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Cultivation line and fruit ripening discriminations of Shiikuwasha (*Citrus depressa* Hayata) peel oils using aroma compositional, electronic nose, and antioxidant analyses

Yonathan Asikin [a], Goki Maeda [b], Hajime Tamaki [c], Masami Mizu [d], Hirosuke Oku [a], Koji Wada [c,*]

[a] Tropical Biosphere Research Center, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan
[b] Regional Agricultural System Section, Okinawa Prefectural Agricultural Research Center, 820 Makabe, Itoman, Okinawa 901-0336, Japan
[c] Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan
[d] Product Development Division, Mitsui Sugar Co., Ltd., 36-2 Nihonbashi-Hakozakicho, Chuo-ku, Tokyo, 103-8423, Japan

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ABSTRACT

Shiikuwasha peel oils from three cultivation lines ('Izumi kugani', 'Katsuyama kugani', and 'Ogimi kugani') and four fruit-ripening stages were differentiated in terms of their aroma and antioxidant profiles. Each cultivation line had a distinctive profile of aroma components, and ripening stages affected the variations in the concentrations of their main constituents such as limonene, γ-terpinene, *p*-cymene, and linalool. Hyphenated total ion masses, obtained by a mass spectrometry (MS)-based electronic nose (e-nose) technique, effectively discriminated the peel oils according to their genetic and seasonal diversities, and generated several discriminant variables such as *m/z* 119, 135, and 149. The e-nose plots were generally alike with regard to the statistical profile of the compositional aroma compounds identified, wherein unripe oils of the early September harvest of the 'Izumi kugani' and 'Ogimi kugani' lines presented distinctive aroma profile clusters compared to the other Shiikuwasha oils. Moreover, despite fruit ripening, the 'Katsuyama kugani' peel oil was closely related to that of the 'Ogimi kugani' line. The antioxidant functions of Shiikuwasha peel oils declined with ripening, as seen from the oxygen radical absorbance capacity (ORAC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity assays. Additionally, the total phenolic contents of the oils were positively associated with their ORAC values.

1. Introduction

Fruit ripening is the most important stage for the development of flavor in commercial citrus fruits, as well for other chemical quality attributes, including their nutritional and biochemical compositions, pigments, and functionalities (Barreca, Bellocco, Caristi, Leuzzi, & Gattuso, 2010; Lan-Phi & Sawamura, 2008; Matsumoto et al., 2007; Pailly, Tison, & Amouroux, 2004). The aroma, taste, and functionality profiles of various known citrus fruits such as mandarin (*Citrus reticulata* Blanco), satsuma (*C. unshiu* Marcovitch), yuzu (*C. junos* Sieb. ex Tan), and Shiikuwasha or Hirami lemon (*C. depressa* Hayata), may also differ, based on the genetic diversity of their species, cultivar, and cultivation line (Asikin et al., 2014; Goldenberg et al., 2014; Lan-Phi, Shimamura, Ukeda, & Sawamura, 2009). Critically, these variations have also been shown to occur in the peel, or exocarp, part of the fruits, where many essential components that contribute to the overall quality of the citrus fruits are present (Asikin et al., 2014; Lan-Phi & Sawamura, 2008).

The citrus peels do not provide only aroma resources due to their oil glands, but also various bioactive compounds and biological activities. In particular, they contain numerous types of beneficial flavonoids that possess antioxidant, antimicrobial, and antidiabetic potential (Park et al., 2013; Yi, Yu, Liang, & Zeng, 2008). Moreover, the polymethoxy flavonoid, nobiletin, from Shiikuwasha peel, which is a plant comprising several cultivation lines that grows naturally in Okinawa, Japan, as well as in other subtropical area of Northeast Asia countries and regions, has promising therapeutic antitumor and anti-inflammatory effects (Aoki, Yokosuka, Mimaki, Fukunaga, & Yamakuni, 2013; Asikin et al., 2012; Choi et al., 2007; Lin, Roan, Lee, & Chen, 2010). However, the present industrial use of citrus peels is primarily in essential oil production for citrus flavor applications (Rouseff, Perez-Cacho, & Jabalpurwala, 2009).

