A Rapid Method for Determining the Pungent Principle in Root of Japanese Radish (*Raphanus sativus* L.)

Kunio **Okano**, Jiro **Asano** and Gensho **Ishii**

*National Research Institute of Vegetables, Ornamental Plants & Tea, Anou, Age, Mie 514-23*

**Summary**

A rapid and simple method for determining 4-methylthio-3-butenyl isothiocyanate (MTB-ITC), a pungent principle in root of Japanese radish, was developed. Fresh root was grated by a food processor, and glucosinolates in the root tissue were hydrolyzed with endogenous myrosinase. Released MTB-ITC was extracted with methylene chloride, and analyzed by a gas chromatograph (GC) equipped with FID at constant column temperature.

Most of added sinigrin was quantitatively recovered as allyl isothiocyanate by the new modified method, indicating that the activity of endogenous myrosinase was high enough to hydrolyze all of glucosinolates to isothiocyanates. MTB-ITC contributed more than 95% of peak area detected on the chromatographic tracing. Methods of crushing root tissue affected the amount of MTB-ITC produced. MTB-ITC in methylene chloride was stable as long as it was kept at \(-20^\circ C\). Differences in MTB-ITC contents among radish cultivars were examined by the method.

**Introduction**

Cruciferous vegetables usually contain various kind of glucosinolates in their leaf, stem, root and seed tissues. When the cells of the tissues are crushed, the isothiocyanates are released from glucosinolates by the action of enzyme myrosinase along with inorganic sulphate and glucose (14). The volatile isothiocyanates are responsible for the flavour and pungency of cruciferous vegetables (4). Pungency is one of the important components contributing to the quality of fresh or grated radish roots. Thus, it is important to determine the amount of isothiocyanates in roots to evaluate the quality of radish. The analytical method should be both rapid and simple.

A number of methods have been reported for the measurement of isothiocyanates or its precursor, glucosinolates (10, 12). As the isothiocyanates are unstable in aqueous solution (4), Esaki and Onozaki (2) measured the isothiocyanates in radish root as stable thiourea derivatives by a colorimetric method. Ishii *et al.* (6) determined the glucosinolate profile in radish root by GC after enzymatic conversion to its isothiocyanates. The procedures for sample preparation employed in these studies are, however, time-consuming and less desirable for routine analysis.

This paper describes a more simple and rapid method for evaluating the pungency of radish root. Glucosinolates in grated radish root were hydrolyzed with endogenous myrosinase. Released isothiocyanates were extracted with methylene chloride and analyzed by GC. Differences in MTB-ITC content among radish cultivars were examined by the modified method.

**Materials and Methods**

**Plant materials**

Fourteen cultivars of Japanese radish (*Raphanus sativus* L.) were grown in the field of the National Research Institute of Vegetables, Ornamental Plants & Tea at Anou, Mie, from August to November. The cultivars were grown using normal amounts of a complete fertilizer (N, P₂O₅, and K₂O; 10, 30 and 10 kg/10a, respectively). Roots with their hypocotyls attached weighing of more than 800 g were successively harvested and prepared for isothiocyanates analysis.

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Sample preparation for isothiocyanate analysis

The root tissue was autolyzed, that is, the glucosinolates in the root were hydrolyzed with the endogenous myrosinase by grating. The fresh root was grated by a food processor (Model HF-60, Hitachi) equipped with a grater. In some cases, a radish grater or a homogenizer (ULTRA-TURRAX T25-S1, IKA-Labortechnik) was used. The homogenate was filtered through two layers of cotton gauze. Three ml of the filtrate were poured into 5 ml of methylene chloride in a glass tube and sealed. The tube was shaken vigorously to dissolve released isothiocyanates into methylene chloride. After the centrifugation (3000rpm, 10min), the lower layer of methylene chloride was recovered by a Pasteur pipette, then dehydrated with anhydrous sodium sulphate. The methylene chloride solution containing isothiocyanates was stored in a refrigerator at -20°C until GC analysis.

