

Volatile Sulfur Components from Fresh Radishes of Korean Origin

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한국산 무우의 휘발성 함유황화합물에 관하여

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요 약

생무우(태백무우, 알타리무우)에 존재하는 휘발성 함유황 화합물을 수증기 증류 및 용매추출(CCl₄) 방법에 의해 추출하여 GC/MS 분석법에 의해 분리 확인하였다.

수증기 증류로 얻은 2종 무우의 휘발성 함유황 성분들의 GC profile은 유사하였으며, 4-methylpentyl, 3-methylthiopropyl, 4-methylthio-3-butenyl, 4-methylthiobutyl, 5-methylthiopentyl isothiocyanate와 5-methylthio-4-pentenitrile, 1-methylthio-3-pentanone, dimethyl sulfides 등이 분리 확인되었다.

한편 CCl₄추출물에는 4-methylsulfinyl-3-butenyl isothiocyanate도 존재하였다.

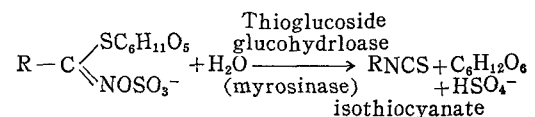
수증기 증류에서는 4-methylthiobutyl isothiocyanate가, 그리고 CCl₄추출에서는 4-methylthio-3-butenyl isothiocyanate가 각각 주된 휘발성 함유황 화합물이었다.

INTRODUCTION

Fresh or processed radishes, possessing a pungent flavor and taste, have been cherished as one of the favorite vegetables¹⁾. The yearly production and the daily consumption of radishes rank second to those of Chinese cabbages in Korean²⁾.

It is well known that the pungent flavor of cabbages belonging to the Cruciferae family, is attributed largely to the isothiocyanates formed from the enzymatic hydro-

lysis of glucosinolates, and partially to the volatile sulfides and thiazoles produced non-enzymatically.



R=alkyl, alkenyl, benzyl, etc.

While the research on the flavor components of cabbages commonly used in the Western countries has been active, the reports concerning those of radishes are limited to a few, and none in Korean.

The earlier researchers who had used the steam distillation method to isolate the pungent sulfur volatiles had reported inconsistent data^{1,3)}, until Friis et al.³⁾ reported the pungent principle in the CCl₄ extract of radish was 4-methylthio-3-butenyl isothiocyanate. Recently, Kjaer et al.^{4,5)} reported the identification of several volatile components from radishes of Japanese and Kenyan origin.

In this investigation, sulfur volatile compounds in radishes were isolated by steam distillation method and CCl₄ extraction method and characterized by GC-mass spectrometry to obtain the basic data for the next study, and to compare the sulfur compounds isolated by two methods.

EXPERIMENTAL

Radish material: Whole, fresh radishes (var. 'Taebak', weight about 1.5 kg and var. 'Altali', weight about 50 g) were purchased in the 'ShinLim' market during summer, 1985.

Preparation of steam distillate from the radish⁴⁾: The radish was ground to a pulp with water (1l/kg radish) in a Waring blender and subsequently subjected to steam distillation. The distillate was received under reduced pressure in ice bath for 3hrs., and then saturated with sodium chloride and extracted with ether.

After drying the ether extract over anhydrous sodium sulfate, the ether was slowly removed by distillation through a 30-cm Vigreux column. The almost ether-free yellow oil residue was transferred into GC vial, dried further under nitrogen gas, and immediately subjected to GC/MS analysis.

Preparation of CCl₄ extract from radish⁵⁾: 300 g of sliced radish in the hot carbon tetrachloride (300 ml) was boiled under reflux

condenser for 30 min, while shaken intermittently. The final extract was vacuum evaporated at 33°C after dehydration over anhydrous sodium sulfate. The yellow oilish residue was dissolved in ether and transferred into small vial. The sample was dried under nitrogen gas.

