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Research Note

GC-MS Analysis of the Extracts from Korean Cabbage (*Brassica campestris* L. ssp. *pekinensis*) and Its Seed

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ABSTRACT: Korean cabbage, a member of the Brassicaceae family which also includes cauliflower, mustard, radish, and turnip plants, is a crucial leafy vegetable crop. Korean cabbage is harvested after completion of the leaf heading process and is often prepared for use in "baechu kimchi", a traditional Korean food. Many of the components in Korean cabbage are essential for proper human nutrition; these components can be divided into two groups: primary metabolites, which include carbohydrates, amino acids, fatty acids, and organic acids, and secondary metabolites such as flavonoids, carotenoids, sterols, phenolic acids, alkaloids, and glucosinolates (GSLs). Using gas chromatography-mass spectrometry, this study examined the variety of volatile compounds (including isothiocyanates) contained in Korean cabbage and its seed, which resulted in the identification of 16 and 12 volatile compounds, respectively. The primary volatile compound found in the cabbage was ethyl linoleolate (~23%), while 4,5-epithiovaleronitrile (~46%) was the primary volatile component in the seed.

Keywords: Korean cabbage seed, volatile compositions, 3-butenyl isothiocyanate, bio-protective effects

INTRODUCTION

Korean cabbage is a member of the Brassicaceae family, which also includes cauliflower, mustard, radish, and turnip plants. Korean cabbage is a crucial leafy vegetable crop harvested after completing the leaf heading process and often prepared for use in "baechu kimchi", a traditional Korean food. Korean cabbage is not only a vegetable crop, but it also serves as an important source of the components essential for proper human nutrition. The nutritional components can be divided into two groups: primary metabolites, which include carbohydrates, amino acids, fatty acids, and organic acids, and secondary metabolites such as flavonoids, carotenoids, sterols, phenolic acids, alkaloids, and glucosinolates (GSLs) (1).

GSLs are usually separated from the endogenous enzyme myrosinase, which catalyzes their hydrolysis. The decomposition products of GSLs include substituted isothiocyanates (ITCs), thiocyanates, nitriles, epithionitriles, and oxazolidinethiones. The composition of these decomposition products can vary depending on the specific substrate and reaction conditions (2). ITCs are biochemically active compounds with a wide variety of medicinal (e.g., anticancer, anticoagulant, anti-inflammatory, anti-asthma, antibiotic, antifungal), pharmaceutical, and industrial (e.g., wood preservative, antifungal) applications (3-8). ITC concentrations differ depending on the plant tissue type. Generally, concentrations in the seed are high, often up to 10% of the dry weight, whereas the levels in the leaf, stem, and root are about 10 times lower (9,10). Much work has been carried out on volatile compound composition and GSL breakdown product identification in various fresh or precooked *Brassica* vegetables (11); however, relatively little information exists regarding the profile of volatile compounds and ITCs in the seed of Korean cabbage. Therefore, the present study investigated the volatile constituents from Korean cabbage and its seed using gas chromatography-mass spectrometry.

MATERIALS AND METHODS

Plant materials preparations

Fresh Korean cabbages (*Brassica campestris* L. ssp. *peckinensis*, cultivar; 'Winter pride') were planted by the direct seeding method in the greenhouse (Duksung Women's University, Seoul, Korea). Korean cabbages were grown for a 15 week growth period under an average temperature of 20~25°C, 60~80% relative humidity and using

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natural fertilizers. Water supply and insect and disease controls were properly maintained as needed. Korean cabbages were harvested, immediately freeze dried and stored at −70°C.

The seeds of 'Winter pride' cultivars of Korean cabbage (*Brassica campestris* L. ssp. *peckinensis*) were obtained from 'Seminis Korea Co.' (Seoul, Korea). The seeds were kept at 100°C for 1 h to inactivate endogenous myrosinase and defatted by hexane several times overnight. After filtration through filter paper, residual solvent was removed under vacuum and immediately stored at −70°C.

Chemical reagents

The standard 2-phenylethyl ITCs was purchased from TCI (Tokyo Chemical Industry Co., LTD., Tokyo, Japan). Myrosinase (E.C. 3.2.1.147; thioglucosidase from *Sinapis alba* seed, 100 units/g solid) was obtained by Sigma-Aldrich (St. Louis, MO, USA). All other reagents of laboratory grade were purchased from Junsei Chemical (Tokyo, Japan).

Analytical profile of hydrolysis products and other volatile constituents by GC-MS

Freeze-dried Korean cabbage (5 g) or defatted seed of Korean cabbage (2.5 g) was mixed with distilled water (250 mL), myrosinase enzyme (5 units) and 5 mg L-ascorbic acid and allowed to hydrolyse for 2 h at ambient temperature. Dichloromethane (100 mL) was added to the mixture, shaken for 30 min and separated by centrifugation for 15 min at 3,500 rpm. The separated organic layer was dried over anhydrous sodium sulfate, and carefully concentrated to a small volume (about 0.5 mL), with a rotary evaporator. The concentrated hydrolysate was kept in a freezer (−20°C) until analysis (12).