Recovery of sinigrin added to grated radish

Recovery of sinigrin (allyl glucosinolate) added to grated radish was examined for evaluating both the activity of endogenous myrosinase and the efficiency of extraction of isothiocyanates with methylene chloride. Three levels (100 to 300 µmol/100 ml) of sinigrin (Sigma Chemical Co. Ltd.) were added to 3 ml of grated radish juice in a sealed glass tube containing 5 ml of methylene chloride. After incubation for 5 min at room temperature, the reaction was stopped by shaking the tube vigorously. The methylene chloride solution containing allyl isothiocyanate was collected, dehydrated, and stored as described above.

GC and GC-MS

Analysis of the isothiocyanates was performed on a gas chromatograph (Model GC-4BMPF, Shimadzu) equipped with a flame ionization detector (FID) and a recorder (Chromatopak C-R1A, Shimadzu). Analytical conditions were primarily based on the methods of Young and Wetter (16) and Ishii et al. (6). The glass column was 3 mm × 2 m, packed with 100-120 mesh Chromosorb WAW DMCS coated with 3% Silicone SE-30. The carrier gas was nitrogen with a flow rate of 30 ml/min. Injector and detector temperatures were 180°C, and the sample volume was 5 µl. The temperature of the column oven was time-programmed for an initial temperature at 80°C, then increased at the rate of 5°C/min to 160°C. After the profile of the isothiocyanates in radish roots had been confirmed, the column oven was maintained at a constant temperature of 135°C to minimize the analysis time.

Identification of isothiocyanates was conducted by a gas chromatograph-mass spectrometer (GC-MS, Hitachi M-2000). A glass column (1.5 mm × 1 m) packed with OV-1 was used. The carrier gas was helium with a flow rate of 30 ml/min. The ionization was performed by electron impact (EI) at 70 eV.

Results and Discussion

Chromatogram of isothiocyanates

The elution profile of isothiocyanates autolytically released from glucosinolates in radish root is shown in Fig. 1 (A). Analysis was conducted under the condition of time-programming of column temperature from 80°C to 160°C. Only two peaks could be detected on the chromatogram. Peaks 1 and 2 had retention times (tR) of 1.9 and 13.9 min, respectively. The identification and quantification of the peaks were conducted by the comparison with an authentic specimen which had been isolated from radish roots by Ishii et al. (6). Peak 2 was identified as 4-methylthio-3-butenyl isothiocyanate (MTB-ITC). The identification was also confirmed by GC-MS. The mass spectral fragmentation of the peak 2 (data not shown) coincided with that of MTB-ITC reported by Friis and Kjaer (5). Peak 1, which appeared on the shoulder of the solvent peak, had not been identified because the quantity was very small.

The MTB-ITC has been well known to be a major species of isothiocyanate in radish root (5, 6, 8, 9). Also in the present study, the peak area of MTB-ITC contributed more than 95% of that detected on the chromatogram. This means that it would be enough to determine only MTB-ITC for the evaluation of pungency of radish roots. Therefore, the following analyses were conducted under the condition of constant column temperature of 135°C to minimize the analysis time. In the analysis at constant temperature, only one peak with tR of 7.3 min could be observed (Fig 1 (B)). The peak was also identified as MTB-ITC by the comparison with the authentic specimen. The
measurement at a constant column temperature reduced the analysis time to less than one third of that at time-programming of temperature. Relative standard deviation of five GC analyses on a same sample was less than 4%.

**Recovery of added sinigrin**

Sinigrin releases allyl isothiocyanate through the hydrolysis with myrosinase. GC analysis was conducted under the condition of time-programming of column temperature from 80° to 160°C. Allyl isothiocyanate had $t_R$ of 2.5 min. Upon addition of 200 and 300 µmol/100 ml of sinigrin to the grated radish samples, 93 to 96% of the added sinigrin were recovered as allyl isothiocyanate (Table 1). When 100 µmol/100 ml of sinigrin were added, the % recovery was lower. This is attributed to experimental error. Addition of sinigrin did not affect the amount of MTB-ITC produced. These results indicate that the activity of endogenous myrosinase was high enough to hydrolyze all glucosinolates present in the root, and that released isothiocyanates were quantitatively extracted with methylene chloride.