Gas Chromatography-mass spectrometry (GC/MS)^{4,5)}: Analyses were performed on a JEOL DX-300 double focusing GC/MS spectrometer, equipped with glass packed column (i.d. 4.6 mm × 1 m) which was packed with 1% OV-1 on Chromosorb W. Temperature of injector were 230°C for both steam distillates and CCl₄ extract. Column temperature for the steam distillates was 20~230°C and the increase of temperature was 10°C/min. Column temperature for the CCl₄- extract was 80~250°C and the increase of temperature was 15°C/min. Temperature for transfer lines and separator was 240°C and that of ionization chamber was 200°C.

RESULTS

When the steam distillates from radishes of both types were subjected to the GC/MS analysis, at least nine compounds containing sulfur atom were obtained as shown in Fig. 1 & 2, and the structural formula of the respective peak is presented in Table 1. In addition, the GC/MS analysis of steam distillates from both types of radishes provided the quite similar GC profiles of the (volatile components, suggesting that these two different varieties of radishes may have the same biogenetic origin. The CCl₄ extract of the "Taebak" radish was also analyzed by GC/MS spectrometry.

Structure of peak I and II: Peak I was identified as dimethyl disulfide by the mass

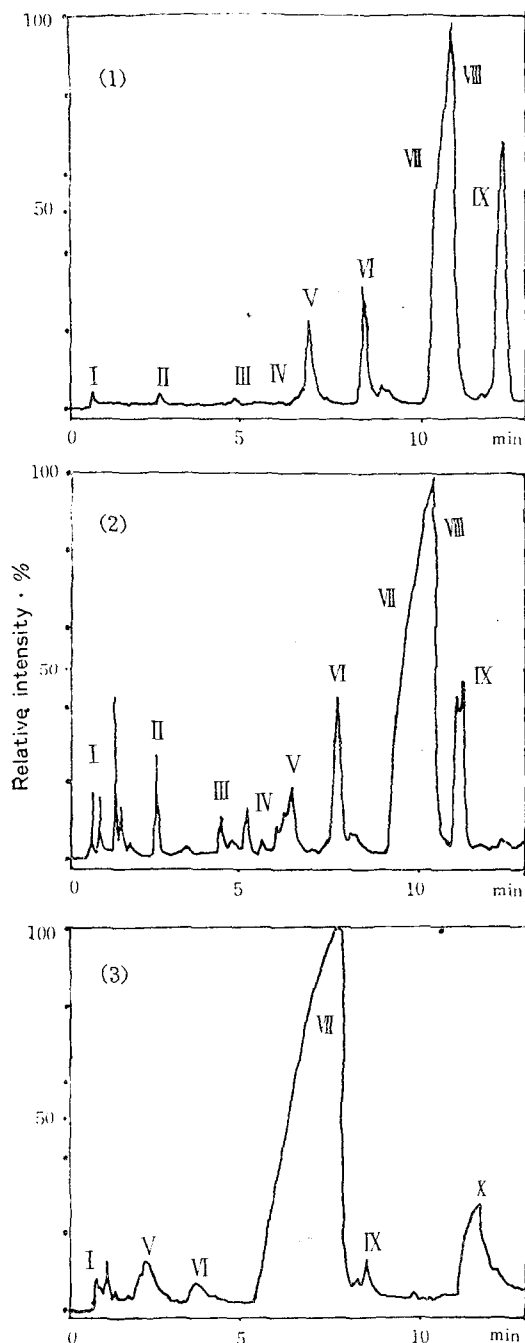


Fig. 1. Gas Chromatograms of Volatiles from Fresh Radishes.