Two μL aliquots of the concentrated dichloromethane extract were analyzed by gas chromatography-mass spectrometry (GC-MS), using an Agilent 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) attached to a JMS-600W mass spectrometer (JEOL Ltd., Tokyo, Japan). The column used was HP DB-5 capillary column (30×0.25 mm×0.25 μm; Agilent Technologies). GC oven initial temperature was 50°C for 2 min and was programmed to 280°C at a rate of 5°C/min, and finally held at 280°C for 2 min. Operating conditions for GC were as follows: hydrogen was used as carrier gas (5 mL/min); the temperature of injector and detector was 250°C and 280°C, respectively; the volume injected was 2 μL in split mode (10 : 1). The mass spectra were performed at 70 eV of the mass range of 35∼400. Three replicates were performed for each sample. The identity of volatile compositions was confirmed by comparison of spectra with published spectra from Wiley's database (13) and literature data (14-16).

RESULTS AND DISCUSSION

Identification of ITCs and other volatile compounds in Korean cabbage and its seed

Tables 1 and 2 summarize the GC-MS data obtained from the analysis of ITCs and other volatile compounds from Korean cabbage and its seed, respectively. Compounds are listed in order of their elution from a DB-5 column.

The major volatile constituents of Korean cabbage extract are ethyl linoleolate (∼23%), benzenepropanenitrile (∼12%), and 2-phenylethyl ITC (∼11%; Table 1). Other minor compounds were observed, including 4-phenylpyridine (∼0.9%) and 2-methyl-6-indolizinecarbonitrile (∼0.7%). The major volatile non-ITC compounds identified in the seed of Korean cabbage were 4,5-epithiovaleronitrile (∼46%) and 9,12-octadecadienal

Table 1. GC-MS analysis of glucosinolates hydrolysis products and miscellaneous volatile compounds of *Brassica campestris* L. ssp. *pekinensis*

Identified compounds (molecular formula)	RT[1)] (min)	$[M]^+$	MS spectral data m/z (% relative abundance)
4-pentenyl isothiocyanate (C_6H_9NS)	8.145	127 (100)	126 (74), 99 (61), 85 (49), 72 (98), 70 (44), 67 (79)
4,5-epithiovaleronitrile (C_5H_7NS)	9.224	113 (100)	98 (5), 86 (23), 80 (17), 73 (51), 67 (22)
Benzenepropanenitrile (C_9H_9N)	12.345	131 (23)	91 (100), 77 (5), 65 (11), 63 (5)
4-amino-2(1H)-pyrimidinethione ($C_4H_5N_3S$)	12.478	127 (100)	94 (41), 80 (11), 68 (19), 57 (7)
2-amino-6-mercapto-4(1H)-pyrimidinone ($C_4H_5N_3OS$)	14.339	143 (45)	128 (3), 96 (35), 82 (8), 69 (21), 61 (100)
2-phenylethyl isothiocyanate (C_9H_9NS)	18.348	163 (47)	105 (12), 103 (5), 91 (100), 77 (10), 72 (8), 65 (11), 63 (4)
4-methylpentyl isothiocyanate ($C_7H_{13}NS$)	20.304	143 (16)	129 (36), 101 (28), 72 (53)
4-phenyl pyridine ($C_{11}H_9N$)	24.456	155 (100)	128 (51), 102 (22), 77 (18)
2-methyl-6-indolizinecarbonitrile ($C_{10}H_8N_2$)	26.240	156 (69)	155 (100), 128 (14), 101 (10), 77 (10), 64 (4)
Neophytadiene ($C_{20}H_{38}$)	27.051	278 (11)	137 (12), 123 (53), 109 (26), 95 (80), 82 (75), 68 (100)
11,14,17-eicosatrienoic acid, methyl ester ($C_{21}H_{36}O_2$)	29.170	320 (64)	250 (14), 108 (31), 93 (46), 79 (100), 67 (62), 55 (48)
Hexadecanoic acid ($C_{16}H_{32}O_2$)	29.523	256 (100)	213 (32), 129 (36), 97 (18), 83 (24), 73 (72), 60 (61)
1-(2-hydroxy-1-naphthalenyl) ethanone ($C_{12}H_{10}O_2$)	30.325	186 (76)	171 (100), 143 (15), 89 (11), 63 (6)
2-hexadecen-1-ol,3,7,11,15,-tetramethyl ($C_{20}H_{40}O$)	32.291	296 (2)	126 (12), 123 (39), 111 (19), 95 (26), 71 (100), 55 (47)
Ethyl linoleolate ($C_{20}H_{36}O_2$)	33.264	308 (31)	135 (14), 108 (45), 95 (59), 79 (100), 67 (75), 55 (62)
25-epiaplysterylacetate-2 ($C_{31}H_{52}O_2$)	49.316	456 (6)	396 (100), 381 (20), 288 (16), 213 (13), 147 (25), 81 (24)

[1)]Retention time (min).

