Korean J. Food Sci. An. Vol. 36, No. 6, pp. 719~728 (2016)
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DOI https://doi.org/10.5851/kosfa.2016.36.6.719
pISSN 1225-8563 eISSN 2234-246X

ARTICLE

Identification the Key Odorants in Different Parts of Hyla Rabbit Meat via Solid Phase Microextraction Using Gas Chromatography Mass Spectrometry

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Abstract

The aim of this study was to explore the volatile compounds of hind leg, foreleg, abdomen and *Longissimus dorsi* in both male and female Hyla rabbit meat by solid phase microextraction tandem with gas chromatography mass spectrometry, and to seek out the key odorants via calculating the odor activity value and principal component analysis. Cluster analysis is used to study the flavor pattern differences in four edible parts. Sixty three volatile compounds were detected, including 23 aldehydes, 4 alcohols, 5 ketones, 11 esters, 5 aromatics, 8 acids and 7 hydrocarbons. Among them, 6 aldehydes and 3 acids were identified as the potential key odorants according to the ratio of concentration and threshold. The contents of volatile compounds in male Hyla rabbit meat were significantly higher than those in female one (p<0.05). The results of principal component analysis showed that the first two principal component cumulative variance contributions reach 87.69%; Hexanal, octanal, 2-nonenal, 2-decenal and decanal were regard as the key odorants of Hyla rabbit meat by combining odor activity value and principal component analysis. Therefore volatile compounds of rabbit meat can be effectively characterized. Cluster analysis indicated that volatile chemical compounds of *Longissimus dorsi* were significantly different from other three parts, which provide reliable information for rabbit processing industry and for possible future sale.

Keywords: rabbit meat odor, solid phase microextraction, gas chromatography mass spectrometry, principal component analysis, cluster analysis

Received May 19, 2016; Revised October 25, 2016; Accepted October 27, 2016

Introduction

Rabbit meat is regarded as a functional food due to its excellent nutritive and dietetic properties benefiting for health (Dalle Zotte and Szendro, 2011). According to the statistics report by the Food and Agriculture Organization of the United Nations, the annual rabbit meat production of China has been increasing gradually in the past decade, from 467,000 tonnes in 2004 to 723,975 tonnes in 2013 (FAOSTAT, 2015). Although taking up one third of the whole production in the world, the average consumption of rabbit meat in China was much lower than other countries especially the Mediterranean countries. Apart from eating habit on rabbit meat of different regions in the world, the special odor affect people's purchasing preference and limit the spending on rabbit meat in China. Therefore, in order to improve the overall consumption of rabbit meat in China, the research on improving flavor and eating quality of rabbit meat was very important. Cookery, which could adjust the flavor of food artificially, is an effective way to cover this odor. That is why people who living in Sichuan Province and Chongqing, southwest city of China, are accustomed to the rabbit meat because of the chili. However, the dietary habit was also various in vast China. Thus, finding the origin of the odor and generated materials is a high priority. From the research about odor of pork meat, people begin to realize that the formation of odor may correlate with sex hormone level (Fischer *et al*., 2014; Weiler *et al*., 2013). Hence, gender is one of the factors must be considered about in this study. Meanwhile, consumers in Sichuan Province and Chongqing have various individual preferences for different edible parts of rabbit meat and the flavor of different edible parts may connect with this preference. The different parts of rabbit may have distinct volatile com-

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pounds affecting human perception. It is the first time to report and to analyze the odor of different gender and different parts of rabbit, to our knowledge. The extraction techniques largely effect the isolation and separation of flavor compounds and also effect the detection and identification of odor compounds from a mixture subsequently. Simultaneous distillation extraction (SDE) was used for studying chicken flavor, although this technique was time consuming and laborious (Ayseli *et al*., 2014). At present, solid phase microextraction (SPME) is considered as an effective isolation method for food aroma analysis (Gu *et al*., 2013). The method was developed in 1990 and was widely used for aroma extraction of meat until now, with its special characteristics of saving preparation time, disposal costs and solvent-free (Domínguez *et al*., 2014; Donadel *et al*., 2013; Kataoka *et al*., 2000; Yang *et al*., 2014). Gas chromatography tandem with mass spectrometry (GC-MS) system has been widely applied to food flavor analysis. However, the contribution of each single compound to overall aroma profile cannot obtain from GC-MS results. In order to make up for it, odor activity value (OAV) is a necessary method for determining the key odorants of the volatile compounds. The OAV is calculated through dividing the concentration of a compound by its odor threshold in air. Thus, the internal standard added in sample is necessary for quantitative analysis. As different parts own different volatile compounds, principal component analysis (PCA) and linear discriminant analysis (LDA) are used to distinguish the samples by means of the data from GC-MS (Sun *et al*., 2014). Cluster analysis (CA) is used to identify the similarity of the samples according to the OVA and to classify the different patterns into groups. In the past few years, researches on the identification of volatile compounds in rabbit meat were rarely published. Also, most of the studies were focused only on relative content of volatile compounds and compounds varieties, such as alcohol, aldehydes, ketones, acids and esters and so on. However, the key odorants of rabbit meat were not clear until now, without using OAV method. The aim of this study was to determine the volatile compounds in four edible parts (hind leg, foreleg, abdomen and *Longissimus dorsi*) of both male and female Hyla rabbit, to ensure the key odorants of rabbit meat by both OAV and PCA, and to classify the different parts into groups by CA as a key point for processing industry and for possible future sale.

