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# Extended aroma extract dilution analysis profile of Shiikuwasha (*Citrus depressa* Hayata) pulp essential oil

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### ABSTRACT

Shiikuwasha pulp is an important raw material for producing citrus essential oils. The volatile aroma composition of pulp essential oil was evaluated using gas chromatography (GC) methods, and its aroma profile was assessed using GC-olfactometry with an extended aroma extract dilution analysis (AEDA) technique in regard to alterations of odor strength and sensorial perception throughout serial dilution steps. The essential oil comprised a mixture of 55 aroma compounds, including monoterpene hydrocarbon, sesquiterpene hydrocarbon, alcohol, aldehyde, ester, and oxide compounds. The predominant compounds were limonene [57.36% (4462.80 mg/100 g of pulp)] and $\gamma$-terpinene [25.14% (1956.21 mg/100 g of pulp)]. However, linalool was identified as one of the key aroma components providing the highest flavor dilution factor in AEDA, whilst three sesquiterpene hydrocarbons ($\delta$-elemene, germacrene B, and bicyclosesquiphellandrene) and two esters (heptyl acetate and decyl acetate) had superior relative flavor activities. The extended AEDA profile identified variations in assessed odor perceptions, intensity, and duration of aroma components over dilution, whereas the 12 most odor-active compounds showed comparable odor strengths.

## 1. Introduction

Shiikuwasha or Hirami lemon (*Citrus depressa* Hayata) is a well-known, small fruit-type citrus cultivar, which grows in subtropical regions of Northeast Asia, spreading from Taiwan, Japan, and Korea [1—3]. In Japan, the cultivar comprises more than 10 major cultivation lines, including "Katsuyama kugani", "Izumi kugani", "Ogimi kugani", "Kaachi", and "Ishikunibu", which vary in their chemical and volatile compositions [3,4]. The fruits also contain numerous bioactive flavonoid and vitamin substances contributing to their potent antioxidant,

anti-inflammatory, and antitumor activities [1,4–6]. Given its distinct flavor characteristics, Shiikuwasha is widely used as flavoring material in various food and beverage products [7].

Citrus flavoring materials in form of essential oil, widely used in food, pharmaceutical, and personal care products for their pleasing aroma qualities, are extracted from citrus fruits with different separation methods. Essential oil is mainly obtained from citrus peels, where oil glands are abundantly present, through cold-pressing or distillation [5,8], and also from by-products of squeezed citrus juice, such as pulp and pomace [9–11]. Citrus essential oils are rich in monoterpenes, sesquiterpenes, and their oxygenated compounds, and also contain small amounts of aldehydes, esters, ethers, and oxides [5,12]. The impact of each citrus aroma component on the aroma profile of essential oil, on finished products in which the oil is applied as flavoring material, and also on human health in aromatherapy has drawn the attention of several recent research studies [13–15].

The chemical composition of citrus essential oils is commonly evaluated using gas chromatography-flame ionization detector/mass spectrometry (GC-FID/MS), and their aroma profiles are characterized with GC-olfactometry (GC-O) instruments followed by odor identification by sensory panels. GC-O techniques for the investigation of the aroma profile of citrus essential oil include aroma extract dilution analysis (AEDA), combined hedonic aroma response measurement (CHARM), and detection frequency technical approaches, but AEDA is the most practical and reliable to identify active or key aroma compounds through a systematic serial dilution of the original concentration of essential oil to the exact threshold of each aroma component [8,12–14]. The AEDA technique provides odor potency as the ratio of concentration to detection threshold, described as flavor dilution (FD) factor, but the method does not provide information on the intensity and duration of the perceived odors [16], and there are missing outcomes on possible alterations in assessed odor strengths and perceptions of volatile aroma components throughout dilution steps [13,14]. Nevertheless, the assessed key compounds with higher FD values represent the most odor-active components that greatly contribute to the overall aroma characteristics of citrus essential oils regardless of their concentrations [13].

To the best of our knowledge, the present study is the first report on GC-O evaluation and key aroma compounds identification of Shiikuwasha essential oil. The aim of this study is to evaluate the aroma profile of Shiikuwasha pulp essential oil and to identify its key aroma compounds using the AEDA technique. Additionally, to investigate the impact of changes in concentration of potent volatile components on odor sensation, we employed an extended approach, in which the odor strengths comprising intensity and duration, and the sensorial description of aroma compounds were assessed applying three-fold dilution factors.

## 2. Materials and methods

### 2.1. Standards and reagents

Standards used for identifying volatile aroma components were purchased from Sigma–Aldrich (St. Louis, MO, USA) and

Tokyo Chemical Industry (Tokyo, Japan). The authentic *n*-hexanol compound was obtained from Tokyo Chemical Industry, and anhydrous sodium sulfate was obtained from Merck KGaA (Darmstadt, Germany). All other reagents were of analytical grade.

