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Volatile flavor constituents in the pork broth of black-pig

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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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(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

concentrate relative to the meat used for the broth preparation. The final results were the averages of three replicates.

2.8. GC-O analysis

An Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a FID detector and a DATU 2000 high-resolution olfactometer (DATU Inc. USA.) was used. The column was DB-5 30 m × 0.25 mm × 0.25 μm. The carrier gas was nitrogen at 1.0 mL/min. The initial oven temperature was 40 °C, then ramped to 280 °C at 5 °C/min. 1 μL of sample was injected in a splitless mode at 250 °C. The GC effluent to the odor port was enclosed with a stream of humidified air of 16 L/min and transferred by one length of stainless steel tube (10 mm i.d) to the Teflon detection cone.

The sample of the concentrate was diluted gradually using dichloromethane to obtain a series of dilutions (1:1, 1:2, 1:4, 1:8, 1:16,. . ., 1:1024, and so on) of the original solutions. Each dilution was submitted to GC-O analysis. The time to effuse dichloromethane was figured out in advance to avoid harm. The sniffing odor characteristics were recorded and each odorant was finally assigned a FD factor representing the highest dilution. Three trained sniffers performed the AEDA/GC-O analyses. Retention times of the odor responses were converted into linear retention indices (RI) relative to the series of *n*-alkanes ($C_6 \sim C_{25}$).

The identification of the odorants were based on the results by GC–MS, the linear retention indices, odor descriptions by GC-O, and the comparison of all the aforementioned analytical parameters with those of the available authentic chemicals listed in Section 2.2.

2.9. Quantitative determination of the odor-active compounds

The Trace 1310-TSR-8000 GC–MS/MS with a TG-5 MS 30 m × 0.25 mm × 0.25 μm column (Thermo Fisher Scientific, Waltham, USA) was used. The carrier gas was helium (⩾99.999%) in 1 mL/min. The mass spectrometry was set in a selected reaction monitoring (SRM) mode. The initial oven temperature was 30 °C, ramped to 60 °C at 5 °C/min; ramped to 70 °C at 0.5 °C/min; ramped to 96 °C at 10 °C/min; and finally ramped to 280 °C at 35 °C/min. The transfer line was at 280 °C. The sample injected was 1 μL at 250 °C in a splitless mode.

The analyzed samples were the concentrates mentioned above by the SAFE treatment. The identified odor-active compounds that were confirmed by the authentic chemicals and in detectable levels (S/N > 10) in the SAFE concentrates were determined. The calibration equation for each compound was obtained using the relative peak area to the internal standard, which was 1, 2-dichlorobenzene dissolved in dichloromethane. The ion pairs (parent ions to daughter ions) for quantitation with collision energies, the concentrations of the standard solutions and the internal standard solution prepared in dichloromethane, and the calibration equations obtained for the odorants were presented in Table 1. By the calibration equation, the concentration of an odor-active compound in the SAFE concentrate was calculated. Then it was converted to micrograms for per kg of wet meat (μg/kg meat), according to the yield of the SAFE concentrate relative to the meat used for the broth. The final results were the averages of three replicates.

2.10. Statistical analysis

All the results were the averages of three replicates. The figures were plotted by Microsoft Excel 2010. In Tables S1 and S2, differences between means were handled by one-way ANOVA with Duncan's multiple range tests using SPSS 19.0 for windows (SPSS Inc., Chicago, IL, USA). A *p*-level less than 0.05 was defined of significant difference.

3. Results and discussion

3.1. Fatty acids and amino acids

Fatty acids and amino acids are important precursors to meat flavor, while the fatty acids in meat is related to the characteristic flavor of different meat species (Mottram, 1998). As shown in Table 2, the total fatty acids was in sum of 52.33 mg/g meat, with the polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA), representing 6.42%, 57.94%, and 35.64%, respectively. The major PUFA, MUFA, and SFA were linoleic acid (5.52%), oleic acid (43.11%), and palmitic acid (21.69%) in turn. Comparing to our former work on the pork of the common white-pig (Wang et al., 2015) (Table S1), the content of crude fat in meat is nearly identical. However, the sum of the MUFA in the black-pig hindquarters (57.94%) was higher, while that of the PUFA (6.42%) was lower than its counterpart, the common white-pig (Table S1).

According to Cameron et al. (2000), the qualities in juiciness, tenderness, and flavor of pork were correlated positively with the MUFA but negatively with the PUFA in intramuscular fat due to the more vulnerable oxidation of the PUFA (Elmore, Campo, Enser, & Mottram, 2002; Elmore, Mottram, Enser, & Wood, 1999). So the higher consumers' acceptability of the pork of the black-pig possibly was related to its relatively higher level of MUFA as well as its relatively lower level of PUFA.

In Table 2, the total content of amino acids was 722.71 mg/g of meat. Glutamic acid was in the highest level (18.07%), followed by aspartic acid (10.33%), leucine (8.73%), arginine (6.90%), and alanine (6.15%). In comparison with the common white-pig (Wang et al., 2015) (Table S1), the black-pig meat had not only a higher total content of all the amino acids but also a higher level (28.40%) of umami taste amino acids including glutamic acid and aspartic acid. This difference in the composition of amino acid was similar to what had been reported between the muscles of Yunan black-pig and Landrace (Dou & Zhou, 2013).

3.2. Volatile compounds by GC–MS

The GC–MS analysis was carried out on both the DB-Wax and the DB-5 columns. The results were shown in Table 3. A total number of 104 compounds were identified, in sum of 333.90 μg/kg of the meat, according to the average results on the two columns (*also referring to the averaged results on the two columns in the following*). They were composed of sulfur-containing compounds, heterocyclic compounds, aldehydes, ketones, alcohols, acids, esters, aliphatic hydrocarbons, and others, of which the acids were in the highest amount, followed by the aldehydes, and the aliphatic hydrocarbons.

