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Volatile flavor constituents in roasted pork of Mini-pig

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Abstract

Volatiles from roasted pork of Mini-pig were first investigated by solid phase microextraction (SPME) as well as simultaneous distillation and solvent extraction (SDE) combined with gas chromatography and mass spectrometry (GC–MS). Total of 86 different compounds were identified with aldehydes being the most abundant followed by the spice components such as estragole, *trans*-anethole, eugenol and so on. Compared with other cooked meat products, much low amount of aliphatic alcohols were present in the roasted Mini-pig pork. The SDE isolation was preferred in the characterization of meat flavor considering that semi-quantitative data was conveniently obtained, more components were revealed, artifact formation was limited and aromatic profile was representative of the meat sample. By gas chromatography and olfactometry (GC–O), 45 olfactory regions were exposed and 43 flavors were located. Finally, 17 important flavor substances in the SDE extract were quantified by gas chromatography and flame ionization detector (GC–FID) using calibration curves of authentic chemicals.

Keywords: Mini-pig; Meat flavor; Solid phase microextraction; Simultaneous distillation and solvent extraction; Olfactometry; Volatile components

1. Introduction

The chemistry of meat flavor has been an attractive subject of many researchers. Several reviews have been published on meat flavor and remarkable progress has been made in meat flavor research (Mottram, 1991; Shahidi, Rubin, & D'Souza, 1986). Meat flavor develops during processing such as roasting or cooking and is a result of complex interaction of precursors in the raw meat, including pyrolysis of amino acids and peptides, sugar degradation, degradation of ribonucleotides, Maillard reactions, thiamine degradation and degradation of lipids. Thus, both chemical composition of animal muscles and preparation procedures influence significantly the resulting meat flavor (Elmore, Mottram, Enser, & Wood, 1999; Gorraiz, Beriain, & Chasco, 2002 ; Mottram, 1985; Verplaetse, 1994).

Although more than 1000 volatiles have been identified in various meat species (Maarse & Visscher, 1989) and some of these components have been reported to be meaty or reminiscent of meat, meat flavor cannot be attributed to a single component or a particular class of compounds thus far (Ramarathnam & Rubin, 1994). The overall idea arising from meat flavor is focusing efforts on compounds that have a real impact on flavor. And for the aim of searching relevant aroma compounds, analytical techniques of gas chromatography (GC), gas chromatography and mass spectrometry (GC–MS) (Wettasinghe, Vasanthan, Temelli, & Swallow, 2001), and gas chromatography and olfactometry (GC–O) (Acree, Barnard, & Cunningham, 1984) are frequently utilized. Recently, Timón, Carrapiso, Jurado, and Lagemaat (2004) discovered characteristic flavors of pyrazines, pyridines and furans from fried bacon and fried pork loin in combination of dynamic headspace with GC–MS and GC–O, and Yu and Sun (2005) identified four new phenols from Chinese traditional smoke-cured bacon using nitrogen purge and steam distillation (NSPD) with GC and GC–MS.

In fact, to perform analysis, the selected extraction method is of significance as the extract should be representative of the original meat flavor (Abbott, Etievant, Langlois, Lesschaeve, & Issanchau, 1993; Moio et al., 1995). For

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example, direct vacuum distillation of ground ham suspended in water had been proved the most correlative with the cooked cured ham flavor, and after GC–FID (flame ionization detector) and sniffing analysis, 14 potent aroma constituents were revealed (Guillard, Le Quere, & Vendeuvre, 1997). Nowadays, extraction based on distillation such as vacuum distillation, hydro distillation (Wettasinghe et al., 2001), simultaneous distillation and solvent extraction (SDE) (Ansorena, Gimeno, Astiasarán, & Bello, 2001) and nitrogen purge and steam distillation (NPSD) (Yu & Sun, 2005), and extraction based on headspace sampling such as dynamic headspace (Dirinck, Van Opstaele, & Vandendriessche, 1997; Hierro, de la Hoz, & Ordóňez, 2004) and solid phase micro extraction (SPME) (Ruiz, Cava, & Ventanas, 1998; Gianelli, Flores, & Toldrá, 2002) have been employed alternatively in meat flavor research, among which simultaneous distillation and solvent extraction (SDE) is common and classic, while solid phase micro extraction (SPME) is relatively new and mild.

Mini-pig is a special pig breed in China, occurring in Chinese south and southwest high altitude mountainous regions of Hainan, Guangxi, Guizhou, Yunnan and Tibet provinces. Famous as its tiny size and excellent flavor, Mini-pig is also called Savory-pig or Radish-pig. Mini-pig's raw meat is not only advantageous in rich pre-flavor amino acids and low intra-muscular fat but also possesses good process performance (Sun & Lu, 2002). Nowadays, meat products of Mini-pig pork are well accepted by the indigenes and travelers far and near. However, studies on Mini-pig pork flavor are scarce.

Here, volatiles of the roasted Mini-pig pork, one popular Mini-pig product, were determined by both SPME and SDE combined with GC–MS. And to find potent contributors to the unique meat flavor, odor evaluation (GC–O) was performed. Finally, some important flavors responsible for aroma of the roasted Mini-pig pork were quantified by GC–FID with calibration curves of authentic chemicals. As far as we know, no previous study on volatile flavor composition of the roasted Mini-pig pork had been reported.