### 2.2. Sample and oil extraction

The fruit pulp was obtained from the Agricultural Production Corporation, Katsuyama Shiikuwasa Ltd. (Okinawa, Japan) as a by-product of the 2012 Shiikuwasha juice production. Shiikuwasha pulp (500 g) was collected from the upper layer of 20 kg of thawed frozen mixture of ripe fruit squeeze that had been placed in a plastic barrel. The essential oil was then extracted from the pulp using a steam-distillation method. Briefly, 50 g of pulp and 400 mL of distilled water were placed into a round-bottomed flask, and extracted by hydro-distillation using a Clevenger-type apparatus for 3 h. Subsequently, the obtained crude extract was centrifuged at 2900 ×g for 5 min at 4 °C (Himac CF-5RX, Hitachi Koki Co. Ltd., Japan), and oils were dehydrated overnight over anhydrous sodium sulfate at 5 °C. The collected Shiikuwasha pulp oil was weighed, and yielded 69.83 g of oil/kg of pulp. The oil was stored in sealed vials at −30 °C prior to analysis. All extractions were performed in triplicate.

### 2.3. Aroma compositional analysis

The analysis of volatile aroma components of Shiikuwasha pulp oil was performed using an Agilent 7890A GC-FID system equipped with a DB-Wax column (60 m × 0.25 mm i.d., 0.25-μm film thickness, Agilent J&W, Santa Clara, CA, USA) [5]. The GC injector and FID were both programmed at 250 °C. Oil samples (1 μL) were injected at a split ratio of 1:50. For the chromatography process, helium was streamed at a flow rate of 32 cm/s, and the oven temperature was set as follows: initial setting at 40 °C for 2 min, increase to 200 °C at 2 °C/min, and final hold for 38 min. Aromatic components were quantified through a calibration of the correlation factor to the FID response of an internal standard (*n*-hexanol) that was prepared at ratio of 1:150 (w/w) to sample prior to injection. Aroma compositions were expressed as the peak relative concentration (%) or mg of aroma compound per 100 g of pulp weight. All assays were performed in triplicate.

Volatile aroma compounds were identified based on linear retention indices (RIs) upon measurement of the homologous series of C7–C30 alkanes, MS patterns of authentic standards, and MS patterns of the peak with the MS data from the National Institute of Standards and Technology (NIST) MS Library, Version 2008. The GC-MS analysis was performed using an Agilent 7890A GC system coupled with an Agilent 5975C MS set with the same GC parameters as described above. For MS detection, the ion source and interface temperatures were both set at 230 °C. The electron impact ionization was generated at 70 eV, and the mass acquisition range (*m/z*) was taken at 29–450 amu.

### 2.4. Aroma profile analysis

The profile of volatile aroma components of Shiikuwasha pulp oil was examined using GC-O in an Agilent 7890A GC

coupled with FID (Agilent J&W) and an olfactory detection port (ODP) (Gerstel, Mülheim, Germany). The odor of each peak was assessed by 5 trained panelists (2 males and 3 females, between 21 and 24 years old) in duplicate through aroma extract dilution analysis (AEDA). The assessors were selected for their sniffing ability using a panel-screening test provided by the Japan Association on Odor Environment. Samples were prepared in three-fold serial dilutions with diethyl ether until no odor was detected. The GC column and conditions were the same as described above except that the injection split ratio was 1:10, and the split ratio for the FID and ODP was 1:1. A panelist was asked to detect odors sniffing at an ODP under humidified air streams 34 min per day, as well as to evaluate the smells and intensity of the odors. The odor potency in which the last dilution of the compound could be detected in AEDA was expressed as the flavor dilution factor (FD factor). The relative flavor activity (RFA) was calculated using the equation: $RFA = \log^{3n}/S^{0.5}$, where n is the FD factor, and S is the peak relative concentration (%) of a compound. Odor intensity and sniffing time were recorded using a four-scale olfactory intensity device (Gerstel) with the levels: 1 mV (weak), 10 mV (moderate), 100 mV (strong), and 1000 mV (very strong), and odor strengths of the aroma compounds were averaged by the number of panelists and duplications, and expressed as millivolt·seconds (mV·s) unit.

## 3. Results and discussion

### 3.1. Volatile aroma components of Shiikuwasha pulp essential oil

A total of 55 volatile aroma components that accounted for the relative percentage of 99.91% and a total content of 7773.64 mg/100 g were identified in Shiikuwasha pulp essential oil through GC-FID/MS analysis (Table 1, Fig. 1a). The essential oil mainly comprised monoterpene hydrocarbons (12 compounds, 93.31%), followed by alcohols (15 compounds, 4.77%) and sesquiterpene hydrocarbons (15 compounds, 1.26%). In detail, the two predominant aroma compounds in Shiikuwasha essential oil were limonene [57.36% (4462.80 mg/100 g of pulp)] and γ-terpinene [25.14% (1956.21 mg/100 g of pulp)]. Shiikuwasha essential oil also contained moderate amounts of an alcohol, α-terpineol (246.98 89 mg/100 g of pulp), followed by the monoterpene hydrocarbons p-cymene, myrcene, α-pinene, and terpinolene (168.62, 148.62, 128.71, and 127.89 mg/100 g of pulp, respectively). These results are in agreement with previously reported data showing that its peel oil and juice comprise two predominant limonene and γ-terpinene compounds in similar ranges that affect their aroma and taste characteristics [4,5]. Aroma formation in Shiikuwasha oil is greatly different from the one in essential oils obtained from known citrus species and other small fruit-type citrus fruits, due to lower levels of limonene and higher levels of γ-terpinene. A literature review revealed the following compositional variations: sweet orange (Citrus sinensis) is characterized by 96.3–97.3% limonene and 0.02–0.04% γ-terpinene, mandarin (Citrus reticulata) by 90.6–92.6% limonene and 3.4–3.5% γ-terpinene, grapefruit (Citrus paradisi) by 75.3%

limonene and 4.9% γ-terpinene, and lemon (Citrus limon) by 69.7% limonene and 8.2% γ-terpinene [17,18].

