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Isolation and Identification of Glucosinolates in Edible Parts of Chinese Cabbages (*Brassica campestris* L. *ssp. Peckinensis*)

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Chinese cabbage (*Brassica campestris* L. *ssp. Peckinensis*) is the most widely consumed *Brassica* vegetable in Asian countries including Korea. *Brassica* vegetables contain glucosinolates, which have been known to contribute health promotion of human being. Glucosinolates are hydrolyzed into isothiocyanates and other breakdown products. Glucosinolates in the Chinese cabbages were characterized using not only GC and GC/MS but also HPLC and LC/MS. The major glucosinolates in Chinese cabbage were identified as 3-butenyl, 4-pentenyl, and 2-phenylethyl glucosinolates, which were gluconapin, glucobrassicanapin, and gluconasturtiin, respectively.

Keywords: Chinese cabbage (*Brassica campestris* L. *ssp. Peckinensis*), glucobrassicanapin, gluconapin, gluconasturtiin, glucosinolates

Introduction

The glucosinolates have been found in many *Brassica* vegetables such as broccolis, cabbages, cauliflowers, kales, horseradishes, mustards, and turnips. They exist as secondary metabolites in many *Crucifer* species. The glucosinolate molecule consists of two parts, a common glycone moiety and a variable aglycone side chain (Fenwick *et al.*, 1983; Rosa *et al.*, 1997). The aglycone part may contain aliphatic, indolyl, or aromatic side chains derived from a corresponding α-amino acid (Halkier and Du, 1997). These sulfur-containing glycosides occur at the highest concentrations in the family of *Brassicaceae* (Mithen *et al.*, 2000).

Thioglucoside glucohydrolysis enzyme, myrosinase, breaks the glucosinolates into isothiocyanates that were considered to prevent the human body from cancers (Fahey and Stephenson, 1999). In recent studies, glucosinolates were focused on anticarcinogenic function. Especially, *Brassica* vegetables that have been used to medical treatments since ancient times were contained high levels of glucosinolates and their breakdown products (Fenwick *et al.*, 1983).

According to the review by Rosa *et al.* (1997), the levels of glucosinolates were varied even in the same study, and were shown in diverse studies. Reasons for these different research reports were caused the use for the various cultivars, the growing conditions and the analytical methods.

Despite of the Chinese cabbages were greatly consumed in Asian countries, few studies on glucosinolates have been conducted in Chinese cabbages.

The objective of this study was to determine glucosinolates using various analytical instruments, and it would be contributed to promote value of Chinese cabbages as functional foods.

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Materials and Methods

Plant materials Plant materials used in this study were 'Winter Pride' (Seminis, Korea) cultivar of Chinese cabbages obtained from a Wholesale market. Six parts of Chinese cabbages: outward leaves, outward midribs, middle leaves, middle midribs, core leaves, and core midribs parts were used.

Chemicals Phosphate buffer (pH 7.0) was prepared according to the method of Gomori (1974). All other reagents of extra pure grade were purchased from Junsei Chemical (Japan). Standard solutions of isothiocyanate were used from Kasei (Japan).

Myrosinase preparation The myrosinase was prepared from white radish that was obtained from the retail market. The chilled white radish (total wt. 500~1,000 g) was homogenized with a waring blender and filtrated with two layers of gauze. One and a half volume of a chilled acetone: water (60:40,v/v) was added to one volume of juice and this mixture was left for 5 minutes at 0~4°C. The mixture was centrifuged at 3,000 rpm for 5 minutes at 0~4°C. Its precipitate was freeze-dried and grinded with a pestle. Then, the acetone powder was stored at –20°C until it was used.