2. Materials and methods

2.1. Materials

Roasted Mini-pig pork products were obtained from Bama region, Guangxi Province, China. They were prepared by local traditional technology. We were informed that Mini-pigs generally about 8 kg from a local market were slaughtered. After 4 h postmortem cleaning and shaping, whole pieces of abdomen and back (composed mainly of M. obliquus abdominis externus) were picked out, and then pickled at 4 °C in a jar for about 10 h, during which manual turnover was given twice. The curing ingredients (based on raw meat weight) included 4% sodium chloride, 2% white granulated sugar, 3.8% yellow cooking wine, 3.7% soybean sauce, 0.01% sodium nitrite, 0.2% spice powder (containing ginger, star aniseed and cinnamon) and some distilled water. Finally, the meat pieces were hung upon bamboo bars and carefully roasted by charcoal fires for 3 h.

Three roasted pork samples from three Mini-pigs taken randomly from the local provider were used in the analysis. On arrival to the lab, the bones were removed; the meat was homogenized by cutting into slices and then stored in a freezer at −20 °C in polyethylene bags. Prior to use, the slices were further ground in frozen by a domestic blender.

2.2. Isolation of volatiles

2.2.1. Solid phase micro extraction (SPME)

The manual SPME holder together with 40 ml vials, Teflon covers and one 75 μm carboxen/polydimethylsiloxane (CAR/PDMS) fiber was purchased from Supelco Inc. (Bellefonte, PA, USA). Before sorption, the fiber was preconditioned for 40 min on an Agilent 6890 gas chromatograph (Agilent Technologies, USA) with the injector temperature of 280 °C.

Ground roasted pork (15 g), 14 ml of water and 0.22 g salt were placed in a 40 ml vial at room temperatures (23 °C). The vial was sealed up with one Teflon cover and kept at 80 °C by a water bath for 40 min while shaken at intervals. After that, the SPME fiber was exposed in the upper space of the vial for 1 h, and then withdrawn and directly introduced to the GC–MS injector for desorption and analysis. Analyses were performed in triplicate.

2.2.2. Simultaneous distillation and solvent extraction (SDE)

Ground roasted pork (120 g) was suspended in 250 ml of water in a 500 ml round bottom flask attached to a modified Likens–Nickerson apparatus. In a 100 ml round bottom flask, 80 ml dichloromethane (purified in advance) was added. The sample mixture and the solvent were heated by one oil bath and one water bath separately. After boiled and refluxed for 1.5 h, the dichloromethane fractions in the solvent flask and the solvent loop were combined and concentrated on a vigreux column to 1.02 g. Isolations were performed in triplicate.

2.3. Gas chromatography and mass spectrometry (GC–MS) analysis

GC–MS analysis was performed on an Agilent 6890 gas chromatograph coupled with a 5973i mass spectrometer (Agilent Technologies, USA). The carrier gas was helium in 1 ml/min. The separation was on a HP-5 MS 30 m × 0.25 mm × 0.25 μm column (Agilent Technologies, USA). The initial oven temperature was at 40 °C, holding for 2 min, then ramped to 220 °C at 4 °C/min; and finally ramped to 280 °C at 20 °C/min. The mass detector was operated at 150 °C in electron impact mode at 70 eV. The ion source temperature was at 230 °C. The transfer line temperature was at 250 °C. The chromatograms were

recorded by monitoring the total ion currents in the 30–450 mass range.

For the SPME analysis, desorption was at 280 °C for 4 min in splitless mode and MS was detected with no solvent delay.

For the SDE concentrates, 2 μl was injected at 250 °C in split mode (1:20 split ratio) and MS was detected with a solvent delay of 2 min. Internal standard 1,2-dichlorobenzene (0.3 mg/ml) in pentane was added, and approximate contents of the volatiles were calculated by relating their peak areas to that of 1,2-dichlorobenzene using a response factor of 1.

Identification was carried out by comparison with Nist 02 library or the published mass spectra (Stenhagen, Abrahamsson, & Mclafferty, 1974), together with personal interpretation and GC retention indices (RI) relative to C_6–C_{23} *n*-alkanes. Besides, authentic chemicals of 3-methylbutanal, 3-hydroxy-2-butanone, hexanal, (*E*)-2-hexenal, 2-heptanone, heptanal, 2,5-dimethylpyrazine, (*E*)-2-heptenal, benzaldehyde, 2-pentylfuran, (*E*,*E*)-2,4-heptadienal, phenyl acetaldehyde, nonanal, (*E*)-2-nonenal, (*E*,*E*)-2,4-nonadienal, (*E*,*E*)-2,4-decadienal and eugenol were applied to further confirm the identifications.

2.4. Gas chromatography and olfactometry (GC–O) analysis

The SDE concentrates were analyzed on an Agilent 6890 gas chromatograph coupled with a HP-5 30 m × 0.32 mm × 0.25 μm column (Agilent Technologies, USA). Chromatographic conditions utilized were identical to those of GC–MS analysis. By one "Y" shape glass splitter, the column effluent was divided (ratio 1:1) between the FID detector and the odour port (Sniffer 9000, Brechbühler Scientific Analytical Solutions INC, Switzerland). The effluent to the odor port was enclosed with a stream of humidified air of 6 ml/min and transferred to the glass detection cone by one length of capillary at the temperature of 260 °C.

By smelling and recording the odour descriptions, five trained assessors evaluated the odors of the GC effluent. Total of 10 assessments were carried out. In order to avoid olfactory fatigue, each assessment took place over four different time intervals (0–10, 10–20, 20–35 and 35–50 min). Retention times of the odor responses were converted into RI values using the retention times of a series of *n*-alkanes (C_6–C_{23}).