Besides α-terpineol, Shiikuwasha essential oil also contained other alcohol components such as terpinene-4-ol, γ-terpineol, linalool, and β-terpineol (44.17, 29.29, 20.45, and 8.24 mg/100 g of pulp, respectively; 0.1–1.0% each). These compounds are known to provide pleasant citrusy and flowery odors in various citrus essential oils [12,13]. Linalool in particular is also known to promote health and wellbeing via an olfactory stimulation path [15,19]. In the sesquiterpene hydrocarbon group, all components were present at a concentration lower than 0.5%, and included γ-elemene, β-caryophellene, δ-cadinene, and β-elemene, resulting in a monoterpene hydrocarbons to sesquiterpene hydrocarbons ratio of 74.06, which is in the specific range of the cultivation line of this Shiikuwasha fruit [5]. This ratio is often used to discriminate among citrus essential oils due to genotypic and geographic variabilities that affect the regulation of terpene biosynthesis pathways in citrus plants [5,18]. Additionally, Shiikuwasha essential oil included seven ester, five aldehyde, and 1-oxide compounds (0.29, 0.26, and 0.02%, respectively) that enrich the mixture with their aroma and functionality potentials. This result thus confirms that essential oil extracted from Shiikuwasha pulp is a by-product of the Shiikuwasha juice industry valuable for the production of citrus-flavoring materials with distinct aroma characteristics.

### 3.2. Extended AEDA profile of Shiikuwasha pulp essential oil

Aroma characteristics, including FD factor, odor description, and odor strength of volatile aroma components of Shiikuwasha pulp essential oil obtained with GC-O analysis are summarized in an extended AEDA profile (Tables 1 and 2; Figs. 2 and 3). Accordingly, an AEDA aromagram comprising the FD factors of 47 sniffable aroma components that ranged from 1 to 729 was plotted to the respective elution orders in the GC-FID chromatogram (Fig. 1b). The highest recorded FD factor of Shiikuwasha essential oil was 729 for linalool, followed by 243 for the compounds α-thujene, limonene, γ-terpinene, p-cymene, terpinolene, nonanal, α-cubebene, δ-elemene, decanal, bicyclosesquiphellandrene, and germacrene B. Only few of these compounds also contribute to the aroma characteristics of the essential oil as shown by their higher RFA values, when their FD factor values were compared to their concentrations (Table 1). Among them were δ-elemene, germacrene B, and bicyclosesquiphellandrene, with identified RFA values of 17.74, 15.70, and 15.00, respectively. On the other hand, linalool had only a smaller RFA value (5.58), and the predominant components limonene and γ-terpinene failed to display superior RFA values. Instead, two ester compounds, heptyl acetate and decyl acetate, with significantly lower concentrations yielded RFA values as high as 16.77 and 15.67, respectively. This result indicated that FD factors of volatile aroma components in AEDA can be altered by their concentrations in the material, and thus FD factors may not always reliably represent the actual contributions of volatile aroma components to the characteristics of citrus essential oil.

Several assessed odors of volatile aroma components were described differently upon dilution in AEDA (Table 2). For

