Chemical Composition and Aroma Evaluation of Essential Oils from Skunk Cabbage

(Symplocarpus foetidus)

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Abstract: Two sample preparation methods, namely hydrodistillation (HD) and solvent-assisted flavor evaporation (SAFE), have been used to investigate the essential oils of the aerial parts (leaves and stems) of Symplocarpus foetidus, a plant with a characteristic odor, by gas chromatography mass spectrometry (GC-MS). Characteristic aroma-active compounds in the oils were detected by GC-Olfactometry (GC-O) and aroma extract dilution analysis (AEDA). From the HD method, the main compounds in the oil were found to be p-vinyl-guaiacol (15.5%), 2-pentyl-furan (13.4%), and (Z)-ligustilide (9.5%). From the SAFE method, the main compounds were 2-butoxy-ethanol (49.6%), ethyl-pentanoate (4.5%), and mesitylene (4.0%). In HD oil, the most intense aroma-active compounds were 2-pentyl-furan (flavor dilution factor (FD) = 32, odor activity value (OAV) = 57), p-vinyl-guaiacol (FD = 16, OAV = 41), and dimethyl disulfide (FD = 16, OAV = 41). In SAFE oil, the main aroma-active compounds were 2-butoxy ethanol (FD = 32, OAV = 16), and 2-methoxy thiazole (FD = 32, OAV = 25).

Key words: Symplocarpus foetidus, essential oil, aroma evaluation, AEDA, OAV

1 INTRODUCTION

Eastern skunk cabbage, Symplocarpus foetidus (L.) or Zazenso in Japanese, is a species of arum lily (Araceae) that grows wild in East Asia and North America. The genus has the unique characteristic of blooming in the freezing weather of early spring. Its inflorescences are thermogenic and thermoregulatory, maintaining a spadix temperature as high as 20°C above its surroundings by increasing the rate of respiratory heat production with the decrease in ambient temperature1–3. Symplocarpus foetidus is grown as an ornamental plant in Japan and North America owing to its characteristic shape. Additionally, Native Americans commonly used it as a medicinal plant and seasoning. The plant is pollinated and brings up a large leaf after flowering. Since the large leaf releases the characteristic odor when damaged, it is left until the plant dies without being harvested.

In spite of this, previous work has focused on the chemical composition of S. foetidus, including reports of antimicrobial activity and cytotoxicity in the root portion, the toxic saponin, and sodium nitrate. In order to toxic components are included in the meditation grassroots, leaves and stems of the root other than not treated as food in general3–5. However, there has been no evaluation of the volatile and the aroma-active compounds in S. foetidus. Detailed identification of the volatile compounds of this plant is imperative.

Volatil profiling, which aims to analyze several predefined volatile targets6, has been used as a tool to assess changes or differences in predefined volatile compounds in several types of foods and biological systems5–9. There are several instrumental methods that can be used to obtain complete profiles of essential oils. In addition, a number of different extraction techniques can be used in the analysis of volatiles. Hydrodistillation (HD) is a process traditionally used for the extraction of essential oils from aroma-active and medicinal plants on a laboratory scale. Indeed, the possibility of hydrolysis and HD of certain compounds is a...
serious obstacle in the reproduction of natural fragrances\(^{10}\). Solvent-assisted flavor evaporation (SAFE) is a good technique for volatile extraction, allowing the careful isolation of volatile compounds from complex matrices\(^{11}\).

In flavor analysis, gas chromatography-olfactometry (GC-O) is the most common method used to evaluate odors; in particular, GC-O combined with aroma extract dilution analysis (AEDA) has been the most useful method for estimating the contribution of most aroma-active compounds. The significant contribution of each odorant to the characteristic flavor can be determined by the odor activity value (OAV). The OAV is the ratio of concentration to the odor threshold of the compound; hence, it is well established that compounds with a high OAV contribute more to the aroma.

The aim of the present study is to analyze the volatile compounds and characterize the aroma-active compounds in the aerial parts (leaves and stems) of \(S. \) foetidus using AEDA and OAV methods.

