Glucosinolates in Chinese Brassica campestris Vegetables: Chinese Cabbage, Purple Cai-tai, Choysum, Pakchoi, and Turnip

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Abstract. Brassica campestris vegetables play an important role in the Chinese diet. The objective of this study was to evaluate the composition and content of glucosinolates (GSs) in five species of Chinese Brassica campestris vegetables by high-performance liquid chromatography. The compositions and contents of GSs varied significantly among and within species and cultivars. The contents of total GSs were 100 to 130 mg/100 g fresh weight (FW) in turnip (B. rapifera), 50 to 70 mg/100 g FW in purple cai-tai (B. chinensis var. purpurea), and 14 to 35 mg/100 g FW in Chinese cabbage (B. pekinensis), choysum (B. chinensis var. utilis), and pakchoi (B. chinensis var. communis). In Chinese cabbage, the predominant individual GSs were glucobrassicin for both cultivars, neoglucobrassicin only for ‘zaoshuwuhao’, and glucopinin only for ‘zaoshuwuhao’. The predominant individual GSs were glucobrassicinapain and glucopinin in purple cai-tai and choysum and glucopinin in pakchoi and turnip. The relative content of total aliphatic GSs was 80% to 90% in purple cai-tai and choysum, 60% to 65% in pakchoi and turnip, and 17% to 50% in Chinese cabbage. The relative content of total indolic GSs was 37% to 75% in Chinese cabbage, 25% to 27% in pakchoi, and 5% to 17% in purple cai-tai, choysum, and turnip. The relative content of aromatic GSs was 28% to 36% in turnip, 8% to 14% in Chinese cabbage and pakchoi, and 2% to 4% in choysum and purple cai-tai. These results suggest that there are significant genotypic variations in composition and content of glucosinolates in Chinese Brassica campestris vegetables.

Epidemiological data show that a diet rich in cruciferous vegetables can reduce the risk from a number of cancers. Several protection mechanisms for the cancer prevention from cruciferous vegetables have been demonstrated for the breakdown products of some glucosinolates (GSs) (Mithen et al., 2000). Some research about glucosinolates has been done in Brassica crops (Carlson et al., 1987; Krumbein et al., 2005; Mullin and Sahasrara-budhe, 1977; Rangkadilok et al., 2002; Rosa et al., 1996; Sang et al., 1984). Brassica campestris vegetables play an important role in the Chinese diet, and so their naturally occurring GSs in edible parts should be monitored. To our knowledge, there is limited information about the comparison of GSs among Chinese Brassica campestris vegetables (He et al., 2000, 2002; Hill et al., 1987; Lewis and Fenwick, 1988). Therefore, the objective of this study was to compare and evaluate the composition and content of GSs in edible parts in five species of Chinese Brassica vegetables, including Chinese cabbage (Brassica campestris L. ssp. pekinensis), choysum (Brassica campestris L. ssp. chinensis var. utilis), purple cai-tai (Brassica campestris L. ssp. chinensis var. purpurea), pakchoi (Brassica campestris L. ssp. chinensis var. communis), and turnip (Brassica campestris L. ssp. rapifera).

Materials and Methods

Plant materials. Five species of Brassica campestris vegetables were used for this experiment. The materials used in this experiment are shown in Table 1. Seeds were sown on 15 Sept. 2004 and transplanted at the farm of Zhejiang University on 15 Oct. 2004 at a randomized complete block design with three replicates of 20 plants each. For analysis, the edible parts of five plants were harvested at commercial maturity stage from each of the three replicates (see Table 1). Edible parts (50 to 200 g of each replicate) were immediately deep frozen with liquid nitrogen, then freeze-dried, and finely ground. The production period and the climatic conditions under a field conditions are shown in Table 1.