**Table 1 – Volatile aroma components of essential oil extracted from Shiikuwasha pulp.**

| Peak No.[a] | RI | Compound | Content (mg/100 g of pulp)[b] | FD factor[c] | RFA | Identification[d] |
|---|---|---|---|---|---|---|
| 1 | 1017 | α-Pinene | 128.71 ± 0.83 | 9 | 0.74 | RI, MS, PC |
| 2 | 1021 | α-Thujene | 72.23 ± 0.95 | 243 | 2.48 | RI, MS, PC |
| 3 | 1102 | β-pinene | 94.30 ± 0.03 | 27 | 1.30 | RI, MS, PC |
| 4 | 1115 | Sabinene | 4.69 ± 0.02 | 1 | 0 | RI, MS |
| 5 | 1159 | Myrcene | 148.62 ± 0.14 | 81 | 1.38 | RI, MS, PC |
| 6 | 1177 | α-Terpinene | 65.89 ± 0.09 | 27 | 1.56 | RI, MS, PC |
| 7 | 1207 | Limonene | 4462.80 ± 4.96 | 243 | 0.31 | RI, MS, PC |
| 8 | 1213 | β-Phellandrene | 10.60 ± 0.10 | nd. | — | RI, MS |
| 9 | 1214 | 1,8-Cineole | 1.86 ± 0.02 | nd. | — | RI, MS, PC |
| 10 | 1251 | γ-Terpinene | 1956.21 ± 2.30 | 243 | 0.48 | RI, MS, PC |
| 11 | 1269 | p-Cymene | 168.62 ± 0.35 | 243 | 1.62 | RI, MS, PC |
| 12 | 1281 | Terpinolene | 127.89 ± 0.17 | 243 | 1.86 | RI, MS, PC |
| 13 | 1285 | Octanal | 6.79 ± 0.02 | 81 | 6.46 | RI, MS, PC |
| 14 | 1370 | Heptyl acetate | 0.57 ± 0.00 | 27 | 16.77 | RI, MS, PC |
| 15 | 1387 | Nonanal | 1.94 ± 0.01 | 243 | 15.10 | RI, MS, PC |
| 16 | 1438 | α-Cubebene | 1.60 ± 0.00 | 243 | 16.61 | RI, MS |
| 17 | 1463 | δ-Elemene | 1.41 ± 0.01 | 243 | 17.74 | RI, MS |
| 18 | 1471 | Octyl acetate | 4.78 ± 0.01 | nd. | — | RI, MS, PC |
| 19 | 1484 | α-Copaene | 4.85 ± 0.01 | 1 | 0 | RI, MS |
| 20 | 1492 | Decanal | 8.80 ± 0.06 | 243 | 7.10 | RI, MS, PC |
| 21 | 1530 | Bicyclosesquiphellandrene | 1.97 ± 0.00 | 243 | 15.00 | RI, MS |
| 22 | 1546 | Linalool | 20.45 ± 0.04 | 729 | 5.58 | RI, MS, PC |
| 23 | 1557 | Octanol | 3.60 ± 0.07 | 9 | 4.43 | RI, MS, PC |
| 24 | 1574 | β-Elemene | 9.97 ± 0.01 | 1 | 0 | RI, MS |
| 25 | 1578 | Bornyl acetate | 5.96 ± 0.01 | 9 | 3.45 | RI, MS, PC |
| 26 | 1581 | α-Bergamotene | 5.10 ± 0.01 | nd. | — | RI, MS |
| 27 | 1587 | β-Caryophellene | 16.08 ± 0.02 | 1 | 0 | RI, MS, PC |
| 28 | 1591 | γ-Muurolene | 0.78 ± 0.02 | 3 | 4.77 | RI, MS |
| 29 | 1597 | Terpinen-4-ol | 44.17 ± 0.06 | 9 | 1.27 | RI, MS, PC |
| 30 | 1629 | γ-Elemene | 32.00 ± 2.06 | nd. | — | RI, MS |
| 31 | 1636 | (E)-2-Decenal | 1.40 ± 0.01 | 9 | 7.12 | RI, MS |
| 32 | 1660 | α-Caryophellene | 4.83 ± 0.02 | 9 | 3.83 | RI, MS |
| 33 | 1667 | Decyl acetate | 0.65 ± 1.02 | 27 | 15.67 | RI, MS |
| 34 | 1681 | β-Terpineol | 8.24 ± 0.01 | 81 | 5.86 | RI, MS, PC |
| 35 | 1691 | Terpinyl acetate | 7.83 ± 0.11 | 27 | 4.51 | RI, MS, PC |
| 36 | 1700 | α-Terpineol | 246.98 ± 0.44 | 27 | 0.80 | RI, MS, PC |
| 37 | 1703 | γ-Terpineol | 29.29 ± 0.11 | nd. | — | RI, MS, PC |
| 38 | 1708 | Dodecanal | 1.45 ± 0.00 | 9 | 6.99 | RI, MS, PC |
| 39 | 1718 | α-Muurolene | 1.97 ± 0.02 | 3 | 3.00 | RI, MS |
| 40 | 1722 | Bicyclogermacrene | 1.12 ± 0.01 | 27 | 11.92 | RI, MS |
| 41 | 1727 | l-Carvone | 19.56 ± 0.07 | 27 | 2.85 | RI, MS, PC |
| 42 | 1744 | α-Farnesene | 2.39 ± 0.00 | 3 | 2.72 | RI, MS |
| 43 | 1750 | δ-Cadinene | 11.83 ± 0.02 | 3 | 1.22 | RI, MS |
| 44 | 1753 | Gerenyl acetate | 2.07 ± 0.01 | 9 | 5.85 | RI, MS, PC |
| 45 | 1763 | Citronellol | 0.89 ± 0.00 | 9 | 8.92 | RI, MS |
| 46 | 1819 | Germacrene B | 1.80 ± 0.01 | 243 | 15.70 | RI, MS |
| 47 | 1848 | Geraniol | 2.07 ± 0.01 | 27 | 8.79 | RI, MS, PC |
| 48 | 2039 | (E)-Nerodiol | 0.85 ± 0.00 | nd. | — | RI, MS |
| 49 | 2051 | Germacrene D-4-ol | 2.93 ± 0.01 | 3 | 2.46 | RI, MS |
| 50 | 2072 | Globulol | 1.94 ± 0.00 | 1 | 0 | RI, MS |
| 51 | 2080 | Elemol | 0.77 ± 0.01 | 1 | 0 | RI, MS |
| 52 | 2165 | γ-Eudesmol | 2.41 ± 0.00 | 3 | 2.71 | RI, MS |
| 53 | 2183 | α-Cadinol | 2.08 ± 0.00 | 9 | 5.84 | RI, MS |
| 54 | 2229 | β-Eudesmol | 4.45 ± 0.01 | 9 | 3.99 | RI, MS |
| 55 | 2248 | Eugenol acetate | 0.60 ± 0.01 | nd. | — | RI, MS |
| NA | | Component groups | | | | |
| | | Monoterpene hydrocarbons (12)[e] | 7260.13 (93.31%)[f] | | | |
| | | Sesquiterpene hydrocarbons (15) | 97.70 (1.26%) | | | |
| | | Alcohols (15) | 371.13 (4.77%) | | | |
| | | Esters (7) | 22.46 (0.29%) | | | |

| Peak No.[a] | RI | Compound | Content (mg/100 g of pulp)[b] | FD factor[c] | RFA | Identification[d] |
|---|---|---|---|---|---|---|
| | | **Table 1 − (continued)** | | | | |
| | | Aldehydes (5) | 20.37 (0.26%) | | | |
| | | Oxides (1) | 1.86 (0.02%) | | | |
| | | Total identified | 7773.64 (99.91%) | | | |

