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Determination of 3-Butenyl Isothiocyanate in Different Parts and Cultivars of Chinese Cabbages

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Abstract: Chinese cabbage (*Brassica campestris* L. *Pekinensis*) is *Brassica* vegetable that contains high amounts of glucosinolates. Glucosinolates and their breakdown products are thought to contribute to health promotion by preventing some cancers. Chinese cabbage is the most commonly consumed vegetable in Asian countries including Korea. In this study, qualitative and quantitative analyses of 3-butenyl glucosinolate (Gluconapin) from different cultivars and different parts of the cabbage were performed. Gluconapin of Chinese cabbage was extracted by hot ethanol (80°C), isolated by an anion exchange column and identified by GC/MS and LC/MS. The levels of glucosinolates in Chinese cabbage varied according to the different parts, cultivars, and blanching time. In general, the concentrations of 3-butenyl isothiocyanate (ITC) were higher in the leaf than in the midribs parts. The cultivar 'Bulam no. 3' had a much greater content of 3-butenyl ITC than the cultivar 'Garak no. 1,' and the levels of butenyl ITC were highest after two weeks of storage. Blanching treatment decreased the concentration of 3-butenyl ITC. The ITC concentration varied extensively among different crops of the same species, and according to the different parts on the cabbage, the storage duration and the boiling time.

Keywords: determination, Chinese cabbage, 3-butenyl isothiocyanate, GC/MS, LC/MS

Introduction

Glucosinolate glycosides are secondary metabolites found in cruciferous vegetables. The glucosinolates are bioactive compounds with anticarcinogenic activity for some cancers including breast cancer (1). *Brassica* vegetables such as broccoli, cabbage, kale, horseradish, and leaf mustard contain high levels of glucosinolates and their breakdown products which have been used for medical treatments since ancient times (2).

The hydrolyzed products of glucosinolates are the isothiocyanates (ITCs), thiocyanates and nitriles which are derived by a rearrangement induced by myrosinase, a thioglucoside glucohydrolase (EC 3.2.3.1) (3). Hydrolyzed glucosinolates, the breakdown products of glucosinolates, have pungent flavor and anticarcinogenic effects. The protective effect of cruciferous vegetables against cancer has been demonstrated due to the action of their relatively high content of bioactive components such as glucosinolates. Thus, glucosinolates occurring in the Cruciferae family might be of importance in the human diet to reduce the risk of cancer (1, 4). ITCs in plants produced by the endogenous myrosinase enzyme have attracted attention as a chemical and dietary inhibitor against cancer. The consumption of *B. oleracea* vegetables reduced the probability of acquiring colon, bladder, rectal, and prostate cancers (5-7). Especially, the pungency of the vegetables associated with the 3-butenyl ITC is considered to be a specific sensory attribute of the food (2). Moreover, certain glucosinolates that are chemically similar to 3-butenyl ITC have been reported to induce mammalian, phase 2 enzymes of detoxification, and the enzyme is a potential metabolite for cancer-prevention (8).

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Although many studies have investigated glucosinolates in the commonly consumed *Brassica* vegetables of many countries, the glucosinolate properties in Chinese cabbage have not received much research attention (*Brassica campestris* L. ssp. *Pekinensis*). Chinese cabbage belongs to a group of cruciferous vegetables that are the most consumed vegetables in Asian countries. They are also the major ingredient in "Kimchi", which is a traditional and ubiquitous fermented food in Korea.

In this study, 3-butenyl glucosinolate was analyzed in Chinese cabbage using GC, HPLC, GC/MS and LC/MS, and substance from different parts and cultivars of the cabbage were determined by GC. Storage and blanching time were also examined as variables related to the concentration of 3-butenyl ITC.

Materials and Methods

Plant materials Two cultivars of Chinese cabbage, 'Garak no. 1' and 'Bulam no. 3', were obtained from the Garak agricultural market in Seoul, Korea. Six parts of the cabbage were used: outward leaves, outward midribs, middle leaves, center midribs, core leaves and core midribs parts.

Chemicals Phosphate buffer (pH 7.0) was prepared by the method of Gomori (9). All other reagents were laboratory grade and were purchased from Junsei Chemical Co. (Japan). The standard solution of 3-butenyl ITC was purchased from Kasei Co. (Japan).

Myrosinase preparation The enzyme solution was prepared from white radish. The juice from chilled white radish (500-1,000 g) was homogenized and filtrated with two layers of gauze. One and a half volumes of chilled acetone: water (60:40) were added to one volume of juice and this mixture was left for 5 min at 0-4°C. The mixture

was centrifuged at 3000 rpm for 5 min at 0-4°C. Its precipitate was freeze-dried and ground with a pestle. The acetone powder was stored at -20°C until use.

