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Characterization of Some Organosulfur Compounds in Shallot Bulbs

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Introduction

Most of organosulfur compounds in *Allium* spp., e.g. garlic and onion, are cysteine sulfoxides, γ-glutamylcysteine and volatile sulfur compounds. Recently organosulfur compounds gain a great interest as natural compounds used in dietary products and as therapeutic agents. Organosulfur compounds are varied in different species and cultivars of plants which are mostly garlic and onion[1]. *S*-alk(en)yl-L-cysteine sulfoxides is a major compound giving a pungent compound of the *Allium* plants. High content of alliin (*S*-allyl-L-cysteine sulfoxide) is found in garlic (*Allium sativum* L.) while isoalliin ((+)-*S*-trans-1-propenyl-L-cysteine) is a major sulfoxide found in onion (*Allium cepa* L.). Organosulfur compounds possess immunomodulatory, anti-cancer and cardioprotective activities *in vitro* and *in vivo*[2,3]. Diallyl disulfide, *S*-allyl cysteine and ajoene showed anti-cancer activity in animal model and suppressed cancer cells *in vitro* and *in vivo*[4,5]. 1-Propylmercaptan, dimethyl disulfide, diallyl disulfide, propyl disulfide and 2,5-dimethylthiophene of *Allium* plants contributed to antioxidant and antimutagenic activities[6]. In addition, propylthiosulfinate oxide (PTSO) is a major organosulfur compound in a commercial *Allium* sp. extract[7]. Due to its antioxidant and antimicrobial activities, it is used as a preservative in food industry. No acute cytotoxicity was found in Caco-2 and HepG2 cells[7]. Dipropyl disulfide and dipropyl sulfide also showed no significant adverse effect in Caco-2 cells[8]. PTSO (0-50 μM) did not showed mutagenicity/genotoxicity *in vitro* assay but it was observed in its metabolites[9].

Chemical constituents found in shallots (*Allium ascalonicum* L.) mainly are flavonoids and their glycosides, sulfur containing compounds and saponins. Flavonol glucosides including quercetin and isorhamnetin mono- and di-glucosides were found in French and Italian shallots[10]. Furostanol saponins were identified from shallot (*Allium ascalonicum* Hort.)[11]. Volatile sulfur compounds in *Allium* plants had been studied for a long times[12]. Shallots planting mostly in the north and north eastern regions of Thailand[13] showed similar flavonoid compounds as found in French and Italian shallots[10]. The γ-glutamylcysteine was found in cytoplasm of shallots as well as other *Allium* plants and was oxidized and hydrolyzed to *S*-alk(en)yl cysteine sulfoxides (ACSO). The ACSO can be further transformed to other organosulfur compounds and thiosulfinates by allinase enzyme.

There are several techniques to determine sulfur containing compounds. A few studies had reported analysis of thiosulfinates in *Allium* plants using GC/MS[12,14,15]. *S*-substituted cysteine was analyzed using liquid chromatography with ultraviolet and mass spectrometer and with some derivatizing agents i.e. dansyl chloride[14,16,17,18]. Although ESI-MS/MS[19] may not be a sensitive method as much as FT ion cyclotron MS[20], it has been used for the analysis of organosulfur compounds. The present study aimed to characterize some non-volatile organosulfur compounds found in fresh shallot bulbs and the shallot extract comparing with garlic using RP-HPLC coupling with ESI-MS/MS technique.

Methods

Sample preparation

Bulbs of garlic (*Allium sativum* L.) were bought from local market and bulbs of shallot (*Allium ascalonicum* L.) were bought from Payao province, Thailand. Fresh samples (10 g each) were removed outer sheath and crushed in the mortar. Then water was added and filtered through 0.45 μ nylon syringe filter. The extract solution (3 μL) was injected into liquid chromatography system. Shallot extract was obtained from maceration of fresh shallot bulbs in 20% ethanol in water bath at temperature of 40 °C for 4 hours. The extract was filtered and centrifuged at 4000 rpm for 10 minutes. After the solvent was removed the aqueous solution was dried in a lyophilizer and kept at 4 °C for the analysis.

