent mustard oils, but there is not much information on the flavor characteristics of GSs (7). Intended selection for particular flavors by breeders may have contributed to the variation of the subspecies; however, there may also be complex genetic associations of particular GSs with certain morphotypes resulting from linkage blocks, for instance, so that GS contents may have diverged inadvertently.

Geographic variation among Group Chinensis (pak choy) cultivars existed in minor GSs, but a comparison of the data on Group Pekinensis (Chinese cabbage) cultivars from Daxenbichler et al. (5) with the data of Chinese cabbage from China presented here suggests that there is variation in the major GS 3-butenyl-GS and in total GS based on the geographic origin of this vegetable. Using the same analytical methodology, they reported the GS contents in cultivars primarily from Japan. Converting the GS data in their paper from parts per million (ppm) to \(\mu\)mol/100 g fresh weight and calculating the relative proportions of the individual GSs shows that the mean relative proportion of 3-butenyl-GS was 13%, compared with 1% found in the cultivars reported here. The mean total GS of the cultivars analyzed by Daxenbichler et al. was 117 \(\mu\)mol/100 g fresh weight, more than twice the amount found in the Chinese cultivars; however, seasonal variation as reported by Ju et al. (9) may have contributed to the variation.

The variation in GS profiles between turnip (Group Rapifera) roots and foliage found in this study is similar to that reported by Carlson et al. (2, 3), who found higher proportions of 3-butenyl- and 4-pentenyl-GS and lower proportions of 3-indolylmethyl-GS in the foliage, except in one crop year where 3-indolylmethyl-GS was higher in the foliage of two cultivars. Significant differences in levels of GS in different plant parts were found also in cabbage, mustard, rapeseed, radish, and swede (16).
Literature Cited