**Various ways of tissue crushing**

The amount of MTB-ITC released from MTB-, glucosinolate in fresh roots depended on the method of crushing the tissue (Table 2). The method, speed, and thoroughness by which the tissues were crushed affected the final degree of autoysis of glucosinolates to isothiocyanates. The values obtained by a food processor and a food processor + homogenizer were lower than those obtained by a radish grater by 14 and 6%, respectively. The peelings (epidermal and subepidermal tissues) of radish root contain more MTB-ITC than do the inner parts (1, 6). The radish grater crushed the peeling completely, whereas the food processor did not. This might be the reason why samples prepared by the food processor gave a lower value than did those obtained by the radish grater. The result indicates that all of the root tissues should be grated thoroughly for the complete hydrolysis of glucosinolates. However, it also seems important to grate a large number of samples rapidly in a same manner within a limited time. For these reasons, we usually used the food processor for the grating even though the method gave relatively lower values.

![Fig. 1. Chromatographic tracings of isothiocyanates auto-lytically released from glucosinolates in radish root (cv. Tensei-Aokubi). (A) Analysis under the condition of time-programming of column temperature from 80° to 160°C. 1, unknown; 2, MTB-ITC; MC, methylene chloride (solvent). (B) Analysis under the condition of constant column temperature of 135°C. 1, MTB-ITC; MC, methylene chloride.](image)

**Table 1.** Recovery of sinigrin added to grated radish.

<table>
<thead>
<tr>
<th>Added sinigrin (µmol/100 ml)</th>
<th>Released allyl-ITC (µmol/100 ml)</th>
<th>Recovery rate (%)</th>
<th>Released MTB-ITC (µmol/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>—</td>
<td>—</td>
<td>261 ± 18</td>
</tr>
<tr>
<td>100</td>
<td>87.4 ± 1.4</td>
<td>87.4</td>
<td>254 ± 43</td>
</tr>
<tr>
<td>200</td>
<td>185.8 ± 8.0</td>
<td>92.9</td>
<td>262 ± 33</td>
</tr>
<tr>
<td>300</td>
<td>289.0 ± 4.9</td>
<td>96.3</td>
<td>258 ± 34</td>
</tr>
</tbody>
</table>

*cv. Akimasa*.

† Values are the mean of 3 roots ± standard deviation.
Stability of MTB-ITC

The MTB-ITC is released from MTB-glucosinolate by the endogenous myrosinase almost instantaneously with the destruction of the tissues (14). The released MTB-ITC was very unstable in the fresh juice, and the content decreased gradually with time (Fig. 2). Approximately 25% of the MTB-ITC disappeared within 60 min after the grating. Thus, it was necessary to extract the released MTB-ITC with methylene chloride as quickly as possible. Esaki and Onozaki (3) reported that MTB-ITC in fresh juice of radish roots might be decomposed to methyl mercaptan and dimethyl disulfide by non-enzymic reaction. The MTB-ITC was, however, stable in methylene chloride as reported by Young and Wetter (16) and Mullin (11). The content of MTB-ITC in methylene chloride solution was scarcely changed during a few months as long as it was kept at -20°C.