- (1) Steam distillate of 'Taebak' Radish
 (2) Steam distillate of 'Altali' Radish
 (3) CCl_4 extract of 'Taebak' Radish

spectrum which showed ions at m/e 94 (M^+ , molecular ion), at m/e 79 (M^+-15 , loss of $\cdot\text{CH}_3$), and at m/e 45 in a good agreement with the spectrum reported previously^{6,7}. The production of dimethyl disulfide was also observed in the CCl_4 extract of radish. Peak II was identified on the ground of the mass spectral fragmentation pattern of dialkyl trisulfide showing the molecular ion at m/e 126 and other characteristic ions at m/e 111 (m^+-15 , loss of $\cdot\text{CH}_3$), at m/e 79 (M^+-47 , loss of $\cdot\text{SCH}_3$), and at m/e 47 ($\cdot\text{SCH}_3$), similar to the one reported by Block et al⁸. Based on these results, peak II was assigned the structure of dimethyl trisulfide. These dimethyl sulfide compounds were observed to be present in both types of radishes.

Structure of peak III and IV: The characterization of peak III, supported by the reports of Kjaer group⁴, was founded on its mass spectrum: molecular ion at m/e 132 and other prominent ions at m/e 103 (M^+-29 , loss of $\cdot\text{C}_2\text{H}_5$), at m/e 85 (M^+-47 , loss of $\cdot\text{SCH}_3$),

Table 1. Mass Spectrometric Identification of Sulfur Volatiles from the Radishes of Korean Origin

Peak No.	Molecular Weight	Structural Formula
I	94	$\text{CH}_3-\text{S}-\text{S}-\text{CH}_3$
II	126	$\text{CH}_3-\text{S}-\text{S}-\text{S}-\text{CH}_3$
III	132	$\text{CH}_3-\text{S}-\text{CH}_2-\text{CH}_2-\text{C}(\text{O})-\text{CH}_2-\text{CH}_3$
IV	143	$\text{CH}_3-\text{CH}(\text{CH}_3)-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NCS}$
V	127	$\text{CH}_3-\text{S}-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_2-\text{CN}$
VI	147	$\text{CH}_3-\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NCS}$
VII	159	$\text{CH}_3-\text{S}-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_2-\text{NCS}$
VIII	161	$\text{CH}_3-\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NCS}$
IX	175	$\text{CH}_3-\text{S}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NCS}$
X	175	$\text{CH}_3-\text{S}(\text{O})-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}_2-\text{NCS}$