Table 1. Ingredients and proportion of the Hyla rabbit diet

Ingredients	Proportion (%)
Alfalfa	35
Corn	24.8
Corn germ cake	4
Dicalcium	0.8
Lysine	0.07
Methionine	0.11
Powder	0.5
Premix[a]	1
Rapeseed	3
Salt	0.5
Soybean	10.11
Wheat bran	20.07

[a]The premix contains (per kg of diet): Vitamin A, 10000 IU; Vitamin D3, 1000 IU; Vitamin E, 30 mg; Vitamin K, 1 mg; Vitamin B1, 1 mg; Vitamin B2, 3.5 mg; Vitamin B6, 2 mg; Vitamin B12, 0.01 mg; niacin, 50 mg; folic acid, 0.3 mg; choline, 1000 mg; Zn, 30 mg; Cu, 5 mg; Mn, 15 mg; Fe, 30 mg; I, 1 mg.

Materials and Methods

Chemicals

Hexanal (GC, >99.3 pure), heptanal (GC, >99.3 pure), octanal (GC, >99.3 pure), butanoic acid (GC, >99.3 pure), 2-nonenal (GC, >99.3 pure), dodecanoic acid (GC, >99.3 pure) and nonanal (GC, >99.3 pure) were supplied by Sigma-Aldrich (USA). C7-C30 Saturated Alkanes Standard (1000 mg/mL in hexane) was purchased from Supelco (USA). Saturated NaCl aqueous was prepared according to the following steps in the lab: 1. 36 g NaCl was weighted by electronic scales precisely; 2. Dissolved in beaker using 100 g water at room temperature; 3. Labeled and stored in narrow-necked bottles. 2, 4, 6-Trimethylpyridine (TMP) was obtained from J&K Scientific Ltd (China).

Sample preparation

Both fifty 75-d old male and female Hyla rabbits were purchased and slaughtered in College of Animal Science and Technology, Southwest University, Chongqing, with the average slaughter weight of 2.53±0.09 kg. Table 1 shows the diet composition of Hyla rabbit (Xue *et al*., 2015). Foreleg, hind leg, abdomen and *Longissimus dorsi* of rabbit meat were segmented and deboned immediately after slaughtering and stored at -20°C respectively until use. The animal experiment was followed by the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China.

Selection of SPME fibers

Carboxen polydimethylsiloxane (CAR/PDMS) fiber and the SPME holder was purchased from Supelco (USA) and

a 75 μm carboxen polydimethylsiloxane (CAR/PDMS) SPME fiber (Supelco, USA) was chosen for this experiment due to better extraction ability than others and lower polarity for volatile organic compounds (Kataoka *et al*., 2000).

Head space (HS)-SPME

Different parts of rabbit meat were well minced and homogenized during 1 min in a household blender. Three grams of the rabbit meat of each part and 3 mL saturated NaCl aqueous solution were placed in a 25-mL vial, sealed with a PTFE-silicone septum and screw cap. After shaking with a lab dance min vortexer for 1 min, the sample was equilibrated in the water bath at 45°C for 15 min. Then fiber which was preconditioned at 250°C for 60 min in the GC injector port was inserted into the vial. The fiber coating was exposed to the sample headspace and the sampling process last at 75°C for 60 min in a thermostat controlled water bath (±1°C). The selection of 75°C as the absorption temperature for HS-SPME was on the basis that requirement of central temperature for meat, poultry and aquatic dishes according to the critical control point in China, so as to simulate the volatiles of boiled rabbit meat. Finally, the fiber was retracted into a needle, transferred to an injection port of the GC system, and desorbed for 5 min at 250°C.

Gas chromatography-mass spectrometry

The volatile compounds in rabbit meat analyzed using an SHIMADZU QP2010 gas chromatograph tandem mass spectrometry. The sample was desorbed in the injection port at 250°C for 5 min. Ultra-pure helium was used as the carrier gas at a flow of 1.1 mL/min. Volatile organic compounds of each sample were separated with DB-5 ms column (122-5532, 30 m length × 250 μm × 0.25 μm film thickness). Each extract was injected by manual with the splitless mode (injector temperature, 250°C). The GC oven temperature was initially held at 40°C for 1 min, then to 180°C at 6°C /min holding for 3 min, to 230°C at 10°C/min with a final hold of 1 min. MS parameters were as follows: ionization energy, 70 eV; ion source temperature, 230°C; quadrupole temperature, 280°C; mass range, m/z 35-350; detector interface temperature, 250°C.

Identification and quantification of compounds

The volatile compounds in rabbit meat were tentatively identified by comparing for mass spectra library to those found in data NIST11.L, by comparing of van den Dool and Kratz indices to those reported in the literature and by comparison of GC retention indices (RI). In this study, 100 μL diluted TMP as an internal standard substance, with a concentration of 0.92 g/mL, was added to 30 g sample. The identified compounds can be calculated by comparing the peak areas with standard substance and the calibration factors were all considered as 1.00. The content of volatile compounds is calculated as follows:

$$M_C(\mu g/kg) = \frac{A_S - C_{TMP}}{A_I} \times \frac{V_{TMP}}{M_S} \times 10^{-3} \quad (1)$$

Where M_C is the content of compound, A_S is the peak area of single compound; A_I is the peak area of internal standard material; C_{TMP} is the concentration of TMP, g/mL; V_{TMP} is the volume of internal standard material, μL; M_S is the weight of meat sample, g.