Rapid measurement of the quality of food materials and products is becoming more important for enhancing quality control and research activities in the food industry. The emerging rapid food analyses also include various aspects of flavor and palatability, which complement the uses of their food compositional data on product formulation, processing, storage, transport, marketing, and so on (Baldwin, Bai, Plotto, & Dea, 2011; Concina et al., 2009). Moreover, accurate information on the composition of foods may still be used to verify the reliability of the rapid measurement technology (Asikin et al., 2014; Hui et al., 2012;

* Corresponding author. Tel.: +81 98 895 8809; fax: +81 98 895 8734.
E-mail address: kojiwada@agr.u-ryukyu.ac.jp (K. Wada).

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Saevels et al., 2004). The instrumentations for this rapid measurement are commonly called as electronic or artificial sensor for mimicking the function of corresponding human sense organs. For instance, electronic nose (e-nose) for the device to evaluate aroma profiles and electronic tongue (e-tongue) for taste characteristic analysis.

Several e-nose techniques have been developed to differentiate aroma components in various food matrices for food aroma analysis, including mass spectrometry (MS) detection and metal oxide semiconductor sensor systems (Di Natale et al., 2001; Park et al., 2011). These two foremost e-nose techniques may give digital fingerprints of aroma through pattern recognition methods. However, MS-based system may also provide another important information of responsible discriminant variables due to differences in ion masses of volatile aroma compounds (Hui et al., 2012; Park et al., 2011). The MS-based e-nose technique has been applied to effectively identify discriminant aroma components and compare aroma profiles of food materials of different origins and production types (Cozzolino, Smyth, Cynkar, Dambergs, & Gishen, 2005; Park et al., 2011; Saevels et al., 2004). MS-based e-nose technology captures the overall aroma profile by hyphenating the total ion masses from the fragmentation of all isolated aroma compounds. The patterns of these meaningful data can be transferred to MS fingerprints in a high throughput manner, using a chemometric technique, and can then be statistically interpreted (Cozzolino et al., 2005; Peña, Cárdenas, Gallego, & Valcárcel, 2002).

This study thus aimed to discriminate Shiikuwasha peel oils based on their cultivation line and fruit-ripening stages, using aroma compositional, MS-based e-nose, and antioxidant analyses. The variation of the aroma profile was characterized using gas chromatography-flame ionization detection/mass spectrophotometry (GC-FID/MS) as well as an MS-based e-nose technique, and was then statistically investigated. The changes in the antioxidant function of the peel oils were monitored in terms of their oxygen radical absorbance capacity (ORAC) values, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activities, and total phenolic contents.

2. Materials and methods

2.1. Fruit sampling and sample preparation

The Shiikuwasha fruits of three cultivation lines, namely 'Izumi kugani', 'Katsuyama kugani', and 'Ogimi kugani', were taken at four sampling times (September, October, November, and December) in 2011 from a farm located in Okinawa, Japan. The fruit characteristics were recorded according to their basic morphological and chemical properties such as weight, size, peel thickness, total soluble solid, titratable acidity, and pH (Table S1, supplementary data). The outermost pigmented layers (flavedo) of the peel were separated from the soft inner white layer (albedo) with a sharp knife.

2.2. Standards and reagents

Authentic standards, used for aroma component identification, were obtained from Sigma-Aldrich (St Louis, MO, USA) and Tokyo Chemical Industry (TCI, Tokyo, Japan). *n*-Hexanol was purchased from TCI. Folin–Ciocalteu reagent was obtained from Kanto Chemical Co. Inc. (Tokyo, Japan). Gallic acid, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Wako Pure Chemical Industries (Osaka, Japan). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Calbiochem (San Diego, CA, USA) and fluorescein sodium salt was obtained from Sigma-Aldrich. All other reagents were of analytical grade.