2 EXPERIMENTAL

2.1 Plant material

Fresh aerial parts (leaves and stems) of \(S. \) foetidus were collected at the skunk cabbage community in Shiga prefecture, Japan in March 2014. Identification of the plant was performed in a biotechnology laboratory at Kinki University (Kindai University), Japan in March 2014. A voucher specimen was deposited at the biotechnology laboratory of Kinki University (Kindai University) in Osaka, Japan.

2.2 Isolation of essential oil

2.2.1 HD

Fresh aerial parts of \(S. \) foetidus (300 g; cut into 1 cm\(^2\) pieces with a pair of scissors) were hydrodistilled for 3 h with diethyl ether, using a Likens-Nickerson-type apparatus, to afford an oil in 0.011% (w/w) yield, which was then dried over anhydrous sodium sulfate. The oil was stored at 4°C in a refrigerator prior to analysis.

2.2.2 SAFE

Fresh aerial parts of \(S. \) foetidus (300 g) were frozen in liquid nitrogen, and then crushed. The crushed frozen parts were added to dichloromethane (300 mL) as solvent, and the mixture was stirred to achieve extraction. After standing for 2 days, the residual substances were removed by passing through filter paper. The volatile compounds were separated from the solvent extracts using SAFE. The filtrate was vacuum distilled using a SAFE apparatus as previously described\(^{11}\). After complete introduction of the filtrate into the SAFE system, distillation was carried out for 2 h at 10\(^{-4}\) Torr. The volatile compounds were collected in a trap submerged in liquid nitrogen to yield 0.015% (w/w) of light greenish oil. The volatile compounds were stored at 4°C in a refrigerator prior to analysis.

2.3 Gas chromatography mass spectrometry (GC-MS)

GC-MS was performed using an Agilent 6890N gas chromatography-5973 MSD mass spectrometer. Samples were analyzed using a fused-silica capillary HP-5MS column (5% phenyl, 95% polydimethylsiloxane, 30 m \(\times\) 0.25 mm i.d., film thickness = 0.25 µm) and a DB-WAX column (polyethylene glycol, 15 m \(\times\) 0.25 mm i.d., film thickness = 0.25 µm). The oven temperature was programmed to increase from 40 to 260°C at a rate of 4°C/min and held at 260°C for 5 min. The flow rate of the carrier gas (He) was 1.5 mL/min. The injector and detector temperatures were 270 and 280°C, respectively. The ionization energy was 70 eV, the mass range was 39–450 amu. One µL of the sample was injected with a split ratio of 1:40.

2.4 Sniffing test by GC-olfactometry

A trained panel of sensory evaluation specialists measured the odor intensities of the main aroma-active constituents of \(S. \) foetidus. Eleven panelists, aged 21–27 years (8 males and 3 females, members of Kinki University (Kindai University), Japan), participated in the study. Sensory-analysis sessions were performed only after suitable training (>30 h). The sniffing test by GC-O was carried out using an Agilent Technologies-6890N gas chromatography-5973 MSD mass spectrometer and sniffing port ODP 2 (Olfactory Detector Port 2, Gerstel). The GC instrument was equipped with a HP-5MS column. The sample was injected into the GC in splitless mode. The GC effluent from the capillary column was split in a 1:1 (v/v) ratio between the MS and the sniffing port. The oven conditions, injector and transfer line temperatures, the carrier gas, flow rate, and ionization mode were the same as those described above for GC-MS.

2.5 AEDA

The results were expressed in terms of the FD factor, which is the ratio of the concentration of the odorant in the initial essential oil to its concentration in the most diluted essential oil in which the odor can be still detected by GC-O. The highest sample concentration (1 mg/mL) was assigned an FD factor of 1. The essential oil was stepwise diluted with diethyl ether (1:1, v/v), and aliquots of the dilutions (1 mL) were evaluated. The process was stopped when the evaluators detected no aroma for a certain dilution.

2.6 Identification of compounds

Identification of the individual compounds was carried out as follows: (i) comparison of their GC-MS retention indices (RI) on apolar and polar columns, determined relative to the retention time of a series of \(n\)-alkanes (C\(_5\)-C\(_{29}\)), with those of authentic compounds; (ii) computer matching
with commercial mass spectral libraries and comparison of spectra with literature data\textsuperscript{16, 17}; and (iii) the calculated RI were compared with the average RI from the literature, obtained from the Aroma Office database ver. 3.0 (Nishikawa Keisoku Co. Ltd., Tokyo, Japan).