2.5. Gas chromatography and flame ionization detector (GC–FID) analysis

When accessible, flavor components found by GC–MS as well as GC–O from the SDE concentrate were further quantified by calibration curves of authentic chemicals using 1,2-dichlorobenzene or tetradecane as internal. Gas chromatography and flame ionization detector (GC–FID) analysis was also performed with the chromatographic conditions of GC–MS analysis.

3. Results and discussion

3.1. Analysis of the volatiles by GC–MS

3.1.1. Solid phase microextraction (SPME) isolation

SPME isolation is dependent on equilibrium between matrix and fiber coating, and results are often influenced by the stationary phase and the extraction conditions used. In this study, CAR/PDMS fiber was utilized (Dufour, Delbecq, & Perez, 2001; Gianelli et al., 2002). Sorption time of 1 h was selected. Prior to extraction, the vial of meat sample was preheated and shaken at intervals to facilitate the volatiles into headspace and to simulate a reheating treatment prior to consumption.

From Table 1, it could be seen that total of 43 compounds were found by SPME, representing 98.68% of the total peak areas, including aldehydes, ketones, alcohols, heterocycles (e.g. furans, pyrazines, thiazoles), terpenes, aromatic and aliphatic hydrocarbons and oxygenous benzene derivatives (e.g. eugenol, estragole). These constituents were characterized by a high content of *trans*-anethole (26.81%), (*E*,*E*)-2,4-decadienal (18.96%) and hexanal (13.61%). Other compounds present in appreciable amounts were (*E*,*E*)-2,4-heptadienal (4.53%), eugenol (4.46%), (*E*)-2-decenal (3.13%), pentadecane (2.17%), benzaldehyde (2.10%) and (*E*)-2-octenal (1.67%).

Among all the identified, aldehyde fraction was the predominant, covering 52.63% of the total, with unsaturated aldehydes, saturated aldehydes and benzene derived aldehydes being 34.17%, 16.13% and 2.33% separately. Followed by the aldehydes was the fraction of oxygenous benzene derivatives, which consisted of estragole, *trans*-anethole, eugenol, ethyl cinnamate, *cis*-anethole and *trans*-ethyl *p*-methoxycinnamate, representing 35.12% of the total. Moreover, the heterocycles of furans, pyrazines, thiazoles and lactones, generally considered as meaty group, only accounted for 3.13%. And ketones were even less, covering 2.04% of the total.

3.1.2. Simultaneous distillation and solvent extraction (SDE) isolation

Also in Table 1, results of the selected SDE analysis were present, and in contrast to SPME analysis, semi-quantitative data was included. Comparison of the results of SDE and SPME in Table 1 illustrated that more volatiles were found by SDE and aroma patterns were indeed influenced by the isolation procedure used. But from a qualitative point of view, the aromatic components, e.g. aldehydes, heterocycles and the oxygenous benzene derivatives identified by the two isolation methods were largely similar.

Total of 81 compounds were found from the SDE extract, amounting to about 185 ng per gram of roasted pork sample, representing 88.31% of the total peak areas. Among them, the aromatic and aliphatic hydrocarbons, which might arise from secondary degradation of compounds from lipid oxidation, were in 5.84% of the total peak areas. Nevertheless, as a result of high odor

Table 1
Volatiles identified in GC–MS by both SPME and SDE from the roasted pork of Mini-pig