Sulfur-containing compounds and heterocyclic compounds can be formed from the Maillard reaction. The sulfur-containing compounds usually have meaty odors that result from the Maillard reaction of sulfur amino acids including cysteine and methionine (Lotfy, Fadel, El-Ghorab, & Shaheen, 2015). Seven sulfur-containing compounds (11.69 μg/kg) were found, with 4-methyl-5-thiazoleethanol being the major one in amount, followed by 2-acetylthiazole and 3-(methylthio)propanal. The heterocyclic compounds in Table 3 included four nitrogen-containing heterocycles (10.42 μg/kg), and three oxygen-containing heterocycles (2.91 μg/kg). The four nitrogen-containing heterocycles were pyridine, pyrazine, 2-ethyl-3-methyl-pyrazine, and 1-methyl-2-pyrrolidinone, which usually have the roasted odors. The

Table 1
The authentic chemicals, the analysis conditions, the concentrations of the prepared standard solutions, and the calibration equations in the quantitative determination of the odor-active compounds by GC–MS/MS in selected reaction monitoring (SRM) mode.

Compounds	RT/min	[1]SRM (*m*/*z*)	[2]Calibration equations	[3]Concentrations (μg/mL)
Pentanal	3.63	58 > 29 (10)	$y = 9.7724\ x$, $R^2 = 0.9948$	1.0, 5, 10, 25, 50
3-Hydroxy-2-butanone	3.84	73 > 45(5); 88 > 45(5)	$y = 17.727\ x$, $R^2 = 0.9996$	5, 10, 25, 50, 500
Hexanal	5.56	56 > 41 (10); 72 > 57(10)	$y = 7.6846\ x$, $R^2 = 0.9959$	1.0, 5, 10, 25, 50
(*E*)-2-hexenal	6.94	69 > 41 (10); 83 > 55 (5)	$y = 7.4210\ x$, $R^2 = 0.9985$	0.1, 0.5, 1.0, 2.0, 5
2-Methyl-3-furanthiol	7.35	85 > 45(5); 114 > 85(5)	$y = 1.8746\ x$, $R^2 = 0.9962$	0.1, 0.5, 1, 5, 10
Heptanal	8.61	55 > 29 (10); 70 > 55 (5)	$y = 2.4138\ x$, $R^2 = 0.9975$	0.1, 0.5, 1, 5, 10
3-(Methylthio)propanal	8.75	76 > 48(5); 104 > 48(10)	$y = 3.6742\ x$, $R^2 = 0.9994$	0.1, 0.5, 1, 5, 10
(*E*)-2-heptenal	11.23	55 > 29 (10); 83 > 55 (5)	$y = 3.8603\ x$, $R^2 = 0.9988$	0.1, 0.5, 1, 5, 10
Dimethyl trisulfide	11.81	79 > 64(15); 126 > 79(15)	$y = 1.2189\ x$, $R^2 = 0.9979$	0.1, 0.5, 1.0, 2.0, 5
1-Octen-3-ol	12.74	57 > 29 (10); 72 > 43 (5)	$y = 4.0858\ x$, $R^2 = 0.9965$	1.0, 5, 10, 25, 50
2-Thiophenecarboxaldehyde	13.88	82.9 > 39(10); 111 > 83(10)	$y = 20.721\ x$, $R^2 = 0.9985$	0.1, 0.5, 1.0, 2.0, 5
2-Ethyl-3-methylpyrazine	14.22	94 > 67(10); 121 > 94(10)	$y = 2.7390\ x$, $R^2 = 0.9989$	0.1, 0.5, 1.0, 2.0, 5
2-Acetylthiazole	15.29	99 > 58(20); 127 > 99(5)	$y = 8.4361\ x$, $R^2 = 0.9990$	0.1, 0.5, 1, 5, 10
Phenylacetaldehyde	17.45	91 > 65 (10); 120 > 91 (10)	$y = 1.3696\ x$, $R^2 = 0.9981$	0.1, 0.5, 1.0, 2.0, 5
Nonanal	24.30	57 > 29(10); 70 > 55 (5)	$y = 8.9647\ x$, $R^2 = 0.9940$	0.1, 0.5, 1, 5, 10
(*E*)-2-nonenal	31.85	83 > 55 (5)	$y = 4.3039\ x$, $R^2 = 0.9918$	0.1, 0.5, 1.0, 2.0, 5
(*E*)-2-decenal	39.72	55 > 29(10); 70 > 41(10)	$y = 15.937\ x$, $R^2 = 0.9902$	0.1, 0.5, 1.0, 2.0, 5
4-Methyl-5-thiazoleethanol	39.94	112 > 85(5); 143 > 112(10)	$y = 1.5534\ x$, $R^2 = 0.9991$	0.1, 0.5, 1.0, 5, 10
(*E*,*E*)-2,4-decadienal	40.45	67 > 41(10); 81 > 53(15)	$y = 2.6367\ x$, $R^2 = 0.9917$	0.1, 0.5, 1.0, 2.0, 5
1, 2-Dichlorobenzene	16.39	111 > 75(10); 146 > 111(15)	**Internal standard**	0.5

[1] The ion pairs used for the quantitation; parent ion > daughter ion (collision energy) (eV).
[2] *x* was the relative peak area relate to that of the internal standard 1,2-dichlorobenzene, *y* was the concentration (μg/mL).
[3] The concentrations of the standard solutions prepared in dichloromethane.

Table 2
Fatty acids and amino acids in meat of hind quarters of the black-pig used for the broth preparation