2.7 Quantification of aroma-active compounds

The odor compounds of the oils were quantitatively analyzed by the internal standard addition method (alkanes C\textsubscript{12} and C\textsubscript{19}). The essential oil was diluted 100 times with diethyl ether to a 1 mL volume, and 5 \mu L of a mixture of C\textsubscript{12} and C\textsubscript{19} (1 mg/mL) was added to the diluted oil. The samples were then subjected to GC-flame ionization detector (FID) analyses. Quantitative analysis was performed based on the calibration curves for (E)-3-hexen-1-ol (peak 2), (E)-2-hexen-1-ol (peak 3), camphene (peak 6), benzaldehyde (peak 7), \beta-\textit{myrcene} (peak 8), limonene (peak 9), linalool (peak 17), borneol (peak 21), bornyl acetate (peak 27), (E,E)-2,4-decadienal (peak 29), \beta-caryophyllene (peak 34), and (E)-\alpha-copaene (peak 33), \beta-cubebene (peak 39), germacrene D (peak 41), (E,E)-\alpha-farnesene (peak 42), \beta-sesquiphellandrene (peak 45) and \tau-muurolol (peak 48) were quantified by the calibration curves of \beta-caryophyllene and \beta-caryophyllene oxide, respectively (Table 3).

2.8 Determination of OAV

OAVs were determined by dividing the concentration of a component by its odor threshold. Odor threshold data was obtained from previous reports\textsuperscript{14-17}.

### Table 1

Chemical composition of essential oils from aerial parts of \textit{S. foetidus}

| No. | RI\textsuperscript{a} | Compounds | Peak area (%)\textsuperscript{b} | ID method\textsuperscript{c} |
|-----|------------------------|------------|----------------------------------|-----------------------------|
|     | HP-5MS | DB-WAX | HD | SAFE |                      |
| 1   | 796 | – | dimethyl disulfide | tr\textsuperscript{d} | – | RI, MS |
| 2   | 803 | – | 2-hexan | – | – | RI, MS |
| 3   | 822 | 1297 | (2E)-octene | – | 2.0 | RI, MS |
| 4   | 825 | 1305 | methyl-pyrazine | – | 1.6 | RI, MS |
| 5   | 844 | 1321 | isopropyl-butanate | – | 0.7 | RI, MS |
| 6   | 849 | – | 2-methyl-butanolic acid | – | tr | RI, MS |
| 7   | 857 | 1328 | ethyl-isovulol | – | 3.0 | RI, MS |
| 8   | 866 | – | p-xylene | – | 1.8 | RI, MS |
| 9   | 892 | – | \alpha-xylene | – | 1.0 | RI, MS |
| 10  | 910 | 1393 | 2-butoxy ethanol | – | 49.6 | RI, MS |
| 11  | 915 | – | ethyl-pentanate | – | 4.5 | RI, MS |
| 12  | 942 | 1362 | 2-acetoxy hexane | – | 0.9 | RI, MS |
| 13  | 946 | 1367 | 2-methoxy thiazole | – | 1.9 | RI, MS |
| 14  | 952 | – | propyl-benzene | – | 0.9 | RI, MS |
| 15  | 960 | – | 2-ethyl toluene | – | 3.1 | RI, MS |
| 16  | 967 | – | 1,2,3-trimethyl benzene | – | 3.2 | RI, MS |
| 17  | 979 | – | 3-ethyl toluene | – | 1.2 | RI, MS |
| 18  | 990 | 1786 | 2-pentyl furan | 13.4 | – | RI, MS |
| 19  | 993 | 1707 | mesitylene | – | 4.0 | RI, MS |
| 20  | 1001 | – | 2-penten-1-yl furan | tr | – | RI, MS |
| 21  | 1018 | – | 3-methyl-1,2-cyclopentanediol | – | 0.4 | RI, MS |
| 22  | 1019 | 1612 | 2-acetyl thiazole | tr | tr | RI, MS |
| 23  | 1033 | 1869 | phenyl alcohol | – | 1.0 | RI, MS |
| 24  | 1043 | – | phenyl acetaldehyde | 8.5 | 0.7 | RI, MS |
| 25  | 1104 | 1889 | nonanal | 9.0 | – | RI, MS |

\textsuperscript{a} Calculated RI based on (E)-3-hexen-1-ol (peak 2) as the internal standard.