Sensory analysis

A sensory test was carried out by a trained panel of 9 members, with 5 male and 4 female students, staffs and teachers. Panelists were asked to point out the intensity of rabbit meat odor of hind leg, fore leg, abdomen and *Longissimus dorsi*. Sensory attribute for rabbit meat odor was assessed with a 5 point intensity line scale, where 1 = no obvious odor and 5 = extremely intensity. All the samples were cooled to room temperature after boiling.

Statistical analyses

All statistical analyses were performed using SPSS 22.0 version. The significant differences ($p<0.05$) among concentrations of volatile compounds in four parts of male and female rabbit were analyzed by ANOVA. PCA was used to determine the key odorants of volatile compounds in rabbit meat, while CA was applied in this study based on OAV data to sort different patterns.

Results and Discussion

GC-MS analyses

A total of 63 volatile compounds, including 23 aldehydes, 4 alcohols, 5 ketones, 11 esters, 5 aromatics, 8 acids and 7 hydrocarbons, were identified in four different parts of both male and female rabbits (Table 2). Among the 63 volatile compounds, 33 and 42 were found in the foreleg of male and female rabbit meat respectively, 31 and 34 were in the hind leg, 33 and 48 were in the abdomen, 27 and 33 were in the *Longissimus dorsi* (Fig. 1). Comparing with other parts, abdomen in both male and female rabbits owned all seven types volatile compounds, and the number of volatile compounds in abdomen was more

Table 2. Thresholds and concentrations of volatile compounds identified in foreleg, hind leg, abdomen and *Longissimus dorsi* of both male and female rabbit meat (n=3)

Code No.	Compounds	Identification	Threshold* (μg/kg)	Concentration (μg/kg)							
				FL		HL		AB		LD	
				M	F	M	F	M	F	M	F
				Aldehydes (23)							
Q1	Pentanal	MS,RI	9[15]	7.34±0.12	9.81±0.23	3.77±0.08	5.24±0.09	7.16±0.10	7.17±0.11	5.21±0.07	1.61±0.03
Q2	2-Pentenal	MS,RI	1500[15]	N.D.	N.D.	N.D.	N.D.	N.D.	0.27±0.01	N.D.	N.D.
Q3	Hexanal	MS,RI, STD	10.5[4]	22.14±0.15	26.46±0.16	27.79±0.17	24.56±0.15	29.56±0.17	31.51±0.19	11.51±0.09	5.46±0.07
Q4	2-Hexenal	MS,RI	19.2[15]	N.D.	0.35±0.01	N.D.	N.D.	N.D.	0.28±0.01	0.57±0.01	N.D.
Q5	Heptanal	MS,RI, STD	3[4]	1.43±0.02	0.90±0.01	1.84±0.02	0.74±0.05	1.54±0.01	1.7±0.01	2.40±0.01	0.21±0.01
Q6	2-Heptenal	MS,RI	13[4]	7.39±0.13	4.49±0.03	7.31±0.03	1.45±0.01	6.21±0.07	5.39±0.02	6.28±0.01	0.35±0.01
Q7	2,4-Heptadienal	MS,RI	15.4[15]	0.68±0.01	N.D.	0.86±0.02	N.D.	0.59±0.01	0.48±0.01	N.D.	N.D.
Q8	Octanal	MS,RI, STD	0.7[4]	0.97±0.02	1.06±0.01	1.50±0.01	1.27±0.02	1.50±0.03	1.90±0.05	1.17±0.01	0.71±0.01
Q9	2-Octenal	MS,RI	3[4]	1.29±0.03	0.77±0.01	1.53±0.01	0.47±0.01	1.25±0.01	1.10±0.01	2.65±0.01	0.14±0.01
Q10	Nonanal	MS,RI, STD	1[4]	1.40±0.02	1.20±0.02	2.16±0.02	3.61±0.02	3.04±0.02	2.64±0.01	2.79±0.01	2.94±0.01
Q11	2-Nonenal	MS,RI	0.08[4]	0.45±0.01	0.18±0.01	0.50±0.04	0.27±0.01	0.68±0.01	0.08±0.01	1.27±0.01	0.13±0.01
Q12	2,4-Nonadienal	MS,RI	0.06[4]	0.27±0.01	0.14±0.01	0.14±0.01	0.12±0.01	N.D.	0.25±0.00	N.D.	N.D.
Q13	2-Decenal	MS,RI	0.4[4]	0.57±0.02	0.30±0.01	0.73±0.01	0.31±0.01	0.75±0.01	0.50±0.01	2.76±0.02	1.23±0.01
Q14	2,4-Decadienal	MS,RI, STD	0.07[4]	0.82±0.02	1.36±0.02	2.23±0.01	1.79±0.01	0.59±0.03	0.75±0.01	0.23±0.01	0.37±0.01
Q15	Decanal	MS,RI	0.1[4]	N.D.	N.D.	N.D.	N.D.	N.D.	0.75±0.01	7.77±0.05	1.11±0.01
Q16	Undecanal	MS,RI	5[15]	0.09±0.01	0.10±0.01	0.21±0.01	0.27±0.01	0.18±0.01	0.14±0.01	0.91±0.06	0.89±0.02
Q17	2-Undecenal	MS,RI	3.16	0.59±0.01	0.21±0.01	N.D.	0.29±0.01	0.93±0.05	0.44±0.02	2.67±0.01	N.D.
Q18	Dodecanal	MS,RI	N.A.	0.20±0.01	0.21±0.01	N.D.	N.D.	0.52±0.01	0.12±0.01	0.84±0.06	0.45±0.01
Q19	Tridecanal	MS,RI	1.00[6]	N.D.	0.03±0.01	N.D.	N.D.	N.D.	0.04±0.01	0.43±0.02	N.D.
Q20	Tetradecanal	MS,RI	0.23	0.18±0.01	0.02±0.01	0.40±0.01	0.45±0.02	0.43±0.01	0.09±0.01	1.11±0.06	N.D.
Q21	Hexadecanal	MS,RI	0.91	N.D.	0.09±0.01	0.06±0.01	N.D.	1.24±0.11	N.D.	N.D.	0.54±0.01
Q22	Octadecanal	MS,RI	N.A.	N.D.	N.D.	1.57±0.01	N.D.	0.38±0.01	0.26±0.01	13.22±0.03	0.07±0.01
Q23	Benzaldehyde	MS,RI	990[5]	N.D.	N.D.	N.D.	0.34±0.03	N.D.	0.53±0.05	N.D.	0.22±0.01
				Alcohols (4)							
C1	n-Tridecan-1-ol	MS,RI	N.A.	0.29±0.01	N.D.	0.57±0.03	N.D.	1.47±0.01	N.D.	N.D.	N.D.
C2	Hexadecen-1-ol	MS,RI	N.A.	0.14±0.01	N.D.	0.25±0.02	N.D.	N.D.	N.D.	N.D.	N.D.
C3	1-Octanol	MS,RI	27[13]	N.D.	0.53±0.03	N.D.	N.D.	N.D.	0.40±0.03	N.D.	N.D.
C4	3,5-Octadien-2-ol	MS,RI	N.A.	N.D.	0.13±0.01	N.D.	N.D.	N.D.	0.12±0.01	N.D.	N.D.
				Ketones (5)							
T1	2-Heptanone	MS,RI	300[13]	N.D.	N.D.	N.D.	N.D.	N.D.	0.14±0.01	N.D.	N.D.
T2	1-Octen-3-one	MS,RI	10[13]	0.57±0.01	0.38±0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
T3	2-Nonanone	MS,RI	100[13]	N.D.	N.D.	N.D.	N.D.	N.D.	0.04±0.01	N.D.	N.D.
T4	3-Methl-2-butanone	MS,RI	N.A.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3.34±0.01
T5	2,3-Octanedione	MS,RI	12[15]	N.D.	N.D.	N.D.	N.D.	0.48±0.01	N.D.	N.D.	N.D.
				Esters (11)							
Z1	Decanoic acid methyl ester	MS	N.A.	0.38±0.01	0.64±0.01	0.30±0.02	0.09±0.00	0.11±0.03	0.07±0.01	N.D.	N.D.
Z2	Tridecanoic acid methyl ester	MS	N.A.	0.18±0.01	N.D.	0.06±0.01	N.D.	0.06±0.01	N.D.	0.13±0.02	N.D.