Methods. Samples were prepared according to the method of Krumbein et al. (2005) with slight modification. Duplicates of the freeze-dried powder (0.25 g) in 10-mL glass tubes were preheated for 5 min in 75 °C water bath. Four milliliters of 70% boiling methanol (75 °C) was added and extracted at 75 °C in a water bath for 10 min. There was 100 μL of 5 mmol-L⁻¹ sinigrin (Sigma-Aldrich Co., St. Louis) as an internal standard in one of the duplicates before extraction. Then 1 mL of 0.4 mol-L⁻¹ barium acetate was rapidly added and vortexed for several seconds. After centrifugation at 4000 rpm for 10 min at room temperature, the supernatants were collected and the pellets were reextracted twice with 3 mL of 70% boiling methanol (75 °C). Three supernatants were combined and made into a final volume of 10 mL with 70% methanol. Five-milliliter extracts were loaded onto a 1-μL mini-column (JT Baker, Phillipsburg, PA) prepared by introducing 500 μL of activated DEAE Sephadex A25 (Amersham Biosciences, Uppsala, Sweden) in a vacuum processor (JT Baker 12) and allowed to desulphate overnight with aryl sulfatase (Sigma-Aldrich Co.). The resultant desulpho (ds)-GS were eluted with 2.5 mL of ultra pure water produced by Milli-Q system (Millipore Co., Milford, CT) and stored at −20 °C before separation by high-performance liquid chromatography (HPLC).

The elution (20 μL) was analyzed in a Shimadzu HPLC system (LC-10AT pump, CTO-10A column oven, SCL-10A VP system controller; Shimadzu, Kyoto, Japan) consisting of an ultraviolet-VIS detector (SPD-10A) set at 229 nm and a protosil ODS2 column (250 × 4 mm, 5 μm; Bischoff, Leonberg, Germany). The mobile phase was ultra pure water (A) and acetonitrile (Tedia, Fairfield, OH) (B) in a linear gradient from 0% to 20% B for 32 min, then constant 20% B for 6 min, and 100% B and 0% B before the injection of the next sample. The flow rate was 1.3 mL-min⁻¹. Each individual ds-GS was identified in the HPLC system coupled with an electro spray ionization ion trap mass detector system (Agilent 1100 series, Agilent Technologies, Palo Alto, CA). The HPLC conditions were the same as described previously, except the flow rate was 1.0 mL-min⁻¹. The nebulizer pressure was 60 psi and the flow rate is nitrogen 13 mL-min⁻¹ at a drying temperature of 350 °C. The scan of the masses ranged from 100 m/z to 600 m/z and helium was used as collision gas for the fragmentation procedure of the isolated compounds in
Brassica campestris

Vegetables (Bradshaw et al., 1987) contained two predominant GSs (glucobrassicin, glucobrassicanapin). From the results of He et al. (2002) also found that the predominant GSs were gluconapin and progoitrin in choy-sum and glucobrassicin and neoglucobrassicin in pakchoi. Hill et al. (1987) reported that the predominant GS was 3-butenyl-GS (gluco-napin) in pakchoi and 3-indolylmethyl-GS (glucobrassicin) in Chinese cabbage and both 3-butenyl-GS and 3-indolylmethyl-GS in turnip. Except the variation in types of predominant GSs, there were differences in quantity of the same predominant GSs. For example, the relative content of predominant GS of glucobrassicin was 69% in purple cai-tai ‘Wushigh’, 40% to 53% in turnip, 31% to 39% in pakchoi, and 23% to 29% in choy-sum.

It was reported that there was great variation in GSs within different plant species (Mithen et al., 2000). Kang et al. (2006) reported that the genotypic effects described most of the phenotypic variation of GSs in Chinese cabbage than the environmental effects. In addition to genetic and environment factors, variation in GS types and concentrations among plant organs was reported in some Brassica vegetables (Bradshaw et al., 1983; Clossais-Besnard and Larher, 1991; Sang et al., 1984). The total amount of GSs was significantly less in the foliage than in the roots of turnip (Hill et al., 1987). The total content of GSs was highest in inflorescences followed by stems and leaves in choy-sum (He et al., 2000). In this study, three corresponding types of tissue (root, bolting stem, and leaves) were analyzed. The total GSs in roots of turnip are nearly two to three times larger than those in bolting stems and inflorescences of purple cai-tai and choy-sum and four to six times larger than those in leaves of Chinese cabbage and pakchoi. Bolting stems and inflorescences of purple cai-tai and choy-sum mainly contained aliphatic GSs, in which relative content was 80% to 90%, whereas the aromatic GSs were found in trace amounts only. In turnip roots, although the greatest GS group was aliphatic GSs (60% relative to total GSs), there were also considerable aromatic GSs in which the relative content reached 28% to 35%. The relative content of three principal GS groups was 69% in purple cai-tai and choy-sum, 40% to 53% in turnip, 31% to 39% in pakchoi, and 23% to 29% in choy-sum.

Data analysis. Differences between means were analyzed by Fisher’s protected least significant difference procedure.

