Evaluation of the Key Odorants in Volatile Oils from Tubers of *Apios americana* Medikus

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Abstract: This study was investigated the chemical composition of volatile oils and aroma evaluation from the tubers of *Apios americana* Medikus. Theses volatile oils were obtained by the hydrodistillation (HD) and the solvent-assisted flavor evaporation (SAFE) methods. These oils were analyzed by Gas chromatography (GC), GC-mass spectrometry (GC-MS), GC-olfactometry (GC-O), aroma extract dilution analysis (AEDA) and odor activity values (OAV) for the first time. The major compounds in the HD oil were palmitic acid (36.5%), linoleic acid (10.5%) and nonadecanol (5.7%). Meanwhile, in the SAFE oil, the major compounds were 4-hydroxy-4-methyl-2-pentanone (34.2%), hexanal (11.0%) and hexanol (7.9%). Through aroma evaluation, 20 (HD) and 14 (SAFE) aroma-active compounds were identified by GC-O. As a result, the most intense aroma-active compounds in both extraction methods were 1-octen-3-ol and hexanal, both of which showed high odor activity values (OAV).

Key words: *Apios americana*, essential oil, hydrodistillation, solvent-assisted flavor evaporation (SAFE), aroma extract dilution analysis (AEDA)

1 INTRODUCTION

*Apios americana* Medikus is a perennial plant belonging to the family Leguminosae. Its underground stem, which becomes edible tubers, forms knots in intervals between 5-10 cm while growing during growth, and they become edible tubers. Attractive feature of *A. americana* is that it is a tuber-production crop with N-fixing tuber production capability. Unlike most other tuberous crops, *A. americana* is able to fix atmospheric nitrogen by using a symbiotic relation with *Bradyrhizobium japonicum* (Buchanan)Jordan. This unique ability of *A. americana* greatly minimizes the use of large quantities of nitrogen fertilizers for crop yield. *A. americana*, which is native to North America, is commonly found in Florida and Texas. Historically, the starchy tubers were consumed by the Native Americans and early settlers. Previously, the Louisiana Agricultural Experiment Station has expressed interest in this crop and initiated a research to study its potential as a food crop. *A. americana* is cultivated as a crop in northern Japan and it is a common belief that it had been imported from the United States with the soil adhered to young apple trees. *A. americana* has attracted significant consumer attentions. The reason is because the result search was described the nutritional value and the traditional health benefits of this tubers. There are some studies that *A. americana* can improve chronic constipation, hypertension, obesity, and diabetes. In previously, the nutrient content, types of lipids, and amino acid composition of *A. americana* tubers were reported. Furthermore, compounds such as 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) conjugated saponin, genistein, and genistein-7-O-genitiobioside have been found in *A. americana* tubers. However, the compounds of this plant have not been reported in detailed so far. Therefore, it is desirable to elucidate aroma-active compounds of *A. americana* owing to its development of uses.

Hydrodistillation (HD) is a process traditionally used for the extraction of essential oils from aroma-active and medicinal plants on a laboratory scale. Indeed, in the case of HD, the possible hydrolysis and solubilisation of certain compounds are serious obstacles in the reproduction of natural fragrances. Solvent-assisted flavor evaporation (SAFE) is a good technique for volatile extraction, and it can allow the careful isolation of volatile compounds from complex matrices.

The purpose of this article is therefore to investigate the...
key odorants from A. americana by gas chromatography-olfactometry (GC-O) and aroma extract dilution analysis (AEDA).

2 Experimental

2.1 Plant material

A. americana tubers were harvested from Aomori prefecture, Japan in August 2009. Identification of the plant was performed at the biotechnology laboratory of Kinki University (Kindai University). A voucher specimen was deposited at the biotechnology laboratory of Kinki University (Kindai University) in Osaka, Japan.

2.2 HD method procedure

The minced tubers of A. americana (500 g) were subjected to hydrodistillation for 3 h using a Likens-Nickerson-type apparatus with diethyl ether as the solvent. The obtained oil was dried over anhydrous sodium sulfate. The yield of the oil was 4 mg (0.0008%). The oil was stored at 4°C in a refrigerator prior to analysis.

2.3 SAFE method procedure

The minced tubers of A. americana (500 g) were frozen in liquid nitrogen. The crushed frozen tubers were added dichloromethane (300 ml) as solvent, and the mixture then being stirred and extracted. After standing for 2 days, the residual substances were removed by passing through filter paper. The filtrate was dried over anhydrous sodium sulfate, and then concentrated to a volume of approximately 50 ml by SAFE method. The whole system was operated under high vacuum (approximately 1.0 × 10^-4 Torr). After distillation, the receiving part of SAFE system was carefully rinsed with dichloromethane, and the rinse was combined with the distillates in the volatile-receiving flask. The volatile compounds were collected in a trap, which was submerged in liquid nitrogen to yield 0.008% (40 mg). The volatile compounds were stored at 4°C in a refrigerator prior to analysis.