Analysis of 3-butenyl isothiocyanate by GC and GC/MS The ITC form of glucosinolate was measured by GC (Agilent, USA) and by GC-Mass (Agilent, USA) spectrometry. Fifty grams of fresh Chinese cabbage were cut into small pieces, mixed well and put into a flask to which 100 mL of hot ethanol was added. The mixture was boiled for 15 min, cooled to room temperature, and then homogenized with a blender. The residue was re-extracted with 100 mL of hot ethanol: water (80:20). After combining the two extracts, the resulting sample was concentrated to 25 mL by an evaporator (EYELA, Tokyo) at 40°C. The concentrated samples were centrifuged at 3000 rpm at 4-5°C for 15 min. The supernatant was increased to 50 mL with distilled water and 20 mL of this solution was passed through an anion exchange column (Dowex 1-X^2, Lancaster, England). The column was washed with sufficient amounts of distilled water until no glucose reaction was detectable by Molisch reagent. The resin of the column was transferred to a 50 mL Erlenmeyer flask containing 5 mL of methylene chloride, 50 mg of crude myrosinase, 1 mL of 10 mM ascorbic acid and 5 mL of 0.1 M sodium phosphate buffer. The flask was shaken in a shaker at room temperature for 18 hr. The enzyme mixture was centrifuged at 4000 rpm at 5°C for 15 min. The methylene chloride layer was transferred to a 5 mL test and then dehydrated with Na_2SO_4 for GC and GC/MS analyses. Gas chromatography was performed by Agilent 4890 (Agilent, USA) with a column (DB-5, 30 × 0.25 mm O.D, 0.25 μm I.D). Mass spectrometry was performed using Agilent 6890 (Agilent, USA) in another column (Ultra II, 230 × 0.25 mm O.D, 0.25 μm I.D) with an FID detector (gas flow 1 mL/min, split 30:1). The oven temperature was ramped from 80 to 180°C at 8°C/min and then from 180 to 255°C at 30°C/min in GC and GC/MS.

Qualitative analysis of 3-butenyl glucosinolate by HPLC and LC/MS The intact form of glucosinolate was used for the LC and LC/MS analyses. Five grams of seeds from the 55-day cultivar Chinese cabbage were used for this investigation. The seeds were placed in a flask to which 100 mL of hot ethanol (80%) was added quickly. The flask was boiled in a hot water-bath for 15 min, cooled and then homogenized with a pestle. The whole samples were re-extracted for 15 min with 100 mL of the same extractive solution. After combining the two extracts, the combined sample was concentrated to less than 40 mL by an evaporator (EYELA, Tokyo, Japan) at 40°C. The supernatant was increased to 50 mL with distilled water and 1 mL of 0.3 M lead-barium acetate solution was then added. The precipitate in the sample solutions was removed by centrifugation at 3000 rpm for 15 min at 5°C. Five milliliters of supernatant were then passed through the QMA cartridge (Sep-pak plus QMA, Waters, USA). After washing with 5 mL of distilled water, the glucosinolates were extracted with 4 mL of 0.3 M potassium sulfate. The sample was analyzed by HPLC and LC/MS. In this study, high performance liquid chromatography (HP1100, Agilent, USA) was performed with a Phenomenex C_{18} column (250 × 4.6 mm, 5 μm, HP). LC/MS was carried out with an HP1100 system in a Quattro LC triple quadrupole tandem MS (Micromass, Manchester, UK) set at an electrospray ionization mode. For the negative mode, the source temperature, desolvation temperature, con voltage and capillary voltage were kept at 70°C, 200°C, 30 V, and 2.5 kV, respectively. The nebulizer and desolvation gas flows were set at 91 and 517 L/hr, respectively.

Results and Discussion

Qualitative analysis of 3-butenyl glucosinolate The peak on the GC chromatogram of 3-butenyl ITC was analyzed by GC/MS (Fig. 1). The GC/MS measurement gave *m/z* signals corresponding to molecular ion [M]+ and fragment ions. The molecular ion of 3-butenyl ITC gave an *m/z* signal at 113.18 and its fragment ions were 87.14, 73.12, 58.08, and 56.11. It was assumed that these fragments came from the intact structure of 3-butenyl ITC.

Three-butenyl glucosinolate, commonly known as gluconapin, extracted from the Chinese cabbages was investigated by LC and LC/MS (Fig. 2). Glucosinolates extracted from Chinese cabbages were injected into the LC/MS. The peaks on the LC chromatogram were analyzed by LC/MS to give results of 373.4 for the molecular weights of 3-butenyl glucosinolate and 266.4, 161.2 and 89.3 for its breakdown products.

