Food Chemistry 226 (2017) 51–60

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Volatile flavor constituents in the pork broth of black-pig

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ARTICLE INFO

Article history:
Received 2 August 2016
Received in revised form 17 November 2016
Accepted 3 January 2017
Available online 4 January 2017

Keywords:
Black pig
Broth
Odor-active
Meat flavor
GC–MS/MS
Quantitation

ABSTRACT

Pork of black-pig in China is well known for its quality and preferred by consumers. However, there is a lack of research on its flavors. By solvent assisted flavor evaporation combined with GC–MS, 104 volatile compounds in the stewed pork broth of black-pig were identified with the dominant amounts of fatty acids, alcohols, and esters. By aroma extract dilution analysis-GC-O method, 27 odor-active compounds were characterized, including 2-methyl-3-furanthiol, 3-(methylthio)propanal, 2-furfurylthiol, γ-decalactone, nonanal, (*E*)-2-nonenal, and (*E*,*E*)-2,4-decadienal that had high FD factors. Compared to the common white-pig, the aroma compounds in both pork broths were almost the same, but the aroma profile of potent odorants for the black-pig pork broth showed less fatty and more roasted notes, which were partially attributed to the higher monounsaturated fatty acids and lower polyunsaturated fatty acids in meat. With aid of authentic chemicals and selected reaction monitoring mode of GC–MS/MS, 19 aroma compounds were quantitated.

1. Introduction

Flavor is one of the most important sensory attributes for consumers to judge the quality and acceptability of foods, including meat. The water-soluble components in meat, such as reducing sugars and amino acids, can induce the Maillard reaction during the thermal processing; Simultaneously, the lipids can undergo the lipid oxidation and degradation, all of which lead to the formation of meat flavor (Mottram, 1998). Up to now, more than 1000 volatiles have been identified from various meats and meat products, including characteristic sulfur-containing compounds, heterocyclic compounds, aldehydes, ketones, alcohols, acids, esters, and hydrocarbons (Shahidi, 1998). However, among a great number of volatile compounds in a food, only a small number of them possess odor activities that truly contribute to the overall aroma. Therefore, it is of significance to elucidate which volatile compounds have odor-activities and play important roles in food flavor.

Gas chromatography-olfactometry (GC-O) is just a method to screen the odor-active compounds in food. By the GC-O analysis, 43 odor-active compounds in the roasted pork of Mini-pig (Xie, Sun, Zheng, & Wang, 2008), 16 in the cooked cured pork ham (Benet et al., 2016), and 41 in the grilled beef of 18 to 19-month-old steers (Resconi et al., 2012), were characterized. Regarding the GC-O, four detection techniques, including frequency detection, time-intensity, aroma extract dilution analysis (AEDA), and charm analysis, are often used to evaluate the significance of a sniffed odorant. For the AEDA, the aroma extract is diluted gradually until no odor is detected in GC-O analysis, in which the higher dilution of a compound suggests its more contribution to the overall aroma. By the AEDA/GC-O, 3-(methylthio)propanal, 3-mercapto-2-methyl-pentan-1-ol, (*E*,*E*)-2,4-decadienal, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, vanillin, (*E*,*E*)-2,4-nonadienal, and (*E*)-2-undecenal had been exposed to be the key aroma constituents in stewed beef and pork vegetable gravies (Christlbauer & Schieberle, 2009).

The Chinese pork market is generally dominated by and produced from the Large White pig, also called common white-pig, which is an exotic breed of pig originating in Yorkshire, England. In addition, there is a special product in local market called black-pig pork, which is preferred by consumers due to its quality, as well as its delicious taste and special flavors of the stewed meat broth, although its sale price is two to three times of the common white-pig pork. The black-pig usually is developed from a Chinese indigenous pig and an exotic pig. However, as far as we know, there had been a lack of research on the flavor of the pork of the black-pigs.

Recently, aroma composition of the pork broth stewed with the loins of the common white-pigs had been analyzed by Xu et al.

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http://dx.doi.org/10.1016/j.foodchem.2017.01.011
0308-8146/

(2011), Wang, Song, Zhang, Tang, and Yu (2016). Our research team also reported the volatile flavor compounds in the pork broth stewed with hind quarters of the common white-pigs (Wang et al., 2015). Furthermore, in the present work, the volatile flavor compounds in the pork broth stewed with the hind quarters of the black-pigs were studied. In addition, comparison of the composition of fatty acids and amino acids in meat and the volatile flavor compounds in the stewed pork broth between the common white-pig and the black-pig were discussed.

2. Materials and methods

2.1. Materials

Different batches of meats from hind quarters of eight Yunan black-pigs (in two slaughtering days) were purchased from Wu-Mart supermarket (Beijing, China). Yunan black pig has the mixed ancestry of purebred Huainan pig and Duroc, produced in Henan province, China. After removing the pork skin, visible fat, and connective tissues, the meat was cut into small cubes of about 0.5 cm^3, which were mixed well, and stored in a refrigerator at −20 °C for 72 h maximum before use.