Qualitative analysis of isothiocyanates using GC and GC/MS Isothiocyanates derived from glucosinolates were measured by GC and GC/MS methods. Fresh Chinese cabbages were cut into small pieces and mixed well and then freeze dried. The sample (5 g) was put into a flask, and 100 ml of hot ethanol was added quickly. The flask was boiled in a hot water-bath for 15 minutes and allowed to cool, then homogenized with a blender and filtered. The residue was re-extracted with 100 ml of hot ethanol: water (80:20,v/v). After combining two extracts, it was concen-

trated to 25 ml using an evaporator (EYELA, Tokyo) at 40°C. Concentrated samples were centrifuged at 3,000 rpm at 4~5°C for 15 minutes. The supernatant was filled up to 50 ml with distilled water and 20 ml of this solution was passed through the anion exchange column (Dowex 1-X^2, Cl form, 50/100 mesh; Lancaster, England). The column was washed with sufficient amount of distilled water until glucose reaction was not detected by Molisch reagent. Resin of column was transferred to 50 ml Erlemmeyer flask that contains 5 ml of methylenchloride, 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 for 18 hours at room temperature and the enzyme mixture was centrifuged at 4,000 rpm for 15 min at 5°C. Methylenechloride layer was transferred to a 5 ml test tube and then dehydrated with Na_2SO_4 for GC and GC/MS analysis. Gas chromatography analysis was performed using Agilent 5890 (Agilent, USA) with a column (stainless, 1 m × 1/8 inch, 2% OV-7 100/120 chromosorb) with a FID detector.

GC/MS was performed using Agilent 6890 (Agilent, USA) with a column (Ultra II, 230 × 0.25 mm O.D, 0.25 μm I.D) and FID detector (gas flows 1 ml/min, split 30:1). Oven temperature was 80°C for 1 min, 80°C~180°C (8°C/min) and then 180°C~255°C (30°C/min) in GC and GC/MS. Injection volumes were 1 μl for GC and 2 μl for GC/MS, respectively.

Qualitative analysis of glucosinolates using HPLC and LC/MS Intact forms of glucosinolates were investigated by high performance liquid chromatography (HPLC) and LC/MS method. Extraction method of Chinese cabbages was same as GC and GC/MS. Supernatant of extraction was filled up to 50 ml with distilled water and 1 ml of 0.3 M lead-barium acetate solution was added. The precipitate in the sample solutions was removed by centrifugation at 3,000 rpm for 15 minutes at 5°C and 5 ml of the supernatant solution was passed through the QMA cartridge (Sep-pak plus QMA, Waters, USA). After washing with 5 ml distilled water, glucosinolates were extracted with 4 ml of 0.3 M potassium sulfate. In this study, HPLC measurement was performed using HP1100 (Agilent, USA) with a Phenomenex C_{18} column (250 × 4.6 mm, 5 μm, HP). LC/MS was carried out by HP1100 system to a Quattro LC triple quadrupole tandem MS (Micromass, Manchester, UK) at an electrospray ionization mode. In negative mode, the source temperature, desolvation temperature, cone voltage, and capillary voltage were kept at 70°C, 200°C, 30 V, and 2.5 kV, respectively. The nebulizer gas flow and desolvation gas flow were set at 91 and 517 L/hr, respectively.

Results

Qualitative analysis of isothiocyanates using GC and GC/MS Using GC and GC/MS, three isothiocyanates from Chinese cabbage could be identified by comparing with the peaks on GC chromatogram of a presumed standard mixture (Fig. 1). Similarly, composition patterns of isothiocyanates were analyzed by GC in various parts of fresh Chinese cabbage. According to these GC chromatograms, four peaks of isothiocyanates in samples from 6 different parts in Chinese cabbage appeared (Fig. 2).