[a] Peak numbers refer to the compounds in Table 2, Figs. 1 and 3.
[b] Each value is expressed as the mean ± standard deviation (n = 3).
[c] FD factor: the last dilution factor of the compound detected in AEDA; nd.: odor was not detected at any dilution step; 1, undiluted extract.
[d] RI: identification based on retention index; MS: identification based on the NIST MS library; PC: identification based on authentic standards that were analyzed by mass spectrometry.
[e] Number of compounds in the component group.
[f] Relative percentage of the component group.

instance, the predominant limonene was perceived as citrusy-lemon, green-herbaceous, and minty from its original or undiluted form up to FD factor 81, but the final sensation was described as citrusy with floral and sweet odors. γ-Terpinene, heptyl acetate, linalool, decyl acetate, and germacrene B were also associated with simpler odor perceptions as they reached their final FD factor thresholds. Moreover, the odor perception of α-thujene changed over dilution steps from floral and sweet scents to green, woody, and oily smells, while nonanal lost its citrus odor over FD factor 27. However, some compounds resulted in similar odor perceptions throughout dilutions, such as terpinolene, octanal, decanal, and bicyclosesquiphellandrene, indicating that their constant aroma traits are less dependent on concentration changes. These findings indicated that dilution can influence specific odor perceptions of the mixture and alter the overall aroma quality of Shiikuwasha essential oil [10,20]. Careful dose measurements have thus to be considered when applying this complex aroma mixture in food, beverage, and nutraceutical products.

The odor strength characterized by intensity and duration of perceived volatile aroma components over systematic dilution steps provided another important GC-O outcome that promoted an extended odor potency feature to the AEDA profile of Shiikuwasha pulp essential oil (Figs. 2 and 3). As the major component group with a concentration of 93.31%, undiluted monoterpene hydrocarbons had a total odor strength as high as 426.94 mV·s. The odor strength significantly dropped to 43.02, 17.05, and 2.29 mV·s for FD factors 3, 9, and 27, respectively, and the aroma components underwent a weakening of their odor potencies to less than 1 mV·s in the last two assessable FD factors (Fig. 2a; Table 1). However, the relative concentrations of volatile aroma components did not always linearly contribute to their odor strengths. For instance, sesquiterpene hydrocarbons, the third largest component group, displayed odor strength at 188.36 mV·s for FD factor 1, and were found at the same levels as monoterpene hydrocarbons after subsequent stepwise dilutions. Moreover, although containing a greater compound number and concentration, alcohols were assessed with considerably weaker odor strength compared to aldehyde components at FD factors 1, 3, and 9 (Fig. 2b). On the other hand, esters were only perceived in four dilution stages as low as 10.24 mV·s (FD factor 1), and then at 8.20, 5.75, and 0.23 mV·s for FD factors 3, 9, and 27, respectively.

Detection of odor strength changes in the AEDA profile of Shiikuwasha pulp essential oil is shown for 12 odor-active
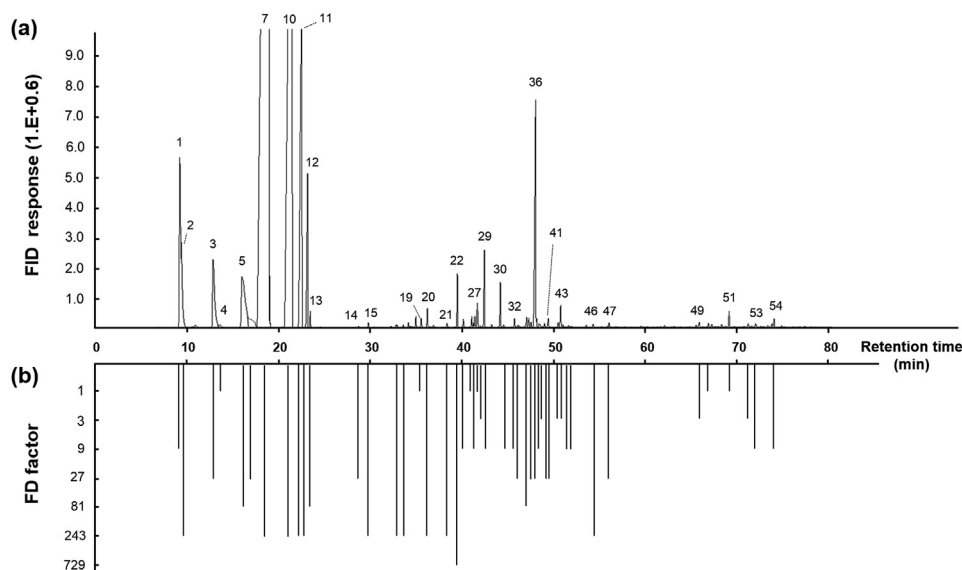


Fig. 1 − (a) GC-FID chromatogram and (b) AEDA aromagram of volatile aroma components of essential oil extracted from Shiikuwasha pulp. Peak numbers refer to the compounds in Tables 1 and 2.

