

TECHNICAL NOTE: CHARACTERIZATION OF KEY VOLATILE ODORANTS IN RABBIT MEAT USING GAS CHROMATOGRAPHY MASS SPECTROMETRY WITH SIMULTANEOUS DISTILLATION EXTRACTION

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Abstract: This study explored the key volatile compounds in both male and female rabbit meat. Simultaneous distillation extraction with dichloromethane was adopted to extract the volatile compounds in Hyla rabbit meat. A total of 35 volatile compounds were identified by gas chromatography–mass spectrometry and quantified with 2, 4, 6-thimethylpyridine as internal standard. Seventeen volatile aldehydes, 4 alcohols, 2 ketones, 2 acids, 1 heterocyclic compound, 2 alkanes and 7 esters were detected. Hexanal, heptanal, octanal, nonanal, (E, E)-2, 4-decadienal, 1-octen-3-ol and (Z)-2-decenal were the key odorant compounds, with high relative odour activity value. Furthermore, the concentration of volatile compounds in male rabbit meat was higher than that in female rabbit meat.

Key Words: rabbit meat, simultaneous distillation extraction, odour active value, gender, gas chromatograph mass spectrometry.

INTRODUCTION

Rabbit meat is a popular food in Europe, Africa, South America, and Asia, especially in China, which produced 727000 tons of rabbit meat in 2013 (FAOSTAT, 2015). Rabbit meat production in China has been steadily increasing in recent years. Meat flavour, which contains a variety of tastes and aromas, affects the meat purchasing behaviour and preference of consumers (Jayasena *et al.*, 2013). Although the flavour of raw rabbit meat is not different from other meats, boiled rabbit meat has a very unpleasant odour, which seriously affects the rabbit meat consumption in most parts of China. In Sichuan province, people are fond of rabbit meat mainly due to dietary history, in which spicy flavours mask the unpleasant odour. In other cuisines without spicy ingredients, however, the popularity of rabbit meat is reduced by its odour. Therefore, identifying the key odorants of boiled rabbit meat is important. Skatole is responsible for boar taint and 4-methylnonanoic acid is the main contributor of goat meat flavour (Wong *et al.*, 1975; Fischer *et al.*, 2014).

The composition of rabbit meat seems no different from other meats such as poultry, but its flavour differs greatly (Gerencsér *et al.*, 2014; Poławska *et al.*, 2016; Li *et al.*, 2016). It is therefore necessary to analyse the main odorants in rabbit meat and determine their source. Aldehydes mainly come from the oxidation of polyunsaturated fatty acids in meat, and the concentration increases as the oxidation level rises (Calkins and Hodgen, 2007). Studies have shown that volatile aldehydes, such as hexanal, heptanal, octanal, (E)-2-heptenal and nonanal, have lower odour thresholds than other compounds (Li *et al.*, 2013; Shi *et al.*, 2013; Ayseli *et al.*, 2014).

Simultaneous distillation extraction (SDE) is an effective extraction method for the extraction of volatile compounds in rabbit meat (Nóbrega *et al.*, 2007; Xie *et al.*, 2008). Although SDE is time-consuming and laborious, one-step isolation-concentration and more flavours acquired are the advantages of SDE, which is still used for evaluating volatile compounds of meat (Watkins *et al.*, 2012). Aldehydes, alcohols, ketones, esters, volatile phenols, acids, and

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terpenes can be obtained through SDE (Yu and Chen, 2010; Ayseli *et al.*, 2014). Hence, this study adopted SDE to extract volatile compounds of rabbit meat. Gas chromatography-mass spectrometry (GC-MS) is used for identifying volatile compounds in many meat species (Lammers *et al.*, 2009; Song *et al.*, 2012; Ma *et al.*, 2013). However, determining the main volatile chemical compounds directly from the mass data may be difficult. Odour activity value (OVA) is applied to evaluate the key odorants in fish, and can be regarded as a simple and efficient method for determining the main chemical volatile compounds (Grosch, 2001; Carrascon *et al.*, 2014; Sun *et al.*, 2014). SDE has not been used to determine volatile compounds of rabbit meat.

Therefore, this study assessed the volatile compounds of rabbit meat by SDE to determine the key odorants of overall flavour using the OAV method.

MATERIALS AND METHODS

Chemical reagents

Dichloromethane (GC, >99.9%), hexanal (GC, >99.3 pure), heptanal (GC, >99.3), octanal (GC, >99.3) and nonanal (GC, >99.3) were supplied by Sigma-Aldrich (St. Louis, MO, USA). C7-C30 saturated alkane standard (1000 µg/mL in hexane) was purchased from Sigma (SUPELCO). Saturated NaCl aqueous was prepared in the laboratory. 2, 4, 6-Trimethylpyridine (TMP) was obtained from J&K Scientific Ltd. (Shanghai, China).