Table 2. Thresholds and concentrations of volatile compounds identified in foreleg, hind leg, abdomen and *Longissimus dorsi* of both male and female rabbit meat (n=3) (Continued)

Code No.	Compounds	Identification	Threshold* (μg/kg)	Concentration (μg/kg)							
				FL		HL		AB		LD	
				M	F	M	F	M	F	M	F
Z3	Hexadecanoic acid methyl ester	MS	N.A.	0.88±0.02	0.17±0.02	0.52±0.02	0.17±0.01	9.93±0.23	0.39±0.02	5.64±0.11	0.30±0.03
Z4	Octadecanoic acid methyl ester	MS	N.A.	1.56±0.01	0.07±0.01	0.19±0.01	0.06±0.01	0.75±0.01	0.21±0.01	1.02±0.01	0.13±0.01
Z5	9-Octadecenoic acid methyl ester	MS	N.A.	5.17±0.03	0.13±0.01	0.41±0.01	0.14±0.01	1.97±0.02	1.23±0.11	N.D.	N.D.
Z6	9,12-Octadecadienoic acid methyl ester	MS	N.A.	1.54±0.01	0.07±0.01	0.14±0.01	0.09±0.01	0.75±0.01	0.15±0.01	0.48±0.04	0.14±0.03
Z7	Dodecanoic acid methyl ester	MS	N.A.	0.29±0.01	0.06±0.01	N.D.	N.D.	0.36±0.01	0.27±0.01	N.D.	N.D.
Z8	Pentadecanoic acid methyl ester	MS	N.A.	4.08±0.02	N.D.	N.D.	N.D.	1.04±0.01	N.D.	N.D.	N.D.
Z9	Hexanoic acid hexyl ester	MS	N.A.	N.D.	0.09±0.01	N.D.	N.D.	N.D.	0.19±0.01	N.D.	N.D.
Z10	Hexanoic acid pentyl ester	MS	N.A.	N.D.	0.07±0.01	N.D.	0.08±0.01	N.D.	0.07±0.01	N.D.	N.D.
Z11	Hexanoic acid octyl ester	MS	N.A.	N.D.	0.03±0.01	N.D.	0.09±0.01	N.D.	N.D.	N.D.	0.16±0.01
	Aromatics (5)										
F1	Naphthalene	MS,RI	60[15]	0.18±0.01	N.D.	0.20±0.07	N.D.	0.27±0.01	N.D.	0.23±0.01	N.D.
F2	Benzene pentyl	MS,RI	N.A.	N.D.	N.D.	N.D.	N.D.	0.11±0.01	0.05±0.01	N.D.	N.D.
F3	Styrene	MS,RI	65[15]	1.19±0.01	0.59±0.01	1.52±0.02	0.57±0.03	1.47±0.07	0.64±0.02	N.D.	N.D.
F4	Toluene	MS,RI	1550[15]	N.D.	N.D.	N.D.	N.D.	0.45±0.01	0.34±0.01	N.D.	N.D.
F5	Ethylbenzene	MS,RI	2205[15]	N.D.	N.D.	N.D.	N.D.	0.46±0.03	0.26±0.01	N.D.	N.D.
	Acids (8)										
A1	Decanoic acid	MS,RI	2800[12]	0.75±0.01	0.76±0.02	0.81±0.02	0.82±0.01	0.98±0.01	0.95±0.01	0.78±0.01	0.77±0.01
