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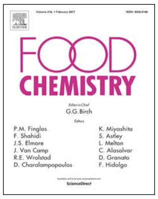

Characterization of the key aroma compounds in mulberry fruits by application of gas chromatography–olfactometry (GC-O), odor activity value (OAV), gas chromatography-mass spectrometry (GC–MS) and flame photometric detection (FPD)

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ABSTRACT

The volatile compounds of mulberries obtained from three cultivars (*M. nigra*, Y1, *M. Macroura*, Y2 and *M. Alba*, Y3) were investigated by gas chromatography–olfactometry (GC-O), odor activity value (OAV), gas chromatography-mass spectrometry (GC–MS) and flame photometric detection (FPD). The results showed that 41, 37 and 41 compounds were detected in Y1, Y2 and Y3 samples, respectively. In addition, 12, 11, and 12 sulfur compounds were identified in three samples by FPD, respectively. Quantitative analysis showed 2-(methylthio) ethanol, methionol, dimethyl sulfide, methional and 3-ethylthiophene were present in relatively high amounts in each of the three samples. Furthermore, benzaldehyde, ethyl butanoate, *(E)*-2-nonenal, 1-hexanol, hexanal, methional, 3-mercaptohexyl acetate and 3-mercaptohexanol were present with much higher OAVs than other compounds. Finally, four compounds (pentanal, ethyl acetate, acetic acid, methionol) were selected to investigate possible effects on six descriptors, which indicated that the volatile compounds at sub-threshold concentrations are likely to contribute to overall aroma.

1. Introduction

Aroma is an important characteristic of fruits and a key indicator for evaluating fruit quality. Although the majority of aroma volatiles in fruits are esters, aldehydes, terpenoid hydrocarbons and terpenols, small quantities of other specific volatiles, such as sulfur compounds, also contribute to the characteristic aromas of fruits. For example, methanethiol, ethanethiol, propanethiol, dimethyl disulfide, 3-mercapto-1-hexanol and 4-mercapto-4-methyl-2-pentanone are important aromas in cranberry fruits (Zhu et al., 2016). Furthermore, 4-mercapto-4-methyl-2-pentanol, 4-mercapto-4-methyl-2-pentanone, 3-(mercapto) hexyl acetate and 3-mercapto-1-hexanol are important contributors to the aroma of Sauvignon blanc (Coetzee & Toit, 2012). Methional, 2-methylthioethanol, 3-mercapto-3-methylbut-1-ol and furfuryl mercaptan are also found in raspberries (Duarte et al., 2010). Sulfur compounds have been widely identified in grape, grapefruit, cheeses, ham, blueberry and cranberry (Bogicevic et al., 2013; Coetzee & Toit, 2012; Du & Rouseff, 2014; Duarte et al., 2010; Jabalpurwala, Gurbuz, & Rouseff, 2010; Sekhon et al., 2010; Zhu et al., 2016). Although the amounts of sulfur compounds in these foods are low, they greatly contribute to the aroma because of their extremely low odor thresholds.

The mulberry fruit is rich in carbohydrates, salts, minerals, vitamins, fatty acids, amino acids and proteins. Due to its high nutrition properties, it is widely used in processing industries all over the world (Ercisli & Orhan, 2007; Jiang & Nie, 2015; Wang, Han, Jia, & Yang, 2016). Recently, studies on mulberry fruits have focused on the antioxidant, chemical and biological properties of the fruit (Ercisli & Orhan, 2008; Natić et al., 2015; Niu, Liu, & Liu, 2014; Sun, Zhang, & Guo, 2016; Tomas et al., 2014; Wang et al., 2016)· There are only a minority of reports aiming to investigate the aroma compounds of mulberry fruits, which remain to be fully characterized (Chen, Kan, & Yang, 2010; Elmaci & Altug, 2002; Feng et al., 2015).