Compounds	SPME	SDE		RI[c]	I method[d]
	Peak area[a] (%)	Peak area[a] (%)	Amount[b] (ng g^{-1})		
Aldehydes					
3-Methylbutanal	0.64 ± 0.03	0.44 ± 0.02	0.92 ± 0.03	657	RI, MS, S
Pentanal	1.27 ± 0.05	1.29 ± 0.09	2.70 ± 0.19	704	RI, MS
(*E*)-2-Pentenal	0.56 ± 0.02	0.32 ± 0.04	0.67 ± 0.09	746	RI, MS
Hexanal	13.61 ± 0.32	9.71 ± 0.87	20.29 ± 1.82	802	RI, MS, S
(*E*)-2-Hexenal	0.71 ± 0.08	0.29 ± 0.07	0.61 ± 0.14	852	RI, MS, S
Heptanal	0.61 ± 0.06	0.61 ± 0.05	1.28 ± 0.11	902	RI, MS, S
(E)-2-Heptenal	1.48 ± 0.05	0.59 ± 0.07	1.23 ± 0.15	957	RI, MS, S
Benzaldehyde	2.10 ± 0.09	0.29 ± 0.05	0.61 ± 0.10	961	RI, MS, S
(*E*,*Z*)-2,4-Heptadienal	0.92 ± 0.07	0.30 ± 0.03	0.63 ± 0.05	998	RI, MS
Octanal	–	0.81 ± 0.04	1.70 ± 0.09	1003	RI, MS
(*E*,*E*)-2,4-Heptadienal	4.53 ± 0.14	0.79 ± 0.12	1.65 ± 0.29	1012	RI, MS, S
Phenyl acetaldehyde	0.23 ± 0.03	0.30 ± 0.03	0.63 ± 0.07	1046	RI, MS, S
(*E*)-2-Octenal	1.67 ± 0.08	1.26 ± 0.12	2.63 ± 0.26	1057	RI, MS
Nonanal	–	1.12 ± 0.15	2.34 ± 0.30	1102	RI, MS, S
(*E*,*E*)-2,6-Nonadienal	–	0.13 ± 0.03	0.27 ± 0.07	1146	RI, MS
(*E*)-2-Nonenal	1.34 ± 0.12	0.54 ± 0.02	1.13 ± 0.05	1163	RI, MS, S
3-Ethylbenzaldehyde	–	0.15 ± 0.02	0.31 ± 0.04	1168	RI, MS
(*E*,*E*)-2,4-Nonadienal	0.87 ± 0.05	0.24 ± 0.06	0.50 ± 0.13	1220	RI, MS, S
4-(1-Methylethyl)benzaldehyde	–	0.29 ± 0.02	0.61 ± 0.04	1232	MS
(*E*)-2-Decenal	3.13 ± 0.07	1.74 ± 0.14	3.64 ± 0.29	1263	RI, MS
(*E*,*Z*)-2,4-Decadienal	–	1.94 ± 0.09	4.06 ± 0.19	1303	RI, MS
(*E*,*E*)-2,4-Decadienal	18.96 ± 0.48	5.32 ± 0.13	11.12 ± 0.26	1327	RI, MS, S
Tetradecanal	–	0.17 ± 0.04	0.36 ± 0.08	1608	RI, MS
Pentadecanal	–	0.39 ± 0.07	0.82 ± 0.14	1714	RI,MS
Hexadecanal	–	5.83 ± 0.13	12.19 ± 0.27	1816	RI,MS
(*E*)-9-Octadecenal	–	2.15 ± 0.11	4.49 ± 0.23	1991	MS
(*E*)-17-Octadecenal	–	1.12 ± 0.15	2.34 ± 0.30	2002	MS
Total	52.63	38.13	79.73		
Ketones					
3-Hydroxy-2-butanone	–	5.68 ± 0.67	11.87 ± 1.40	714	RI, MS, S
2-Heptanone	–	0.19 ± 0.07	0.40 ± 0.15	891	RI, MS, S
2,5-Octanedione	0.54 ± 0.02	0.87 ± 0.07	1.82 ± 0.14	985	RI, MS
2-Nonanone	–	0.17 ± 0.04	0.36 ± 0.07	1087	RI, MS
3,5-Octadien-2-one	0.88 ± 0.02	0.19 ± 0.02	0.40 ± 0.05	1098	RI, MS
2-Pentadecanone	0.62 ± 0.02	1.20 ± 0.13	2.51 ± 0.27	1698	RI, MS
Total	2.04	8.30	17.36		
Alcohols					
1-Penten-3-ol	0.25 ± 0.02	0.14 ± 0.06	0.29 ± 0.12	685	RI, MS
3-Methyl-1-butanol	–	0.25 ± 0.02	0.52 ± 0.05	732	RI, MS
1-Pentanol	–	0.42 ± 0.05	0.88 ± 0.11	771	RI, MS
1-Hexanol	–	0.18 ± 0.05	0.38 ± 0.10	869	RI, MS
3,5-Octadien-2-ol	–	0.16 ± 0.03	0.33 ± 0.06	1039	MS
Phenethyl alcohol	–	0.14 ± 0.06	0.29 ± 0.12	1114	RI, MS
Total	0.25	1.29	2.69		
Heterocycles					
2-Ethylfuran	0.14 ± 0.03	–	–	712	RI,MS
2-Methylpyrazine	0.36 ± 0.02	0.12 ± 0.08	0.25 ± 0.16	824	RI, MS
Furfural	0.32 ± 0.04	0.05 ± 0.01	0.10 ± 0.03	831	RI, MS
2-Furanmethanol	–	0.03 ± 0.01	0.06 ± 0.03	856	MS
2,5-Dimethylpyrazine	0.57 ± 0.10	0.35 ± 0.04	0.73 ± 0.09	912	RI, MS, S
2-Pentylfuran	1.18 ± 0.22	0.92 ± 0.12	1.92 ± 0.26	994	RI, MS, S
2-Acetylthiazole	0.09 ± 0.02	0.07 ± 0.02	0.15 ± 0.05	1016	MS
3-Ethyl-2,5-dimethylpyrazine	–	0.54 ± 0.06	1.13 ± 0.13	1078	RI, MS
Benzothiazole	–	0.02 ± 0.00	0.04 ± 0.00	1230	MS
γ-Dodecalactone	0.47 ± 0.06	1.12 ± 0.06	2.34 ± 0.13	1684	RI, MS
Total	3.13	3.22	6.72		

Table 1 (*continued*)

