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Characterization of aroma-active volatiles in three Chinese bayberry (*Myrica rubra*) cultivars using GC–MS–olfactometry and an electronic nose combined with principal component analysis

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ABSTRACT

Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) is one of the most popular and valuable fruits in China because of its unique and exquisite flavor. In this study, headspace solid-phase micro-extraction (HS-SPME) coupled with gas chromatography–mass spectrometry and olfactometry (GC–MS–O) analyses were used to characterize the aroma-active profiles of the fruits from three different bayberry cultivars. The aim was to differentiate the bayberry cultivars by their aroma. Fifty-five volatile components, composed of aldehydes (10), alcohols (9), esters (8), terpenes (17), and others (11), were identified by optimized HS-SPME/GC–MS. Meanwhile, 36 aroma-active compounds were detected by olfactometry using detection frequency analysis (DFA). Hexanal (grass-like), (E)-2-hexenal (green), nonanal (fruit, flower), 1-hexanol (flower), and isocaryophillene (wood) were identified in all three cultivars. Further principal component analysis (PCA) of the active aromas revealed their contributions to the odor differences among the bayberry cultivar groups. The BQ bayberry was characterized by having a stronger "herb" odor, which is mainly caused by benzoic acid and methyl ester. DK bayberry had a stronger "grass" odor, which is mainly caused by 2,6-dimethyl-2,4,6-octatriene, while FHZ bayberry had a stronger "pine" odor, which is caused mainly by α-pinene. The GC–MS–O and electronic nose techniques, when combined with PCA, could be used to successfully distinguish between different bayberry cultivars.

1. Introduction

Chinese bayberry (*Myrica rubra* Sieb. et Zucc) fruits are one of the most appealing fruits in the markets because of their unique flavor, taste, and high levels of nutritional components such as carbohydrates, sugars, organic acids, minerals, vitamins, and polyphenols (Cheng et al., 2009; Fang, Zhang, Lü, Liu, & Ye, 2009; Zhou et al., 2009). However, they are quite perishable and have short fruiting seasons. Therefore, in order to increase their consumption and their shelf life, the flesh from bayberry fruits is processed into sweets, jam, juice, and wine, or canned in syrup (Shao & He, 2007). Bayberry fruit juice and wine are also important Chinese export products, which means that bayberry has a relatively high export value (Fang & Bhandari, 2012).

Aroma is one of the most valued attributes of Chinese bayberry fruits and can greatly influence the consumers' acceptance of the bayberry fruit and related fruit-products. Gas chromatography–mass spectrometry (GC–MS) has been widely used to analyze the aroma composition of fruits. However, not all volatile compounds are aroma-active in some products due to different odor thresholds and interactions between compounds. One of the major difficulties when studying odors is the identification of those compounds that really contribute to the food flavor. A combination of GC–MS with olfactometry and detection frequency analysis (DFA) has been used to identify odor-active compounds (Pang, Chen, Hu, Zhang, & Wu, 2012; Pang et al., 2012). It has been extensively used in the flavor analysis of a number of fruits, especially oranges (Plotto, Margaria, Goodner, & Baldwin, 2008), cherry (Sun, Jiang, & Zhao, 2010), muskmelon (Pang, Guo, et al., 2012b), guava (Pino & Bent, 2013), papaya (Pino, 2014), and banana (Pino & Febles, 2013). However, there is limited information available on the volatile compositions of fruits from different bayberry varieties and cultivars, and very few studies have focused on the aroma-active components of Chinese bayberry. Kang, Li, Xu, Jiang, and Tao (2012) reported that ethyl acetate, 1-hexanol, (Z)-3-nonen-1-ol, and β-caryophyllene were the predominant flavor volatiles. However, studies on the aroma-active volatiles, instead of volatiles in general, are more important when it comes to the development of Chinese bayberry products.

Chinese bayberry cultivars and plants that have been grown in different locations have different flavors, which hinder product development and reduces economic value. Furthermore, it is quite difficult to differentiate between the flavors of different Chinese bayberry cultivars, especially when it has been processed into a product. To our best knowledge, there have been no studies that have classified the volatile profiles and the aroma-active compounds produced by different Chinese

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bayberry (*M. rubra* Sieb. et Zucc.) cultivars using GC–MS–O, with or without PCA analysis, which is a multivariate statistics method.

GC–MS studies have mainly focused on the measurement of certain volatile components, while the electronic nose (e-nose) is an instrument that uses chemical sensors to detect volatiles and then provide a pattern output showing the component combinations contributing to a defined smell. It has been successfully used to classify olive oil (Haddi et al., 2013), differentiate between ginseng made from varieties that have different varietal origins (Li et al., 2012), identify different milk flavorings (Wang, Xu, & Sun, 2010), authenticate different cherry tomato juices (Xuezhen, Jun, & Shanshan, 2014), and characterize different famous liquors (Xiao, Yu, & Niu, 2014). Thus, it could potentially be used to classify different bayberry samples.

The aim of this study was to detect differences in the volatile composition of three bayberry cultivars. These differences could then be used to identify the different bayberry cultivars. Principal component analysis (PCA) grouped the samples according to their volatile profile. The data from the GC–MS–O analysis and the electronic nose were then statistically analyzed in order to detect the volatile compounds that could be used to differentiate the bayberry cultivars according to their cultivar group.

