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*Full Length Research Paper*

# Screening of Chinese brassica species for anti-cancer sulforaphane and erucin

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**Natural sulforaphane and erucin have been of increasing interest for nutraceutical and pharmaceutical industries due to their anti-cancer effect. The sulforaphane and/or erucin contents in seeds of 43 different Chinese *Brassica oleracea L.* varieties were analyzed by HPLC and GC-MS. Among them, 21 cultivars seed meal (in 5 species, including Chinese kale, cabbage, kohlrabi, kale and broccoli) yielded sulforaphane and/or erucin. The results indicated that erucin was found mainly in cabbage and kohlrabi, and sulforaphane was found mainly in broccoli. The highest content of sulforaphane (1575.5 mg/kg) and erucin (2528.1 mg/kg) were found in Green Dragon I (broccoli) and Dongchunbao (cabbage), respectively. Crude sulforaphane and erucin were extracted in gram-scale; 1.266 g sulforaphane (purity: 78.2%) and 0.972 g erucin (purity: 65.6%) were obtained.**

**Key words:** Sulforaphane, erucin, brassica seed, diet supplement.

## INTRODUCTION

In addition to their high nutritional value, cruciferous vegetables (e.g. broccoli, cabbage, kale) have a potential role in cancer chemoprevention (Cohen et al., 2000; Vermeulen et al., 2006), since they are rich in aromatic and aliphatic glucosinolates. Some glucosinolates break-down products, especially some isothiocyanates such as sulforaphane (4-methylsulphinyl-3-butenyl) (Fahey and Talalay, 1999; Zhang et al., 1992) are associated only with induction of Phase II detoxification enzymes and have been termed mono-functional inducers, which are hypothesized to be of health benefits because of their role in detoxification of carcinogens. Several other mechanisms of sulforaphane were also demonstrated, such as inhibition of tumor cell proliferation, induction of apoptosis, protection of DNA from damage induced by different xenobiotics, antimetastatic potential, etc (Higdon et al., 2007; Fimognari and Hrelia, 2007). Erucin (4-methylthiobutyl), which is an analogue of sulforaphane, is also of interest to health researchers. It has also been identified as an *in vivo* metabolite of sulforaphane in rat (Fimognari et al., 2004). Erucin has been shown to arrest cell cycle on Jurkat T-leukemia cells and holds a promise for future development as a chemo-preventive agent (Kassahun et al., 1997). Many laboratory animal feeding studies have shown that these isothiocyanates indeed possess anti-carcinogenic properties (Hwang and Jeffery, 2004; Chung et al., 2000).

Although people are increasingly more aware of the relationship of cruciferous vegetables to health and disease, many people do not gain these benefits because they do not like the taste of the vegetables or they find them cumbersome to eat. Yet since cruciferous vegetables are the major source of isothiocyanates in the diet which distinguishes them from other vegetables, they may pursuade themselves to eat. Therefore, a diet supplemented with beneficial isothiocyanates, especially sulforaphane and erucin is necessary.

Several investigations have focused on the glucosinolates composition of cruciferous seeds (Daxenbichler et al., 1991). However, the information in current literature regarding the kinds and contents of isothiocyanates in seeds is somewhat sketchy. Although the isothiocyanates pattern in certain species is similar, there are significant differences in the contents of individual isothiocyanates within the different cultivars. Moreover, the capacity of

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isothiocyanates in different plants depends on the climate and soil. In most of those literature there is lack of data about Chinese plants. In this work, the contents of sulforaphane and/or erucin in seeds of 21 different Chinese *Brassica oleracea L.* varieties were determined, and the sulforaphane and erucin were extracted to produce diet supplement in a gram-scale without the need of chromatographic separation.

## MATERIALS AND METHODS

### Materials

The seed samples analyzed in our study were as follows: Chinese cabbage (*Brassica Pekinensis Rupr*), Chinese kale (*B. Capitata* L. var *alboglabra*), root mustard (*Brassica Juncea* var. *megarrhiza Tsen et lee*), Zacai (preserved Szechuan pickle, *B. Juncea* var. *tumida*), bok choy (*B. chinesnses L.*), cauliflower (*B. oleracea* L. var. *botrytis* L.), cabbage (*B. Oleracea* var. *capitatal*), kohlrabi (*B. Oleracea* L. var. *caulorapa* DC.), kale (*B. Oleracea* var. *acephala*), broccoli (*B. Capitata* L. var. *italica*) and shepherd's purse (*Capsella bursapastoris* L.). Kale seeds and two varieties of broccoli seeds (Green Broccoli and Green Broccoli 80 Day) were obtained from Wenzhou First-Finger Seed Co., Wenzhou city. Others were obtained from some seed companies in Hangzhou. Sulforaphane and erucin standards were purchased from Sigma Chemical Co (St. Louis, USA). Chemical reagents (sodium chloride, anhydrous sodium sulfate) and solvents (hexane, ethyl acetate, ethanol) were of analytical grade and used without further purification. Methanol was HPLC grade.