Sampling

The diet composition was 24.2% corn, 19% wheat bran, 10.8% soybean meal, 36% alfalfa meal, 4% corn germ cake, 3% rapeseed, 0.5% powder, 0.8% dicalcium, 0.1% lysine, 0.1% methionine, 0.5% salt and 1% premix (more details of the diet in Xue *et al.*, 2015). One hundred 75-d-old Hyla rabbits were purchased from College of Animal Science and Technology, Southwest University, Chongqing, China. Fifty male and female Hyla rabbits were slaughtered and subcutaneous fat, viscera, and glands were removed. They were all segmented at the same day under the same conditions, and the slaughter weight was recorded as 2.51 ± 0.09 kg. Then, the meat was stored immediately in the freezer at -20°C before use. The animal experiment was conducted in accordance with the Regulations on Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China.

Simultaneous distillation extraction

The Hyla rabbit meat was well minced and homogenized for 1 min in a household blender. Then, 50 g of rabbit meat without subcutaneous and intermuscular fat tissues, and 150 mL of saturated NaCl aqueous solution were placed in a 500 mL round-bottom flask attached to the appropriate arm of the SDE apparatus. TMP was added at the concentration of 4 µg/kg of rabbit meat as internal standard. Another 50 mL round-bottom flask containing 25 mL of dichloromethane was connected with the other arm of the SDE apparatus. The contents in both sample and solvent flasks were heated up to boiling. The steams were cooled by the circulation of water at a moderate temperature. Solvent flask was heated to reach 55°C in water bath, with sample flask heated up to boiling by an electric heater. The distillation extraction was continued for 3 h. The volume of the extract was evaporated to 5 mL by a Soxhlet extraction apparatus, and then to 200 µL under a gentle stream of nitrogen. The extract was stored at -20°C in a glass vial with a polytef cap until analysis. Extraction of volatile compounds of rabbit meat was conducted in triplicate.

Gas chromatography-mass spectrometry

The volatile compounds in rabbit meat were analysed using an Agilent 7890A-5975C GC-MS. An extracted liquid (1 µL) was injected into a DB-WAX column (J&W 122-7032, 30 m length \times 250 µm \times 0.25 µm film thickness) by an automatic sampler, with splitless mode. The GC oven temperature was held at 40°C for 2 min initially, increased at a rate of $6^{\circ}\text{C}/\text{min}$ to 230°C and held for another 3 min. The injector and detector temperatures were kept at 250 and 230°C , respectively. The injector was held in the splitless mode for the first 2 min of the analysis, and then in the split mode (50:1) for the remainder of the analysis. Helium was used as the carrier gas at a flow rate of 1.1 mL/min. MS

was operated in electron ionization mode (70 eV), and data were acquired in full scan mode for range of 40 to 350 Da. The temperature of the source and the detector was 230°C, while that of the MS transfer line was 250°C. Volatile compounds were tentatively identified by comparing their mass spectral data with those in the Wiley library, and the retention indices was compared with those reported in the literature. Peak areas were calculated using the total ion chromatogram represented for each compound. The content of each compound was reported as percentages, representing the relative amount of each identified peak to the total area of identified peaks in each chromatogram.

Identification and quantification of compounds

The identified compounds can be calculated by comparing the peak areas with standard substance according to Equation 1:

$$\text{Concentration } (\mu\text{g/kg}) = \text{area}_{\text{ratio}} \times \text{Concentration}_{\text{IS}} \quad (1)$$

where $\text{area}_{\text{ratio}}$ represents the ratio of analyte peak area and internal standard substance peak area and $\text{Concentration}_{\text{IS}}$ was the concentration of the internal standard in the sample. Then, the calibration factors were all considered as 1.00. The standard solution of alkenes (C7–C30) 40 mg/L in hexane was injected into GC-MS and analysed under similar conditions to those in previous literature to identify the volatile compounds. The retention indices (RI) of volatile compounds in extraction were calculated using Equation 2:

$$RI = 100Z + 100 \times \frac{tR(X) - tR(Z)}{tR(Z+1) - tR(Z)} \quad (2)$$

where X, Z, and Z+1 represent the retention time of analyte and the time of alkanes of efflux before and after the analyte, respectively.

Statistical analyses

Analysis of variance was carried out using IBM SPSS Statistics 22.0. The means and standard errors were calculated by Microsoft Excel 2010. The differences between means were compared through independent-samples T tests ($P < 0.01$).