2.3. Peel oil extractions

The Shiikuwasha peel oils were extracted from the flavedo peel part using a steam distillation method. Briefly, flavedo peels (50 g) were cut into approximately 5 mm^2 pieces and placed in a 1 L round-bottomed flask. Subsequently, the peels were extracted with 500 mL of distilled water in a Clevenger-type apparatus for 5 h. The collected crude oils were purified by centrifugation at 2900 ×*g* for 15 min at 4 °C, and the resulting oils were then dehydrated over anhydrous sodium sulfate for 24 h at 5 °C. The extraction yields were expressed as grams of oil per kg of fruit, and the peel oils were stored at −30 °C prior to analysis. All extractions were carried out in triplicate.

2.4. Aroma component analysis

The aroma components of the peel oils were analyzed using gas chromatography-flame ionization detection/mass spectrophotometry (GC-FID/MS), according to the previously described method (Asikin et al., 2012). The aroma compounds were separated through a DB-Wax column (60 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent J&W, Santa Clara, CA, USA) in an Agilent 6890N GC-FID system. The samples were mixed with an internal standard, *n*-hexanol, at a ratio of 150:1 (w/w), and 1 μL of the mixture was injected into the system using a split ratio of 1:50. The GC injector and FID were both set at 250 °C, and the oven was programed from 40 °C (held for 2 min) to 200 °C at 2 °C/min, then isothermally held for 38 min. Helium was used as the carrier gas at a flow rate of 32 cm/s.

Each aroma compound was identified by comparison of the linear retention indices (RIs) from the calculation of a homologous series of *n*-alkanes (C7–C30), the MS patterns of the authentic standards, and the MS patterns of the peak. The GC–MS analysis was performed using an Agilent 7890A GC coupled with an Agilent 5975C MS with the same column and oven conditions as described above. For MS detection, the ion source and interface were both programmed at 230 °C, the electron impact ionization at 70 eV, and the acquisition range (*m*/*z*) at 29–450 amu. The aroma compositions were expressed as the peak weight relative concentration (%) or milligrams of aroma compound per 100 g of fresh flavedo weight, according to the internal standard calibration. All analyses were carried out in triplicate.

2.5. MS-based electronic nose (e-nose) analysis

The e-nose profile of the peel oils was determined using an Agilent 7890A GC–5975C MS (Agilent J&W) equipped with a GERSTEL Chemsensor (GERSTEL, Mülheim, Germany) and Pirouette 4.5 (Infometrix, Bothell, WA, USA) (Cozzolino et al., 2005; Peña et al., 2002). The samples (0.2 μL) were injected with a split ratio of 1:200 into an HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent J&W). The oven was set from 100 °C (held for 1 min) to 300 °C at 20 °C/min, then isothermally held for 5 min. The MS was programmed to acquire a full scan in selective ion monitoring (SIM) mode at ion source and interface temperatures of 250 °C, an electron impact ionization of 70 eV, and an acquisition range (*m*/*z*) of 33–300 amu. All analyses were carried out in triplicate. The scanned total ion masses of the peel oils were then outlined in principal component analysis (PCA) and hierarchical cluster analysis (HCA) statistical plots.

2.6. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was performed according to the previously described method (Asikin et al., 2014; Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002) with slight modifications. Briefly, 25 μL of samples at different concentrations (diluted in ethanol) and 150 μL of 90 nM fluorescein solution were transferred into inner wells of a black 96-well microplate (Nunc, Roskilde, Denmark). The microplate was placed in a Synergy HT microplate reader (BioTek Instruments, Winooski, VT, USA), and left to stand for 10 min. The microplate incubator was prewarmed and maintained entirely at 37 °C and 25 μL of 160 mM AAPH-induced peroxyl radicals was immediately added to the wells. The fluorescence signal was recorded every minute for 60 min at