### Seed extraction

Three replicates (of 15.0 g each) of seed were ground in a Chinese herbal medicine grinder for 30 to 60 s to get seed meal. The seed meal was subsequently defatted with excess hexane in an incubator shaker for 24 h at 20°C. Then the seed meal was allowed to dry overnight in a fume hood. Defatted seed meal was mixed with 50.0 mL methylene chloride and 25.0 mL potassium phosphate buffer (0.05 mol/L, pH 7.0). The mixture was placed in an incubator shaker to autolyze for 8 h at 25°C. Following this, 10.0 g sodium chloride and 10.0 g anhydrous sodium sulfate were added and mixed thoroughly. The $CH_2Cl_2$ layer was filtered and the residual paste was extracted three times with equal volumes of $CH_2Cl_2$, which was combined and dried at 32°C under vacuum. The residue was dissolved in 50.0 mL methanol, and filtered through the 0.45 µm membrane. The extracts were analyzed on the GC-MS for compound content. Purity of individual compounds present in the extracts was determined by GC-FID or HPLC.

### Seed extraction in gram-scale

1.0 kg seed was ground in a Chinese herbal medicine grinder to get seed meal. The seed meal was subsequently defatted with excess hexane in an agitator for 24 h at 20°C. Then the seed meal was allowed to dry overnight in a fume hood. Defatted seed meal was mixed with 3.5 L ethyl acetate and 1.6 L potassium phosphate buffer (0.05 mol/L, pH 7.0). The mixture was placed in an agitator to autolyze for 8 h at 25 °C. Thereafter, 600 g sodium chloride and 600 g anhydrous sodium sulfate were added and mixed thoroughly by an agitator. The ethyl acetate layer was filtered and the residual paste was extracted one more time with 3.0 L ethyl acetate, which were combined and condensed at 40°C under vacuum in a rotary evaporator. The residue was partitioned between 0.5 L hexane and 1.5 L water. The water fraction was further partitioned against 0.5 L ethyl acetate, after which the ethyl acetate fraction was dried at 40°C under vacuum in a rotary evaporator.

### HPLC

The content of sulforaphane and erucin was analyzed by Waters 1525 HPLC system. The column employed in our experiment was a ZORBAX Eclipse XDB-$C_{18}$ (4.6×150 mm, 5 µm). The mobile phase consisted of 10% methanol in water, then changed linearly over 40 min to 90% methanol, and maintained 2 min to purge the column. Column oven temperature was set at 30°C. The flow rate was 1 mL/min. Isothiocyanates were detected by Waters 2996 detector at the wavelength of 241 nm.

### GC-FID

The content of sulforaphane nitrile was quantified by gas chromatography using a flame ionization detector (GC-FID). GC analyses were performed on an Agilent 6820 system. Column employed was fused-silica HP-5MS capillaries (0.25 µm film thickness, 30 m × 0.32 mm i.d.). The operating conditions were as follows: injector and detector temperature was set at 250°C; the injections were performed in a split ratio of 10:1; injection volume, 1 µL; the injector and detector temperature was set at 250°C; column oven temperature began at 50°C for 2 min, progressed to 190°C (ramp 5°C/min), then progressed to 315°C (ramp 25°C/min), and was held at 315°C for 5 min.

### GC-MS

A TRACE DSQ GC/MS (Thermoelectron Company, USA) was used for the identification of glucosinolate hydrolysis products. An HP-5 capillaries (0.25 µm film thickness, 30 m × 0.32 mm i.d.) delivered compounds to the mass spectrometer. Ultra-high purity helium was used as the carrier gas at flow rate of 1 mL/min. The injector and detector temperature was set at 270 and 280°C, respectively. The injection volume was 1.0 µL, all the injections were performed in a split ratio of 10: 1. Column oven temperature began at 50°C for 2 min, progressed to 190°C (ramp 10°C/min), then progressed to 300°C (ramp 20°C/min), and was held at 300°C for 5 min. Mass spectra were obtained by electron impact ionization (EI) over the range of 35-500 amu at a rate of 2.0 scans/s. The ion source temperature was 200°C, and the electronic impact energy was 70 eV.