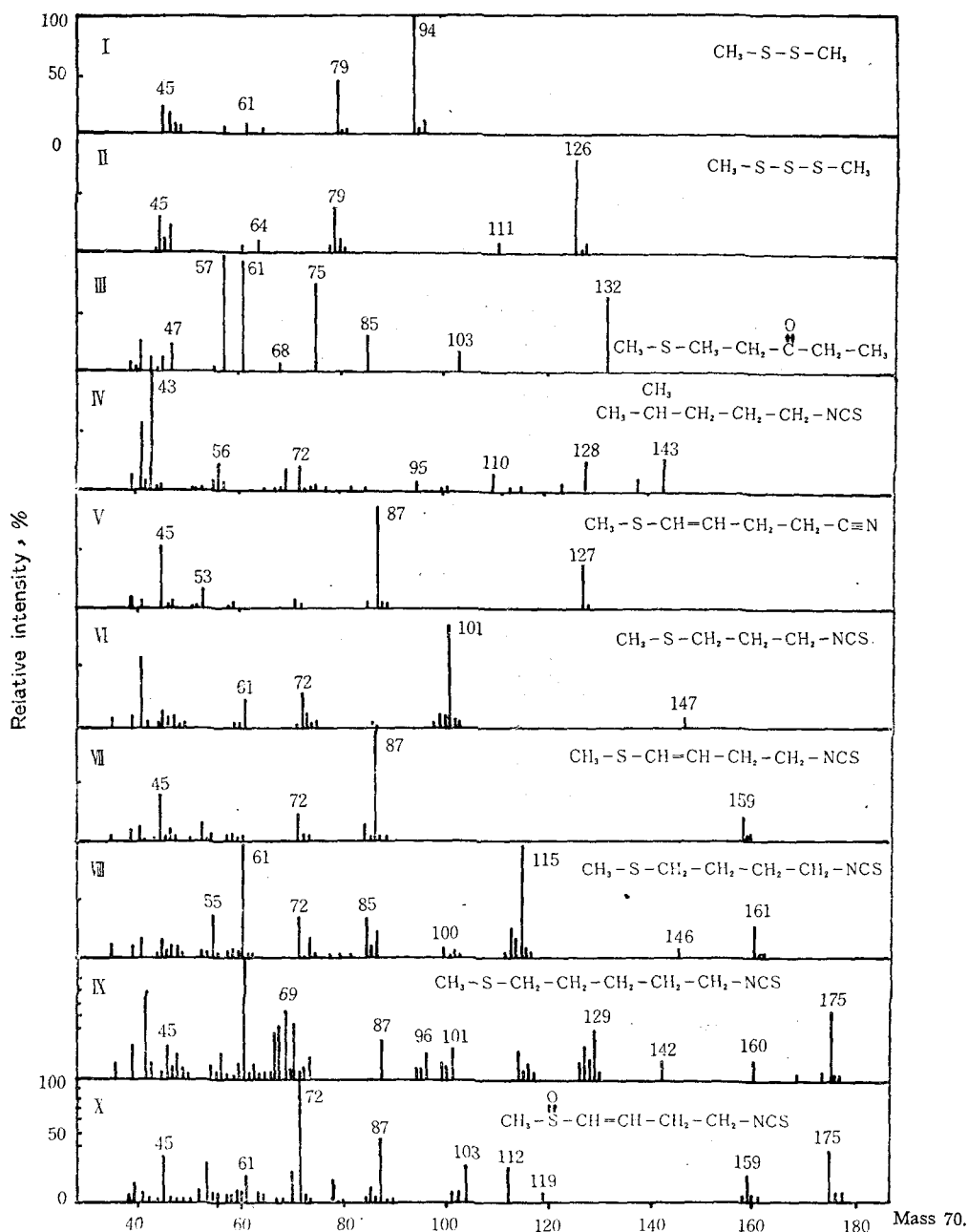


Fig. 2. Mass Spectra of Volatile Sulfur Components from Radishes.

at m/e 75 ($M^+ - 57$, loss of $\cdot\text{COC}_2\text{H}_5$), at m/e 61 ($\cdot\text{CH}_2\text{SCH}_3$), at m/e 57 ($\cdot\text{COC}_2\text{H}_5$), and at m/e 47 ($\cdot\text{SCH}_3$), compatible with the mass spectrum of the synthetic specimen, 1-methylthio-3-pentanone.

The identity of peak IV was established on the basis of the comparison of its mass spectrum with that of an authentic sample⁴⁾: molecular ion at m/e 143 and other prominent ions at m/e 128 ($M^+ - 15$, loss of $\cdot\text{CH}_3$), at

m/e 100 ($M^+ - 43$, loss of $\dot{C}H_3-CH-CH_3$), at m/e 72 ($\cdot CH_2NCS$) and m/e 43 ($\dot{C}H_3-CH-CH_3$). Based on these results, it was named as 4-methylpentyl isothiocyanate.

Structure of peak V and VI: Peak V was characterized as 5-(methylthio)-4-pentenitrile by comparison of its mass spectrum with that of authentic sample⁴⁾ which exhibited the prominent peaks at m/e 127 (molecular ion), at m/e 87 ($M^+ - 40$, loss of $\cdot CH_2CN$), and at m/e 53 ($\cdot CH_2=CH-CN$). The occurrence of the nitrile suggests the presence of the corresponding isothiocyanate. The identification of peak VI as 3-methylthiopropyl isothiocyanate was founded on the comparison of its mass spectrum with that of an authentic specimen⁹⁾: molecular ion at m/e 147 and other characteristic ions at m/e 101 ($M^+ - 46$, $C_4H_7NS\cdot$, rearrangement fragment), at m/e 72 ($\cdot CH_2SCN$), and at m/e 61 ($\cdot CH_2SCH_3$). The presence of the compound in both types of radishes suggests that two varieties of radishes have the same biosynthetic pathway.