A2	Butanoic acid	MS,RI	240[4,19]	1.23±0.02	1.21±0.01	1.45±0.02	1.41±0.01	0.98±0.01	0.95±0.01	1.79±0.01	1.68±0.02
A3	Tetradecanoic acid	MS,RI	N.A.	N.D.	0.26±0.01	N.D.	0.17±0.01	N.D.	0.26±0.01	N.D.	0.63±0.01
A4	Hexadecanoic acid	MS,RI	N.A.	N.D.	1.84±0.02	N.D.	3.31±0.02	N.D.	2.66±0.02	N.D.	5.47±0.03
A5	Octadecanoic acid	MS,RI	N.A.	N.D.	1.31±0.01	N.D.	2.43±0.01	N.D.	1.91±0.01	N.D.	0.04±0.01
A6	Oleic Acid	MS,RI	N.A.	N.D.	2.42±0.01	N.D.	4.81±0.01	N.D.	3.87±0.01	N.D.	7.52±0.02
A7	Dodecanoic acid	MS,RI	9153	0.41±0.02	0.42±0.02	0.35±0.01	0.33±0.01	0.78±0.01	0.74±0.01	0.28±0.01	0.27±0.01
A8	Heptadecanoic acid	MS,RI	N.A.	N.D.	0.02±0.01	N.D.	0.12±0.01	N.D.	0.05±0.01	N.D.	0.11±0.01
	Hydrocarbon (7)										
H1	Dodecane	MS,RI	2040[15]	0.15±0.01	0.12±0.01	0.09±0.01	0.07±0.01	N.D.	N.D.	N.D.	N.D.
H2	Tridecane	MS,RI	2140[15]	N.D.	N.D.	1.13±0.03	0.91±0.02	N.D.	N.D.	0.14±0.01	0.12±0.01
H3	Tetradecane	MS,RI	N.A.	N.D.	N.D.	0.24±0.01	1.26±0.02	0.38±0.01	N.D.	0.23±0.01	0.73±0.01
H4	Pentadecane	MS,RI	N.A.	0.35±0.03	0.29±0.01	0.51±0.01	0.99±0.01	1.20±0.01	0.14±0.01	0.52±0.01	0.72±0.02
H5	Hexadecane	MS,RI	N.A.	4.81±0.01	0.46±0.01	3.41±0.01	0.16±0.01	6.18±0.01	0.19±0.01	2.03±0.01	0.44±0.01
H6	Octadecane	MS,RI	N.A.	N.D.	N.D.	0.62±0.01	0.45±0.02	N.D.	N.D.	N.D.	N.D.
H7	Nonadecane	MS,RI	N.A.	N.D.	0.02±0.01	N.D.	0.09±0.01	N.D.	0.07±0.01	N.D.	1.03±0.01

*Odor threshold of each component was determined in water.

MS, mass spectrum; RI, retention index; STD, standard substance; FL, foreleg; HL, hind leg; AB, abdomen; LD, *Longissimus dorsi*.

N.A., not available; N.D., not detectable.

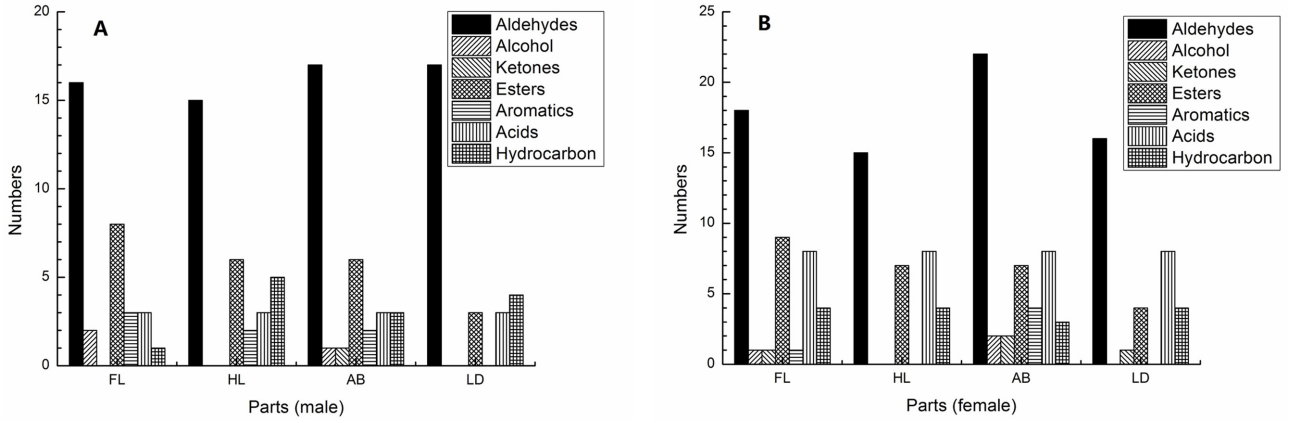


Fig. 1. Number differences of volatile compounds among four parts of both male (A) and female (B) Hyla rabbit meat. FL, foreleg; HL, hindleg; AB, abdomen; LD, *Longissimus dorsi*.