[1]Fatty acids	[2]mg/g dry meat	[2]Total%	[3]Amino acids	[2]mg/g dry meat	[2]Total%
C14:0	3.05 ± 0.26	5.83 ± 0.17	Valine	35.15 ± 0.24	4.86 ± 0.02
C16:0	11.35 ± 0.10	21.69 ± 1.07	Isoleucine	32.91 ± 0.16	4.55 ± 0.03
C18:0	4.12 ± 0.37	7.87 ± 0.25	Leucine	63.06 ± 0.37	8.73 ± 0.04
C20:0	0.13 ± 0.01	0.25 ± 0.00	Phenylalanine	32.60 ± 0.63	4.51 ± 0.04
C16:1	3.12 ± 0.02	5.96 ± 0.34	Histidine	32.37 ± 0.32	4.48 ± 0.04
C18:1	22.56 ± 1.86	43.11 ± 1.07	Tyrosine	27.58 ± 0.99	3.82 ± 0.11
C20:1	4.63 ± 0.05	8.85 ± 0.44	Tryptophan	5.49 ± 0.13	0.76 ± 0.02
C22:1	0.01 ± 0.00	0.02 ± 0.00	Arginine	49.87 ± 0.39	6.90 ± 0.03
C14:2	0.02 ± 0.00	0.04 ± 0.00	Methionine	17.98 ± 0.14	2.49 ± 0.02
C18:2	2.89 ± 0.30	5.52 ± 0.25	Glutamic acid	130.63 ± 1.84	18.07 ± 0.07
C20:2	0.14 ± 0.02	0.27 ± 0.02	Aspartic acid	74.65 ± 0.94	10.33 ± 0.05
C20:3	0.01 ± 0.01	0.02 ± 0.01	Threonine	36.33 ± 0.30	5.03 ± 0.07
C20:4	0.20 ± 0.02	0.38 ± 0.01	Serine	32.00 ± 0.27	4.43 ± 0.02
C22:3	0.03 ± 0.01	0.06 ± 0.01	Glycine	34.50 ± 0.14	4.77 ± 0.03
C22:4	0.06 ± 0.00	0.11 ± 0.01	Alanine	44.48 ± 0.59	6.15 ± 0.02
C22:6	0.01 ± 0.00	0.02 ± 0.01	Lysine	38.50 ± 0.21	5.33 ± 0.04
SFA	18.65	35.64	Cysteine	4.97 ± 0.14	0.69 ± 0.01
MUFA	30.32	57.94	Proline	29.63 ± 0.90	4.10 ± 0.10
PUFA	3.36	6.42	Umami taste amino acids	205.28	28.40
Total	52.33	100	**Total**	722.71	100

[1] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[2] Means ± standard derivations (n = 3).
[3] Umami taste amino acids included glutamic acid and aspartic acid.

condensation reaction of α-aminoketones in the Maillard reaction can produce the pyrazine compounds (Li, Yang, & Yu, 2016; Mottram, 1998). The three oxygen-containing heterocycles were furfural, furfuryl alcohol, and 2-pentylfuran. Furfural and furfuryl alcohol usually have caramel odors. The 1,2-enolization and cyclization of pentose in the Maillard reaction could produce furfural, and thus furfuryl alcohol (Yahya, Linforth, & Cook, 2014). 2-Pentylfuran could be formed from the lipid oxidization and degradation (Elmore et al., 1999). It is often found in cooked meat (Elmore & Mottram, 2006; Roldán, Ruiz, del Pulgar, Pérez-Palacios, & Antequera, 2015; Yang, Pan, Zhu, & Zou, 2014) and meat products (Benet et al., 2015).

In comparison, the amounts of the sulfur-containing compounds and the heterocyclic compounds identified in the pork broths of the black-pig and the common white-pig were similar in a large extent (Table S2) (Wang et al., 2015). However, the amount of the sulfur-containing compounds found in the roasted pork of Mini-pig (Xie et al., 2008) was rather less compared to either of the two pork broths. Despite the presence of multiple factors such as processing methods that can influence the formation of meat flavor, it was suggested that such kind of differences in sulfur-containing flavor compounds was mainly related to the breed difference between the Mini-pig and the black-pig (or the common white-pig) (Feng, Li, Lv, Zhang, & Ren, 2010). On the other hand, the nitrogen-containing heterocyclic flavors are usually produced during the roasting or grilling of meat under high temperature (Mottram, 1998; Shahidi, 1998). It was reported that eleven pyrazine compounds were found in the grilled beef (Frank et al., 2016). In contrast, only a couple of pyrazine compounds were found in the black-pig and the common white-pig broths shown

Table 3
GC–MS results of volatile compounds in the stewed pork broth of the black-pig after solvent assisted flavor evaporation (SAFE).