2.4 GC-MS

GC-MS was performed using an Agilent 6890 gas chromatography-5973 MSD mass spectrometer. The samples were analyzed using a fused-silica capillary column, HP-5MS (5% phenyl 95% polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness = 0.25 μm) and DB-WAX (polyethylene glycol, 15 m × 0.25 mm i.d., film thickness = 0.25 μm). The oven temperature was programmed 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. On the DB-WAX column, the oven temperature was programmed from 40 to 240°C at a rate of 4°C/min and held at 240°C for 5 min. The injector and transfer line temperatures were 230°C, and the ionization energy was 70 eV. The mass range was 39-450 amu. 1 μL of the sample was injected, and the split ratio was 1:20.

2.5 Sniffing Test by GC-O

A trained panel of sensory evaluation specialists measured the odor intensities of the main aroma-active compounds of A. americana. Ten panellists, aged 21 to 27 years (8 males and 2 females, members of Kinki University (Kindai University), Japan), participated in this study. Sensory-analysis sessions were performed only after suitable training (>30 h). The sniffing test by GC-O was carried out using an Agilent 6890N gas chromatography-5973 MSD mass spectrometer and coupled to a sniffing port ODP 2 (Olfactory Detector Port 2, Gerstel). The GC instrument was equipped with a HP-5MS column (5% phenyl 95% polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness = 0.25 μm). The sample was injected into the GC in the 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 the GC-MS.

2.6 AEDA

The highest sample concentration (10 mg/mL) was assigned a flavor dilution (FD) factor of 1. The volatile oil was stepwise diluted with diethyl ether (1:1, v/v), and aliquots of the dilutions (1 μL) were evaluated. The process stopped when aromas ceased to be detected by the assessors. The result was expressed as the FD factor, which is the ratio of concentration of the odorant in the initial volatile oil to its concentration in the most diluted volatile oil in which the odor is still detectable by GC-O.

2.7 Identification and Quantitative of Compounds

Identification of the individual components was based on: (i) comparison of their GC-MS retention indices (RIs) on apolar and polar columns determined relative to the retention times of a series of n-alkanes (C12-C26) with those of authentic compounds or literature data; (ii) computer matching with commercial mass spectral libraries (Wiley, NIST02, Mass Finder, and Aroma Office version 3.0), which includes 72,120 entries of RIs of aroma compounds and literature sources. Then, all the identifications were confirmed by comparing their RIs with those of the references standards or with the published data. The components of the oils were quantitatively analyzed by internal standard addition method (alkanes C12 and C19). The volatile oil was diluted 100 times with diethyl ether to a 1 mL volume, and 5 μL of a mixture of C12 and C19 (1 mg/mL) was added to the diluted oil. Then, the samples were subjected to GC-flame ionization detector (FID) analyses. The quantitative analysis was
Table 1  Chemical components of the volatile oils from the tubers of *A. americana*.

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<th>DB-WAX RIs</th>
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performed on the based on the calibration curves for 1-penten-3-one (peak 1), 1-penten-3-ol (peak 2), pentanol (peak 4), hexanal (peak 6), furfural (peak 8), (2E)-hexenal (peak 11), (3Z)-hexenol (peak 12), hexanol (peak 13), 2-butoxyethanol (peak 15), 2,6-dimethylpyrazine (peak 16), 2,3-dimethylpyrazine (peak 17), (2E)-heptenal (peak 19), 1-octen-3-ol (peak 20), 2-pentylfuran (peak 22), 2-ethyl-6-methylpyrazine (peak 24), 2,3,5-trimethylpyrazine (peak 26), 2-ethylhexanol (peak 27), 2,3-dimethyl-5-ethylpyrazine (peak 33), sabine hydrate (peak 35), terpinen-4-ol (peak 39), citronellol (peak 42), carvone (peak 43), (2E, 4E)-decadienal (peak 46), γ-nonanal (peak 52), neryl acetone (peak 54) within the concentration range of (0.5-1000 μg/ml). Because of the lack of a proper standard, epι-cubenol (peak 58) was quantified by the calibration curves of β-eudesmol (peak 61).