\textsuperscript{b} Calculated peak area based on diarylpropene calibration.

\textsuperscript{c} Identification method: RI = retention index; MS = mass spectra; HD = hexane; SAFE = safe.

\textsuperscript{d} Tr = trace.
RESULTS AND DISCUSSION

3.1 Chemical constituents of *S. foetidus*

Table 1 lists the identified volatile compounds separated by two sample preparation methods, their concentrations, and RIs on the HP-5 MS and DB-WAX columns. The oils had a yield of 0.011% (w/w) from HD and 0.0115% (w/w) from SAFE. The essential oil obtained by HD had a sweet-sulfur-woody-green odor, and that from the same plant by SAFE had a sulfur-green odor.

In the HD oil, a total of twenty-two compounds (90.3%) were identified. The main compounds were *p*-vinyl guaiacol (peak 37: 15.5%), 2-pentyl-furan (peak 18: 13.4%), and (Z)-ligustilide (peak 42: 9.5%). In the SAFE oil, a total of thirty-one compounds (90.5%) were identified. The main compounds were 2-butoxy ethanol (peak 10: 49.6%), ethyl-pentanoate (peak 11: 4.5%), and mesitylene (peak 19: 4.0%).

The characteristic compounds in the HD oil were sulfur-containing compound, namely: dimethyl disulfide (peak 1) and sulfur- and nitrogen-containing compounds, such as...
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Table 2 Classification of the compounds of the essential oils from S. foetidus.

| Compound Type                        | Peak area (%)<sup>a</sup> |
|--------------------------------------|---------------------------|
|                                      | HD<sup>b</sup> | SAFE<sup>c</sup> |
| Hydrocarbons                         | 26.8         | 18.2          |
| Alcohols                             | 20.0         | 52.2          |
| Aldehydes                            | 17.5         | 1.4           |
| Ketones                              | 18.0         | 1.8           |
| Acids                                | tr<sup>d</sup> | 2.2           |
| Ethers                               | 13.4         | –             |
| Esters                               | –            | 11.6          |
| Lactones                             | 12.7         | –             |
| Sulfur-containing compounds          | tr.          | –             |
| Nitrogen-containing compounds        | –            | 1.6           |
| Sulfur- and Nitrogen-containing      | tr.          | 2.0           |

<sup>a</sup> These values were calculated from GC peak area.

<sup>b</sup> HD = hydrodistillation

<sup>c</sup> SAFE = solvent assisted flavor evaporation

<sup>d</sup> tr. < 0.1%

2-acetyl thiazole (peak 22). In the SAFE oil, four compounds were detected; one nitrogen-containing compound, namely: methyl-pyrazine (peak 4), and three compounds containing sulfur- and nitrogen, namely 2-methoxy thiazole (peak 13), 2-acetyl thiazole (peak 22), and 5-methyl thiazole (peak 39). These sulfur- and/or nitrogen-containing compounds are considered important flavor compounds in foods, and can be synthesized by microorganisms, the Maillard reaction and other chemical reactions at room temperature.<sup>18–21</sup>

Comparing the two pretreatment methods, Table 2 shows the class and percentage of each compound found in the essential oils obtained from S. foetidus. By GC analysis, HD efficiency furnished hydrocarbons (26.8%), alcohols (20.0%), and ketones (18.0%), which are the three classes with relatively high contents in the oil. SAFE extraction resulted in a higher percentage of alcohols (52.2%), hydrocarbons (18.2%), and esters (11.6%). On comparing the two sample preparation methods, samples obtained from the HD method had hydrocarbon levels about 1.5 times higher than those from SAFE. On the other hand, the alcohol levels in the SAFE oil were twice those in HD oil. Moreover, two ethers and two lactones were identified in the HD oil, but not in the SAFE oil. Seven esters, one nitrogen-containing compound, and three sulfur- and nitrogen-containing compounds were identified in the SAFE oil, but not in the HD oil. The SAFE method was superior for extracting esters, nitrogen-containing compound, and sulfur- and nitrogen-containing compounds. These differences in the HD extract were possibly due to thermal degradation of compounds resulting from the high temperatures required, whereas, the SAFE method utilizes vaporization of volatiles from nonvolatile materials at relatively low temperature ranges (40 ± 20°C) under ultra-high vacuum. Thus, formation of thermal artifacts can be avoided in the SAFE extraction procedure and it has a high extraction efficiency and recovery<sup>11,22</sup>. From these results, the HD method, oxidized compounds (aldehyde, ketone, carboxylic acid) where the resolution or alcohol was oxidized by heat contained a lot it. On the other hand, it is thought that many ingredients, such as alcohols, were retained without being oxidized by the SAFE method. To the best of our knowledge, no other study has been conducted to identify the volatile composition of S. foetidus.