### Results and Discussion

**Total glucosinolates.** There were significant differences in total GSs among and within these Brassica campestris vegetables (Fig. 1). The contents of total GSs in turnip were the highest range from 102.43 mg/100 g fresh weight (FW) to 124.57 mg/100 g FW, followed by purple cai-tai (50 to 70 mg/100 g FW), and the lowest in Chinese cabbage, choy-sum, and pakchoi (14 to 35 mg/100 g FW). This result was consistent with that of Carlson et al. (1987) who reported the considerable differences in total GS levels among and within seven Brassica species. There was consistent reports that total GS content was 198 mg/kg FW in Chinese cabbage and 534 mg/kg FW in pakchoi (Lewis and Fenwick, 1988). He et al. (2002) reported the content of total GSs was 71.49 μmol/100 g FW (29.17 mg/100 g FW) in pakchoi and 295.65 μmol/100 g FW (120.63 mg/100 g FW) in choy-sum. Although the same edible parts were analyzed, the differences in total GSs of Chinese cabbage, pakchoi, and choy-sum in different experiments may be caused by the variety, plant age, and the growing conditions.

Aliphatic, indolic, and aromatic glucosinolates. According to the chemical structure, different GSs can fall into three principal groups comprising the aliphatic group, indolic group, and aromatic group. Except for the difference in total GS levels, there was also considerable variation in the composition and content of aliphatic, indolic, and aromatic GSs (Table 2). Turnip and purple cai-tai had the highest content of total aliphatic GSs (42 to 73 mg/100 g FW); Chinese cabbage and purple cai-tai ‘Wushihong’ had the highest content of total indolic GSs (11 to 13 mg/100 g FW); and turnip had the highest content of aromatic GSs (29 to 46 mg/100 g FW). The relative content of three principal GS groups also can illuminate the differences in GS levels among and within these Brassica campestris vegetables (Table 3). For example, the relative content of total aliphatic GSs in purple cai-tai and choy-sum were 80% to 90%, 60% to 65% in pakchoi and turnip, and 17% to 50% in Chinese cabbage. Furthermore, the relative content of aromatic GSs in turnip and indolic GSs in Chinese cabbage were markedly higher than in other Brassica campestris. This result was consistent with those of Lewis and Fenwick (1988) and He et al. (2002).

**Individual glucosinolates.** The content of total GSs could not predict the levels of individual GSs. There were greater differences in the levels of individual GSs than total GSs and three principal GS groups. Twelve kinds of individual GSs, including seven aliphatic, four indolic, and one aromatic GSs, were detected. The variation in composition of GSs mainly occurred in aliphatic GSs. For example, Chinese cabbage ‘Huanyacai’ contained seven kinds of individual GSs and pakchoi ‘Wuyoudong’ only contained four individual GSs.

**Predominant glucosinolates.** The percentage differences of individual GSs indicate the type and quantity of predominant GSs within species and even within cultivars (Table 3). For example, gluconapin and glucosinasturin were the predominant GSs in both cultivars of turnip, whereas glucobrassicin and neoglucobrassicin were the predominant GSs in Chinese cabbage ‘Huanyacai’ and ‘Zaozhu- wuhao’, respectively. The major GSs in choy-sum ‘Siju’ were identified as sinigrin, glucosin, glucobrassicanpin, and progoitrin, with the latter three generally predominating. In contrast, choy-sum ‘Youqing’ only contained two predominant GSs (gluconapin and glucobrassicanpin). From the results of Lewis and Fenwick (1988), glucobrassican pin was predominant in Chinese cabbage and a mixture of glucobrassicin and glucobrassicin (not separated) were predominant GSs. He et al. (2002) also found that the predominant GSs were gluconapin and progoitrin in choy-sum and glucobrassicin and neoglucobrassicin in pakchoi. Hill et al. (1987) reported that the predominant GS was 3-butenyl-GS (gluco-napin) in pakchoi and 3-indolylmethyl-GS (glucobrassicin) in Chinese cabbage and both 3-butenyl-GS and 3-indolylmethyl-GS in turnip. Except the variation in types of predominant GSs, there were differences in quantity of the same predominant GSs. For example, the relative content of predominant GS of glucobrassicin was 69% in purple cai-tai ‘Wushihong’, 40% to 53% in turnip, 31% to 39% in pakchoi, and 23% to 29% in choy-sum.