HPLC ESI MSMS Method

Liquid chromatography was performed on an Ultimate 3000, Dionex coupling with UV-visible detector and autosampler. Data analysis was carried out using Chromelon software. Samples were separated on a Poroshell 120 C18 (2.1 x 150 mm, 4 μm) column at 25 °C. The mobile phase composition was 5% acetonitrile with 0.2% formic acid (A) and 95% acetonitrile with 0.2% formic acid (B). The gradient elution was 0-10 min, 0-5%B; 10-20 min, 5-15%B; 20-28 min, 15-40%B; 28-35 min, 40-80%B; 35-38 min, 80%B; 38-40 min return to 0%B and stayed for 5 minutes[18]. The flow rate was 0.3 mL/min and monitored at the wavelength of 195 nm. ESI-MS/MS was performed on an ion trap Amazon SL mass spectrometer (Bruker) using Hystar and trap control software. ESI-MS was equipped with quadrupole ion trap. Capillary voltage was set at 4,500 V, nebulizer gas was set at 2 bars, and drying gas temperature was 220 °C with a flow rate of 7.0 L/minute. MS was evaluated in both positive and negative modes and scanned at the mass range of m/z 70-900 amu. MS/MS fragmentation was performed with MRM in positive mode and the precursor ions were 162, 178, 264, 291, 305 and 307.

Results

As shown in Table 1, the molecular ions and fragment ions of constituents in fresh shallot bulbs were detected. MS spectra of shallot extract showed similar fragmentation pattern except that the fragment ion of *m/z* 178 was rarely seen in the extract compared with fresh bulbs. In addition, organosulfur compounds detected in shallots were similar to those found in garlic. The fragment ions obtained from *m/z* 162 were 145 and 73 which corresponded to *S*-allyl-L-cysteine and clearly seen in garlic but not in shallot. In comparison, the molecular ion 177 of isoalliin gave a fragment ion m/z 88 but could not be detected further because the limitation of the lowest mass range of the instrument was 70 amu. The abundance of this fragment ion at *m/z* 88 was relatively low in shallot compared with garlic. The fragmentation pattern of *m/z* 291 showed the fragment ions of 234, 170, 162, 145 which corresponded to γ-glutamyl-*S*-propenylcysteine. This profile was seen in both shallot and garlic but the other fragmentation pattern which showed about equal relative abundance of fragment ions at *m/z* 162 and 145 was observed only in garlic. The molecular ion of *m/z* 305 gave some fragment ions that indicated a structure of γ-glutamyl-*S*-propenylcysteine-*S*-oxide. The molecular ion of *m/z* 307 gave a fragment ion of *m/z* 217 that indicated loss of 90 mass unit (C_3H_7SO), suggesting the structure of γ-glutamyl-*S*-propylcysteine-*S*-oxide. MS spectra of fragment ions detected in shallot bulbs are shown in Figures 1 and 3.

Table 1 Molecular ions and fragment ions detected in shallot bulbs using ESI-MS/MS

Compounds	Molecular weight	Molecular ions $[M+H^+]^+$, $[M-H^+]^-$	Fragment ions
S-Propenyl-L-cysteine-*S*-oxide	177	175	88
γ-Glutamyl-*S*-methylcysteine	264	265, 263	136, 133, 119, 86
γ-Glutamyl-*S*-propenylcysteine	291	291	245, 234, 170, 162* 145, 122, 99, 84.6
γ-Glutamyl-*S*-propenylcysteine-*S*-oxide	305	305	248, 189, 147, 130, 116, 98.5
γ-Glutamyl-*S*-propylcysteine-*S*-oxide	307	307	217*, 178, 130, 88.5