## RESULTS

### Hydrolysis products from seed meal

Altogether, 43 kinds of cultivars in 10 species were analysed in our study. We obtained very low amounts of isothiocyantates from four cultivars of bok choy seeds, two cultivars of Zacai (preserved Szechuan pickle) seeds and two cultivars of Shepherd's Purse seeds, hence the isothiocyanates pattern of these seed meal were not identified by GC-MS since they were not suitable for production of isothiocyanates. The main breakdown products of the glucosinolates identified in our study, particularly the isothiocyanates, were allyl isothiocyanate (2-propenyl), 3-BITC (3-butenyl), iberverin (3-methylthiopropyl),

**Table 1.** Concentration (mg/kg) of aglucones from seeds of 28 varieties of brassica.

| Species | Cultivars | Erucin | Sulforaphane | Goitrin | Sulforaphane nitrile |
|---|---|---|---|---|---|
| Broccoli | Blue Sea | 60.4±1.2[a] | 119.6±9.5 | 188.1±12.1 | 607.4±53.6 |
| | Blue Band | 101.1±10.3 | 101.7±9.4 | 60.7±6.3 | 583.5±62.9 |
| | Blue King | 82.4±5.0 | 62.4±7.1 | 267.7±22.4 | 637.6±51.8 |
| | Green Dragon I | 69.1±3.2 | 1575.5±108.2 | 158.3±13.9 | 168.4±17.2 |
| | Green Dragon II | 106.7±9.4 | 1391.0±96.7 | 62.2±2.0 | 121.6±12.5 |
| | Green Broccoli | 75.7±3.6 | 1126.9±102.8 | 360.4±26.9 | 107.4±9.5 |
| | Green Broccoli 80 | 76.4±2.1 | 588.3±56.8 | 270.7±19.7 | 98.5±10.4 |
| Cabbage | Xinfeng | 1326.7±124.3 | 64.8±3.1 | 1044.5±89.1 | -[b] |
| | Summer Cap | 1064.5±112.0 | 68.7±1.6 | 206.7±22.6 | - |
| | Jingfeng | - | - | 76.6±2.3 | - |
| | Dongchunbao | 2528.1±213.6 | 74.5±8.4 | 1462.1±103.1 | - |
| | Cattle's Heart | 66.5±3.1 | - | - | - |
| | Early Spring | 82.8±2.8 | - | 794.4±58.4 | - |
| | Yousheng | 80.5±4.3 | - | 232.4±27.6 | - |
| | Xiaguang | 76.7±4.7 | - | 184.9±10.2 | - |
| | Jiashi | 72.4±4.4 | - | 118.5±12.4 | - |
| Kohlrabi | Chunqi | 65.7±1.3 | 146.3±10.8 | - | - |
| | Chunqiu | 800.1±10.3 | 65.9±1.5 | 206.5±23.1 | - |
| Kale | Weitasa | 70.6±6.7 | - | 550.3±39.4 | - |
| | Nagoya | 84.6±5.1 | - | 572.7±41.2 | - |
| Chinese Kale | Chenghai | 70.0±5.8 | - | 119.2±17.2 | - |

[a]Mean values of three independent extraction ± standard deviation.
[b]Not detectable or not detected.

erucin (4-methylthiobutyl), iberin (3-methylsulphinyl-propyl), sulforaphane (4-methylsulphinyl-butyl), PEITC (2-phenylethyl), dehydroerucin (4-Methylthio-3-butenyl) and goitrin (L-5-vinyloxazolidine-2-thione). Some nitriles and other chemicals that degraded from these isothiocyanates were also identified. Among the 43 cultivars, only 21 cultivars' seed meal yielded sulforaphane and/or erucin. The content of these isothiocyanates as well as sulforaphane nitrile and goitrin in the 21 cultivars is listed in Table 1. Other cultivars that did not contain these compounds are not listed here. As shown in Table 1, sulforaphane was mainly found in broccoli, especially in Green Dragon I, Green Dragon II and Green Broccoli. The hydrolysis of kohlrabi and some cultivars of cabbage seed meal also produced sulforaphane. Erucin and goitrin were found in many species. We also found that in some species, there were significant differences in the contents of isothiocyanates within the different cultivars, especially in broccoli, cabbage and kohlrabi. Mass spectral data of glucosinolate hydrolysis products are presented in Table 2.