Compounds	SPME	SDE		RI[c]	I method[d]
	Peak area[a] (%)	Peak area[a] (%)	Amount[b] (ng g^{-1})		
Terpenes and oxygenated terpenes					
α-Pinene	0.23 ± 0.02	0.14 ± 0.04	0.29 ± 0.09	931	RI, MS
Camphene	–	0.18 ± 0.03	0.38 ± 0.06	943	RI, MS
Sabinene	–	0.09 ± 0.02	0.19 ± 0.05	975	RI, MS
β-Pinene	–	0.16 ± 0.01	0.33 ± 0.03	976	RI, MS
3-Carene	–	0.44 ± 0.01	0.92 ± 0.02	1007	RI, MS
Limonene	0.17 ± 0.04	1.25 ± 0.09	2.61 ± 0.19	1025	RI, MS
Fenchone	–	0.17 ± 0.01	0.36 ± 0.02	1085	RI, MS
Borneol	–	0.16 ± 0.02	0.33 ± 0.04	1173	RI, MS
Terpinen-4-ol	–	0.37 ± 0.10	0.77 ± 0.21	1177	RI, MS
Geranial	–	0.12 ± 0.02	0.25 ± 0.04	1268	RI, MS
δ-Elemene	–	0.11 ± 0.03	0.23 ± 0.07	1325	RI, MS
Copaene	0.36 ± 0.01	0.23 ± 0.05	0.48 ± 0.11	1367	RI, MS
β-Elemene	–	0.18 ± 0.09	0.38 ± 0.19	1395	RI, MS
Caryophyllene	0.22 ± 0.07	1.61 ± 0.18	3.34 ± 0.38	1421	RI, MS
β-bisabolene	1.15 ± 0.09	0.85 ± 0.03	1.78 ± 0.07	1504	RI, MS
γ-Cadinene	–	0.77 ± 0.04	1.61 ± 0.04	1508	RI, MS
E-Nerolidol	–	0.28 ± 0.01	0.59 ± 0.02	1556	RI, MS
Total	2.13	7.10	14.84		
Aromatic and aliphatic hydrocarbons					
Toluene	0.42 ± 0.02	–	–	764	RI,MS
1,3-*trans*,5-*cis*-Octatriene	0.26 ± 0.07	–	–	880	MS
Styrene	0.32 ± 0.05	–	–	892	MS
p-Cymene	–	0.16 ± 0.02	0.33 ± 0.04	1021	RI, MS
Undecane	–	0.34 ± 0.06	0.71 ± 0.13	1100	RI, MS
Naphthalene	0.21 ± 0.03	0.23 ± 0.07	0.48 ± 0.15	1190	RI, MS
Tetradecene	–	0.14 ± 0.05	0.29 ± 0.11	1381	MS
1-(1,5-dimethyl-4-hexenyl)-4-Methylbenzene	–	0.74 ± 0.16	1.55 ± 0.33	1480	MS
Pentadecane	2.17 ± 0.26	3.54 ± 0.18	7.40 ± 0.38	1496	RI, MS
8-Heptadecene	–	0.35 ± 0.07	0.73 ± 0.15	1670	MS
Triphenylmethane	–	0.34 ± 0.05	0.71 ± 0.10	1997	MS
Total	3.38	5.84	12.20		
Oxygenous benzene derivatives					
Estragole	1.56 ± 0.07	1.57 ± 0.12	3.28 ± 0.26	1206	RI, MS
cis-Anethole	0.74 ± 0.02	0.32 ± 0.01	0.69 ± 0.03	1245	RI, MS
trans-Anethole	26.81 ± 0.36	14.53 ± 1.20	30.37 ± 2.51	1290	RI, MS
Eugenol	4.46 ± 0.23	3.74 ± 0.17	7.82 ± 0.36	1361	RI, MS, S
1-(4-Methoxyphenyl)-2-propanone	–	0.35 ± 0.04	0.73 ± 0.09	1374	RI, MS
Ethyl cinnamate	0.79 ± 0.11	0.83 ± 0.07	1.73 ± 0.15	1466	RI, MS
cis-Ethyl *p*-methoxycinnamate	–	0.45 ± 0.05	0.94 ± 0.11	1653	MS
trans-Ethyl *p*-Methoxycinnamate	0.76 ± 0.02	2.64 ± 0.35	5.52 ± 0.73	1765	MS
Total	35.12	24.43	51.08		
Total volatiles	98.68	88.31	184.62		

[a] Means and standard errors of the identified compounds expressed as percentage of peak areas, means derived from three replicate samples.
[b] Means and standard errors of the identified compounds expressed as ng g^{-1} sample, means derived from three replicate samples.
[c] Linear retention index on a HP-5 low-bleed MS column.
[d] RI, agrees with retention index of literatures; MS, compared with Nist 02 Mass Spectral Database or the published mass spectra; S, agrees with retention index and mass spectrum of standard chemical.

thresholds, they are generally believed to have little contribution to meat flavor.

Similar to SPME analysis, aldehydes were again in the largest level, with hexanal (9.71%) and 2,4-decadienal isomers (7.26%) being the majors. The aldehydes were mostly of linear saturated and unsaturated aldehydes with more than five carbon atoms such as hexanal, (*E*)-2-hexenal, (*E*)-2-octenal, nonanal, (*E*,*E*)-2,6-nonadienal, (*E*,*E*)-2,4-nonadienal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E*,*Z*)-2,4-decadienal and (*E*,*E*)-2,4-decadienal, all of which were produced from fat oxidative degradation (Belitz & Grosch, 1987, chap. 3). On the other hand, the identified methyl-branched

aldehyde (3-methylbutanal) and phenyl acetaldehyde were probably related to amino acid degradation. Due to low odor thresholds and distinctive odors, the above volatile aldehydes should belong to potent contributors to the meat flavor. Besides, long chain aliphatic aldehydes, e.g. tetradecanal, pentadecanal and hexadecanal were also found in the SDE extract. However, these high molecular weight aldehydes probably just acted as precursors of the volatile saturated and unsaturated aldehydes, since lower volatility makes them less important to meat flavor.