3.2 GC-O, AEDA, and OAV

To clarify the most potent odors contributing to the characteristic green and sweet aromas, the AEDA method was performed via GC-O analysis. The aroma-active compounds of the essential oils from S. foetidus were identified by GC-O and AEDA. In both oils, fifteen compounds were detected, including four alcohols, three esters, three aldehydes, one ether, one sulfur-containing compound, one nitrogen-containing compound, and two sulfur- and nitrogen-containing compounds. All of the aroma-active compounds, which were identified for the first time as the key aroma compounds in S. foetidus, were satisfactorily identified based on their retention index (RI) and their mass spectra. HD oil and SAFE oil contained seven and nine compounds, respectively (Table 3). Hence, SAFE was deemed the most suitable method for GC-O analysis<sup>22</sup>.

A comparison of the gas chromatogram from GC and the corresponding FD chromatogram of the odor-contributing compounds can be seen in Figs. 1 and 2. As seen in Fig. 1, dimethyl disulfide (peak 1: sulfur) and 2-pentyl-furan (peak 18: sweet) showed the highest FD factor of 32, and p-vinylguaiacol (peak 37: woody, FD = 16) showed a high FD factor of 16. Other FD factors were as follows: 2-hexanal (peak 2: green, FD = 8), phenyl acetaldehyde (peak 24: green, FD = 4), nonanal (peak 25: green, FD = 8), and (Z)-ligustilide (peak 42: green, FD = 4). The AEDA results revealed that 2-pentyl-furan emitted a sweet odor, and dimethyl sulfide contributed a sulfur odor, p-vinylguaiacol make a woody odor, and 2-hexanal, phenyl acetaldehyde, nonanal, and (Z)-ligustilide all produced a green odor in the HD oil. In the SAFE oil, 2-butoxy-ethanol (peak 10: green) and 2-methoxy thiazole (peak 13: sulfur) had the highest FD factors of 32 (Fig. 2). Other compounds were methyl-pyrazine (peak 4: green, FD = 2), ethyl isovalerate (peak 7: fruity, FD = 2), ethyl pentanoate (peak 11: fruity, FD = 4), phenyl alcohol (peak 23: sweet, FD = 1), maltol (peak 27: sweet, FD = 2), and 5-methyl thiazole (peak 39: sulfur, FD = 2). These compounds seemed to make up the characteristic odor of the SAFE oil. In order to determine the rela-

J. Oleo Sci. 64, (12) 1329-1336 (2015)
The contribution of each compound to the odor, OAVs were used. OAVs were obtained by taking into account the concentration and odor threshold of each compound. The results are shown in Table 3. In the HD oil, 2-pentyl furan had the highest OAV of 57, followed by $p$-vinyl guaiacol (OAV = 41) and dimethyl disulfide (OAV = 30). 2-Methoxy thiazole (OAV = 25) and 2-butoxy ethanol (OAV = 16) had the highest OAVs in the SAFE oil. These compounds also had high FD factors, and were therefore considered to be the main aroma-active compounds in the oils. Generally,