### Extraction in gram-scale

The seed of Green Dragon II (broccoli) and Summer Cap (cabbage) (of 1.0 kg each) were extracted. The results are presented in Table 3. Altogether 1.266 g sulforaphane and 0.972 g erucin were obtained, the purity of which were 78.2 and 65.6%, respectively. Except for sulforaphane and Erucin, allyl isothiocyannate, 3-BITC, iberverin, iberin, and low concentration of goitrin were also yielded. The average yield of sulforaphane from Green Broccoli as determined by HPLC was 1391.0 ± 96.7 mg/kg of defatted seed meal. When extracted in gram-scale, the yield was 1266.0 mg/kg. Therefore, the percentage extraction rate of sulforaphane is (1266.0/1391.0)×100% = 91.0%. Similarly, the percentage extraction rate of erucin is 91.3%.

## DISCUSSION

Suitable source of plant materials for the isolation of glucosinolate hydrolysis products are chosen such that the starting material is known to be high in certain parent glucosinolates. In another separate study, we found that when seed sprouted, the concentration of isothiocyanates decreased so sharply that very low peaks of isothiocyanates were found in autolysis of seedling meal on FID detector (unpublished observations). This result is consistent with previous report (Pereira et al., 2002). It was

**Table 2.** Mass spectral data of glucosinolate hydrolysis products.

| Common name | Molecular formula | MS spectral data m/z (%) |
|---|---|---|
| Allyl isothiocyanate | $CH_2$=CH-$CH_2$-NCS | 99($M^+$, 97), 72(27), 59(5), 45(7), 41(100), 39(60) |
| 3-BITC | $CH_2$=CH-$(CH_2)_2$-NCS | 113($M^+$, 50), 85(5), 72(100), 59(4), 55(23), 41(14), 39(26) |
| Iberverin | Me-S-$(CH_2)_3$-NCS | 147($M^+$, 20), 130(1), 101(100), 72(39), 61(37), 45(55), 41(72) |
| Erucin | Me-S-$(CH_2)_4$-NCS | 161($M^+$, 13), 115(78), 85(36), 72(45), 61(100) |
| Dehydroerucin | Me-S-CH=CH-$(CH_2)_2$-NCS | 159($M^+$, 28), 112(2), 87(100), 72(40), 53(20), 45(95), 39(35) |
| Iberin | Me-SO-$(CH_2)_3$-NCS | 163($M^+$, 3), 130(11), 116(26), 100(22), 86(13), 72(100), 41(84) |
| Sulforaphane | Me-SO-$(CH_2)_4$-NCS | 177($M^+$, 1), 160(64), 114(10), 85(7), 72(100), 64(13), 55(44), 39(16) |
| PEITC | Φ-$CH_2$-$CH_2$-NCS | 163($M^+$, 42), 105(19), 91(100), 77(10), 65(12), 51(7), 39(5) |
| Goitrin | $CH_2$=CH-OZT | 129($M^+$, 100), 101(3), 86(10), 73(13), 68(42), 57(22), 45(30), 43(40) |
| Sulforaphane nitrile | Me-SO-$(CH_2)_4$-CN | 145($M^+$,19), 128(9), 82(42), 64(54), 55(100) |
| Iberverin nitrile | Me-S-$(CH_2)_3$-CN | 115($M^+$, 30), 88(1), 74(11), 68(12), 61(100), 48(17), 45(26), 44(46), 41(35) |
| Erucin nitrile | Me-S-$(CH_2)_4$-CN | 129($M^+$,78), 115(2), 114(11), 102(3), 87(16), 82(80), 61(100), 48(50), 41(41) |

**Table 3.** Major hydrolysis products (g) in gram-scale.

| Hydrolysis Products | Green Dragon II (broccoli) | Summer Cap (cabbage) |
|---|---|---|
| 3-Bitc | 0.091[a] | 0.249 |
| Iberverin | -[b] | 0.028 |
| Erucin | 0.096 | 0.972 |
| Iberin | - | 0.017 |
| Sulforaphane | 1.266 | 0.026 |
| Sulforaphane nitrile | 0.109 | - |
| Goitrin | 0.057 | 0.189 |
| Total | 1.619 | 1.480 |

[a]Extracted from 1.0 kg seeds.
[b]Not detectable or not detected.

reported that crucifer seed contained approximately 10 times the total glucosinolate concentration in the edible portion of the vegetable (Tookey et al., 1980). Therefore, seed was chosen as the most concentrated plant source of isothiocyanates.