### 2.8 OAV

OAVs were calculated by dividing the concentration of the compound by its recognition odor threshold. The determination of FD factors gives a first impression of potent odor active compounds. Nevertheless, the flavor contribution of these odorants can be better estimated by calculating their OAVs. These values are defined by dividing the concentrations of the compound by its recognition threshold in a suitable matrix. For OAVs > 1, an impact of

#### Table 1

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<th>No.</th>
<th>RIs&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>-</td>
</tr>
<tr>
<td>78</td>
<td>2282</td>
<td>squalene</td>
<td>2.2</td>
<td>-</td>
</tr>
<tr>
<td>total identified</td>
<td></td>
<td></td>
<td>total identified</td>
<td>84.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Retention indices (RIs) relative to C<sub>10</sub>-C<sub>30</sub> alkanes on HP-5MS and DB-WAX columns. <sup>b</sup> Compounds are listed in order of their elution times from a HP-5MS column. Presence of compound is indicated by its GC-MS. <sup>c</sup> Peak area (%) was related to total detected compounds by GC-MS. <sup>d</sup> Identification methods: RI, retention index; MS, mass spectrum. tr: trace (<0.1%)
Aroma Evaluation of the volatile oils from Apios americana


the respective compound on the overall flavor can be assumed.  

3 RESULTS AND DISCUSSION

The HD and SAFE oils from the tubers of A. americana were obtained in yields of 0.0008 and 0.008% (w/w), respectively. The HD oil had a mushroom-earthly-nutty-green odor, while the SAFE oil had mushroom-green-earthy odor. Sixty-two and forty-two compounds were identified by their mass spectrums and the RIs on the HP-5MS and DB-WAX columns, which comprised 84.1% (HD) and 88.7% (SAFE), respectively (Table 1).

In the HD oil, the main compounds were palmitic acid (36.5%) and linoleic acid (10.5%). Other predominant compounds were some nitrogen-containing compounds (2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2,3,5-trimethylpyrazine, 1H-pyrrole-2-carboxaldehyde, and 2,3-dimethyl-5-ethylpyrazine), or oxygen-containing compounds (furfural and 2-pentylfuran). On the other hand, the main components of the SAFE oil were 4-hydroxy-4-methyl-2-pentanone (34.2%) and hexanal (11.0%). The SAFE oil was characterized by including acyclic C6 compounds (3-hexanone, hexanal, (2E)-hexenal, (3Z)-hexenol, and hexanol) in high content. Comparing the two methods, Table 2 shows the classes and percentages of the compounds in the oils from A. americana. By GC analysis, it contained the ethers (55.3%), alcohols (21.6%), and hydrocarbons (10.5%) at high percentage in the HD oil. Meanwhile, the SAFE oil resulted in

### Table 2 Classification of the compounds in the oils from tubers of A. americana

<table>
<thead>
<tr>
<th>Compound</th>
<th>HD (%)</th>
<th>SAFE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocarbons</td>
<td>10.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Alcohols</td>
<td>21.6</td>
<td>67.1</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>2.9</td>
<td>15.1</td>
</tr>
<tr>
<td>Ketones</td>
<td>1.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Esters</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Acids</td>
<td>5.7</td>
<td>8.2</td>
</tr>
<tr>
<td>Lactones</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Others</td>
<td>1.0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*These values were calculated from GC peak area.

### Table 3 Odor-active compounds in the volatile oils from tubers of A. americana

<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds</th>
<th>FD factor</th>
<th>OD</th>
<th>OAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-penten-3-one</td>
<td>spicy</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1-penten-3-ol</td>
<td>green</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>pentanal</td>
<td>fruity</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>hexanal</td>
<td>green</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>furfural</td>
<td>sweet</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>(2E)-hexenal</td>
<td>green</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>(3Z)-hexenal</td>
<td>green</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>hexanol</td>
<td>green, floral</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>2-butoxyethanol</td>
<td>sweet</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>2,6-dimethylpyrazine</td>
<td>nutty</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>17</td>
<td>2,3-dimethylpyrazine</td>
<td>nutty</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>(2E)-heptenal</td>
<td>nutty</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>1-octen-3-ol</td>
<td>potato, earthy</td>
<td>128</td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td>2-pentylfuran</td>
<td>fruity</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>24</td>
<td>2-ethyl-6-methylpyrazine</td>
<td>earthy</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>26</td>
<td>2,3,5-trimethylpyrazine</td>
<td>nutty</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>27</td>
<td>2-ethylhexanol</td>
<td>green</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>33</td>
<td>2,3-dimethyl-5-ethylpyrazine</td>
<td>nutty</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>sabine hydrate</td>
<td>green, woody</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>39</td>
<td>terpinen-4-ol</td>
<td>green</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>42</td>
<td>citronellol</td>
<td>floral</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>43</td>
<td>carbonyl</td>
<td>mint</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>46</td>
<td>(2E,4E)-decadienal</td>
<td>fatty</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>52</td>
<td>n-octanal</td>
<td>sweet</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>54</td>
<td>neryl acetate</td>
<td>green</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>58</td>
<td>c-epi-cubenol</td>
<td>spicy</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

* Odor quality perceived at the sniffing port. * OD: odor thresholds measured in water solution and represented as mg of compound/g of water (ppb).