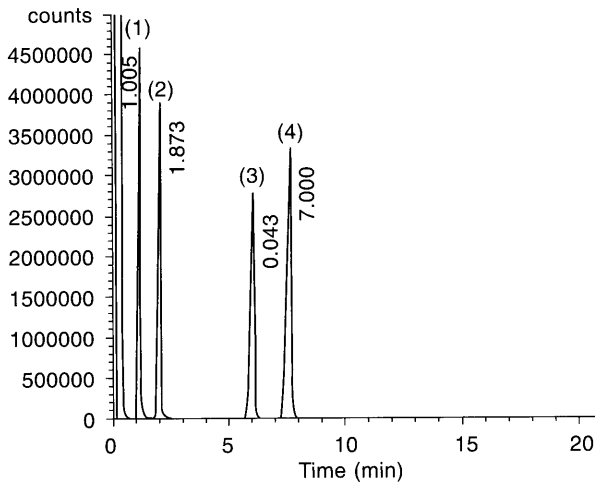

Fig. 1. GC spectrum of isothiocyanate standard mixtures. Peak identification: (1) 3-butenyl isothiocyanate, (2) 4-pentenyl isothiocyanate, (3) benzyl isothiocyanate, (4) 2-phenylethyl isothiocyanate.

The GC/MS measurement gave *m/z* signals corresponding to molecular ion [M]+ and fragmentation ions of each isothiocyanate. On the GC/MS measurement of these peaks, the first peak was identified as 3-butenyl isothiocyanate, and its molecular ion due to at *m/z* 113.18 and fragment ions at *m/z* 87.14, 73.12, and 56.11 (Fig. 3). The second peak was identified as a 4-pentenyl isothiocyanate due to its molecular ion at *m/z* 127.21 and fragment ions at *m/z* 73.12, 56.11, and 42.08. The third peak was identified as a 2-phenylethyl isothiocyanate its molecular ion at *m/z* 163.24 and fragment ions at *m/z* 106.17, and 92.14. The last peak was not detected in GC/MS analysis and remained as an unknown peak.

Qualitative analysis of glucosinolates using HPLC and LC/MS Using LC and LC-Mass spectroscopy, three different glucosinolates were also investigated that were extracted from Chinese cabbages. At first, glucosinolates extracted from Chinese cabbages were injected to LC and identification was conducted by LC/MS.

3-Butenyl glucosinolate was confirmed with a protonated $[M-H]^-$ ion at *m/z* 372.6 and a weak solvent adduct $[M+CH_3CN-H]^-$ ion at *m/z* 414.4. Characterizing fragmentation ions at *m/z* 266.4, 161.2, 113.2, and 89.3 is also supporting the correct structure (Fig. 4).

The mass spectrum of 4-pentenyl glucosinolate has a protonated molecular ion $[M-H]^-$ at *m/z* 386.6 and fragmentation ions at *m/z* 280.4, 144.2, and 89.4 (Fig. 4).
The mass spectrum of 2-Phenylethyl glucosinolate showed a protonated molecular ion $[M-H]^-$ at *m/z* 422.8, a weak solvent adduct $[M+CH_3CN-H]^-$ ion at 485.4 and characteristic major fragmentation ions at *m/z* 161.2 and 121.2 (Fig. 4).

Discussion

According to studies, more than 120 different glucosinolates have been characterized, of which only a few have