Compounds	[1]RI		[2]Amount (μg/kg meat)			[4]Identification methods
	DB-Wax	DB-5	DB-Wax	DB-5	[3]Averaged	
Sulfur compounds						
Dimethyl disulfide	1073	737	0.23 ± 0.03	0.49 ± 0.02	0.36 ± 0.02	MS/RI/S
3-(Methylthio)propanal	1424	906	1.16 ± 0.03	1.12 ± 0.04	1.14 ± 0.04	MS/RI/S
2-(Methylthio)ethanol	1523	823	0.31 ± 0.01	0.65 ± 0.05	0.48 ± 0.03	MS/RI
2-Acetylthiazole	1650	1040	1.78 ± 0.03	4.19 ± 0.37	2.98 ± 0.20	MS/RI/S
2-Thiophenecarboxaldehyde	1679	–	0.65 ± 0.03	–	0.33 ± 0.02	MS/RI/S
Benzothiazole	1961	–	0.69 ± 0.01	–	0.34 ± 0.01	MS/RI/S
4-Methyl-5-thiazoleethanol	2275	1266	6.41 ± 0.97	5.69 ± 0.27	6.05 ± 0.62	MS/RI/S
Subtotal			11.23	12.14	11.69	
Heterocyclic compounds						
Furfural	–	818	–	0.51 ± 0.25	0.26 ± 0.12	MS/RI/S
Pyridine	1178	752	1.69 ± 0.17	2.12 ± 0.22	1.91 ± 0.20	MS/RI
Pyrazine	1206	–	0.51 ± 0.06	–	0.26 ± 0.03	MS/RI
2-Pentylfuran	1234	993	1.21 ± 0.01	2.71 ± 0.08	1.96 ± 0.05	MS/RI/S
2-Ethyl-3-methylpyrazine	1356	981	8.42 ± 0.20	3.64 ± 0.28	6.03 ± 0.24	MS/RI/S
Furfuryl alcohol	1635	864	1.10 ± 0.17	0.29 ± 0.01	0.69 ± 0.09	MS/RI/S
1-Methyl-2-pyrrolidinone	1660	1033	1.73 ± 0.13	2.71 ± 0.03	2.22 ± 0.08	MS/RI
Subtotal			14.66	11.98	13.32	
Aldehydes						
Pentanal	1002	693	3.11 ± 0.07	5.01 ± 0.09	4.06 ± 0.08	MS/RI/S
Hexanal	1098	789	22.65 ± 1.27	43.28 ± 1.20	32.97 ± 1.24	MS/RI/S
2-Methyl-2-butenal	1103	738	3.27 ± 0.09	0.54 ± 0.04	1.90 ± 0.06	MS/RI/S
Heptanal	1192	881	0.52 ± 0.05	1.34 ± 0.05	0.93 ± 0.05	MS/RI/S
(*E*)-2-hexenal	1206	856	0.15 ± 0.01	0.23 ± 0.01	0.19 ± 0.01	MS/RI/S
(*E*)-2-heptenal	1316	970	1.30 ± 0.08	0.69 ± 0.07	0.99 ± 0.08	MS/RI/S
Nonanal	1388	1090	6.28 ± 0.21	11.66 ± 0.39	8.97 ± 0.30	MS/RI/S
(*E*)-2-octenal	1416	1065	1.04 ± 0.08	1.05 ± 0.02	1.05 ± 0.05	MS/RI/S
Benzaldehyde	1530	939	7.16 ± 0.13	15.93 ± 0.68	11.55 ± 0.40	MS/RI/S
(*E*)-2-nonenal	–	1157	–	0.64 ± 0.03	0.32 ± 0.02	MS/RI/S
Phenylacetaldehyde	1648	1031	0.93 ± 0.04	2.04 ± 0.01	1.48 ± 0.02	MS,RI/S
(*E*)-2-decenal	–	1247	–	0.49 ± 0.03	0.24 ± 0.02	MS/RI/S
(*E*,*E*)-2,4-nonadienal	–	1204	–	0.11 ± 0.00	0.05 ± 0.00	MS/RI/S
(*E*,*E*)-2,4-decadienal	1781	1306	0.76 ± 0.14	1.46 ± 0.05	1.11 ± 0.10	MS/RI/S
Tetradecanal	–	1609	–	0.62 ± 0.04	0.31 ± 0.02	MS/RI
Hexadecanal	2133	1791	6.90 ± 0.49	12.46 ± 0.52	9.68 ± 0.50	MS/RI
Octadecanal	2380	1998	8.62 ± 0.45	5.57 ± 0.43	7.09 ± 0.44	MS/RI
Subtotal			62.69	103.12	82.91	
Ketones						
2-Heptanone	–	892	–	1.12 ± 0.04	0.56 ± 0.02	MS/RI
2,3-Octanedione	1333	983	0.38 ± 0.01	1.03 ± 0.02	0.71 ± 0.01	MS/RI
3-Methyl-2-cyclopenten-1-one	1498	976	1.88 ± 1.29	0.77 ± 0.05	1.33 ± 0.67	MS/RI
2-Pentadecanone	2008	1688	1.85 ± 0.03	0.89 ± 0.05	1.37 ± 0.04	MS/RI
Subtotal			4.11	3.81	3.96	
Alcohols						
1-Butanol	1155	–	1.23 ± 0.09	–	0.62 ± 0.04	MS/RI
1-Penten-3-ol	1172	–	0.27 ± 0.01	–	0.14 ± 0.00	MS/RI/S
1-Pentanol	1250	761	1.67 ± 0.08	8.62 ± 1.44	5.14 ± 0.76	MS/RI/S
3-Methyl-3-buten-1-ol	1254	733	0.20 ± 0.01	0.80 ± 0.03	0.50 ± 0.02	MS/RI
3-Methyl-2-buten-1-ol	1325	767	0.64 ± 0.05	1.20 ± 0.03	0.92 ± 0.04	MS/RI
1-Hexanol	1358	–	0.81 ± 0.38	–	0.40 ± 0.19	MS/RI/S
1-Octen-3-ol	1451	960	4.39 ± 0.11	8.04 ± 0.14	6.22 ± 0.12	MS/RI/S
1-Heptanol	–	970	–	0.64 ± 0.03	0.32 ± 0.02	MS/RI/S
2-Ethyl-1-hexanol	1493	–	0.90 ± 0.01	–	0.45 ± 0.00	MS/RI
1-Octanol	1553	1072	2.62 ± 0.03	5.84 ± 0.20	4.23 ± 0.12	MS/RI/S
2,3-Butanediol	1568	778	3.02 ± 0.04	10.85 ± 0.38	6.93 ± 0.21	MS/RI
1-Phenethyl alcohol	1821	1039	1.07 ± 0.01	3.00 ± 0.08	2.04 ± 0.05	MS/RI
Benzyl alcohol	1848	1045	1.30 ± 0.08	3.45 ± 0.47	2.37 ± 0.27	MS/RI
Phenylethyl alcohol	1883	1112	1.95 ± 0.03	0.61 ± 0.00	1.28 ± 0.02	MS/RI/S
Pentadecanol	–	1765	–	1.64 ± 0.12	0.82 ± 0.06	MS/RI
Subtotal			20.07	44.69	32.38	
Acids						
Acetic acid	1431	–	4.10 ± 0.05	–	2.05 ± 0.03	MS/RI/S
Propanoic acid	1539	–	2.27 ± 1.82	–	1.14 ± 0.91	MS/RI
Butanoic acid	1635	–	13.99 ± 7.60	–	6.99 ± 3.80	MS/RI
Pentanoic acid	1732	–	11.59 ± 9.41	–	5.80 ± 4.71	MS/RI
Hexanoic acid	1857	1036	5.60 ± 0.70	0.57 ± 0.00	3.08 ± 0.35	MS/RI
Heptanoic acid	1952	1071	0.90 ± 0.02	0.81 ± 0.06	0.86 ± 0.04	MS/RI
Octanoic acid	2049	–	2.39 ± 0.11	–	1.19 ± 0.05	MS/RI
Nonanoic acid	2153	–	2.55 ± 0.07	–	1.27 ± 0.04	MS/RI
Decanoic acid	–	1376	–	1.24 ± 0.00	0.62 ± 0.00	MS/RI
Dodecanoic acid	–	1574	–	3.08 ± 0.04	1.54 ± 0.02	MS/RI
Tetradecanoic acid	–	1769	–	9.34 ± 0.66	4.67 ± 0.33	MS/RI
Pentadecanoic acid	–	1856	–	3.08 ± 0.11	1.54 ± 0.06	MS/RI

(*continued on next page*)

Table 3 (*continued*)