Table 3  Aroma-active compounds from aerial parts (leaves and stems) of *S. foetidus*

| No. | Compounds<sup>a)</sup> | Odor<sup>b)</sup> | Conc. (ppm) | OT (ppm)<sup>c)</sup> | FD-factor<sup>d)</sup> | OAV<sup>e)</sup> |
|-----|----------------------|----------------|-------------|----------------|----------------|-------------|
|     |                      |                | HD | SAFE | HD | SAFE | HD | SAFE | HD | SAFE |
| 1   | dimethyl disulfide   | sulfur         | 0.01 | –    | 0.03 | –    | 32 | –    | 30 | –    |
| 2   | 2-hexanal           | green          | 0.05 | –    | 0.03 | 8    | –  | 1    | –  | –    |
| 4   | methyl-pyrazine      | green          | –   | 2.39 | 0.35 | –    | 2  | –    | 7  | –    |
| 7   | ethyl isovalerate    | fruity         | –   | 4.48 | 0.55 | –    | 2  | –    | 8  | –    |
| 10  | 2-butoxy ethanol     | green          | –   | 74.42 | 4.59 | –    | 32 | –    | 16 | –    |
| 11  | ethyl pentanate      | fruity         | –   | 6.72 | 0.98 | –    | 4  | –    | 7  | –    |
| 13  | 2-methoxy thiazole   | sulfur         | –   | 1.28 | 0.05 | –    | 32 | –    | 25 | –    |
| 18  | 2-pentyl furan       | sweet          | 0.16 | –    | 9.06 | 32   | –  | 57   | –  | –    |
| 23  | phenyl alcohol       | sweet          | –   | 1.44 | 0.62 | –    | 1  | –    | 2  | –    |
| 24  | phenyl acetaldehyde  | green          | 4.00 | –    | 9.23 | 4    | –  | 2    | –  | –    |
| 25  | nonanal              | green          | 2.15 | –    | 9.82 | 8    | –  | 5    | –  | –    |
| 27  | maltol               | sweet          | –   | 2.47 | 2.50 | –    | 2  | –    | 1  | –    |
| 37  | $p$-vinyl guaiacol   | woody          | 0.36 | –    | 14.77 | 16  | –  | 41   | –  | –    |
| 39  | 5-methyl thiazole    | sulfur         | –   | 0.16 | 0.05 | –    | 2  | –    | 3  | –    |
| 42  | (Z)-ligustilide      | green          | 1.85 | 0.16 | 0.87 | 4    | 2  | 1    | 3  | –    |

<sup>a</sup> Compounds are listed in order to their elution time from a HP-5MS column

<sup>b</sup> Odor description at the GC-sniffing port

<sup>c</sup> Odor detection threshold (ppm)

<sup>d</sup> The sample concentration (1 mg/ml) was assigned on FD-factor of 1

<sup>e</sup> FD-factor on HP-5MS column

<sup>f</sup> OAV = Odor activity value (concentration divided by odor threshold)

Fig. 1  Gas chromatogram and FD-factor (aromagram) of essential oils from *S. foetidus* using HD method: peak 1, dimethyl disulfide; peak 18, 2-pentyl furan; peak 37, $p$-vinyl guaiacol; peak 51, squalene.
compounds with a high FD factor also have a high OAV; these results confirm the positive correlation between the FD factor and OAV.

4 CONCLUSION
To the best of our knowledge, the study represents the first detailed analysis of the chemical composition and the key aroma-active compounds in essential oils extracted from the aerial parts (leaves and stems) of *S. foetidus*. A total of fifteen compounds were detected by AEDA method. These results imply that the oils of *S. foetidus* need to be investigated further to determine their potential application in perfumes and pharmaceutical industries.