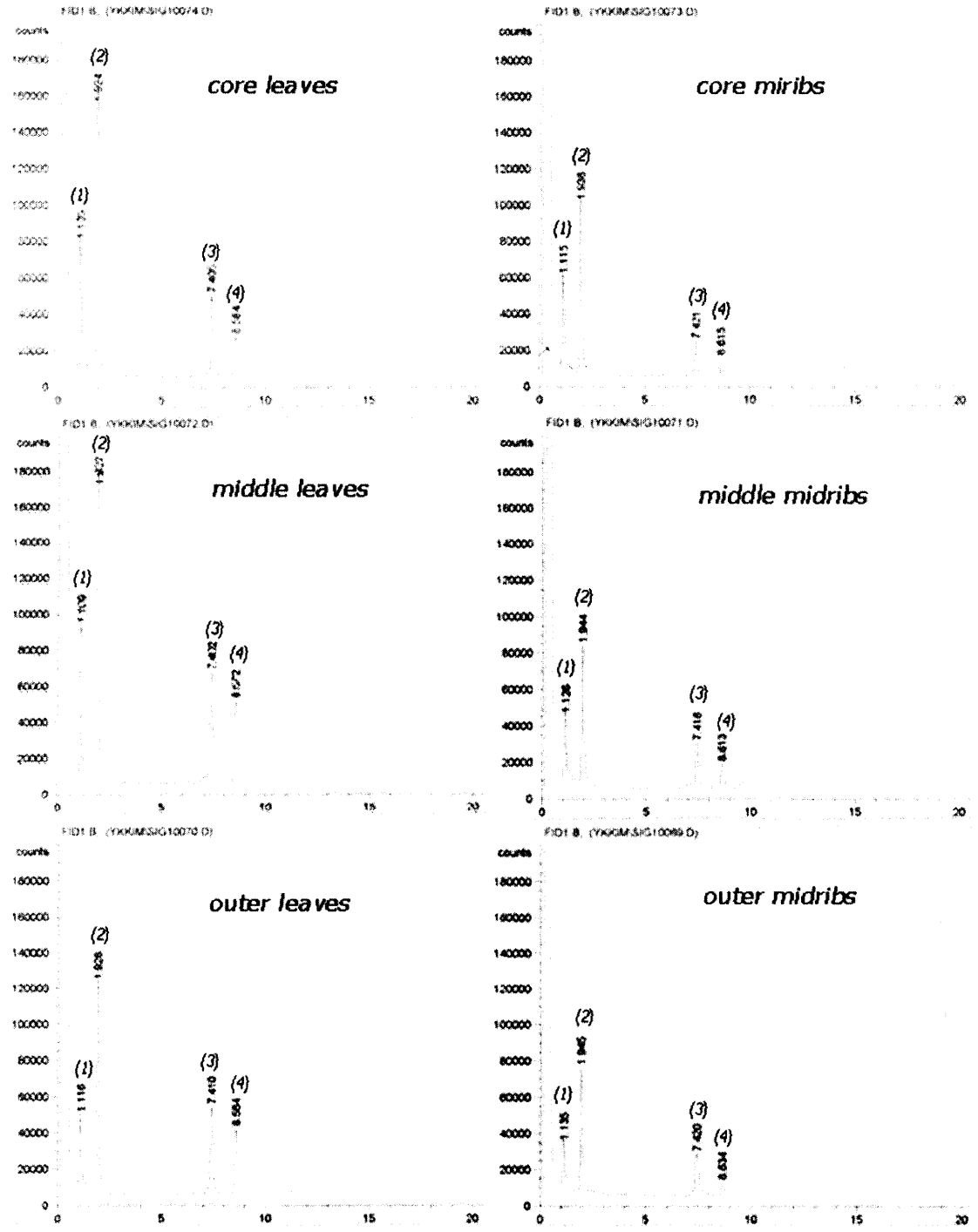

Fig. 2. GC spectra of isothiocyanates in six parts of Chinese cabbage 'Winter Pride' cultivar. These peaks are; (1) 3-butenyl, (2) 4-pentenyl, (3) 2-phenylethyl isothiocyanates, and (4) is unknown peak.

been investigated thoroughly (Verkerk *et al.*, 1998). There is a need for analysis of glucosinolates in common vegetables in Asia. This study found 3-butenyl, 4-pentenyl, and 2-phenylethyl glucosinolates and their breakdown products in Chinese cabbages. These results agree with the finding of Daxenbichler and others who conducted using GC and GC/MS only (1979). In general, plant species could contain up to four different glucosinolates in significant amounts (Verkerk *et al.*, 1998). Similarly, higher concentrations are usually found in the seeds except for indol-3-ylmethyl and N-methoxyindol-3-ylmethyl glucosinolates that are rarely found in the seeds (Tookey *et al.*, 1980).