Table 2. GC-MS analysis of glucosinolates hydrolysis products and miscellaneous volatile compounds of Korean cabbage seed

Identified compounds (molecular formula)	RT[1)] (min)	$[M]^+$	MS spectral data m/z (% relative abundance)
2-methylenebutyronitrile (C_5H_7N)	2.410	81 (100)	54 (80)
3-butenyl isothiocyanate (C_5H_7NS)	5.521	113 (80)	85 (8), 72 (100), 55 (18)
4-pentenyl isothiocyanate (C_6H_9NS)	8.145	127 (100)	99 (48), 85 (46), 72 (81), 67 (76), 55 (38)
4,5-epithiovaleronitrile (C_5H_7NS)	9.329	113 (100)	86 (23), 73 (44), 67 (22), 60 (34)
Benzenepropanenitrile (C_9H_9N)	12.287	131 (25)	91 (100), 65 (9)
2-phenylethyl isothiocyanate (C_9H_9NS)	18.290	163 (46)	105 (11), 91 (100), 77 (10), 65 (9)
2,6-dimethyl-3-(methoxymethyl)-p-benzoquinone ($C_{10}H_{12}O_3$)	20.476	180 (100)	165 (44), 137 (32), 122 (15), 105 (8)
2-methyl-4-isopropyl-1,3,4-thiadiazine ($C_7H_{16}N_2S$)	25.228	160 (93)	115 (23), 72 (100), 55 (46)
Propionic acid, 3-(allythio)-,ethyl ester ($C_8H_{14}O_2S$)	28.034	174 (100)	128 (26), 72 (84), 69 (59), 61 (44)
Hexadecanoic acid ($C_{16}H_{32}O_2$)	29.409	256 (100)	213 (30), 129 (31), 97 (16), 73 (73), 60 (57)
9,12-octadecadienal ($C_{18}H_{32}O$)	33.035	264 (34)	123 (16), 111 (26), 97 (48), 83 (51), 55 (100)
Octadecanoic acid ($C_{18}H_{36}O_2$)	33.210	284 (100)	241 (22), 129 (27), 97 (16), 73 (56)

[1)]Retention time (min).

(~7%; Table 2). Other minor compounds observed in the seed include octadecanoic acid (~5%), hexadecanoic acid (~4%), and benzenepropanenitrile (~2%).

GLS hydrolysis products identified in Korean cabbage include 2-phenylethyl ITC derived from gluconasturtiin (~11%), 4-pentenyl ITC derived from glucobrassicanapin (~3%), and 4-methylpentyl ITC derived from 4-methylpentyl GSL (~1%). 3-Butenyl ITC derived from gluconapin (~7%), 2-phenylethyl ITC derived from gluconasturtiin (~2%), and 4-pentenyl ITC derived from glucobrassicanapin (~1%) were also found in the Korean cabbage seed. Of all ITC compounds identified, 2-phenylethyl ITC and 3-butenyl ITC derived from the aliphatic GSL were present in the highest concentrations in Korean cabbage and its seed, respectively.

ITCs are stored in abundant quantities in the seeds, roots, stems, and leaves of cruciferous vegetables as relatively stable precursors known as GSLs (β-thioglucoside *N*-hydroxysulfates). GSLs can break down to form ITCs by the action of the endogenous enzyme myrosinase. ITCs are utilized by plants as protection from herbivore attacks and pathogens. The compositions of the ITCs vary depending on the plant species studied, side-chain substitutions, cellular pH, and iron concentration (2,17). The concentrations and profiles of hydrolysis products and volatile compounds in *Brassica* plants also vary according to cultivars and vegetable part, and depend on the development stage of the plant (18).

ITCs are naturally occurring small molecules characterized by the presence of a reactive ITC (R−N=C=S) group and are absorbed across intestinal cell membranes by passive diffusion. A wide spectrum of ITCs is possible given the variety of R-groups on the parent GSL. The R-group can be an aliphatic, aromatic, or heteroaromatic residue (19). Previous research detected 10 different ITCs in 22 varieties of cabbages, including allyl, phenyl, benzyl, and 2-phenylethyl ITCs (20). Recent reports indicated the presence of 3-indolymethyl, 2-hydroxy-3-butenyl, and 3-indolymethyl ITCs from different *Brassica* species (21). However, the identification of GSL degradation products and other volatile compounds in the seed of Korean cabbage has never been reported. In the present study, the primary volatile compounds contained in Korean cabbage and its seed were isolated and identified. Although the current study is qualitative, further research will focus on monitoring individual ITCs in various plants based on the tentative identifications presented herein.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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