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Characterization of aroma-active compounds in Chinese quince (*Pseudocydonia sinensis* Schneid) by aroma dilution analyses

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ABSTRACT

Aroma-active compounds in the peel and pulp of Chinese quince fruits were extracted by high-vacuum distillation (HVD) and headspace solid-phase microextraction (HS-SPME) methods and identified by gas chromatography-olfactometry (GC-O) combined with aroma dilution analyses. Ethyl 2-methylpropanoate, ethyl (*E*)-2-butenoate, ethyl 2-methylbutanoate, methional, (*Z*)-3-hexenyl acetate, β-ionone, ethyl nonanoate, and γ-decalactone were detected as the potent aroma-active compounds ($\log_3$FD factors ≥ 5) in the peel of Chinese quince, while hexanal, (*Z*)-3-hexenal, and (*Z*)-3-hexenol, which have a green odor note, were potent aroma-active compounds with high $\log_3$FD factors (≥ 3) in the pulp of Chinese quince. In particular, ethyl propanoate, ethyl (*E*)-2-butenoate, and (*Z*)-3-hexenyl acetate—which had sweet and fruity aroma notes with relatively high FD factors—were detected in the samples extracted by HS-SPME.

1. Introduction

Chinese quince (*Pseudocydonia sinensis* Schneid.) and its fruits are important crops that have been traditionally used in the production of various foodstuffs as well as pharmaceutical materials in many Asian countries, including Korea, Japan, and China (Hamauzu, Yasui, Inno, Kume, & Omanyuda, 2005). Chinese quince belongs to the rose family, which is native to China and widely distributed throughout Korea (Mihara, Tateba, Nishimura, Machii, & Kishino, 1987; Yin, Liu, & Liu, 2017). Chinese quince and quince (*Cydonia oblonga* Mill.) are not normally consumed raw and fresh due to their flesh hardness, strong acidity, and astringency. Instead, they are processed before being consumed in forms such as fruit liquor, syrup, jam, jelly, and candy (Hamauzu, Inno, Kume, Irie, & Hiramatsu, 2006; Hamauzu, Kume, Yasui, & Fujita, 2007; Nowicka, Wojdylo, Teleszko, & Samoticha, 2016). In particular, the fruits have long been consumed as tea using all their parts as raw materials, including their peel and pulp, and are also used in pharmaceutical medicines in Korea for relieving coughs and removing phlegm from the throat (Chung, Cho, & Song, 1988; Moradi, Saba, Mozafari, & Abdollahi, 2016).

The fruit of Chinese quince has a strong and characteristic aroma, and the characteristic aroma and flavor of Chinese quince are more easily released than those of other quince fruits (*Cydonia oblonga* Mill. and *Cydonia vulgaris* Pers.; Marmelo in Japanese) during maturation (Mihara et al., 1987). There have been several reports on the volatile compounds of Chinese quince fruits (Chung et al., 1988; Mihara et al., 1987). Chung et al. (1988) detected volatile compounds in Chinese quince fruits using high-resolution gas chromatography (GC) and GC–mass spectrometry (GC–MS). In addition, Mihara et al. (1987) compared the volatile compounds in Chinese quince oil from the peel and flesh. (*E*,*E*)-α-farnesene, isobutyl octanoate, ethyl octanoate, isobutyl 7-octenoate, and hexyl hexanoate were identified as the main volatile compounds in Chinese quince peel, which were recognized to be important contributors to the typical flavor of Chinese quince. They also explained that the flavor of Chinese quince might be determined more by the volatile compounds in the peel than those in the flesh.

The key odorants—so-called aroma-active compounds—can be determined by GC-olfactometry (GC-O) combined with aroma dilution methods (Grosch, 1993). In addition, the volatile profiles and determination of aroma-active compounds can be affected by the extraction method used. The aroma compounds are evaluated by aroma extract dilution analysis (AEDA), and each such compound is quantified by the flavor-dilution (FD) value, which corresponds to the maximum dilution in solvent at which it can no longer be detected by the nose (Grosch, 1993). Therefore, AEDA is normally accompanied by a solvent extraction method, such as high-vacuum distillation (HVD). Also, HVD has some advantages such as high extraction efficiency and low bias during sample preparation (Cho, Choi, & Kim, 2006). On the other

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hand, solid-phase microextraction (SPME) can also be employed for determining aroma-active compounds using various dilution methods, such as verifying sample concentrations, the length of the SPME fiber, and GC split ratios (Deibler, Acree, & Lavin, 1999; Kang & Baek, 2014; Kim et al., 2003; Zhang, Hartung, Fraatz, & Zorn, 2014). In particular, SPME can be applied to aroma-active compounds that are eluted earlier and together with the solvent peak during solvent extraction. In addition, a different aroma dilution method using SPME has been used for the successive dilution of extracted volatiles by increasing the GC injector split ratio in headspace (HS)-SPME/GC-O (Kim et al., 2003).

It is well known that only certain key odorants present at concentrations above their respective odor thresholds can contribute to the overall aroma perception of a foodstuff (Schieberle & Hofmann, 2012). However, the contribution of each single odorant to the overall aroma of Chinese quince has not been reported previously. Therefore, the aim of present study was to investigate and compare characteristic aroma active compounds from both peel and pulp in Chinese quince. Two different extraction methods such as HVD and HS-SPME, combined by GC-O and GC–MS, were used to compensate for each other in the analyses of aroma-active compounds and volatile compounds, as explained earlier. The potent aroma-active compounds were determined by GC-O with AEDA (Grosch, 1993) and an aroma dilution method using HS-SPME, including varying the GC injector split ratio (Kim et al., 2003). The determination of aroma-active compounds will help in the understanding of organoleptic properties of the peel and pulp in Chinese quince.