In recent studies, many researchers conducted that analysis of glucosinolates in various vegetables. Mithen *et al.* (2000) reported that gluconapin was detected in Brussels sprouts, savoy cabbage, broccoli, and red cabbage. Broccoli also contained 3-butenyl, and 2-phenylethyl glucosinolate (Rosa & Rodrigues, 2001). Using GC and LC, it was found that various canola seeds included gluconapin,

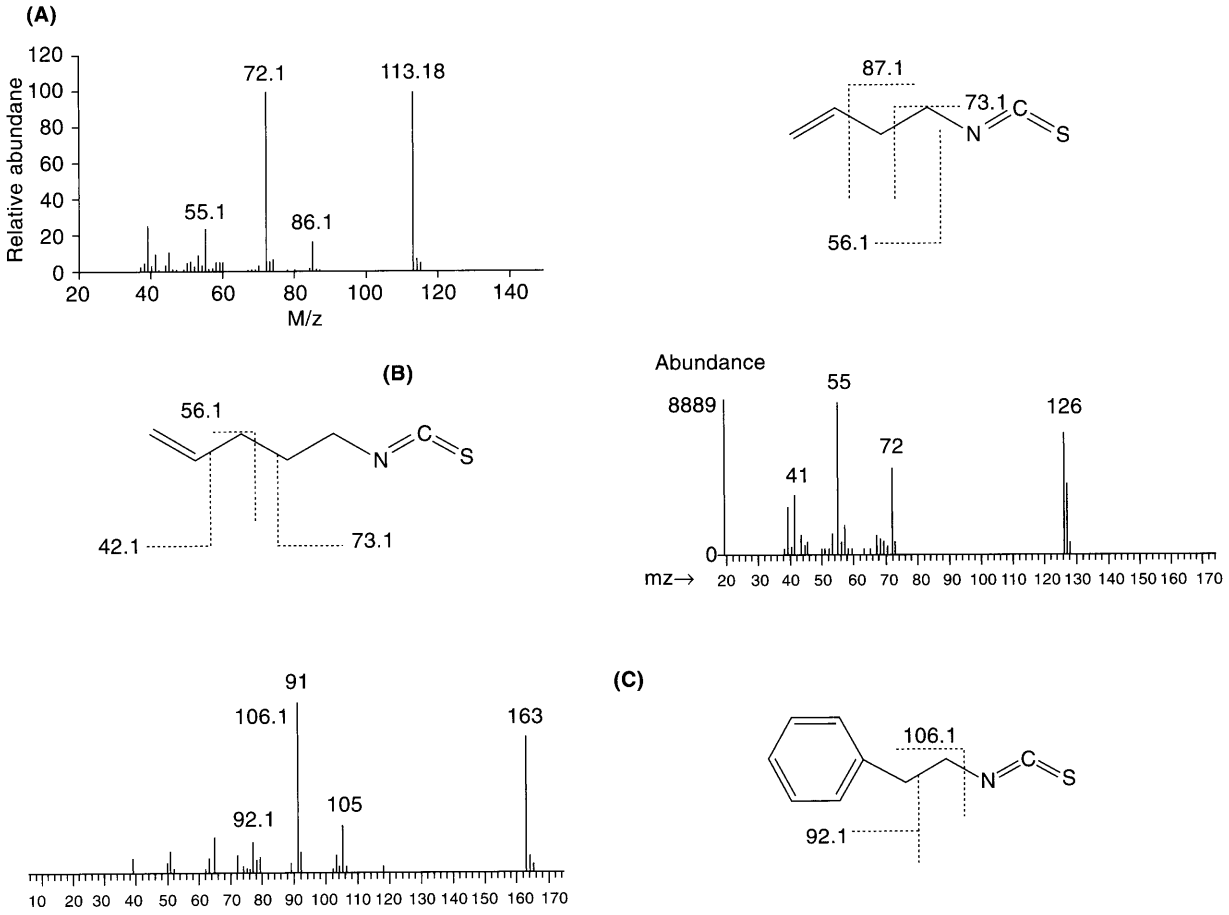

Fig. 3. Structure and mass spectra of 3-butenyl isothiocyanate (A), 4-pentenyl isothiocyanate (B), and 2-phenylethyl isothiocyanate (C).

glucobrassicanapin, progoitrin, and napoleoferin (Szmigielska & Schoenau, 2000). Szmigielska and Schoenau (2000) performed quantitative analysis of gluconapin in Indian mustard. Similarly, Verkerk *et al.* (2001) investigated gluconapin in broccoli, white cabbage, and red cabbage and Branca *et al.* (2002) also detected it in cauliflower, broccoli, and kale. On the other hand, Agerbirk *et al.* (2001) studied 2-phenylethyl glucosinolate and other glucosinolate in *Barbarea vulgaris* ssp. *Arcuata*.