Others that generally contribute to meat flavor by SDE present in Table 1 were heterocyclic compounds, ketones and alcohols. Heterocycles especially the sulfur-containing ones commonly appear as impact odorants. The heterocycles found were furfural, 2-furanmethanol, 2-pentylfuran, 2-methylpyrazine, 2,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 2-acetylthiazole, benzothiazole and γ-dodecalactone, accounting for 3.22% of the total peak areas. Though the sulfured 2-acetylthiazole and benzothiazole only fractioned in 0.09%, famous for their low thresholds and characteristic odors, they could contribute to the meat flavor significantly. Moreover, the ketones consisted of polyfunctional ketones and methyl ketones, representing 8.30% of the total. The polyfunctional ketones (6.74%) identified were 3-hydroxy-2-butanone, 2,5-octanedione and 3,5-octadien-2-one. Among them, 3-hydroxy-2-butanone possesses buttery and creamy characteristics. The methyl ketones identified were 2-heptanone, 2-nonanone and 2-pentadecanone; they are generally defined as sweet, fruity and fatty notes. The alcohols were fractioned as low as 1.29% of the total. Like the aliphatic aldehydes, both the methyl ketones and the aliphatic alcohols could arise from lipid degradation.

Noticeably, also analogous to SPME analysis, amount of the oxygenous benzene derivatives (eugenol, 1-(4-methoxyphenyl)-2-propanone, estragole, anethole, ethyl cinnamate and ethyl *p*-methoxycinnamate) in the SDE extract still ranked the second largest, representing 24.43% of the total. These spice components typically have herbaceous and woody odors and can play roles as blend and append the meat flavor. Together with the terpenoids (7.10%), they probably originated from spices used in the meat curing.

Conclusively, in comparison with the SPME analysis, the SDE approach resulted in a relatively complete picture of more representative volatiles found in the roasted pork aroma, and the semi-quantitative data were available. What was more, artifact formation and aroma degradation resulting from the SDE distillation were limited since the majority of aromatic components were identical between the SPME and the SDE and the SDE extract proved representative of the meat sample (assessed by a five member trained panel). This just agreed with observations of Raes et al. (2003) that for the type of roasted meat flavor analysis, Likens–Nickerson extraction is good "total volatile" practice, since roasting meat was already a severe heat treatment and distillation can eliminate the problem of aroma release from the heterogeneous meat samples, which usually presents in headspace sampling procedures. Thus in the following GC–O and GC–FID analysis, the SDE isolation was utilized.

3.1.3. Volatile profile of the roasted pork of Mini-pig

The procedure selected for the isolation of volatiles from meat is generally recognized as a crucial aspect of objective analysis of its aroma characteristics. To investigate the meat aroma completely, both the headspace SPME technique, which took into account the release of top volatiles from matrix, and a relatively total volatile isolation based on SDE were adopted in this study.

As shown in Table 1, in total, 86 different compounds were identified from the roasted pork of Mini-pig, which included 28 aldehydes, 6 ketones, 6 alcohols, 10 heterocycles, 16 terpenoids, 11 aromatic and aliphatic hydrocarbons and 9 oxygenous benzene derivatives. Volatile composition was characterized by major quantities of the lipid degradation aldehydes and the oxygenous benzene derivatives from spices.

The flavor of the roasted pork of Mini-pig, like that of other cooked meats, is mainly derived thermally, and the most important mechanisms responsible for the volatile formation are lipid degradation and Maillard reactions. Yet, in comparison, volatile profile of the roasted pork of Mini-pig was highly different from those of the others. For instance, 1-octen-3-ol was reported as the most abundant in the roasted Chinese-style pork jerky (Chen, Liu, & Chen, 2002) while hexanal was the highest in our analysis. And what is more, in fried bacon and fried pork loin (Timón et al., 2004), 19 short chain (C3–C9) aliphatic alcohols had ever been found, and their contribution to the total volatiles was as high as about 19% and 10% separately (derived by the amount of the alcohol fraction divided by the total). Nevertheless, the kinds of aliphatic alcohols were much less here, and the contribution in the SDE extract was as low as 1.5%. Besides, differences of the aliphatic hydrocarbons from fried bacon and fried pork loin also appeared prominent. Anyway, this can be ignored as hydrocarbons often contribute little to meat aroma. Otherwise, distinction could also be observed for components from Maillard reactions. Raes et al. (2003) found a large number of various heterocycles especially 14 pyrazines and 5 pyrrole derivatives from grilled Belgian retail beef (corresponding to *longissimus lumborum*), whereas only 3 pyrazines were found in this roasted Mini-pig pork.

As we know, specific aroma for one meat product is concerned with its meat composition of flavor precursors, e.g. fatty acids and amino acids as well as the process that it undergoes. For examples, in cured meat, alkanenitriles and pyridines are often in appreciable amount when nitrites or nitrates were used to develop cured meat flavor (Timón et al., 2004); in dry-cured hams, various methyl branched aldehydes, aliphatic alcohols and aliphatic carboxylic acids and their esters are usually detected resulting from microbial activity present in the long ripening stage (Flores et al.,1997; Huan, Zhou, Zhao, Xu, & Peng, 2005); and in smoke-cured meat products, often various

phenol derivatives and cyclopentenones exist (Hierro et al., 2004). For the present work, star aniseed and cinnamon were used in the curing ingredients, thus high amount of spice compounds, e.g. eugenol, 1-(4-methoxyphenyl)-2-propanone, estragole, anethole, ethyl cinnamate, and ethyl *p*-methoxycinnamate were contained in volatiles of the roasted Mini-pig pork.

3.2. Characterization of aromatic components by GC–O and GC–FID

Gas chromatography and olfactometry, which is used to evaluate the aromas eluted from GC, often results in the detection of some characteristic odors related to meat flavor. Besides, with the effluent splitter, GC chromatograms can be recorded simultaneously. By the correlation of odors and sniffing RI values in GC–O with chromatographic analysis, existences of some flavors can be further confirmed.