Structure of peak VII and VIII: The major peak was expected to compose of two components as the shoulder of the peak demonstrates in Fig. 1. This observation was supported by selected ion monitoring at m/e 159 and 161. The front part (peak VII) of the peak was found to correspond to 4-methylthio-3-butenyl isothiocyanate upon the comparison of its mass spectrum with that of an authentic specimen. The molecular ion at m/e 159 and other characteristic fragmentation ions at m/e 144 ($M^+ - 15$, indicative of the loss of methyl group), at m/e 87 ($CH_3-S-CH=CH-CH_2\cdot$) corresponding to the base peak, and at m/e 72 characteristic of alkyl isothiocyanate were observed in a good agreement with the mass spectrum published by Kjaer et al.^{3,10)} On the other hand, the back part of the peak

was identified as 4-methylthiobutyl isothiocyanate on the basis of the mass spectrum analysis: molecular ion at m/e 161 and other ions at m/e 115 ($M^+ - 46$, $C_5H_9NS\cdot$, rearrangement fragment), at m/e 100 ($M^+ - 61$, loss of $\cdot CH_2SCH_3$), at m/e 72 ($\cdot CH_2SCN$), and at m/e 61 ($\cdot CH_2SCH_3$). In the distillates of two types of radishes, it turned out that peak VII and peak VIII was overlapped. Selected ion monitoring of that peak at m/e 159 and 161 indicated that the ratio of 4-methylthio-3-butenyl to 4-methylthiobutyl isothiocyanate was approximately 1 to 12 for the 'Taebak' radish and 1 to 4 for the 'Altali' radish. It was also observed that the amount of 4-methylthio-3-butenyl isothiocyanate in the distillate from the 'Altali' radish is above four times higher than in the distillate from the 'Taebak' radish. On the other hand, the predominant product in the CCl_4 extract of the 'Taebak' radish was found to be 4-methylthio-3-butenyl isothiocyanate by recording the mass spectrum at various scan numbers of the major peak, similar to the observation by Friis et al.³⁾

Structure of peak IX and X: Peak IX, which came out last in the GC profile of the steam distillate, was characterized as 4-methylthiopentyl isothiocyanate in its mass spectrum analysis¹⁰⁾: molecular ion at m/e 175, and other fragmentation ions at m/e 160 ($M^+ - 15$, loss of $\cdot CH_3$), at m/e 129 ($M^+ - 46$, $C_6H_{11}NS\cdot$, rearrangement fragment), at m/e 114 ($M^+ - 61$, loss of $\cdot CH_2SCH_3$), at m/e 101 ($M^+ - 46 - 28$, additional loss of $\cdot C_2H_4$), and at m/e 61 ($\cdot CH_2SCH_3$). Although the peak IX from the steam distillates was contaminated with the unidentified compound the peak IX from the CCl_4 extract showed the presence of the single component of 4-methylthiopentyl isothiocyanate. Peak X, which is more polar

than peak IX, was obtained as one of major peaks in the CCl_4 extract only. The identification of the peak as 4-methylsulfinyl-3-butenyl isothiocyanate, confirmed by the mass spectrum of authentic specimen¹¹⁾, was based on the mass spectrum analysis: molecular ion at 175, and other ions at m/e 159(M^+-16 , loss of oxygen), at m/e 119($M^+-C_4H_8^+$), at m/e 112($M^+-CH_3SO^+$), at m/e 87($\cdot\text{CH}_2-\text{CH}=\text{CH}-\text{S}-\text{CH}_3$), and at m/e 72($\cdot\text{CH}_2\text{SCN}$).

DISCUSSION

Comparisons of the sulfur volatiles present in the 'Taebak' and 'Altali' radishes, as shown in Fig. 1, exhibit a similarity in their components. Whereas the sulfides, dimethyl disulfide and dimethyl trisulfide, are present as a minor part, the isothiocyanates constitute the major portion of the total sulfur volatiles.