**Table 2 – Odor description of volatile aroma components of essential oil extracted from Shiikuwasha pulp in AEDA.**

| Peak No.[a] | Compound | FD factor dilution step | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 (undiluted) | 3 | 9 | 27 | 81 | 243 | 729 |
| 1 | α-Pinene | Pine, woody | Woody | Woody | — | — | — | — |
| 2 | α-Thujene | Floral, sweet | Green, minty, woody | Green, sweet, woody, oily | Green, fruity, sweet, oily | Woody, green | Woody | — |
| 3 | β-pinene | Citrus, herbaceous, pine | Citrus, herbaceous, fruity, pine | Pine, oily | Green-grassy | — | — | — |
| 4 | Sabinene | Citrus, green, woody | — | — | — | — | — | — |
| 5 | Myrcene | Citrus, grassy, musty, woody | Citrus, pine, oily, woody | Musty, pine, oily, woody | Herbaceous, pine, oily, woody | Musty, oily, woody | — | — |
| 6 | α-Terpinene | Citrus, green, oily, woody | Citrus, green, sweet, woody | Citrus, green, herbaceous, woody | Green, herbaceous | — | — | — |
| 7 | Limonene | Citrus, green, minty | Lemon, green, minty | Lemon, green, herbaceous, minty | Citrus, green, herbaceous, minty | Lemon, green, woody | Citrus, floral, sweet | — |
| 8 | β-Phellandrene | — | — | — | — | — | — | — |
| 9 | 1,8-Cineole | — | — | — | — | — | — | — |
| 10 | γ-Terpinene | Chewing gum, oily, waxy, woody | Dampness, green, oily, woody | Chewing gum, green, oily, woody | Green, waxy, woody | Waxy, woody | Green | — |
| 11 | p-Cymene | Green, minty, oily, woody | Green, herbaceous, oily, woody | Citrus, green, oily, woody | Citrus, green | Woody, dampness | Woody, musty | — |
| 12 | Terpinolene | Floral, fruity, herbaceous, sweet | Citrus, grassy, floral, sweet | Citrus, herbaceous, floral, sweet | Citrus, grassy, floral, sweet | Citrus, floral, fruity, sweet | Green, floral, sweet | |
| 13 | Octanal | Citrus, fruity, oily, woody | Citrus, fruity, oily | Citrus, fruity, oily | Citrus, floral, oily | Citrus, sweet, oily, woody | — | — |
| 14 | Heptyl acetate | Floral, woody | Floral, oily, woody | Grassy | Woody | — | — | — |
| 15 | Nonanal | Citrus, floral, oily, woody | Citrus, floral, herbaceous, oily | Citrus, floral, oily | Floral, sweet, oily | Floral, grassy, oily | Floral, grassy | — |
| 16 | α-Cubebene | Citrus, herbaceous, oily, woody | Citrus, fruity, green, oily | Citrus, floral, green, sweet, oily | Citrus, green, oily | Citrus, oily | Citrus, green, oily | — |
| 17 | δ-Elemene | Herbaceous, floral, fruity, sweet | Fruity, sweet | Citrus, sweet | Citrus, sweet | Citrus, sweet | Citrus, green | |
| 18 | Octyl acetate | — | — | — | — | — | — | — |
| 19 | α-Copaene | Chewing gum, oily, woody | — | — | — | — | — | — |
| 20 | Decanal | Floral, grassy, herbaceous, oily | Citrus, floral, fruity, grassy | Floral, grassy, oily | Floral, grassy, oily | Floral, green, herbaceous | Floral, grassy | — |
| 21 | Bicyclosesquiphellandrene | Green, herbaceous, minty, woody | Citrus, green, herbaceous, oily | Citrus, herbaceous, sweet, oily | Citrus, green, oily | Citrus, green | Citrus, green | |
| 22 | Linalool | Citrus, floral, sweet | Citrus, floral, sweet | Citrus, floral, fruity, sweet | Citrus, floral, green, sweet | Citrus, floral, green, sweet | Citrus, floral, sweet | Floral, sweet |
| 23 | Octanol | Citrus, sweet, oily | Citrus, green, oily, woody | Oily, woody | — | — | — | — |
| 24 | β-Elemene | Grassy, woody | — | — | — | — | — | — |
| 25 | Bornyl acetate | Green, herbaceous, woody | Green, herbaceous, woody | Green, herbaceous, woody | — | — | — | — |
| 26 | α-Bergamotene | — | — | — | — | — | — | — |