In fact, the SDE extract revealed aroma of the roasted pork, with green, roasted, meaty, mould and fatty but not oily characteristics (assessed by a five member trained panel). The GC–O results containing odor description, possible compound and odor occurrence were listed in the order of sniffing RI in Table 2. Total of 45 odor active regions were perceived, mainly corresponding to garlic and

Table 2
GC–O analysis of volatiles isolated by SDE from the roasted pork of Mini-pig

RI[a]	Odor description	Possible compound	Odor occurrence[b]
644–650	Odious, strong garlic	Unknown	10
656	Unpleasant, pungent, sour	3-Methylbutanal	10
690	Garlic, onion	Unknown	10
701	Pungent, green	Pentanal	10
714	Buttery, sour	3-Hydroxy-2-butanone	10
750	Sweet, herbaceous	(*E*)-2-Pentenal	10
759	Roasted meat	Unknown	9
804	Green, pungent	Hexanal	10
810	Garlic, onion	Unknown	10
822	Popcorn	2-Methylpyrazine	9
832	Sweet, caramel like	Furfural	6
853–859	Rancid, green, roasted	(*E*)-2-Hexenal + 2-furanmethanol	8
894	Green, sweet, spicy	2-Heptanone	7
904	Green, roasted, sweet	Heptanal	9
911	Roasted, popcorn	2,5-Dimethylpyrazine	10
941	Garlic, onion	Unknown	10
958–970	Roasted, green, sweet, almond	(*E*)-2-Heptenal + benzaldehyde	10
974–980	Green, woody	Sabinene + β-pinene	7
986	Green, sweet, buttery	2,5-Octanedione	6
993	Beany-greenish	2-Pentylfuran	6
998	Green, fatty, roasted	(*E*,*Z*)-2,4-Heptadienal	9
1001	Oily	Octanal	9
1013–1020	Green, roasted, meaty	(*E*,*E*)-2,4-Heptadienal + 2-acetylthiazole	10
1022	Green, spicy	*p*-Cymene	7
1025	Floral, green, sweet	Limonene	7
1046	Green, pungent, floral	Phenyl acetaldehyde	9
1075–1084	Nutty, roasted sunflower seeds	3-Ethyl-2,5-dimethylpyrazine	9
1100	Oily	Nonanal	7
1146	Green, cucumber	(*E*,*E*)-2,6-Nonadienal	9
1150	Onion, garlic	Unknown	10
1162	Green, fatty	(*E*)-2-Nonenal	9
1178	Roasted, woody	Terpinen-4-ol	6
1218	Fatty, fried, sweet	(*E*,*E*)-2,4-Nonadienal	8
1228–1235	Pungent, roasted, musty, sweet, nutty	Benzothiazole + 4-(1-methylethyl)benzaldehyde	9
1264	Green, roasted, earthy	(*E*)-2-Decenal	8
1292	Green, spicy	*trans*-Anethole	8
1325	Green, roasted	(*E*,*E*)-2,4-Decadienal	10
1329	Burnt rubber	δ-Elemene	7
1364	Burnt, woody	Eugenol	6
1425	Woody, herbaceous, spicy	Caryophyllene	6
1464	Woody, burnt rubber	Ethyl cinnamate	7
1536	Burnt rice	Unknown	8
1554	Sweet, burnt rice	*E*-nerolidol	7
1601	Roasted, fried meat	Tetradecanal	7
1652	Woody, spicy	*cis*-Ethyl *p*-methoxycinnamate	6

[a] Sniffed retention index.
[b] Assessment was repeated total of 10 times.

Table 3
GC–FID quantitative data of some flavors in the SDE extract from the roasted pork of Mini-pig (expressed as ng g^{-1} sample)

Flavor	Amount[a] (ng g^{-1})	Flavor	Amount[a] (ng g^{-1})
3-Methylbutanal	0.847 ± 0.002	2-Pentylfuran	1.276 ± 0.008
3-Hydroxy-2-butanone	8.001 ± 0.008	(*E*,*E*)-2,4-Heptadienal	1.582 ± 0.003
Hexanal	18.902 ± 0.002	Phenyl acetaldehyde	0.632 ± 0.002
(*E*)-2-Hexenal	0.432 ± 0.002	Nonanal	1.697 ± 0.001
2-Heptanone	0.322 ± 0.002	(*E*)-2-Nonenal	0.875 ± 0.003
Heptanal	0.883 ± 0.010	(*E*,*E*)-2,4-Nonadienal	0.414 ± 0.001
2,5-Dimethylpyrazine	0.450 ± 0.001	(*E*,*E*)-2,4-Decadienal	10.692 ± 0.013
(*E*)-2-Heptenal	0.851 ± 0.002	Eugenol	5.301 ± 0.004
Benzaldehyde	0.560 ± 0.004		

[a] Means and standard errors of the flavors, means derived from three replicate samples.

onion, green, roasted, sweet and spicy notes. Relating the sniffed RI values with the identifications of GC–MS, 39 odor active regions were assigned to 43 flavors. According to chemical family, these flavors contained 17 aliphatic aldehydes, 3 benzene derived aldehydes, 3 ketones, 7 terpenoids, 3 furans, 4 oxygenous benzene derivatives, 2 sulfur compounds, 3 pyrazines and 1 aromatic hydrocarbon. Due to no detection in GC–MS, contributors were not found for several odor active regions. For examples, in RI 644–650, RI 690, RI 810, RI 941 and RI 1150 were there obvious garlic and onion odors, in RI 759 were there roasted and meat like odors, and in RI 1536 were there burnt rice odors, but corresponding to the above RI regions, no peaks appeared in the chromatograms of both GC and GC–MS. However, as for the garlic and onion odors, they probably resulted from the low threshold impact sulfur-containing meaty flavors (Sun, Tian, Zheng, Liu, & Xie, 2005).