The presence of dimethyl disulfide, reportedly derived from methanethiol, in both the steam distillates and the CCl_4 extract suggests that dimethyl disulfide or at least methanethiol may be present in the fresh radishes.

Since the amount of dimethyl disulfide or dimethyl trisulfide is small, and the dimethyl sulfides are known to contribute little to the pungent flavor, the isothiocyanates are regarded as responsible for the typical fresh radish flavors.

Among isothiocyanates, 4-methylthio-3-butenyl isothiocyanate and 4-methylthiobutyl isothiocyanate were found to be major constituents, and the other isothiocyanates, namely, 4-methylpentyl 3-methylthiopropyl and 5-methylthiopentyl isothiocyanates were minor ones.

In earlier papers^{1,3,4)}, 4-methylthio-3-butenyl isothiocyanate was reported to be responsible for the pungent flavor as a major

one. However, our results with the steam distillate show that the steam distillation resulted in the loss of the pungent flavor on the basis of the sensory test, and the quantitatively predominant component turned out to be 4-methylthiobutyl isothiocyanate rather than 4-methylthio-3-butenyl isothiocyanate. This discrepancy might be explained by the assumption that steam distillation may cause the hydrolytic decomposition of 4-methylthio-3-butenyl isothiocyanate. This was supported by the observations that the organic solvent (CCl_4) extraction gave rise to the very high yield of 4-methylthio-3-butenyl isothiocyanate, as a major one, and the pungency was maintained during extraction procedure. In further studies, the pungent compound was found to be unstable in the acidic or neutral aqueous system¹²⁾. These results reaffirm that the pungent flavor of radishes is due to the presence of 4-methylthio-3-butenyl isothiocyanate.

Consistent with the sensory evaluation that the 'Altali' radish had more pungent flavor than the "Taebak" radish, the amount of 4-methylthio-3-butenyl isothiocyanate was found to be higher in the 'Altali' radish than in the 'Taebak' radish, based on the GC/MS analysis. It is interesting that 4-methylsulfinyl-3-butenyl isothiocyanate was isolated as one of major products in the CCl_4 extract. Also, it is first observation that radish roots contain the significant amount of 4-methylsulfinyl-3-butenyl isothiocyanates, although this compound was reported as a major isothiocyanate constituent in the radish seeds¹³⁾. This S-oxidation pathway may be one of the catabolic routes of 4-methylthio-3-butenyl isothiocyanate. Interestingly, 4-methylthiopentenyl nitrile was not found in Japanese radish, but was observed to be present in

Korean radishes.

In addition, the detection of this nitrile compound is an indicator that isothiocyanate glucosinolate is hydrolyzed in part to nitrile aglycone, as explained by Macleod et al.¹⁴.

The present study shows that two dimethyl sulfides at least five isothiocyanates are components of the volatile flavor in both types of radishes, and that the patterns of major isothiocyanate volatiles vary with the method of sample preparations, namely steam distillation and CCl₄ extraction.

Further studies concerned with the flavors of processed radishes and the catabolism of the pungent principle according to the processing methods are encouraged.

Summary

The volatile sulfur components from two varieties of radishes ('Taebak' and 'Altali'), obtained by steam distillation and solvent (CCl₄) extraction, were isolated and characterized by GC/MS analysis.

The GC profile of the volatile sulfur compounds in the steam distillates from two types of radishes was quite similar. 4-Methylpentyl, 3-methylthiopropyl, 4-methylthio-3-butenyl, 4-methylthiobutyl and 5-methylthiopentyl isothiocyanates, 5-methylthio-4-pentenenitrile, 1-methylthio-3-pentanone, and dimethyl sulfides were commonly identified. In the CCl₄ extract, 4-methylsulfinyl-3-butenyl isothiocyanate was obtained as another major constituent.

Whereas 4-methylthiobutyl isothiocyanate was found to be a predominant component

in the steam distillate, 4-methylthio-3-butenyl isothiocyanate was a major one in the CCl₄ extract.

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