Table 1. Species, cultivars, and edible parts of five Brassica campestris vegetables and the production period and climatic conditions.

| Common name       | Latin name                          | Cultivars                | Edible part | Production period          | Avg day/night temp (°C) |
|-------------------|-------------------------------------|--------------------------|-------------|---------------------------|-------------------------|
| Chinese cabbage   | B. campestris ssp. pekinensis var. purpurea | Huayingcai Zaoshuwu Hao | Leaves      | 15 Sept. to 10 Dec.       | 21.6/12.7               |
| Purple cai-tai    | B. campestris ssp. chinensis var. purpurea | Wushihong and Xiangyang Hong | Bolting stems and inflorescences | 15 Sept. to 20 Nov. | 23.3/14.6               |
| Choy-sum          | B. campestris ssp. chinensis var. utilis | Youqing and Siju | Bolting stems and inflorescences | 15 Sept. to 20 Nov. | 23.3/14.6               |
| Pakchoi           | B. campestris ssp. chinensis var. communis | Huaying and Wuyoudong | Leaves | 15 Sept. to 20 Nov. | 23.3/14.6               |
| Turnip            | B. campestris ssp. rapifera          | Wenzhouhai and Wenzhouhong | Roots       | 15 Nov. to 10 Dec.        | 21.6/12.7               |

![Fig. 1. Total glucosinolates in Chinese Brassica campestris vegetables 1-1 Chinese cabbage ‘Huanyacai’; 1-2 Chinese cabbage ‘Zaozhuwuhao’; 2-1 Purple cai-tai ‘Wushihong’; 2-2 Purple cai-tai ‘Xiangyanghong’; 3-1 Choy-sum ‘Youqing’; 3-2 Choy-sum ‘Siju’; 4-1 Pakchoi ‘Huaying’; 4-2 Pakchoi ‘Wuyoudong’; 5-1 Turnip ‘Wenzhouhai’; and 5-2 Turnip ‘Wenzhouhong’](image-url)
### Table 2: Composition and content of glucosinolates in Chinese cabbage varieties

| Variety | Aliphatic GSs | Indolic GSs | Aromatic GSs |
|---------|---------------|-------------|-------------|
| Chinese cabbage | Glucoerucin (1.35 ± 0.21 b) | 4-Hydroxyglucobrassicin (0.05 ± 0.01 c) | Gluconasturtiin (4.41 ± 0.99 c) |
| | Gluconapin (6.57 ± 1.52 de) | Glucobrassicin (10.31 ± 1.72 a) | 4-Methoxyglucobrassicin (0.78 ± 0.20 cd) |
| | Total aliphatic GSs (15.42 ± 3.80 e) | Total indolic GSs (11.67 ± 2.02 a) | Total aromatic GSs (64.80 ± 10.39 a) |

### Table 3: Relative content of glucosinolates in Brassica campestris vegetables

| Variety | Aliphatic GSs | Indolic GSs | Aromatic GSs |
|---------|---------------|-------------|-------------|
| Chinese cabbage | Glucoerucin (4.34 ± 0.42 a) | Glucoerucin (2.34 ± 0.24 b) | Gluconasturtiin (13.99 ± 0.74 c) |
| | Gluconapin (20.83 ± 0.66 e) | Glucobrassicin (32.96 ± 1.59 a) | 4-Methoxyglucobrassicin (0.78 ± 0.20 cd) |
| | Total aliphatic GSs (48.75 ± 1.74 a) | Total indolic GSs (37.27 ± 1.59 b) | Total aromatic GSs (25.55 ± 2.00 c) |

According to our data, roots of turnip had a stronger ability for synthesis and storage of GSs than stems and inflorescences of purple cai-tai and choy sum; leaves of Chinese cabbage and pakchoi, especially on aromatic GSs; and bolting stems and inflorescences of purple cai-tai and choy sum had preference on synthesis and storage of aliphatic GSs.

Detailed analysis from five Chinese *Brassica campestris* vegetables has shown considerable variation in total GS levels and in the content and relative content of individual GSs among species and among varieties within a species. The wide ranges of variation will offer important information for conventional breeding or genetic engineering with enhanced health benefits. The breakdown products from glucoraphanin, sinigrin, indole glucosinolates, and gluconasturtiin had been proven with potential beneficial effects on human health (Mithen et al., 2000). Considering these beneficial effects, Chinese cabbage, which contains high relative content of indolic GSs, and turnip, which contains high relative content of gluconasturtiin, are of high dietary value in all of these five *Brassica campestris* vegetables.

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