However, cruciferous seed had toxic (e.g. goitrogenic) effects in higher animals and humans. The toxic effects had been generally attributed to goitrin (L-5-vinyloxazolidine-2-thione), which would interfere with thyroid hormone synthesis and would therefore be goitrogenic irrespective of the iodine status. Moreover, goitrin had been shown to be nitrosated by treatment with nitrite under stomach conditions to form N-nitroso-oxazolidone, a mutagen (Luthy et al., 1984). In other studies, a few isothiocyanates and indoles showed mutagenic and carcinogenic properties (Sasagawa and Matsushima, 1991; Musk et al., 1994). High intake of glucosinolates and their hydrolysis products might also result in toxic effects. For example, rats fed diets with a high content of isothiocyanates caused decreased food intake and growth depression (de Groot et al., 1991). However, no toxic (but positive) effects were found when humans take in high content of isothiocyanates (Verhoeven et al., 1997). Therefore, seed suitable for production of food supplement with some effective isothiocyanates must have three important characteristics. Firstly, the seed must be common and commercially available in bulk quantities; secondly, the seed meal must contain sulforaphane or erucin in high levels; thirdly, no discernable peaks of goitrin and other toxic chemicals should be found in the seed meal.

Obviously, broccoli seed was the most suitable source for production of food supplement since they contained high content of sulforaphane, a major inducer of quinone reductase and glutathione S-transferase (Phase II enzymes). But the breakdown products of some cultivars (e.g. Blue Sea) produced low content of sulforaphane with high amount of 3-BITC (3-butenyl) and sulforaphane nitriles also present. This difference might be attributed to the distinct myrosinase in these cultivars. In the study of Matusheski and colleagues, exogenous myrosinase added during hydrolysis might have favored

sulforaphane production (Matusheski et al., 2001). So it is possible to find some exogenous myrosinase that could increase the ratio of sulforaphane in these cultivars. The seed meals of Green Dragon I and Green Dragon II yielded high amount of sulforaphane, and Green Dragon II yield very low amount of goitrin. Moreover, no threshold content of indolyl isothiocyanates was found in Green Dragon II since the extraction of this cultivar contained only one major peak and three small peaks in UV detector when analyzed by HPLC. Though hydrolysis of the seed meal of Green Broccoli contained relatively fewer amount of sulforaphane, its price was cheaper than other broccoli seeds, suggesting that Green Broccoli also would be the suitable source for production of pure sulforaphane or diet supplement that contains sulforaphane in a large scale. Erucin is present in some cultivars of cabbage and kohlrabi in high level. But as concerned the concentration of goitrin, Summer Cap (cabbage) should be the best material to produce diet supplement. Accordingly, the raw material of diet supplement should contain Green Dragon II (Broccoli) and Summer Cap (cabbage) or other seeds that contain high content of beneficial isothiocyanates (Table 1).

Isothiocyanate can be extracted by weak polar fluid, such as methylene chloride, ethyl acetate, etc. In gram-scale extraction, we prefer to use ethyl acetate extraction because this solvent can be used for food-grade extraction of isothiocyanate. The extraction rate was lower than 100%, and the reasons include: (i) the extraction rate of methylene chloride is higher than ethyl acetate; (ii) in gram-scale extraction, heat and mass transfer rate is lower; (iii) solvent partitioning procedure was included in the gram-scale extraction. The purpose of solvent partitioning procedure is to discard non-polar components. However, most of isothiocyanates were extracted. Besides sulforaphane and/or erucin, other isothiocyanates, though the concentration of which was low, were also extracted. But we need not take in pure individual isothiocyanates, since study showed that a mixture of isothiocyanates was more effective as chemoprotectants than the individual components (Staack et al., 1998). So as food supplement that has anticancer ability, there is no need to purify them by expensive and time-consuming chromatographic methods. Our future research will focus on the best mixing proportions of the two chemicals to produce food supplement, and the food supplement's toxicity and anticancer ability by animal feeding experiments.

## Conclusions

The content of sulforaphane and erucin in various brassica seeds was determined in our work. The results showed that the content of these compounds in different cultivars is quite different even in the same species. Thus, it is very important to select several cultivars with high sulforaphane or erucin level. The cultivars that have high content of these compounds and low content of toxic compounds were chosen as raw materials to produce diet supplements, and then sulforaphane and erucin were extracted in gram-scale. Our method offers a procedure to produce relatively large amounts of sulforaphane and erucin at low costs, which are suitable for production of food additives.

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