Compounds	[1]RI		[2]Amount (μg/kg meat)			[4]Identification methods
	DB-Wax	DB-5	DB-Wax	DB-5	[3]Averaged	
(*Z*)-9-hexadecenoic acid	–	1948	–	3.21 ± 0.16	1.60 ± 0.08	MS/RI
(*Z*)-11-hexadecenoic acid	–	1958	–	7.42 ± 0.19	3.71 ± 0.09	MS/RI
Hexadecanoic acid	–	1952	–	33.87 ± 0.32	16.93 ± 0.16	MS/RI
Heptadecanoic acid	–	2068	–	10.19 ± 0.45	5.10 ± 0.23	MS/RI
(*E*)-9-octadecenoic acid	–	2125	–	42.54 ± 0.84	21.27 ± 0.42	MS/RI
Octadecanoic acid	–	2148	–	32.79 ± 0.53	16.40 ± 0.27	MS/RI
Subtotal			43.39	148.14	95.77	
Esters						
Acetic acid, butyl ester	1089	–	0.13 ± 0.00	–	0.07 ± 0.00	MS/RI
Formic acid, octyl ester	1544	–	0.71 ± 0.06	–	0.35 ± 0.03	MS/RI
γ-Butyrolactone	1610	908	0.15 ± 0.00	2.01 ± 0.01	1.08 ± 0.01	MS/RI
γ-Hexalactone	1693	1063	1.26 ± 0.00	0.41 ± 0.04	0.84 ± 0.02	MS/RI
δ-Hexalactone	1800	1068	0.99 ± 0.05	1.45 ± 0.08	1.22 ± 0.07	MS/RI
Hexadecanoic acid, methyl ester	2196	1923	1.28 ± 0.02	3.82 ± 0.23	2.55 ± 0.13	MS/RI
15-Octadecenoic acid, methyl ester	2379	–	3.11 ± 0.07	–	1.56 ± 0.03	MS
Subtotal			7.63	7.69	7.66	
Aliphatic hydrocarbon						
2,3,5-Trimethyl-hexane	–	803	–	3.29 ± 0.15	1.64 ± 0.07	MS/RI
2,4-Dimethyl-heptane	823	844	0.49 ± 0.21	0.29 ± 0.01	0.39 ± 0.11	MS/RI
2,4-Dimethyl-1-heptene	–	851	–	0.71 ± 0.06	0.36 ± 0.03	MS/RI
4-Methyl-octane	–	863	–	4.03 ± 0.29	2.01 ± 0.15	MS/RI
Nonane	–	896	–	1.14 ± 0.05	0.57 ± 0.02	MS/RI
Decane	–	993	–	3.45 ± 0.26	1.73 ± 0.13	MS/RI
3,7-Dimethyl-decane	1085	1100	2.08 ± 0.04	3.72 ± 0.21	2.90 ± 0.12	MS/RI
Dodecane	1189	1203	0.19 ± 0.01	6.93 ± 0.15	3.56 ± 0.08	MS/RI
Tridecane	1301	1293	1.06 ± 0.07	2.26 ± 0.07	1.66 ± 0.07	MS/RI
Tetradecane	1399	1397	0.23 ± 0.00	0.84 ± 0.04	0.53 ± 0.02	MS/RI
Pentadecane	1495	1498	0.33 ± 0.01	0.82 ± 0.01	0.57 ± 0.01	MS/RI
Hexadecane	–	1605	–	0.80 ± 0.02	0.40 ± 0.01	MS/RI
1-Heptadecene	1759	1685	0.23 ± 0.00	1.48 ± 0.07	0.85 ± 0.04	MS/RI
2,6,10,14-Tetramethyl-pentadecane	–	1 689	–	0.76 ± 0.03	0.38 ± 0.01	MS/RI
Octadecane	–	1792	–	0.83 ± 0.03	0.42 ± 0.01	MS/RI
Nonadecane	–	1896	–	2.18 ± 0.09	1.09 ± 0.05	MS,RI
Eicosane	–	1998	–	3.83 ± 0.07	1.92 ± 0.04	MS/RI
Docosane	2193	2199	7.09 ± 0.12	19.91 ± 0.90	13.50 ± 0.51	MS/RI
Tricosane	2287	2289	11.42 ± 0.00	32.30 ± 0.56	21.86 ± 0.28	MS/RI
Tetracosane	2405	2397	12.74 ± 0.29	29.94 ± 0.71	21.34 ± 0.50	MS/RI
Subtotal			35.86	119.51	77.68	
Others						
p-Xylene	1118	875	0.36 ± 0.01	1.47 ± 0.08	0.91 ± 0.04	MS/RI
Ethylbenzene	1132	–	0.40 ± 0.01	–	0.20 ± 0.01	MS/RI
D-limonene	1170	1 014	1.45 ± 0.00	3.09 ± 0.09	2.27 ± 0.05	MS/RI
o-Xylene	1176	–	0.42 ± 0.01	–	0.21 ± 0.01	MS/RI
Styrene	–	895	–	0.34 ± 0.11	0.17 ± 0.06	MS/RI
1,2,3,4-Tetramethyl-benzene	–	1144	–	0.62 ± 0.01	0.31 ± 0.00	MS/RI
Butylated hydroxytoluene	1902	1484	0.88 ± 0.07	0.54 ± 0.19	0.71 ± 0.13	MS/RI
p-Cresol	2071	1093	1.17 ± 0.05	2.59 ± 0.13	1.88 ± 0.09	MS/RI
2,4-Bis(1,1-dimethylethyl)-phenol	2248	1487	1.07 ± 0.05	2.67 ± 0.09	1.87 ± 0.07	MS/RI
Subtotal			5.75	11.32	8.53	
Total			205.39	462.40	333.90	

[1] The linear retention indices related to n-$C_5 \sim C_{25}$ alkanes on the DB-Wax or the DB-5 column.
[2] Means ± standard derivations (n = 3), semi-quantitated based on the internal standard 1,2-dichorobenzene using a calibration factor of 1; "–", undetected in the GC–MS analysis.
[3] The amounts on the DB-Wax and the DB-5 columns were averaged.
[4] MS, identified by NIST 15 mass spectral database; RI, agreed with the retention indices published in literatures; S, the analytical parameters of MS and RI both agreed with those of the authentic chemicals injected.

in Table S2; Similar phenomenon in the common white-pig broths was also reported by Wang et al. (2016) and Xu et al. (2011), where all the broths were obtained by stewing of meat in water under a temperature of no more than 100 °C.