References
1) Ito, K. Heat-production and respiration control in eastern skunk cabbage. *Seikagaku* **84**, 853-857 (2012).
2) Takahashi, K.; Ito, T.; Onda, Y.; Endo, T.; Chiha, S.; Ito, K.; Osada, H. Modeling of the thermoregulation system in the skunk cabbage: *Symplocarpus foetidus*. *Nat. Prod. Res.* **76**, 15-18 (2007).
3) Knutson, R. Chemotaxonomy of Marsypianthes based on essential oil. *Nat. Prod. Res.* **25**, 1504-1511 (2014).
4) Fiehn, O.; Kristal, B.; Ommen, B.; Sumner, L. W.; Sansone, S.-A.; Taylor, C.; Hardy, N.; Kaddurah-Daouk, R. Establishing reporting standards for metabolomic and metabonomic studies: a call for participation. *OMICS* **10**, 158-163 (2006).
5) Cho, I. H.; Choi, H-K.; Kim, Y-S; Difference in the volatile composition of pine-mushrooms (*Tricholoma matsutake* Sing.) according to their grades. *J. Agric. Food Chem.* **54**, 4820-4825 (2006).
6) Pongsuwan, W.; Fukusaki, E.; Bamba, T.; Yonetani, T.; Yamahara, T.; Kobayashi, A. Prediction of Japanese green tea ranking by gas chromatography/mass spectrometry-based hydrophilic metabolite fingerprinting. *J. Agric. Food Chem.* **55**, 231-236 (2007).
7) Beleggia, R.; Platani, C.; Spano, G.; Monteleone, M.; Cattivelli, L. Metabolic profiling and analysis of volatile composition of durum wheat semolina and pasta. *J. Cereal Sci.* **49**, 301-309 (2009).
8) Ko, B-K.; Ahn, H-J.; Berg, F.; Lee, C-H.; Hong, Y-S. Metabolomic insight into soy sauce through 1H NMR spectroscopy. *J. Agric. Food Chem.* **57**, 6862-6870 (2009).
9) Namgung, H-J.; Park, H-J.; Cho, I. H.; Choi, H-K.; Kwon, D-Y; Kim, Y-S. Metabolite profiling of doenjang, fermented soybean paste, during fermentation. *J. Sci. Food Agric.* **90**, 1926-1935 (2010).
10) Nejia, H.; Séverine, C.; Jalloul, B.; Mehrez, R.; Stéphane, C. J. Extraction of essential oil from *Cupressus sempervirens*: comparison of global yields, chemical composition and antioxidant activity obtained by hydrodistillation and supercritical extraction. *Nat. Prod. Res.* **27**, 1795-1799 (2013).
11) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation-a new versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Tech.* **209**, 237-241 (1999).
12) Adams, R. P. Identification of essential oil components by gas chromatography/mass spectroscopy, 4th edn., Allured Publishing Corp., Carol Stream, IL (2007).
13) Kim, M.; Iwai, K.; Oodera, A.; Matsue, H. Identification
and antiradical properties of anthocyanins in fruit of Viburnum dilatatum Thunb. J. Agric. Food Chem. 51, 6173-6177 (2003).

14) Kurihara, T.; Kikuchi, M. Studies on the constituents of the fruits of Viburnum dilatatum Thunb. Annu. Rep. Tohoku Coll. Pharm. 24, 123-127 (1997).

15) Pino, J.; Mesa, J. Contribution of volatile compounds to mango (Mangifera indica L.) aroma. Flav. Fragr. J. 21, 207-213 (2006).

16) Schieberle, P. New developments in methods for analysis of volatile flavor compounds and their precursors. Characterization of Food. Emerging Methods, Gaonkar AG (ed.). Elsevier Science Amsterdam, pp.403-431 (1995).

17) Wang, P.; Francis, F. A new anthocyanins from Viburnum dilatatum. L. Hortic. Sci. 7, 87 (1972).

18) Cao, J.; Chen, Y.; Fu, C.; Wu, P. GC-MS analysis of essential oil components from flowers of Edgeworthia chrysantha Lindl. Yaowu Fenxi Zazhi 25, 1211-1214 (2005).

19) Castioni, P.; Kapetanidh, I. Volatile constituents from Brunfelsia grandiflora. Qualitative analysis by GC-MS. Scientia Pharmaceutica 64, 83-91 (1996).

20) Seitz, E. W. Fermentation production of pyrazines and Terpenoids for flavors and fragrance, and color ingredients. New York: Wiley. 95-134 (1994).

21) Mega, J. A. Mushroom flavor. J. Agric. Food Chem. 29, 1-4 (1981).

22) Werkhoff, P.; Brennecke, S.; Bretschneider, W.; Bertram, H.-J. Modern methods for isolating and quantifying volatile flavor and fragrance compounds. In Flavor, Fragrance, and Odor Analysis, Marsili R (ed.), New York: Marcel Dekker. pp.139-204 (2002).

23) Majcher, H. H. J. Comparison of suitability of SPME, SAFE and SDE methods for isolation of flavor compounds from extruded potato snacks. J. Food Compos. Anal. 22, 606-612 (2009).

24) Grosch, W. Determination of potent odorants in foods by aroma extract dilution analysis (AEDA) and calculation of odor activity values (OAV). Flav. Fragr. J. 9, 147-158 (1994).