than that of other three parts. This indicated that the stronger odor may generate in abdomen than in other parts of rabbit meat. On the contrary, the volatiles numbers in *Longissimus dorsi* were the least among all four parts, which means weak odor intensity. Aldehydes, of all detected volatile compounds, owned largest amount in four parts of both male and female rabbits. Esters and hydrocarbon owned the second and third largest amount in male rabbit meat, while esters and acids took up the second and third largest quantity in female rabbit meat. As we all know that aldehydes play a significant role in overall flavor of meat, due to its very low threshold. For example, saturated aldehydes have the second important relation with lamb's overall flavor (Bueno *et al*., 2014). Pentanal, hexanal, heptanal, 2-heptenal, octanal, 2-octenal, nonanal, 2-nonenal, 2-decenal, undecanal, 2, 4-decadienal, decanal, butanoic acid, dodecanoic acid, hexadecanoic acid methyl ester, octadecanoic acid methyl ester and 9,12-octadecadienoic acid methyl ester were detected in all four parts of both male and female rabbit meat. It was shown that no unique substance was found exclusively in both male and female rabbit meat, but the concentrations of those substances were significantly different ($p<0.05$). This distinction may elucidate the strength of the odor in male rabbits was stronger than that of female rabbits. From Fig. 2, significant difference of detected volatile compounds between genders was found in FL, AB and LD, except HL ($p<0.05$). Thus, we confirmed that the odor of rabbit meat had closely relationship with genders. These significant differences may relate with the amount of intramuscular lipids in different parts and between genders (Neethling *et al*., 2016). Moreover, the peak areas of male rabbit meat are larger than those of

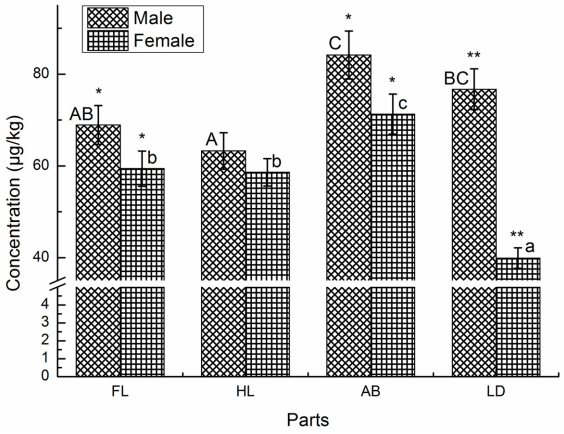


Fig. 2. Concentration of detected volatile compounds of different parts in both male and female Hyla rabbit meat. FL, foreleg; HL, hindleg; AB, abdomen; LD, *Longissimus dorsi*.

female one, representing that the odor of male rabbit meat is stronger than female one (Fig. 3). However, the reason is yet unknown and is worth further studying.

Selection of key odorants

Eight volatile compounds, with OAV greater than one, were found among the whole 63 volatile compounds. All eight volatile compounds were aldehydes. Decanal owns the highest OAV (77.7) among the aldehydes, and 2, 4-decadienal (35.8) is the second. Although the concentration of hexanal is larger than other seven aldehydes, the OAV is the smallest in all eight volatile compounds. Pentanal and hexanal were positively related with liver and rancid off-flavor in various beef muscles (Stetzer *et al*., 2008). Both pentanal and hexanal showed closely rela-

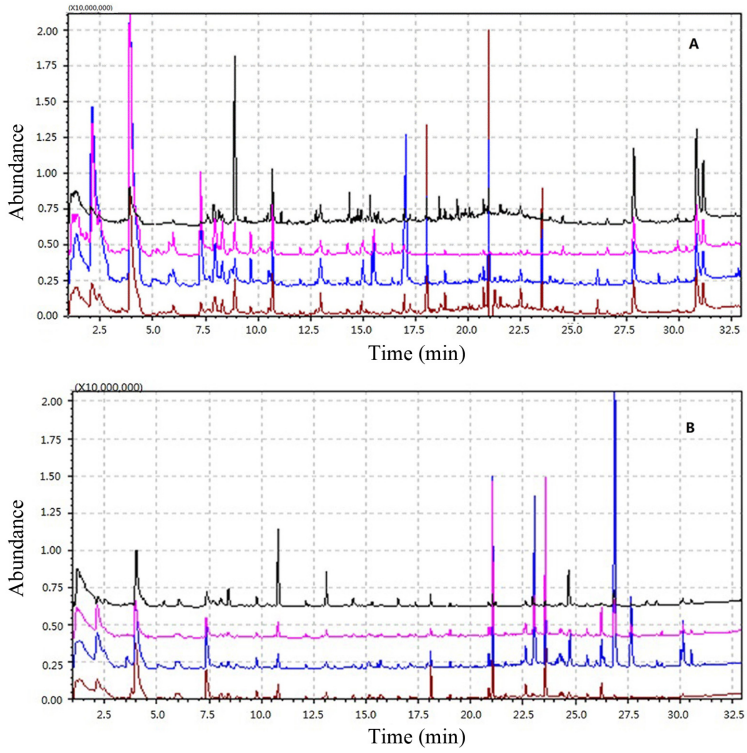


Fig. 3. Comparisons of volatile compounds in four edible parts of both male (A) and female (B) Hyla rabbit meat. FL, black line; HL, pink line; AB, blue line; LD, brown line.