As what had been reported on meat flavor (Elmore, Mottram, & Hierro, 2000; Xie et al., 2008), the aldehydes shown in Table 3 were not only in a high total amount (82.91 μg/kg), but also contained a large number, including fifteen aliphatic aldehydes, and two aromatic aldehydes. Among them, hexanal, benzaldehyde, hexadecanal, nonanal, octadecanal, and pentanal were particularly in a considerable level (>4 μg/kg). Regarding the two aromatic aldehydes, benzaldehyde could be formed from the degradation of α-linolenic acid (Elmore & Mottram, 2006), while phenylacetaldehyde could be formed from the Maillard reaction of phenylalanine (Lotfy et al., 2015).

Only four ketones (3.96 μg/kg) were found in the black-pig broth, including 2-heptanone, 2, 3-octanedione, 3-methyl-2-cyclopenten-1-one, and 2-pentadecanone, all in low amounts. Similarly, few ketones were found in the common white-pig broths stewed with the hindquarters (Table S2) (Wang et al., 2015) and the loins (Wang et al., 2016; Xu et al., 2011). Hitherto the reports of meat flavor had been mainly focused on the cooked meat and meat products (Machiels, Ruth, Posthumus, & Istasse, 2003; Madruga, Elmore, Dodson, & Mottram, 2009; Rivas-Cañedo,

Juez-Ojeda, Nuñez, & Fernández-García, 2011; Roldán et al., 2015), while those on the stewed broth of meat were scarce. In comparison, it seemed that relatively more number of volatile ketones were usually found in the cooked pork than in the stewed pork broths due to the difference in processing method. For instances, nineteen ketones were found in the *longissimus* muscle of a typical Chinese hybrid pig (Duroc × Landrace × Large White) after being heated at 130 °C for 45 min (Lu, Li, Yin, Zhang, & Wang, 2008), and fifteen ketones were found in the M. *longissimus* lumborum of pork chops after being heated at 140 °C for 30 min (Elmore et al., 2000).

Fifteen alcohols (32.38 μg/kg) were also identified, including three aromatic alcohols, and twelve aliphatic alcohols. The aromatic alcohols were 1-phenethyl alcohol, benzyl alcohol, and phenylethyl alcohol. Except for the pentadecanol, all other aliphatic alcohols were short-chain alcohols. Among the alcohols, 2, 3-butanediol (6.93 μg/kg) was in the highest level, followed by 1-octen-3-ol (6.22 μg/kg), and 1-pentanol (5.14 μg/kg).

In Table 3, the largest amount (95.77 μg/kg) of compounds found in the broth were the acids, including nine short-chain fatty acids ($C_2 \sim C_{10}$), and nine long-chain fatty acids ($C_{11} \sim C_{18}$). Owing to differences in molecular polarities, the short-chain fatty acids such as butanoic acid mainly were detected by the DB-Wax column, while the long-chain fatty acids such as hexadecanoic acid all were detected by the DB-5 column. For the long-chain fatty acids, (*E*)-9-octadecenoic acid (21.27 μg/kg) was in the highest level, followed by hexadecanoic acid, and octadecanoic acid. For the short-chain fatty acids, butanoic acid (6.99 μg/kg) was in the highest level, followed by pentanoic acid, and hexanoic acid.

The esters usually have sweet and typical fruity odors which can be formed by the esterification of acids and alcohols. In particular, the lactones can be formed by the intramolecular esterification of the hydroxy acids (Sánchez-Sevilla, Cruz-Rus, Valpuesta, Botella, & Amaya, 2014). Seven esters (7.66 μg/kg) were found, including two long-chain esters (hexadecanoic acid, methyl ester, and 15-octadecenoic acid, methyl ester), two short-chain esters (acetic acid, butyl ester, and formic acid, octyl ester), and three lactones (γ-butyrolactone, γ-hexalactone, and δ-hexalactone). Among them, hexadecanoic acid methyl ester (2.55 μg/kg) was in the highest level, followed by 15-octadecenoic acid, methyl ester, and δ-hexalactone.

Most of the aldehydes, ketones, alcohols, acids and esters mentioned above belonged to the aliphatic compounds that arose from the lipid oxidization and degradation. Among them, the short chain aliphatic aldehydes usually are of importance in meat flavor (Lorenzo & Domínguez, 2014; Mottram, 1998; Shahidi, 1998; Xie et al., 2008; Yang et al., 2015). As shown in Table S2, the distribution of the short-chain aliphatic aldehydes ($C_5 \sim C_{10}$) in the two broths was different; The common white-pig broth had a higher summed amount of the short-chain aliphatic aldehydes ($C_5 \sim C_{10}$), which might be one of the reasons for certain aroma difference of the two broths. On the other hand, compared to the usual cooked pork (Elmore et al., 2000; Lu et al., 2008; Meinert, Andersen, Bredie, Bjergegaard, & Aaslyng, 2007; Yang et al., 2014), much greater number and higher amount of short-chain fatty acids, alcohols, and esters were found in either the black-pig or the common white-pig broth (Table S2). Besides, the same phenomenon was also reported by Wang et al. (2016), where twelve short-chain aliphatic alcohols and eight short-chain aliphatic esters (no fatty acids) were found in the pork broth stewed with the loins of the common white-pig. However, only six aliphatic alcohols but no fatty acids and esters were found in the heated *longissimus* muscles of both a typical hybrid pig and five Chinese indigenous pigs (Lu et al., 2008). Despite the differences in analytical methods, nature of meat, etc., as discussed above on the ketones, the distinctions shown in the short-chain fatty acids, alcohols, and esters between the stewed pork broth and the usual cooked meat of pork were again considered in a large extent related to the differences in processing method. It might be that when stewing of meat in water for broth, the lipids in meat were readily hydrolyzed into fatty acids. The fatty acids would undertake oxidization and degradation, giving the short-chain fatty acids, alcohols and thus esters, instead of the ketones.

Besides, as shown in Table 3, the aliphatic hydrocarbons were also found in a great number and high amount, but they only had marginal impacts on overall aroma because of their high odor thresholds. Moreover, there were nine other compounds that seemed unrelated to the typical meat flavor (see Table 3). Among them, *p*-cresol, d-limonene, butylated hydroxytoluene (BHT, antioxidant), and 2,4-bis(1,1-dimethylethyl)-phenol (2,4-DTBP, antioxidant) could come from the animal feeds, while styrene, ethylbenzene, *p*-xylene, *o*-xylene, and 1,2,3,4-tetramethylbenzene, which are the benzene derivatives, usually were recognized as volatile organic contaminants in food (del Olmo, Calzada, & Nuñez, 2014; Fleming-Jones & Smith, 2003). *p*-Xylene, limonene, *o*-xylene, and *p*-cresol had been reported present in the low-acid fermented sausage "espetec" and the sliced cooked pork shoulder (Rivas-Cañedo, Juez-Ojeda, Nuñez, & Fernández-García, 2012) and some other meats or meat products (Corral, Salvador, & Flores, 2015; Lorenzo & Domínguez, 2014).