One study found that, 3-butenyl glucosinolate and other glucosinolates in Brussels sprouts was isolated and identified by LC/MS (Mellon *et al.*, 2002). Canola crops have 2-phenylethyl isothiocyanate and they have been shown to resist soil-borne pathogens (Rumberger & Marschner, 2003).

In conclusion of this study, glucosinolates were identified by GC, GC/MS, HPLC, and LC/MS in Chinese cabbages. Through the GC chromatogram, three forms of isothiocyanates were assumed and were identified by GC/MS. In results of LC and LC/MS analyses, three kinds of intact glucosinolates were identified to be 3-butenyl glucosinolate (gluconapin), 4-pentenyl glucosinolate (glucobrassicanapin), and 2-phenylethyl glucosinolate (gluconasturtiin). Matching these results, three kinds of isothiocyanates were proven the breakdown products of intact glucosinolates in Winter Pride Chinese cabbage cultivar, namely, 3-butenyl, 4-pentenyl, and 2-phenylethyl isothiocyanates.

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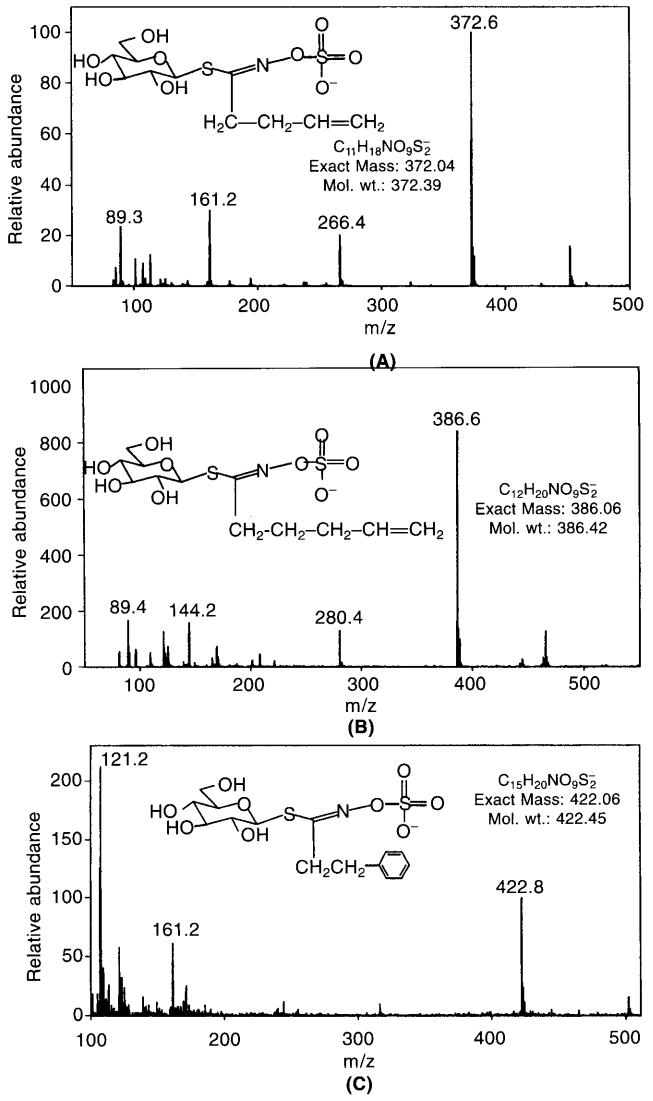

Fig. 4. Structure and LC/MS spectra of 3-butenyl glucosinolate (A), 4-pentenyl glucosinolate (B), and 2-phenylethyl glucosinolate (C).

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