In a previous study, the flavor characteristics of three black mulberry (*Movws nigva*) cultivars were investigated using GC–MS and sensory analysis techniques (Elmaci & Altug, 2002). The results revealed that ethyl linolenate was the most important contributor to the unique flavor of black mulberry. Another study which investigated the aroma of mulberry wine detected a total thirty-one volatile compounds

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(Feng et al., 2015), amongst which 3-methyl-1-butanol, 2-methyl-1-propanol, 1-octanol and hexyl acetate were present in relatively high amounts. Moreover, fruity and floral odor compounds contributed to the aroma of mulberry wine. In addition, the aroma compounds in different varieties of mulberries were extracted by solvent extraction and analyzed by gas chromatography-mass spectrometry (Chen et al., 2010). The results indicated that long chain fatty acids, fatty acid esters, fatty alcohols, aromatic alcohols, fatty aldehydes and fatty ketones were the major aromatic components.

The studies described above focused mainly on the identification of aroma compounds of mulberry. However, sulfur compounds are also considered to be important to the aroma of fruit yet less attention has been paid to these compounds in mulberry.

The aims of the current study were to identify the key aroma compounds in mulberry fruit using gas chromatography–olfactometry (GC-O) and odor activity value (OAV), to further identify the volatile and sulfur compounds by gas chromatography-mass spectrometry (GC–MS) and flame photometric detection (FPD), and finally, to investigate interactions between different aroma compounds in mulberry.

2. Materials and methods

2.1. Chemicals

Acetaldehyde, ethyl acetate, 2-methylbutanal, 3-methylbutanal, pentanal, 1-penten-3-one, 2-pentanone, methyl butanoate, ethyl butanoate, hexanal, pentanoic acid, ethyl 2-methylbutanoate, (*E*)-2-pentenal, 1-butanol, 2-heptanone, heptanal, (*E*)-2-hexenal*,* (*Z*)-2-hexenal, ethyl hexanoate, 2-octanone, 3-methyl-1-butanol, 1-pentanol, (*E,E*)-2,4-hexadienal, hexyl acetate, octanal, 6-methyl-5-hepten-2-one, 1-hexanol, nonanal, (*Z*)-3-hexen-1-ol, 2-nonanone, (*E*)-2-octenal, 1-octen-3-ol, ethyl octanoate, furfural, acetic acid, (*E,E*)-2,4-heptadienal, 2-ethyl-1-hexanol, benzaldehyde, (*E*)-2-nonenal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,6-nonadienal, 2,3-butanediol, butanoic acid, ethyl decanoate, acetophenone, 1-nonanol, phenethyl acetate, γ-nonalactone, 3-hydroxy-2-butanone*,* (*Z*)-2-heptenal and 1,3-dichlorobenzene were purchased from Alfa Aesar Corporation (Tianjin, China). Methanethiol, dimethyl sulfide, ethanethiol, propanethiol, 2,5-dimethylthiophene, 3-ethylthiophene, methyl 1-propenyl disulfide, 3-mercapto-2-butanone, 3-mercapto-2-pentanone, methional, 2-(methylthio)-1-ethanol, methionol, dipropyl disulfide, 3-mercaptohexyl acetate and 3-mercapto-1-hexanol were purchased from Sigma-Aldrich (St Louis, MO). All of the chemical standards used above were of GC quality.

2.2. Materials

Ripe samples consisted of three varieties of mulberry (Y1, *Morus nigra* L. cv. 'Dashi', Y2, *Morus macroura* M. cv. 'Changshang', Y3, *Morus alba* L. cv. 'Baishang'), collected in Qingpu Mulberry Fruit Field of Shanghai on May 5, May 5 and May 7, 2017, respectively. Mature fruit without any physical damage, nor fungal infection, were selected. One kilogram of mulberries was crushed and blended in a glass container at 100 rpm for 5 min. Then, the mulberry musts were immediately employed in the next experiment.