2. Materials and methods

2.1. Materials

Chinese quince (*Pseudocydonia sinensis* Schineid) was purchased at a local farm in Cheongdo-si, Gyeongsangbuk-do, Korea. All samples were separated into pulp and peel and each part was cut into thin slices. All Chinese quince samples were stored at − 70 °C in a deep freezer prior to the extraction of volatile compounds. Authentic compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA), Aromaline (Seongnam-si, Gyeounggi-do, Korea), Seoul Aromatics (Seoul, Korea), and Borak (Seoul, Korea).

2.2. Extraction of volatile flavor compounds from Chinese quince

2.2.1. HVD

The peel and pulp of sample (30 g) were directly extracted with 300 mL of dichloromethane (Fisher Scientific Korea Ltd., Seoul, Korea). The each sample suspended in dichloromethane was magnetically stirred at 200 rpm for 3 h and then filtered (paper no. 41, Whatman, Maidstone, U.K.) under vacuum. After direct extraction, the extracts were evaporated to 50 mL using a Vigreux column (50 cm × 3 cm) in a 45 °C water bath. Following the procedure of extraction reported previously, the volatiles were separated using high-vacuum distillation (HVD) (Cho et al., 2006). The extraction system was composed of a high vacuum pumping system (Model VPC-250F, ULVAC KIKO, Inc., Yokohama, Japan) connected with custom made glassware (Chang Young Scientific, Seoul, Korea). The solvent extract was placed in an addition funnel which was added drop by drop into bottom flask when the operating vacuum level reached below 3×10^{-5} Torr at 45 °C with magnetic stirring of 300 rpm. The HVD extract was collected in two liquid nitrogen traps. After that, the collected extract was concentrated to 10 mL with Vigreux column in 45 °C water bath, dried over anhydrous sodium sulfate (Na_2SO_4) and then concentrated to 0.2 mL using a gentle stream of nitrogen (N_2) gas. All extractions were performed in triplicate.

2.2.2. HS-SPME

Each of the cut samples (4 g, peel and pulp of Chinese quince) was added in a 40 mL glass vial equipped with an open center screw cap and a Teflon/silicon septum (Supelco, Bellefonte, USA), and then equilibrated for 1 h at 40 °C. Volatiles were adsorbed on SPME fiber which was coated with 75 μm Carboxen/PDMS (CAR/PDMS) (Supelco, Bellefonte, PA, USA) for 20 min with 20 mm of needle length. After adsorption, the extracted volatiles were desorbed at 200 °C for 5 min in a gas chromatograph (GC) injection port.

In the preliminary experiment, diverse SPME fibers coated with different layers of stationary phases, such as 100 mm PDMS, 75 mm Carboxen (CAR)/PDMS, 65 mm PDMS/divinylbenzene (DVB), and 50/30 mm DVB/CAR/PDMS, were tested. Among them, CAR/PDMS fiber showed the best performance in terms of reproducibility and recovery for the analysis of volatile compounds in the peel and pulp of Chinese quince fruits. Also, other SPME conditions including adsorption/desorption times and needle length of fiber exposed were optimized. The fiber was cleaned by baking in the GC injector for 30 min before use. All experiments were conducted in triplicate.

2.3. Analysis of volatile flavor compounds from Chinese quince

2.3.1. GC-mass spectrometry (GC–MS)

GC–MS analysis was performed using an HP 5890 Series II GC/5972 mass selective detector (MSD) (Hewlett-Packard, Palo Alto, USA) equipped with a capillary column, DB-5ms and DB-wax (30 m length × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, Folsom, USA) for analyzing HVD extracts. For HS-SPME/GC–MS analysis, DB-5ms column was used. The carrier gas was helium at a constant flow rate of 0.8 mL/min. One microliter of the extracts from HVD was injected (splitless mode) into each column at 40 °C and then held for 5 min. The oven temperature was held at 40 °C for 1 min, then increased to 200 °C at 4 °C/min, and held at 200 °C for 20 min. The temperatures of injector and ion source were 200 and 250 °C, respectively. The mass detector was operated in the electron impact mode with an ionization energy of 70 eV and a scanning range of 33–550 amu.

2.3.2. GC-olfactometry (GC-O)

The aroma-active compounds of peel and pulp from Chinese quince were analyzed by GC-O with AEDA. GC-O was performed using a Varian 3900 GC (Walnut Creek, USA) equipped with a flame ionization detector (FID) and a sniffing port (ODO II, SGE, Ringwood, Australia). The Effluent collected from the end of GC column was split equally between the FID and the sniffing port. After the 1 μL aliquot was injected into the GC column, the GC oven temperature was held at 40 °C for 5 min and then increased to 200 °C at 10 °C/min and held there for 24 min. The injector and detector temperatures were 200 and 250 °C, respectively.

2.3.3. Aroma dilution analyses

The odor descriptions and flavor dilution (FD) factors of each odorant were determined by AEDA (Grosch, 1993). The original extracts (final volume = 0.2 mL) from HVD were diluted stepwise by dichloromethane [each dilution was 1:3 (v/v)], and each dilution was analyzed until no odor was perceivable at the sniffing port. The FD factors were defined as the highest dilution at which a compound could be still perceived. Three trained panelists determined the odor descriptions. Their maximum value was then provided as the FD factor of that compound.

The volatiles extracted by HS-SPME were stepwise diluted by controlling the split ratio through an electronic flow control system (Kim et al., 2003). The split ratios used for aroma dilution were 1 (splitless), 9, 27, 81, 243, 729 and 2187. In this case, FD factor was defined as the maximum split ratio at which a compound could be detected at the sniffing port by three panelists familiar with Chinese quince aroma. The other GC conditions were the same as those for the analysis of HVD extracts.