**Table 2 — (continued)**

| Peak No.[a] | Compound | FD factor dilution step | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 (undiluted) | 3 | 9 | 27 | 81 | 243 | 729 |
| 27 | β-Caryophellene | Chewing gum, oily, woody | — | — | — | — | — | — |
| 28 | γ-Muurolene | Citrus, oily | Waxy | — | — | — | — | — |
| 29 | Terpinen-4-ol | Green, herbaceous, waxy | Green, herbaceous, waxy | Waxy | — | — | — | — |
| 30 | γ-Elemene | — | — | — | — | — | — | — |
| 31 | (E)-2-Decenal | Citrus, floral | Fruity | Fruity | — | — | — | — |
| 32 | α-Caryophellene | Green, herbaceous, minty, oily | Green, oily, woody | Green, floral, oily | — | — | — | — |
| 33 | Decyl acetate | Citrus, fruity, oily | Citrus, oily | Sweet, floral, fruity, oily | Oily | — | — | — |
| 34 | β-Terpineol | Citrus, green, herbaceous, woody | Citrus, green, herbaceous | Green, musty, woody | Green, woody | Oily, woody | — | — |
| 35 | Terpinyl acetate | Citrus, grassy, oily, woody | Grassy, herbaceous, oily | Grassy | Green, floral | — | — | — |
| 36 | α-Terpineol | Green, waxy | Herbaceous, floral, sweet | Fruity, sweet, oily, woody | Green, floral, fruity, oily | — | — | — |
| 37 | γ-Terpineol | — | — | — | — | — | — | — |
| 38 | Dodecanal | Grassy, oily, woody | Grassy, oily, woody | Oily, woody | — | — | — | — |
| 39 | α-Muurolene | Oily, woody | Fresh, fruity, woody, minty | — | — | — | — | — |
| 40 | Bicyclogermacrene | Fruity, sweet | Fruity, sweet | Citrus, minty | Citrus, fruity, sweet | — | — | — |
| 41 | l-Carvone | Green, herbaceous, minty | Green, fresh, woody | Green, herbaceous, minty | Green, fresh | — | — | — |
| 42 | α-Farnesene | Green, herbaceous, minty | Green, herbaceous, minty | — | — | — | — | — |
| 43 | δ-Cadinene | Grassy, woody | Grassy | — | — | — | — | — |
| 44 | Gerenyl acetate | Citrus, floral, sweet | Fruity, sweet | Citrus, oily | — | — | — | — |
| 45 | Citronellol | Citrus, fresh, green, sweet | Citrus, fresh, green, sweet | Floral, sweet | — | — | — | — |
| 46 | Germacrene B | Chewing gum, dampness, musty | Chewing gum, dampness, oily | Waxy | Waxy | Waxy | Oily | — |
| 47 | Geraniol | Citrus, floral, sweet | Citrus, floral, sweet | Citrus, floral, sweet | Citrus | — | — | — |
| 48 | (E)-Nerodiol | — | — | — | — | — | — | — |
| 49 | Germacrene D-4-ol | Citrus, green, sweet, oily | Citrus, oily | — | — | — | — | — |
| 50 | Globulol | Fruity | — | — | — | — | — | — |
| 51 | Elemol | Grassy, herbaceous | — | — | — | — | — | — |
| 52 | γ-Eudesmol | Green, herbaceous, minty, woody | Grassy | — | — | — | — | — |
| 53 | α-Cadinol | Citrus, green, oily, woody | Citrus, green, oily, woody | Citrus, oily | — | — | — | — |
| 54 | β-Eudesmol | Grassy, herbaceous | Grassy, woody | Grassy | — | — | — | — |
| 55 | Eugenol acetate | — | — | — | — | — | — | — |

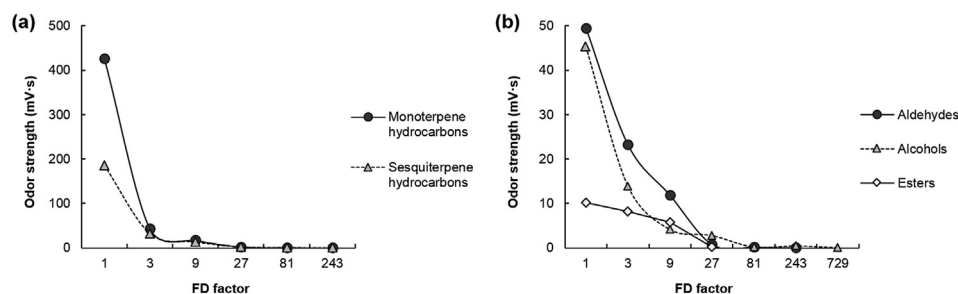[a] Peak numbers refer to the compounds in Table 1, Figs. 1 and 3.