Comparison of the results of GC and LC analysis confirmed the identity of 3-butenyl glucosinolate isolated in Chinese cabbage. Szmigielska et al. (10) used GC and HPLC to determine that many varieties of canola seeds contained gluconapin and glucobrassicanapin, which was similar to our study. Broccoli contains 3-butenyl and 2-phenylethyl glucosinolates (11). Among different varieties of nine mustard seeds, gluconapin was found only in the Indian mustard seeds (12). Some researchers have also reported that gluconapin was found in cauliflower, broccoli and kale (13, 14). It should be noted that the 3-butenyl glucosinolate content in fresh vegetables is higher than that in cooked vegetables (15).

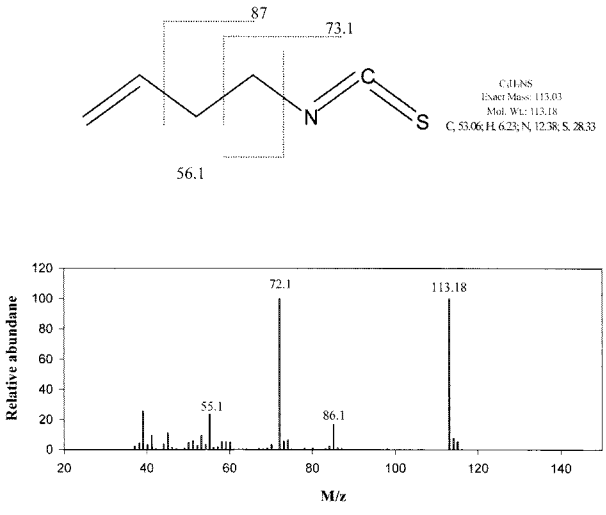

Fig. 1. Structure of 3-butenyl isothiocyanate and mass spectrum of GC/MS analysis.

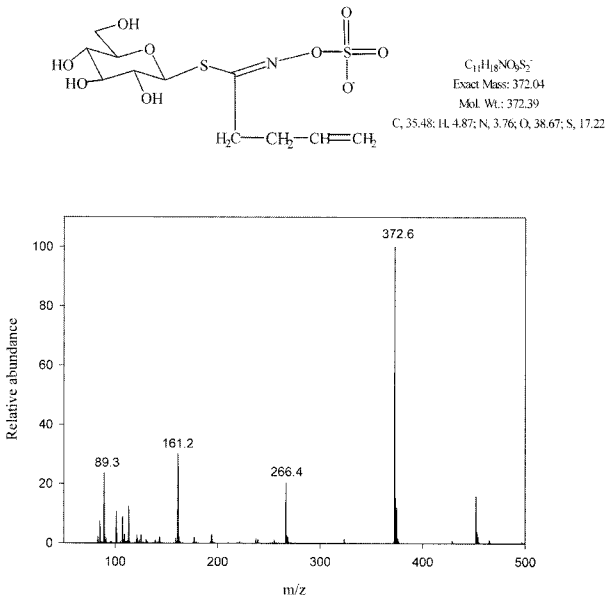

Fig. 2. Structure of 3-butenyl glucosinolate and mass spectrum of GC/MS analysis.

Quantitative analysis of 3-butenyl isothiocyanate The GC quantitative analysis of 3-butenyl ITC was performed and compared to the ITC standard solution calibration curves.

In fresh Chinese cabbages, the concentration of 3-butenyl ITC in 'Bulam no. 3' cultivar was much higher than that in 'Garak no. 1' cultivar. The core and the leaf parts had a higher concentration of 3-butenyl ITC than the other parts. In both cultivars, the concentration of 3-butenyl ITC increased up to 2 weeks of storage, after which it decreased. This was especially notable in 'Bulam no. 3' cultivar, for which the level peaked at 2 weeks of storage (Figs. 3, 4). Using a blanching treatment (a basic cooking method in Asia), the levels of 3-butenyl ITC in different cultivars and portions decreased rapidly after 1-min blanching and were maintained at lowered levels until 5-min blanching. 'Bulam no. 3' and 'Garak no. 1' cultivars showed similar graphical patterns of 3-butenyl ITC content (Figs. 5, 6). On the other hand, the concentration

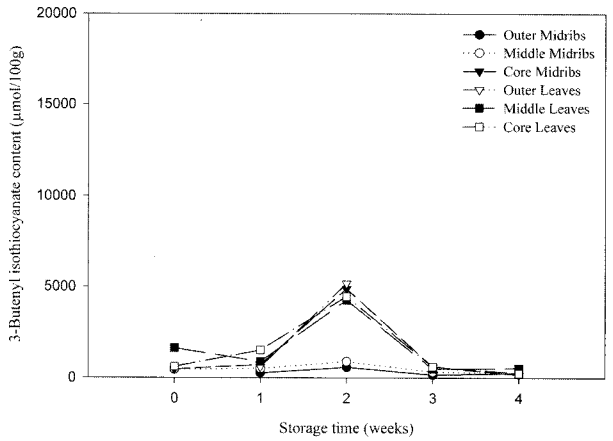

Fig. 3. Patterns for 3-butenyl ITC in Chinese cabbages, 'Garak no. 1' cultivar, during storage.