2. Materials and methods

2.1. Fruit materials

Bayberry cultivars that produced fruits with good edible qualities and had been planted over large areas of land were collected in Zhejiang Province, China, during May and June, 2013. The following cultivars were collected: BQCX (Biqi, CiXi), BQXJ (Biqi, XianJu), DKNH (Dongkui, NingHai), DKXJ (Dongkui, XianJu), and FHZ (Fenhongzhong, ShangYu). This meant that there were three cultivar groups: BQ, DK, and FHZ. After the fruit had been picked, they were kept cold in baskets and immediately transported to the laboratory where they were frozen in liquid nitrogen within 12 h and stored at −80 °C until needed for analysis.

2.2. Chemicals

A mixture of n-alkanes (C_8–C_{20}) was used for the retention index (RI) analyses. The experimental procedure and the RI calculation were carried out according to the chemical manufacturer's instructions (Sigma Chemical Co., St. Louis, MO, USA). The cyclohexanone (9.46 mg/kg of fruit juice), used as the internal standard, was purchased from J&K Chemical Ltd (Shanghai, China). The sodium chloride used for volatile extraction and the other reagents were all either analytical grade or the highest purity that was commercially available.

Four different coating fibers for headspace solid-phase microextraction (HS-SPME) were tested. These were 100 μm polydimethylsiloxane (PDMS), 75 μm carboxen/polydimethylsiloxane (CAR/PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), and 50/30 μm DVB/CAR/PDMS. They were purchased from Supelco, Inc. (Bellefonte, PA, USA).

2.3. Optimal conditions for headspace sampling and SPME

A manual SPME (Supelco, Inc.) fiber was used for volatile extraction after the fiber had been conditioned. The flesh was ground to pulp by a commercial blender. Then the bayberry pulp (4 g) and the internal standard (10 μL) were immediately transferred to a 15 mL headspace bottle containing 4 g of sodium chloride (NaCl) saturated solution (Aprea et al., 2012). The mix was then equilibrated with a laboratory stirrer/hot plate (model PC-420, Corning Inc. Life Science, Acton, MA, USA). After the solution had equilibrated, a stainless steel needle, housing the SPME fiber, was placed through a hole in the top of the headspace bottle and fed through until it was 1 cm above the liquid surface. Then the samples were magnetically stirred at 800 rpm. Three independent extractions were carried out for each bayberry sample.

In order to improve volatile compound absorption, the following experimental parameters were investigated: four coating fibers (PDMS, CAR/PDMS, PDMS/DVB and DVB/CAR/PDMS); incubation times between 5 and 20 min; extraction temperatures between 35 °C and 65 °C; and extraction times between 10 and 40 min. The analysis was conducted in triplicate for each parameter investigated.

2.4. GC–MS analysis

The SPME extract was injected into the port of an Agilent 7890A-5975C GC/MSD equipped with a DB-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness) (Agilent Technologies) and desorbed at 250 °C for 3 min. The injection port was operated in split mode (1:10), and 99.999% pure helium was used for vial pressurization and as the carrier gas. The helium flow rate was 1.4 mL/min. The initial oven temperature was 40 °C (2 min), which was ramped up at 5 °C/min to 170 °C and held there for 2 min. Then it was ramped up at 10 °C/min to 250 °C and held there for 5 min. The mass detector was operated in the electronic impact (EI) mode at 70 eV and the source temperature was set at 250 °C. The mass spectra were scanned in the m/z 29–350 amu range at 1 s intervals.

2.5. Detection frequency analysis by GC–MS–O

The odor-active compounds were characterized by a sniffing port (Sniffer 9000, Brechbühler, Switzerland) coupled to a GC–MS (7890A-5975C, Agilent Technologies, Inc.). At the exit of the capillary column, the effluents were split 1:1 (by volume) into a sniffing port and an MS detector by employing Agilent capillary flow technology. The transfer line to the GC–O sniffing port was held at 260 °C. The GC–MS conditions were the same as those described above.

An eight-member panel of assessors was required to individually sniff the GC effluent and report their results by the GC–O analyses. The panel consisted of a mixed group of both sexes, aged between 20 and 30 years. The panelists were trained prior to the sensory analysis so that they could become familiar with the odor descriptions for solutions of artificial odorants and different bayberry samples. In total, eight GC–O runs were performed (one run for each assessor). The aroma-active compounds perceived by the panelists were recorded when sniffing the effluent from the sniffing mask. The panelists also noted the perceived odor characteristics and intensities. At the sniffing port, any odorant that had total detection frequencies ≥ 2 was arbitrarily considered to have potential aroma activity (Pang et al., 2012a).

2.6. Electronic nose system

An electronic nose (e-nose, PEN2, Airsense Analytics, GmBH, Schwerin, Germany) was used to discriminate between the odor patterns produced by the different cultivars. The e-nose consists of a sampling apparatus that is exposed to the volatiles, an array of sensors composed of ten different metal-oxide semiconductors (MOS) enclosed in a small chamber, and computer-controlled pattern recognition software.

Each sample was placed in an airtight 500 mL glass jar (concentration chamber). The glass jar was then closed and the headspace inside was equilibrated for 30 min. The measurement process consisted of three different phases: concentration, measurement, and standby. Airflow was always kept constant throughout the concentration chamber during the three phases. The measurement phase lasted 70 s and the collected data interval was 1 s so that the sensors could reach a stable value. When a measurement was completed, a standby phase was activated (70 s) in order to clean the circuit and return the sensors to their baseline. All the e-nose measurement procedures were carried out at 25 ± 1 °C. Each analysis was repeated more than four times, and all of the sensor response data were analyzed by the e-nose software.