3.3. Odor-active compounds by GC-O

In Table 4, 28 odor-active regions in retention indices were found for the black-pig pork broth in the GC-O analysis, of which 27 compounds were identified but one remained unknown. Among the 27 compounds, 25 compounds were positively identified with their authentic chemicals. Worth mentioning, some identifications presented were neither detected in the DB-Wax nor in the DB-5 column by the GC–MS, due to their low levels in the samples or being co-eluted with other compounds. As shown in Table 4, the sniffed odors consisted of meaty, sesame-like, green, fatty, fruity, sweet, caramel, roasted aromas, and so on. Noticeably, the fatty acids found by the GC–MS gave no odor-activities at all in the GC-O analysis, in spite of the large numbers and high amounts. The same phenomenon was also observed in regards of the aliphatic acids in the pork broth of the common white-pig (Table S3) (Wang et al., 2015).

The principle of AEDA indicates that a compound has a higher FD factor will give a greater contribution to the overall aroma. In Table 4, 23 odorants, which mainly included the aliphatic short chain aldehydes and the sulfur-containing compounds, showed high FD values ($\log_2 FD \geqslant 5$). In descending FD value, the most potent aroma compounds ($FD \geqslant 11$) were ranked in an order of 2-methyl-3-furanthiol, 3-(methylthio)propanal, γ-decalactone, 2-furfurylthiol, nonanal, (*E*)-2-nonenal, (*E*,*E*)-2,4-decadienal, 2,4-bis(1,1-dimethylethyl)-phenol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 1-octen-3-ol, and 3-hydroxy-2-butanone. Worth mentioning, 2,4-bis(1,1-dimethylethyl)-phenol, which is an antioxidant, also gave a high FD value ($\log_2 FD = 15$), suggesting it could have an effect on the overall aroma.

In comparison, 21 odorants in Table 4 of the black-pig pork broth also were identified in its counterpart, the common white-pig pork broth (Table S3) (Wang et al., 2015). Yet they were present in different FD values. Besides, (*E*)-2-undecenal and bis (2-methyl-3-furyl)disulfide were only found in the common white-pig pork broth (Table S3), while 2,4-bis(1,1-dimethylethyl)-phenol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, (*E*)-2-hexenal, 2-acetyl-1-pyrroline, (*E*)-2-decenal, and 4-methyl-5-thiazoleethanol were only found in the black-pig pork broth (Table 4). As the most relevant aroma attributes of a food can be explained by a limited number of odor potent compounds (Aceña, Vera, Guasch, Busto, &

Table 4
GC-O results for the stewed pork broth of the black-pig after solvent assisted flavor evaporation (SAFE) and the quantities of the odor-active compounds determined by GC–MS/MS in selected reaction monitoring (SRM) mode with aid of authentic chemicals.

Compounds	[1]RI	Odor descriptors	[2]Amounts (μg/kg meat)	log_2FD	[3]Identification Methods
Sulfur compounds					
Methanthiol	455	Sulfur, gasoline	/	5	RI/odor
2-Methyl-3-furanthiol	875	Cooked meat	3.393 ± 0.181	18	RI/S/odor
3-(Methylthio)propanal	912	Cooked potato	6.649 ± 0.332	18	RI/S/odor/MS
2-Furfurylthiol	916	Sesame	/	17	RI/S/odor
Dimethyl trisulfide	984	Sulfur, meaty	0.888 ± 0.034	6	RI/S/odor
2-Mercaptothiophene	986	Sesame	/	6	RI/S/odor
2-Thiophenecarboxaldehyde	1005	Sulfur	0.453 ± 0.024	2	RI/S/odor/MS
2-Acetylthiazole	1023	Sesame, roasted	2.172 ± 0.017	9	RI/S/odor/MS
4-Methyl-5-thiazoleethanol	1299	Sesame	2.398 ± 0.092	4	RI/S/odor/MS
Heterocyclic compounds					
2-Acetyl-1-pyrroline	950	Nutty, roasted	/	8	RI/S/odor
2-Ethyl-3-methylpyrazine	1023	Roasted	0.195 ± 0.004	9	RI/S/odor/MS
Aldehydes					
Pentanal	687	Green	14.295 ± 0.025	7	RI/S/odor/MS
Hexanal	794	Green	26.654 ± 0.092	6	RI/S/odor/MS
(*E*)-2-hexenal	835	Green, fatty	0.663 ± 0.042	8	RI/S/odor/MS
Heptanal	886	Sweet melon	4.758 ± 0.102	5	RI/S/odor/MS
(*E*)-2-heptenal	960	Fatty	2.667 ± 0.023	1	RI/S/odor/MS
Phenylacetaldehyde	1049	Rose	0.884 ± 0.008	4	RI/S/odor/MS
Nonanal	1104	Sweet melon	3.198 ± 0.064	15	RI/S/odor/MS
(*E*)-2-nonenal	1150	Cucumber	0.317 ± 0.011	15	RI/S/odor/MS
(*E*,*E*)-2,4-nonadienal	1218	Fatty	/	6	RI/S/odor/MS
(*E*)-2-decenal	1261	Fatty	0.680 ± 0.018	5	RI/S/odor/MS
(*E*,*E*)-2,4-decadienal	1319	Fatty	0.345 ± 0.014	15	RI/S/odor/MS
Ketones					
3-Hydroxy-2-butanone	705	Yogurt	560.500 ± 12.150	11	RI/S/odor
4-Hydroxy-2,5-dimethyl-3(2 *H*)-furanone	1055	Caramel	/	13	RI/S/odor
Alcohols					
1-Octen-3-ol	982	Mushroom	11.737 ± 0.352	13	RI/S/odor/MS
Esters					
γ-decalactone	1430	Peach	/	18	RI/S/odor
Others					
Unknown	1174	Fatty	/	6	
2,4-Bis(1,1-dimethylethyl)-phenol	1407	Woody	/	15	RI/odor/MS

[1] The linear retention indices sniffed in the GC-O analysis on the HP-5 column.
[2] Means ± standard derivations (n = 3), quantitated using the calibration equations of the authentic chemicals; "/", not quantitated due to no available authentic chemicals or presence in a very low level (S/N ⩽ 10).
[3] MS, identified by NIST 15 mass spectral database; RI, agreed with the retention indices published in literatures; Odor, agreed with the odor descriptors published in literatures; S, the analytical parameters including MS, RI, and odor characteristics all agreed with those of the authentic chemicals used.