tionship with TBARS in meat volatile flavor. However, only the female rabbit foreleg's concentration of pentanal was larger than its threshold. This indicated that pentenal made no contribution to the overall flavor of rabbit meat. Hexanal represents the the lipid oxidation status of the meat better than any other volatile component (Brunton *et al*., 2000), and it was regarded as an indicator of flavor deterioration in various meat volatile compounds (Goodridge *et al*., 2003; Shahidi and Pegg, 1994). However, the OAV of hexanal in rabbit meat is the lowest of all eight aldehydes, which means it is just one of the key odorants. The concentration of hexanal in abdomen of both male and female rabbits showed highest, with lowest in both male and female *Longissimus dorsi*. The odor description of octanal is solvent, lemon and bitter. It can be produced during oxidation of saturated or unsaturated fatty acids from tallow, and also had relevance with off-flavors in cooked chicken (Kang *et al*., 2013; Shi *et al*., 2013). The concentration distribution of octanal showed that hind leg and abdomen were higher than foreleg and *Longissimus dorsi*. Nonanal which is one of the major aldehydes found in boiled beef, was also found in rabbit meat (Ruan *et al*., 2015). According to the research on the "Hanwoo" beef, what is different is that octanal and nonanal derived from oleic acid shows pleasant flavor (Hoa *et al*., 2013). The concentration of nonanal in foreleg was lowest in both male and female rabbit meat among four parts. The OAV of 2-nonenal (15.9) which smells like cardboard in rabbit meat is third highest in our research, and it was one of the main contributors to the overall off-flavor of porcine liver (Im *et al*., 2004). Like octanal, 2-nonenal was also common product generated from thermal oxidation of tallow, and the concentration of 2-nonenal in male rabbits was higher than in female rabbits. From aspect of distribution in different parts, the content of 2-nonenal in *Longissimus dorsi* of male rabbits was highest, whereas that in abdomen of female rabbits was lowest. 2, 4-Decadienal was detected as one of the key volatile compounds in many kinds of meat (Chen *et al*., 2009; Christlbauer and Schieberle, 2009; Madruga *et al*., 2009). In our study, the concentration of 2, 4-decadienal in hind leg was highest for both male and female rabbit, and they were lowest in *Longissimus dorsi* of both male and female rabbits. Moreover, decanal was not detected in hind leg while its concentration was highest in part of *Longissimus dorsi*. As compounds of decanal and 2, 4-decadienal has first two highest OAV, it means that the flavor pattern of hind leg and *Longissimus dorsi* were obviously different.

The concentrations of butanoic acid, decanoic acid and dodecanoic acid in all four parts of rabbit meat were lower than their odor thresholds, so acids were not responsible for the odor of rabbits here. The same situation was also found in ketones, esters, alcohols, aromatics and hydrocarbon. It can be seen that aldehydes were the main characterization odor of rabbit meat. So, this means that the oxidation of lipid may be most responsible for the odor of rabbit meat.

Principal component analysis of volatile compounds of rabbit meat

From Fig. 4, the number of acids in female rabbit meat is more than male one. Apart from *Longissimus dorsi*, three other parts owned various kinds of volatile compounds between male and female rabbit meat. In order to find out the key odorants target of rabbit meat, all detected 16 chemical compounds were choose to analyze via PCA with software of SPSS22.0. The results of two-axis analysis were PC1 being 54.35% and PC2 being 32.41%, respectively. The first and second principal component can explain 86.76% of the total variance. From PC1 (Fig. 4), dodecanoic acid (13), hexanal (2), decanal (12) and octanal (4) represented the rabbit meat flavor, meanwhile heptanal (3), 2-heptenal (7), 2-nonenal (8) and 2-decenal (9) were the main effective volatile compounds in PC2. This means those eight chemical volatile compounds seriously affected the flavor of rabbit meat, and represented the key odorants of rabbit meat flavor. Combining with method of OAV, hexanal (2), octanal (4), 2-nonenal (8), 2-decenal (9) and decanal (12) were seen as key odorants, with OAV>1. The odor description and origination of those five odorants can be seen from Table 3.

Cluster analysis of different parts of rabbit meat

Four different parts of both male and female rabbit meat were divided into two groups according to CA of the key odorants (Fig. 5). The first group contained fore leg, hind leg and abdomen of both male and female rabbit, and the second group included both the male and female *Longissimus dorsi* of Hyla rabbit meat. This indicated that the flavor of *Longissimus dorsi* significantly different from other three parts. It was accordance with sensory analysis results which showed that the intensity of rabbit meat odor of *Longissimus dorsi* was obviously less than other three parts (Table 4). And it was clear that the content of intramuscular phospholipids in *Longissimus dorsi* was lower than both abdominal muscle and hind leg. Hence, this may relate with the content of intramuscular lipid and the profile of fatty acid composition (Xue *et al*., 2015). In the first sub cluster, case 3 was close to case 4 and case 5 was close to case 6, suggesting that flavor of hind leg and abdomen in both male and female rabbit meat were the same. In the second sub cluster, case 1 was separated from other cases, manifesting that flavor of male fore leg was greatly different from other parts. Overall, cluster analysis results provide reliable information for rabbit processing industry and for possible future sale.