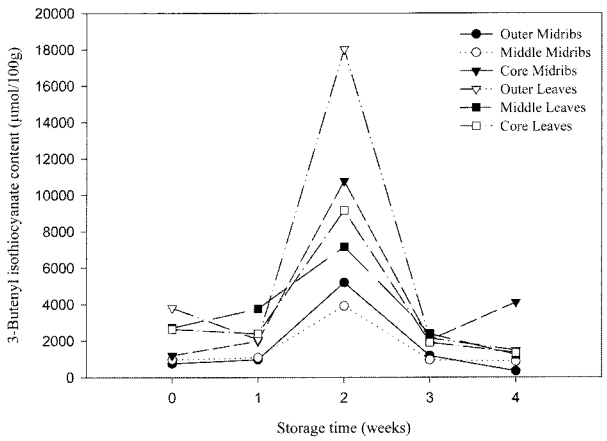

Fig. 4. Patterns for 3-butenyl ITC in Chinese cabbages, 'Bulam no. 3' cultivar, during storage.

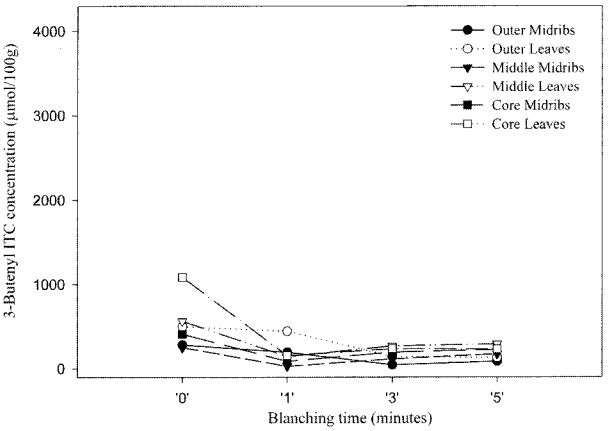

Fig. 5. Patterns of 3-butenyl ITC in Chinese cabbages, 'Garak no. 1' cultivar with boiling time.

of 3-butenyl ITC in 'Bulam no. 3' cultivar was 2.7 times higher than that of 'Garak no. 1' cultivar. The ITC content in cooked vegetables was less than that of fresh vegetables, which agreed with the results of Vos and Blijleven (15).

The major glucosinolate in the seed of Chinese cabbage

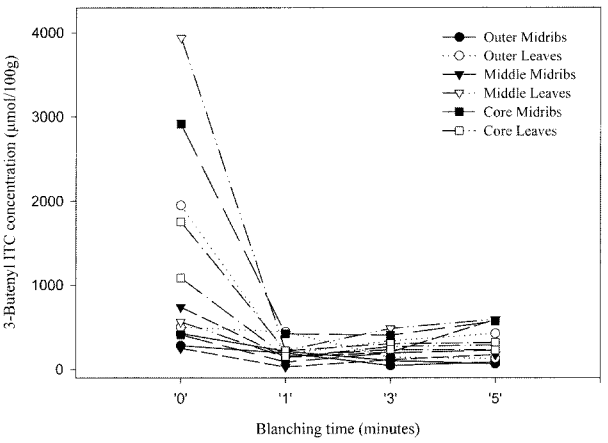

Fig. 6. Patterns of 3-butenyl ITC in Chinese cabbages, 'Bulam no. 3' cultivar with boiling time.

was 3-butenyl glucosinolate and its concentration in 14 varieties of Chinese cabbage ranged from 0.9 ppm to 68.5 ppm (16). Three-butenyl glucosinolates in Chinese cabbages were present at concentrations ranging from 0.6 to 23 μmol/100 g of fresh weight (17).

Recently, a lot of research on gluconapin content has been conducted in different *Brassica* vegetables. Mithen et al. (18) reported that the concentrations of gluconapin in Brussel sprouts, savoy cabbage, broccoli, and red cabbage were 7.0, 0.4, 0.6, and 0.4 μmol/g dry weight, respectively. Verkerk et al. (19) also reported that the concentrations of gluconapin in broccoli, white and red cabbages were 26, 127, and 300 μmol/100 g of dry weight, respectively.

Conclusion

Gluconapin analysis in GC, HPLC, GC/MS, and LC/MS showed that the composition of glucosinolates varied extensively among crops of the same species and among different portions such as leaves, midribs, outwards, and core parts. In addition, the concentrations of 3-butenyl ITC in fresh Chinese cabbages were higher in the leaves than in the midribs. On the other hand, the concentration of 3-butenyl ITC was decreased rapidly in blanched vegetables. These results indicated that the 3-butenyl ITC level in Chinese cabbages varied with different cultivars, portions, and blanching time.

Acknowledgments

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