* OAV = odor activity value (concentration divided by odor threshold). * N/A = Dot no available. * N/D = Not determined.
a relatively high percentage of alcohols (67.1%), aldehydes (15.1%), and acids (8.2%). The results of the HD and SAFE oil indicated that the HD oil had about ten times the percentages of hydrocarbons and ethers compared to those in the SAFE oil. On the other hand, the percentages of alcohols and aldehydes in the SAFE oil were above three and five times relative to those in the HD oil, respectively.

The aroma-active compounds of the oils (HD and SAFE...
methods) from the tuber parts of A. americana were identified by GC-O and AEDA analyses. These key odorants and their odor properties were showed in Table 3 and Fig. 1. In the HD oil, twenty compounds were identified as aroma active compounds. These compounds are including 8 alcohols, 5 nitrogen-containing compounds, 3 aldehydes, 2 ketones, 1 ether and 1 lactone. The strongest aroma-active compound, 1-octen-3-ol (mushroom, earthy odor, FD = 128) played an important role in the characteristic odor of the HD oil. A large number of nitrogen-containing compounds (2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2,3,5-trimethylpyrazine, and 2,3-dimethyl-5-ethylpyrazine) were represented by a nutty or earthy odor, therefore, the HD oil had these odors. The aliphatic compounds (hexanal, hexanol, and 2-ethylhexanol) and monoterpene (sabinene hydrate, terpinen-4-ol, and neryl acetone) were associated with green odor of the HD oil. Among them aforementioned compounds, hexanal (green odor, FD = 32) was concerned with linked to the faint scent of a green. The another aroma-active compound, furfural (sweet odor, FD = 32) also played a role in the characteristic aroma of the oil. It seems that these compounds are responsible for the mushroom-earthy-nutty-green odor of the HD oil.

On the other hand, fourteen compounds were identified, including 8 alcohols, 3 aldehydes and 3 ketones from the SAFE oil as aroma-active compounds. Hexanal (green odor, FD = 128) and 1-octen-3-ol (mushroom-earthly odor, FD = 128) were also the strongest odorants of the SAFE oil. In addition, the other strong aroma-active compounds, 1-penten-3-one (spicy odor, FD = 64) also played an important role in the characteristic odor of the SAFE oil. Furthermore, it was indicated that (2E)-hexenal (FD = 16) and (3Z)-hexenol (FD = 2), known as the leaf odor, played an important role in the strong green odor of the SAFE oil. It seems that these compounds are responsible for making the green-mushroom-earthy odor of the SAFE oil.

The higher FD factor was often related to the aroma-active compounds, but the high FD factor of the compounds may be caused by their high content in oils. In order to determine the relative contribution of each of the compounds to the overall odor, the OAV method was used. The resulting OAVs of the both oils were presented in Table 3, respectively. The OAV was indicated that 1-octen-3-ol (OAV = 7080), hexanal (OAV = 1856), and furfural (OAV = 9330) played an important role in the characteristic aroma of the HD oil. Carvone and (2E, 4E)-decadienal had a low FD factor. However, these components played an important role in the characteristic odor. On the other hand, it was indicated that 1-octen-3-ol (OAV = 282720), hexanal (OAV = 392891), 1-penten-3-one (OAV = 16590), and (2E)-hexenol (OAV = 35119) played an important role in the characteristic aroma of the SAFE oil.

As compared with the HD and the SAFE oil, the HD oil had the heavy earthy-nutty odor, because of a number of nitrogen-containing compounds were included in the oil. Furthermore, the SAFE oil was the strong green odor, because of owing to the presence of (2E)-hexenal, (3Z)-hexenol and 1-penten-3-ol were included in the oil. Generally, compounds with high FD factor also had high OAVs which confirms the positive relationship previously found between the FD factor and OAVs.

CONCLUSION

We have investigated the key odorants of the oils from the tubers of A. americana by GC-O and using the concept of OAVs. On the basis of AEDA, OAVs, and GC-O, 1-octen-3-ol was estimated as the strongest aroma-active compound of the two extraction methods. The chemical compositions of the oils were also described in the details. These results provide a fundamental starting point imply that the oils from the tubers of A. americana deserve to facilitate further investigations of the oils for practical applications in the phytochemical and food industries.

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