2.2. Chemicals

1,2-Dichlorobenzene (99%) (internal standard), and n-alkanes ($C_6 \sim C_{25}$) for retention indices, were purchased from Beijing Chemical Reagents Co. Ltd. (Beijing, China). The authentic chemicals used for identification and/or quantitation were mainly in a purity over 95% (GC), including dimethyl disulfide (98%), dimethyl trisulfide (98%), 2-furfurylthiol (98%), 2-thiophenecarboxaldehyde (98%), 4-methyl-5-thiazoleethanol (98%), bis(2-methyl-3-furyl) disulfide (98%), furfural (98%), (*E*)-2-heptenal (95%), benzaldehyde (98%), phenylacetaldehyde (98%), 3-hydroxy-2-butanone (95%), 1-penten-3-ol (98%), 1-octen-3-ol (98%), phenylethyl alcohol (99%), furfuryl alcohol (98%), acetic acid (98%), and γ-decalactone (98%) were purchased from *J&K* Chemical Ltd. (Beijing, China). 2-Methyl-3-furanthiol (95%), 3-(methylthio)propanal (97%), 2-mercaptothiophene (96%), 2-ethyl-3-methylpyrazine (98%), benzothiazole (96%), 2-pentylfuran (98%), 2-methyl-2-butenal (96%), pentanal (95%), hexanal (95%), heptanal (95%), nonanal (95%), (*E*)-2-hexenal (95%), (*E*)-2-octenal (95%), (*E*)-2-decenal (93%), (*E,E*)-2,4-nonadienal (95%), (*E,E*)-2,4-decadienal (90%), (*E*)-2-undecenal (93%), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (98%), 1-pentanol (98%), 1-hexanol (98%), 1-heptanol (98%), 1-octanol (99%), 2-acetylthiazole (99%), 2-acetyl-1-pyrroline (98%), and (*E*)-2-nonenal (97%) were purchased from Sigma-Aldrich Co. Ltd (Shanghai, China).

2.3. Fatty acid analysis

The crude fat, extracted by the classical Soxhlet extraction using diethyl ether as a solvent, was 1.81% of the wet meat determined by the method of GB/T 5009.6-2003 (China National Standards: determination of fat in food). The analysis of fatty acids was conducted according to the method reported by Yang et al. (2015) by an Agilent 7890A/5975B gas chromatograph and mass spectrometer (Agilent Technologies, Santa Clara, USA) using a capillary column DB-5 MS (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Santa Clara, USA). The fatty acid methyl esters were identified by injection of the standards and the NIST 15 mass spectra database, and quantitated using C13:0 as an internal standard. Three replicates were performed.

2.4. Amino acid analysis

The crude protein content of the wet meat was 22.85%, which was analyzed by the method of the Association of Official Analytical Chemists (AOAC, 1990). The wet meat samples were dried in a vacuum-freeze dryer, and then finely ground to pass a 60-mesh sieve. After being hydrolyzed by 6 N HCl at 110 °C for 24 h, the amino acid composition was analyzed on a 30$^+$Automatic Amino Acid Analyzer (Biochrom Technologies, Cambridge, UK) equipped with a Biochrom Na^+ cation exchange resin column (20 cm × 4.6 mm ID, 5 μm). The detector wavelength for detection of amino acids was set at 570 nm, except the proline at 440 nm. The flow rates of ninhydrin and the Biochrom buffer solutions of mobile phase were 25 mL/h and 35 mL/h, respectively. The standards of eighteen amino acids were used for the construction of calibration curves. The content of an amino acid was expressed as mg/g dry meat. Three replicates were analyzed.

2.5. Pork broth preparation

Two hundred grams of the meat and 200 mL of water were placed in a 1000 mL 3-neck flask, which was fitted with a reflux condenser and a mechanical stirrer. The meat was stewed at ca. 100 °C for 3 h by an oil bath. Three replicates were performed and subjected for the following analyses.

2.6. Solvent-assisted flavor evaporation

With the meat residue filtered out, the broth was extracted three times by dichloromethane (3 × 200 mL). The volatiles in the extract solution were carefully isolated at 40 °C using the solvent-assisted flavor evaporation (SAFE) apparatus. The high vacuum (10^{-4} to 10^{-5} Pa) was achieved by an Edwards vacuum pump system (Edwards Abatement & Integrated Systems, Clevedon, United Kingdom). Liquid nitrogen was used to condense the distillate. The distillate was dried over anhydrous sodium sulfate, concentrated to about 1 mL in a Vigreux column (50 cm × 1 cm) and finally to 0.35 mL under a stream of gentle nitrogen gas.

2.7. Gas chromatography and mass spectrometry (GC–MS)

The same GC–MS mentioned above for *fatty acid analysis* was used. Two capillary columns were used, including DB-Wax (30 m × 0.25 mm × 0.25 μm) and DB-5 MS (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Santa Clara, USA). For the DB-Wax, the initial oven temperature was 40 °C, then ramped to 180 °C at 2.5 °C/min, and finally ramped to 230 °C at 10 °C/min. For the DB-5 MS, the initial oven temperature was 40 °C, then ramped to 150 °C at 2.5 °C/min, and finally ramped to 280 °C at 10 °C/min. Ultra high purity helium (⩾99.999%) was used as the carrier gas at flow rate of 1 mL/min. The sample was injected in 1 μL at 250 °C in a splitless mode.

The ion source temperature was at 150 °C in electron impact mode at 70 eV. The transfer line temperature was at 230 °C. The MS was detected in the 50 ∼ 450 mass range with a solvent delay of 2.5 min.

The compounds were identified by comparing their mass spectra with NIST 15 mass spectra database and their linear RI (retention index) values relative to $C_6 \sim C_{25}$ n-alkanes with those published, and also confirmed by the injection of available authentic chemicals. The quantity of a compound in the concentrate after SAFE treatment was approximately calculated by its peak area to that of 1,2-dichlorobenzene (internal standard, 200 μg/mL in dichloromethane) using a calibration factor of 1. Then it was converted into μg/kg of wet meat, according to the yield of the SAFE