* Base peak

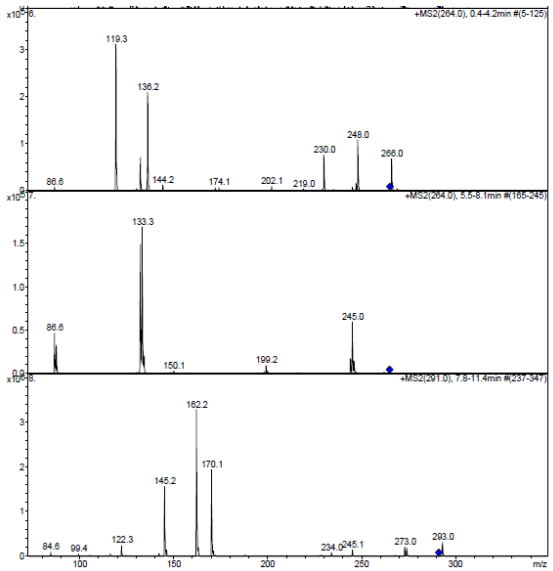
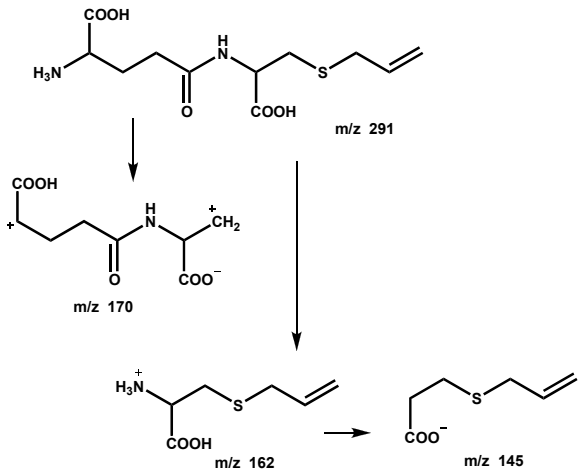


Figure 1. MS spectra of fragment ions of molecular ions *m/z* = 264 and 291 detected in shallot bulbs

Figure 2. Fragmentation pattern of *m/z* 291



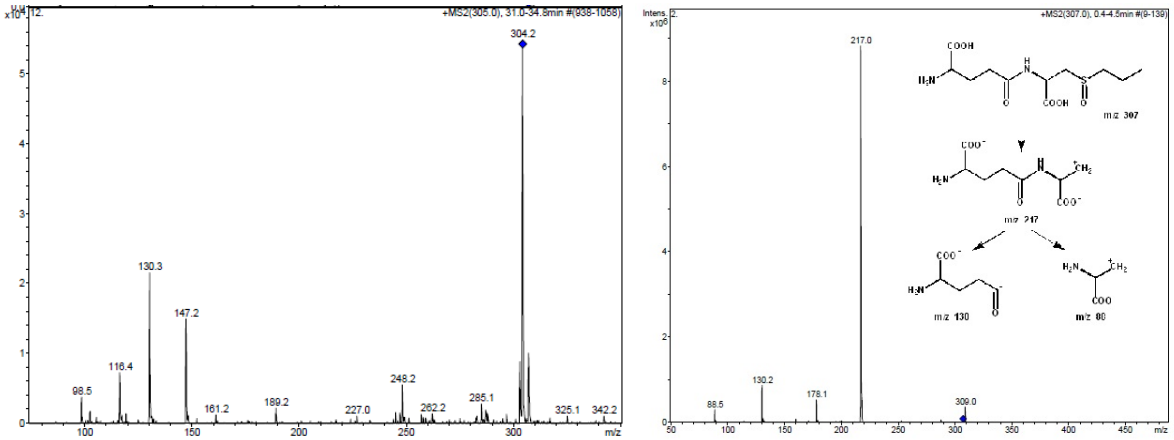


Figure 3. MS spectra of fragment ions of molecular ions *m/z* = 305 and 307 detected in shallot bulbs