VARIETAL DIFFERENCE

VARIETAL difference in MTB-ITC contents of radish root was examined by the proposed method. As there is a distinct gradient in MTB-ITC content along the root axis (2), the mid-section of a root of each cultivar was used for comparison. The fresh root was grated by the food processor, and the autolytically released MTB-ITC was analyzed by gas chromatography at a constant column temperature of 135°C. Relative standard deviation of five measurements on a root was less than 8%. Among the 14 cultivars examined, 'Wase-Ohkura', 'Hayabutori-Shougoin' and 'Akimasari' showed a higher content of MTB-ITC, reaching more than 200 µmol/100 ml, than the other cultivars (Fig. 3). Carlson et al. (1) and Ishii et al. (7) also noticed that the variety group 'Shougoin' had a higher content of MTB-glucosinolate among the cultivars. In contrast, 'Hakushu' and 'Taibyou-Soubutori' exhibited the lowest contents. The contents of MTB-ITC in 14 cultivars ranged from 125 to 323 µmol/100 ml. Carlson et al. (1) measured the glucosinolates content of roots on 41 cultivars of Japanese radish. They concluded that the contents of MTB-glucosinolate in more than half of the cultivars examined fell into the 200 to 299 µmol/100 g FW. Ishii et al. (7) also reported that the contents of MTB-glucosinolate were in the range from 41 to 345 µmol/100 g FW among 20 cultivars of radish. Assuming that most of glucosinolates in the root tissue might be hydrolyzed to isothiocyanates by

![Fig. 2. Changes in MTB-ITC content in fresh juice with time after grating (cv. Wase-Ohkura, lower part of the root). Values are means of three roots.](image-url)
the grating, these reported values are in accord with those obtained in the present study, though the units of expression are different.

General Discussion

The main purpose of the present study was to develop a rapid method for evaluating the pungency of radish root. Efforts were focused on the analysis of MTB-ITC, a pungent principle in root of Japanese radish. Some radish cultivars such as the Chinese type, however, may contain not only MTB-glucosinolate but also other kinds of glucosinolates (7). According to the data reported by Sang et al. (13), most of these would be indole glucosinolates. The indole isothiocyanates are not volatile in nature, and cannot be detected by the method employed in the present study. Thus, evaluation of pungency in such types of radish cultivars should be conducted by another method.

The amount of isothiocyanates released by the endogenous myrosinase is considered to be an indicator of "apparent pungency". On the other hand, content of total glucosinolates present in the root tissue can be regarded as an indicator of "potential pungency". Thus, it is necessary to know the conversion rate of MTB-glucosinolate to MTB-ITC in grated radish for a better understanding of radish pungency. Unfortunately, the conversion rate was not determined in this study. However, most of sinigrin added to grated radish could be hydrolyzed to allyl isothiocyanate by the endogenous myrosinase. This result suggests that the activity of endogenous myrosinase was high enough so that most of MTB-glucosinolate present in the radish root were converted to MTB-ITC by grating. Wilkinson et al. (15) reported that radish had the greatest myrosinase activity among 12 cruciferous vegetables examined. However, direct measurement of the conversion rate should be conducted in future studies.

Recently, Kim et al. (9) determined the aroma components in fresh root of Japanese radishes by GC-MS. The aroma components, including isothiocyanates, in the grated radish were extracted with ether by shaking. The procedure for sample preparation is relatively simple, but not as rapid as compared with one proposed here. Our method is not only simple but also reliable, and is readily available for the rapid screening of MTB-ITC content in roots of Japanese radish.

Acknowledgment

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Literature Cited


ダイコン搾汁液中の辛味成分の簡易定量法

岡野邦夫・浅野次郎・石井現相

野菜・茶業試験場 514-23 三重県安芸郡安濃町草生 360

摘 要

ダイコンの主要な辛味成分である4-メチルチオ-3-プテニルイソチオシアネート(MTB-ITC)の迅速かつ簡単な定量法を開発した。新鮮ダイコンをおろし金付きのフードプロセッサーですりおろし、内生ミロシナーゼの働きで、組織内のグルコースノレート（辛子油配糖体）を加水分解し、生成したMTB-ITCは塩化メチレンに転溶し、ガスクロマトグラフィー(FID)で定量した。

ダイコンおろしに添加したシジリグリンの大部分は、アリルイソチオシアネートとして回収された。このことは内生ミロシナーゼの活性が十分高いことを示している。MTB-ITCはクロマトグラム上のピーク面積の95%以上を占めた。ダイコン組織の破壊方法や破壊程度はMTB-ITCの生成量に影響を与えた。塩化メチレン中のMTB-ITCは-20℃以下で保存すれば長期間安定であった。この方法を用いて、14品種のダイコンのMTB-ITC含量の比較を行った。