Otherwise, owing to the strong complexity of the analyte, which led to very rich chromatographic profiles and made it quite difficult to associate one single label to one single compound, odor descriptions of the sniffers were prone to differ from the literatures. Besides, the evaluation of a single peak or a given chromatographic region was often affected by the former eluents. Anyway, in Table 2, odor perception of 28 flavors pertaining to 26 odor active regions was basically similar to the reported (He & Sun 1995; Sun, 2003). They were 3-methylbutanal, pentanal, 3-hydroxy-2-butanone, hexanal, 2-methylpyrazine, furfural, (*E*)-2-hexenal, 2-heptanone, heptanal, 2,5-dimethylpyrazine, (*E*)-2-heptenal, benzaldehyde, 2-pentylfuran, octanal, (*E*,*E*)-2,4-heptadienal, 2-acetylthiazole, limonene, phenyl acetaldehyde, 3-ethyl-2,5-dimethylpyrazine, nonanal, (*E*,*E*)-2,6-nonadienal, (*E*)-2-nonenal, (*E*,*E*)-2,4-nonadienal, benzothiazole, (*E*)-2-decenal, *trans*-anethole, (*E*,*E*)-2,4-decadienal and eugenol.

So far, GC–O study on aroma of cooked meat is not so sufficient. Similarly, pentanal (rancid, grassy), 2-pentenal (rancid), hexanal (rancid, grassy oily), methylpyrazine (meaty), furfural (meaty), 2-heptanone (chemical bitter), heptanal (fruity), 2-heptenal (oily), 2,4-heptadienal (wet earth), phenyl acetaldehyde (biscuit rancid), nonanal (fruity, fatty), 2-nonenal (cooked meat, grassy), 3-hydroxy-2-butanone (buttery) and 2,4-nonadienal (meaty) had ever been sniffed from fried bacon and fried pork loin by GC–O (Timón et al., 2004). And so had 3-methylbutanal (pungent, sweet, roasty) and nonanal (fragrant, sweet, fatty, green, pungent) from shallow-fried beef (Specht & Baltes, 1994), and also 3-methylbutanal (chocolate, chemical), 2-heptanone (gas gravy), benzothiazol (stewed, gravy, roasted) and nonanal (gravy, green) from one commercial Irish beef meat (Machiels, van Ruth, Posthumus, & Istasse, 2003). As mentioned above, the aliphatic alcohols should play tiny roles in the roasted Mini-pig pork aroma as none of them were detected in the GC–O analysis, though from fried bacon and fried pork loin six aliphatic alcohols had been perceived.

In order to grasp and imitate the roasted Mini-pig meat flavor, when accessible, flavor compounds validated by GC–MS as well as GC–O in the SDE extract were further quantified by GC–FID with calibration curves of authentic chemicals using 1, 2-dichlorobenzene or tetradecane as internal, and the results were expressed as amount (ng) in per gram of meat sample. In Table 3, the quantities of 17 flavors, including 3-methylbutanal, 3-hydroxy-2-butanone, hexanal, 2-hexenal, 2-heptanone, heptanal, 2,5-dimethylpyrazine, (*E*)-2-heptenal, benzaldehyde, 2-pentylfuran, phenyl acetaldehyde, (*E*,*E*)-2,4-heptadienal, (*E*)-2-nonenal, nonanal, (*E*,*E*)-2,4-nonadienal, (*E*,*E*)-2,4-decadienal and eugenol were present, among which hexanal, (*E*,*E*)-2,4-decadienal and 3-hydroxy-2-butanone were the dominators, amounting to 18.902 ng g^{-1}, 10.692 ng g^{-1} and 8.001 ng g^{-1} separately.

4. Conclusions

For the first time, volatiles in the roasted pork of Mini-pig were studied. Sampling technologies of both headspace (SPME) and "total volatile analysis" (SDE) were performed. In total, 86 different compounds were identified. Volatile profile appeared as the highest amount of aldehydes from lipid oxidation followed by the spice components. In comparison, volatiles isolated by SPME and SDE were essentially similar, whereas a more complete and typical number of aromatic volatiles together with semi-quantitative data was obtained by SDE. And artifact formation during SDE was limited as roasting was already

a severe thermal treatment. Therefore, the SDE extract was selected in the succedent research work. Despite complexity of olfactometry and description differences present, 45 odor active regions were sniffed from the SDE extract. And by relating the sniffing RI values of GC–O with identifications in GC–MS, 43 contributors were found, among which the volatile saturated and unsaturated aliphatic aldehydes, the furans, the pyrazines and the sulfur compounds should be components of main note of the roasted Mini-pig pork aroma. The perception of strong garlic and onion odors in five RI regions could substantiate the contribution of some low threshold sulfur flavors in the SDE extract though few sulfur identifications were revealed in GC–MS. It seemed that the aliphatic alcohols probably functioned little in the roasted meat aroma since its amount was rather low and none of them was perceived in GC–O. Finally, to simulate the roasted Mini-pig pork flavor, 17 important flavor substances in the SDE extract were further quantified on GC–FID by calibration curves of authentic chemicals.

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