Fig. 2 – Odor strength (mV·s) of component groups of essential oil extracted from Shiikuwasha pulp in AEDA: (a) monoterpene hydrocarbons and sesquiterpene hydrocarbons, and (b) aldehydes, alcohols, and esters.
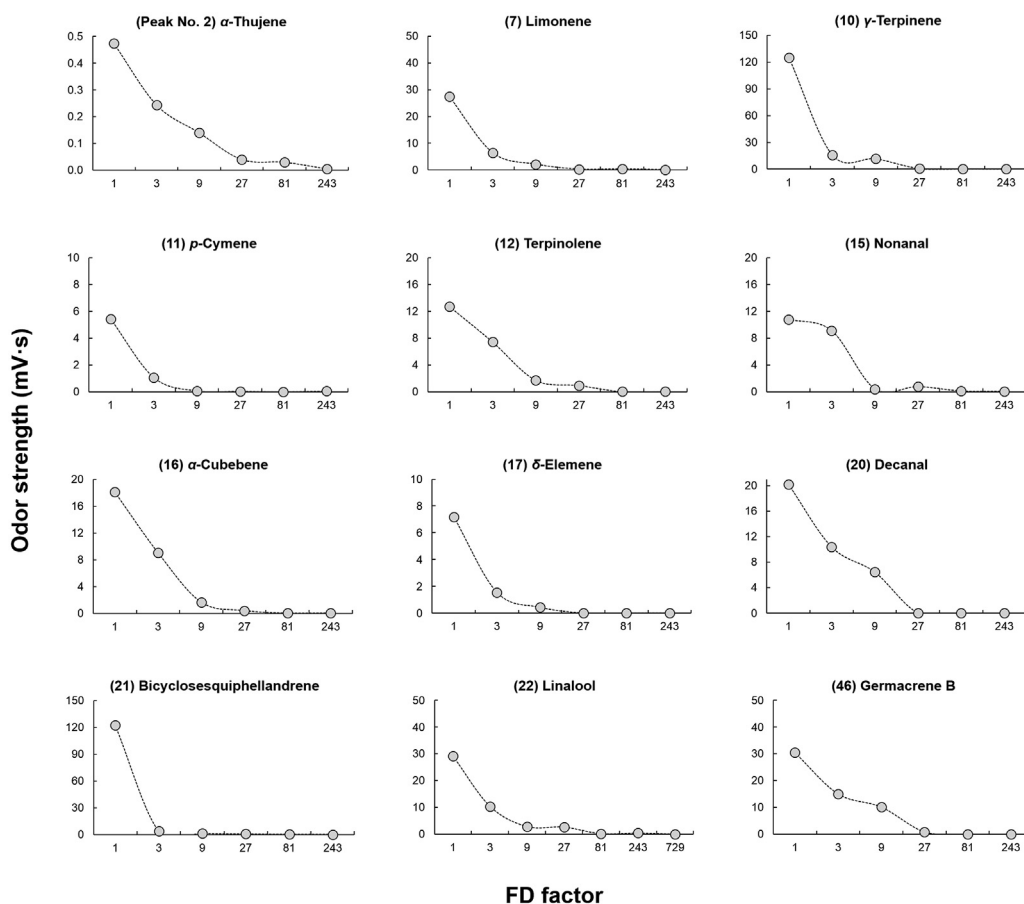


Fig. 3 – Odor strength (mV·s) of most odor-active compounds (FD factor ≥ 243) of essential oil extracted from Shiikuwasha pulp in AEDA. Peak numbers (shown in brackets) refer to the compounds in Tables 1 and 2.

compounds with an FD factor ≥243 (Fig. 3). The compounds were characterized by odors with high potencies at FD factor 1; odor strengths of almost all components subsequently dropped upon dilution. At FD factor 1, $\gamma$-terpinene and bicyclosesqui-phellandrene possessed superior odor strengths as 124.96 and 122.20 mV·s, respectively, than the ones of other compounds. In contrast, the predominant component limonene only presented a moderate potency of 27.46 mV·s, which was lower than the one of germacrene B and linalool (30.50 and 29.14 mV·s, respectively) but stronger than the one of decanal (20.20 mV·s). Moreover, these extended AEDA data clearly exhibit the decline

in odor strength of lower potency aroma compounds like $\alpha$-thujene with a starting strength at just 0.47 mV·s up to its last assessed dilution stage at FD factor 243. As to linalool, albeit possessing the highest FD factor, its extended AEDA pattern also shows constant potency alteration at the last three dilution stages, from FD factor 81 to 729, as for other odor-active components, indicating that Shiikuwasha essential oil has comparable odor strengths when it is diluted at the approximate range of 1:25 to 1:250. Odor potency measurements should therefore be thoroughly evaluated in the final concentrations when applying the AEDA technique in GC-O analysis.

The AEDA profile of Shiikuwasha pulp essential oil identified key aroma components based on their prolonged perceivable odors over systematic dilution procedures, as described by FD factor values, and subsequently by their RFA values that further highlighted their potent contribution to overall aroma quality with regards to compositional formation. The profile also revealed additional data on the changes at each dilution stage in odor and strength of perceived aroma components that can be used to describe the detection potency and differentiation with the AEDA technique. This extended AEDA profile thus provided a thorough aroma characterization of Shiikuwasha essential oil that may have implications in food and pharmaceutical production with widely different flavor insights.

## 4. Conclusion

Essential oil extracted from Shiikuwasha pulp possesses a distinct aroma composition comprising a unique balance of monoterpene hydrocarbon, sesquiterpene hydrocarbon, alcohol, aldehyde, ester, and oxide compounds, in which the predominant components limonene and γ-terpinene are different from those in other known citrus essential oils. The key aroma components in the essential oil are linalool, having the highest FD factor, and δ-elemene, germacrene B, bicyclosesquiphellandrene, heptyl acetate, and decyl acetate, yielding superior RFA values. The extended AEDA profile also described variations in assessed odor perceptions, intensity, and duration of volatile aroma components throughout serial dilution steps. These compositional and aroma profile data are necessary for developing a unique citrus flavoring material to employ for food, beverage, and nutraceutical products.

## Conflicts of interest

All authors declare no conflicts of interest.

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