Mestres, 2011; Souza, Vásquez, del Mastro, Acree, & Lavin, 2006), the different combination of odor-active compounds in the two stewed broths inevitably led to their different aroma profiles, as shown in Figs. 1 and 1S, which were plotted according to their respective potent aroma compounds by GC-O. It shows that aroma of the black-pig pork broth has less fatty notes but more roasted notes compared to that of the common white-pig pork broth, which enhances the desirable meaty aroma and, as a result, probably was one of the reasons why people prefer the black-pig pork.

Based on what had been reported previously (Cameron et al., 2000; Mottram, 1998), the different aroma profile for the two broths should be mainly due to the dissimilar fatty acid composition between the two-pig meats, which, as discussed in Section 3.1, was attributed to the higher MUFA and lower PUFA in the black pig meat than those in the common white-pig meat. Benet et al. (2016) had also observed in the cooked cured pork ham that a high content of PUFA in fatty acid composition decreased the roasted and pleasing aroma attributes. Besides, studies on "roasted" notes of meat flavor revealed the different aroma profiles of cooked meat might also be related to the different composition in inosine monophosphate (IMP), ribose, glucose, and glucose-6-phosphate between the meat species (Farmer, Hagan, & Paraskevas, 1999; Meinert, Schäfer, Bjergegaard, Aaslyng, & Bredie, 2009; Meinert et al., 2007). However, whether or not the carbohydrates could determine the aroma difference between the pork broths of the black-pig and the common white-pig, it is still remained unclear and needs to be answered in our future work.

3.4. Quantities of the odor-active compounds by GC–MS/MS

So far, many volatile flavor compounds in various meat and meat products had been discovered, but accurate quantitation of those compounds using authentic chemicals was rare due to the laborious work. Rather than the usual GC–MS measurement, SRM detection in tandem mass spectrometry (GC–MS/MS) was applied in the present quantitative analysis, since the latter is better in specificity, sensitivity, and detection limit. As shown in Table 4, except for 2-furfurylthiol, 2-mercaptothiophene, (*E*,*E*)-2,4-nonadienal, γ-decalactone, etc., which were found in very low levels (S/N ⩽ 10), all the other odorants with authentic chemicals available including those undetected in the GC–MS analyses (e.g. 2-methyl-3-furanthiol, and dimethyl trisulfide) were quantitated. In a descending order, the compounds in high amounts (>10 μg/kg) were 3-hydroxy-2-butanone, hexanal, pentanal, and 1-octen-3-ol. In agreement with the GC–MS results, the total amount of the sulfur compounds was rather less than that of the aliphatic compounds (see Table 4). However, in contrast to the abundant amounts of hexanal, and pentanal, due to the nature of low odor thresholds, the sulfur-containing compounds including 2-methyl-3-furanthiol, 3-(methylthio)propanal, and 2-furfurylthiol actually were more powerful in regards of their FD values.

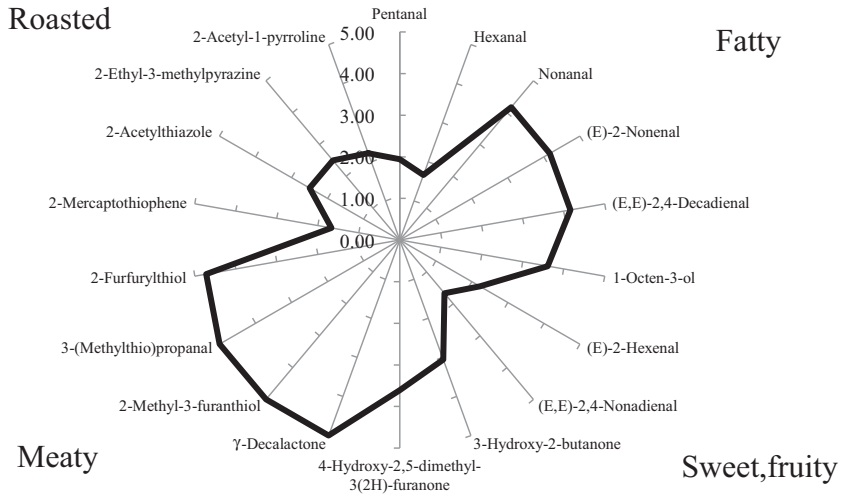

Fig. 1. Spider-web for the top eighteen odorants in the stewed pork broth of the black-pig. The full distance on the scale was defined to be 5, distance from the origin for a compound is 5 times the ratio of its $\log_2$FD value divided by the highest $\log_2$FD value of the odorant presented.

4. Conclusions

In this study regarding the analysis of flavors in the stewed pork broth of black-pig, 104 volatile compounds were identified by GC–MS, 27 compounds were revealed of odor-activities by AEDA/GC-O, and 19 odor-active compounds were quantitated with aid of authentic chemicals by GC–MS/MS in the SRM detection. The major volatiles were the aliphatic aldehydes as well as the aliphatic acids, alcohols, and esters. The potent aroma compounds found were 2-methyl-3-furanthiol, 3-(methylthio)propanal, γ-decalactone, 2-furfurylthiol, etc. The aroma compounds determined in high quantities were 3-hydroxy-2-butanone, hexanal, pentanal, and 1-octen-3-ol. Compared to our former work on the stewed pork broth of the common white-pig, most of the odor-active molecules found in the stewed pork broth of the black-pig were nearly as same as those in its counterpart, but with different FD values. However, according to aroma profile of the potent odorants, the black-pig broth shows the more desirable meaty flavor with less fatty but more roasted notes, which were mainly attributed to the higher MUFA but lower PUFA in fatty acid composition and the possible difference in the carbohydrates of meat, and considered to be one of the reasons why people prefer the black-pig pork.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.01.011.

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