2.3. Solid phase microextraction (SPME) absorption of aroma compounds

The manual SPME holder, together with 20-mL vials, Teflon covers and one 100 µm Carboxyl-divinylbenzene-polydimethylsiloxane- (CAR-PDMS-DVB) fiber were purchased from Supelco, Inc. (Bellefonte, PA). The length of the fiber was 1 *cm*. The fiber penetration depth into the headspace was 2 *cm*, which was consistent in the whole experiment. Before chemical absorption, the fiber was preconditioned for 60 min on an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) with an injector temperature of 250 °C. The main parameters that were known to influence the methodology were investigated. The optimized SPME experimental conditions were established, i.e. 45 min of extraction time, a sample volume of 5 g, 30 °C extraction temperature. The vials with 5 g of musts and 0.1 g of the internal standard solution containing 10 mg kg^{-1} of 1,3-dichlorobenzene (or 0.01 g of the internal standard solution containing 10 µg kg^{-1} of dipropyl disulfide) were kept at 30 °C in a water bath with shaking at regular intervals. The equilibration time was 10 min. After the SPME fiber had been exposed in the headspace of the vial for 45 min, it was then withdrawn and directly introduced to the GC-FID-O, GC–MS and GC-FPD for desorption and analysis. The desorption time was 3 min; then the fiber was cleaned for 60 min in the 6890 gas chromatograph injector at 250 °C.

2.4. SPME-GC-FID-O analysis of mulberry

An Agilent 6890 chromatograph equipped with an ODP-2 olfactory detector port (Gerstel, Mulheim an der Ruhr, Germany). provided an FID signal alongside the odor characteristics of each compound detected by sniffing port. GC effluent was split 1:1 between the FID and sniffing port. Samples were separated using both a DB-Wax analytical fused silica capillary column and a DB-5 analytical fused silica capillary column (both 60 m × 0.25 mm × 0.25 µm; Agilent, Santa Clara, CA). Conditions for GC-FID-O analysis were as follows: The flow rate of carrier gas (nitrogen) was 1.8 mL min^{-1}. The oven temperature was held at 40 °C maintained for 2 min, then ramped at a rate of 2 °C/min to 60 °C, and ramped to 230 °C at a rate of 4 °C/min (the final temperature maintained for 5 min). The injection port was set in splitless mode for 3 min at 250 °C. The desorption time was 3 min. Moist air was pumped into the sniffing port at 40 mL min^{-1} to quickly remove the odorant eluting from sniffing port. Each of the GC-O users was trained to recognize odors by using solutions of artificial odorants. The aroma-active compounds perceived by ten assessors were recorded as the time for onset and end while sniffing the effluent from the sniffing port. The assessors also noted the perceived odor characteristics and intensities of aroma extracts by SPME-GC-FID-O, to obtain an aroma profile for the mulberry must. The frequency was also calculated to evaluate the number of times odors were perceived by assessors. At the same time, the computer automatically recorded the retention time and sniffing time of aroma-active compounds individually. The aroma intensities (AIs) were evaluated using 10-point intensity scale from 0 to 10; "0" was none, "5" was moderate, and "10" was extreme. The experiment was carried out in triplicate by each assessor. Finally, the aroma intensity was the average from ten assessors.

2.5. SPME-GC–MS of volatile compounds in mulberry musts

A 6890 gas chromatograph with SPME and a 5975 mass selective detector (MSD) (Agilent Technologies, USA), DB-Wax and DB-5 analytical fused silica capillary column (60 m × 0.25 mm × 0.25 µm; Agilent, Santa Clara, CA) were used for analyzing the volatile compounds. The injection port was set in splitless mode for 3 min at 250 °C. The carrier gas was helium at a constant flow rate of 1 mL min^{-1}. The MSD was used for chemical identification. Its electron ionization energy was 70 eV. The ion source temperature was set at 230 °C. The quadrupole mass filter was operated at 150 °C. The transfer line temperature was 250 °C. The chromatograms were recorded by monitoring the total ion currents from *m/z* 30–450. The temperature program was identical to that used for SPME-GC-O. The volatile compounds were identified by comparing retention indices, retention times with those obtained for authentic standards, or with mass spectra in the W8N08 Database (Hewlett-Packard, Palo Alto, CA). The retention indices (RIs) of unknown compounds were determined via sample injection with a homologous series of straight-chain alkanes (C_6–C_{30}) (Sigma-Aldrich, St. Louis, MO).