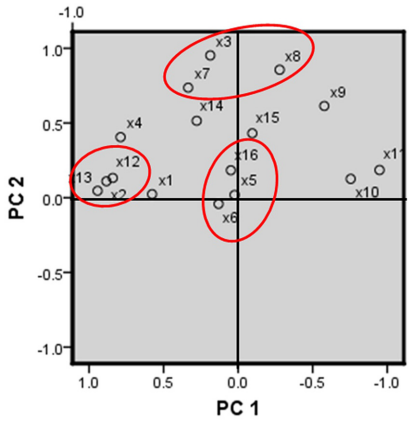


Fig. 4. Principal component analysis of all 16 detected volatile compounds in Hyla rabbit meat. x1-x16: pentanal, hexanal, heptanal, 2-heptenal, octanal, 2-octenal, nonanal, 2-nonenal, 2-decenal, undecanal, 2, 4-decadienal, decanal, butanoic acid, dodecanoic acid, hexadecanoic acid methyl ester, octadecanoic acid methyl ester, 9,12-octadecadienoic acid methyl ester.

Table 3. Potential key odorants in Hyla rabbit meat

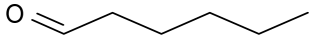
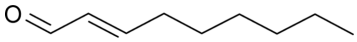
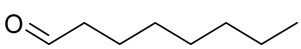
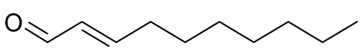
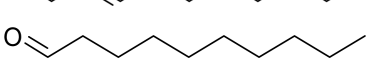
Compounds	CAS	Molecular formula	Odor description[13,20]	Origination[16,21,23,26]
Hexanal	66-25-1		Green, grassy, fatty	Lipid degradation or decarboxylation
2-Nonenal	18829-56-6		Fatty, waxy	Oxidation of arachidonic acid
Octanal	124-13-0		Pungent, orange	Oxidation of fatty acids
2-Decenal	3913-81-3		Metallic, soap	Thermal oxidation of tallow
Decanal	112-31-2		Green, oily	Autoxidation of unsaturated fatty acids

Table 4. Sensory scores of intensity of rabbit meat odor in four different parts of Hyla rabbit

	Fore leg	Hind leg	Abdomen	*Longissimus dorsi*
Scores	3.22±0.83	3.44±0.88	4.33±0.71	2.44±0.88
Odor intensity	normal	intense	extreme	little

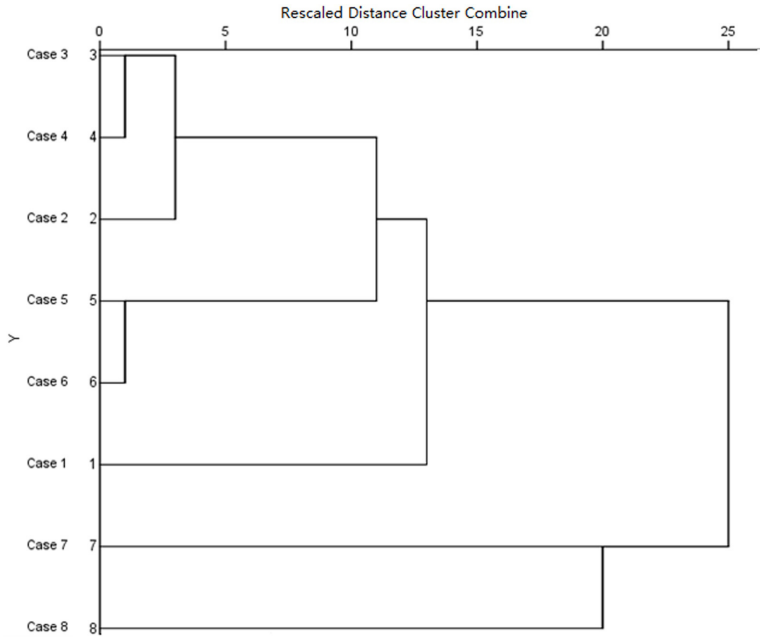


Fig. 5. Clustering results of four edible parts based on the key odorants in both male and female Hyla rabbit meat. case 1, 3, 5 and 7: sample of FL, HL, AB and LD of male Hyla rabbit respectively; case 2, 4, 6 and 8: sample of FL, HL, AB and LD of female Hyla rabbit respectively.

Conclusions

Considering the consumer's acceptance of rabbit meat odor, it is necessary to know the volatile chemical compounds of four different edible parts. SPME which is a useful and simple extraction technology for volatile and semi-volatile organic compounds in food samples was used to extract volatiles of Hyla rabbit meat. Although the whole volatile compounds among four parts of rabbit meat were different, the flavor pattern of rabbit meat was affected by dodecanoic acid, hexanal, decanal, octanal, heptanal, 2-heptenal, 2-nonenal and 2-decenal from the analysis of PCA. Based on OAV, there were 5 key odorants in all four parts, including hexanal, octanal, 2-nonenal, 2-decenal and decanal. The flavor pattern of *Longissimus dorsi* was different from other three parts according to key odorants by CA.

Acknowledgements

This study was supported by National Natural Science Foundation of China (31671787), Rabbit Industry System of Chinese Ministry of Education (100030-40305411) and Fundamental Research Funds for the Central Universities (XDJK2014D042). I was full of gratitude to the staff in Experimental Rabbit Farm of College of Animal Science and Technology Southwest University for raising and slaughtering.

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