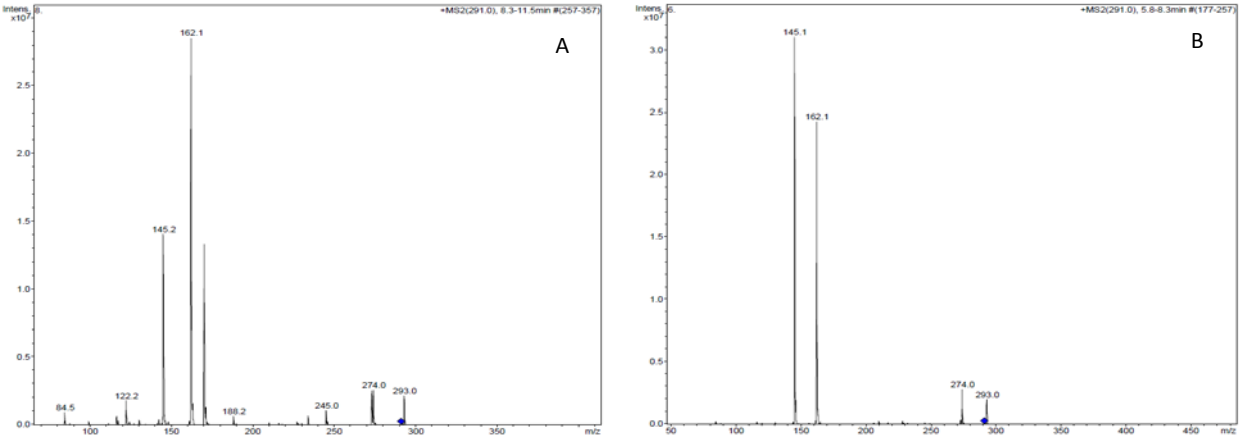


Figure 4. MS spectra of fragment ions of molecular ions *m/z* = 291 detected in garlic bulbs

Discussion

Freshly prepared shallot bulb sample and 20% ethanol shallot extract showed similar organosulfur compounds as presented in Table 1. Since organosulfur compounds were less ultraviolet absorption, some studies used derivatization step prior to HPLC analysis[14,17]. In this study, MRM (multiple reaction monitoring) technique in positive mode was used to perform fragmentation. A fragment ion at *m/z* 88 from a precursor ion m/z 178 suggested a propenyl sulfoxide moiety $[C_3H_5SO]^+$ or a loss of $[C_3H_5O_2N]^+$ ion. If a fragment ion could further fragmented and gave an ion of m/z 41, it would confirm a propenyl moiety $[C_3H_5]^+$. This finding was similar to a report by Kubec[14] that isoalliin was found in shallot sample. A molecular ion of *m/z* 264 corresponded to γ-glutamyl-*S*-methylcysteine and a fragment ion of *m/z* 119 suggested

a moiety of $[C_4H_8SNO_2]^+$ which loss NH ion. Two fragment ions at *m/z* 133 and 136 could be a γ-glutamyl moiety which was obtained from cleavage of a peptide bond in the structure and there might be isotope of oxygen atoms. A major fragment ion at *m/z* 170 suggested loss of *S*-allyl and – NH_3 moieties (Figure 2). The presence of a fragment ion *m/z* 162 obtained from a hydrolysis of a peptide bond and further fragmented to a product ion at *m/z* 145. The MS/MS spectra of a molecular ion m/z 291 was similar to the one that reported by Nakabayashi[20] and the pattern of three fragment ions (*m/z* 145, 162, 170) suggested a structure of γ-glutamyl-*S*-1-propenylcysteine. The compound that showed molecular ion at *m/z* 291 in shallot gave only one fragmentation profile whereas in garlic there were two different fragmentation patterns for *m/z* 291 which were eluted with different retention times. The results suggested that two organosulfur compounds, γ-glutamyl-*S*-1-propenylcysteine and γ-glutamyl-*S*-2-propenylcysteine, were observed in garlic sample (Figure 4A and 4B).

The molecular ion at *m/z* 305 was observed and the fragment ions suggested a structure of γ-glutamyl-*S*-propenylcysteine-*S*-oxide. However, low resolution of the instrument cannot give more detail about isomers of these compounds (γ-glutamyl-*S*-1-propenylcysteine-*S*-oxide or γ-glutamyl-*S*-2-propenylcysteine-*S*-oxide). In addition, the molecular ion at *m/z* 307 gave a major fragment ion at *m/z* 217 suggesting a loss of a propyl sulfoxide moiety (C_3H_7SO-). Cleavage of a peptide bond also yielded an ion at *m/z* 130. Diallyl sulfide, di-, tri- and tetra sulfides are oil soluble organosulfur compound and can generally be detected by GC/MS; therefore, they were not observed in this shallot sample and the extract which used polar solvent.

Conclusion

This study demonstrated the presence of organosulfur compounds in fresh shallot and its extract in polar solvent. MS/MS profiles of fragmentation showed similar patterns with those of garlic which were characterized to organosulfur compounds. Although the resolution of ESI-MS/MS was not very high, five organosulfur compounds can be characterized. γ-Glutamyl-*S*-propenylcysteine and γ-glutamyl-*S*-propylcysteine-*S*-oxide were detected in high relative abundance. Further modified HPLC method is needed to get clear separation and will be used for quantification analysis.

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