RESULTS AND DISCUSSION

Volatil compounds in rabbit meat

Rabbit meat flavour is characterized by the presence of a variety of volatiles belonging to several classes of compounds, such as aldehydes, esters, acids, alkanes, alcohols, ketones, and furans. A total of 35 volatile compounds were identified and quantified in rabbit meat (Table 1). The total concentration of volatile compounds was 162.00 $\mu\text{g/kg}$, including 62.65 $\mu\text{g/kg}$ of aldehydes, 19.00 $\mu\text{g/kg}$ of ketones, 42.15 $\mu\text{g/kg}$ of esters, 17.40 $\mu\text{g/kg}$ of volatile acids, 17.60 $\mu\text{g/kg}$ of alcohols, 1.40 $\mu\text{g/kg}$ of heterocyclic compounds and 1.80 $\mu\text{g/kg}$ of alkanes. Aldehydes and esters were the first and second highest in amount among the volatile compounds. Although generation of aldehydes is common in many meat species, especially when they are cooked, aldehydes are not the key odorants in most meat species, as other special volatile compounds have stronger characteristic odours (Kang *et al.*, 2013; Duan *et al.*, 2015). Ketones formed by auto-oxidation are also important food odorants. In addition, ketones can be formed in β -oxidation and decarboxylation of fatty acids. Methyl ketones, such as 2-butanone, are formed by chemical reactions in the presence of a large number of microorganisms (Marušić *et al.*, 2014). 2-Pentyl-furan is a product of auto-oxidation of linoleic acid and has been detected in many fats, oils, and lipid-containing foods, as well as in rabbit meat (Xie *et al.*, 2008; Ma *et al.*, 2013). Hence, 2-pentyl-furan was generated from the oxidation of lipids in rabbit and the intramuscular fat. The formation of hexanal, heptanal, octanal, nonanal, (E, E)-2, 4-decadienal, 1-octen-3-ol, and (Z)-2-decenal could have a significant impact on the final aroma of the product, as the contents of these volatile compounds were above their respective thresholds (Souza and Bragagnolo, 2014).

Table 1: Volatile compounds detected via gas chromatograph mass spectrum in rabbit meat.

Compounds	LRI	Content ($\mu\text{g}/\text{kg}$)	Retention time (min)	Method of identification
Aldehydes				
Hexanal	1081	15.00	5.997	A,B,C
Heptanal	1182	3.05	8.242	A,B,C
(E)-2-Heptenal	1198	3.25	10.676	A,B,C
Octanal	1207	1.65	10.802	A,B,C
Nonanal	1392	9.15	13.460	A,B,C
(E)-2-Octenal	1345	2.55	11.684	A,B,C
2-Dodecenal	1523	1.25	16.966	A,B
(Z)-2-Decenal	1644	3.05	19.526	A,B
2-Undecenal	1751	2.10	21.965	A,B
(E,E)-2,4-Decadienal	1214	1.70	22.251	A,B
2-Methyl-undecanal	1376	0.35	23.125	A,B
Tetradecanal	1927	1.25	25.570	A,B
Pentadecanal	2042	5.10	27.692	A,B
(Z)-14-Methyl-8-hexadecenal	2219	0.75	32.097	A,B
Octadecanal	2357	1.15	33.506	A,B
(Z)-9-Octadecenal	2693	8.65	33.920	A,B
(Z)-9,17-Octadecadienal	2734	2.65	34.773	A,B
Esters				
Octyl chloroformate	1585	1.80	17.632	A
Dimethyl silanediol	1637	0.70	19.820	A
Hexadecanoic acid methyl ester	2114	0.55	31.252	A
Butyl octyl phthalate	2841	4.55	36.755	A
2-Chloropropionic acid octadecyl ester	2901	18.10	37.171	A
Dibutyl phthalate	2985	12.5	39.185	A
Octaethylene glycol monododecyl ether	3102	3.95	46.320	A
Alcohols				
1-Pentanol	1256	2.25	9.924	A,B
1-Octen-3-ol	1687	2.25	14.978	A,B
2-Eicosanol	2937	1.65	38.268	A,B
2-Hexyl-1-decanol	2980	11.45	39.690	A,B
Acids				
Nonahexacontanoic acid	2634	0.25	33.822	A,B
trans-13-Octadecenoic acid	2915	17.15	37.112	A,B
Ketone				
3-Hydroxy-2-butanone	1243	18.55	10.682	A,B
3-Pentadecanone	1984	0.45	26.854	A,B
Heterocyclic compounds				
2-Pentyl-furan	1241	1.40	9.362	A,B
Alkane				
1-Iodo-tridecane	1249	1.35	9.594	A
1-Iodo-hexadecane	2713	0.45	34.368	A

A: Mass spectrum (identified according to the mass spectra of the compounds). B: LRI Liner Retention Index (the LRI of the compound identified on the column of DB-Wax). C: Standard material.

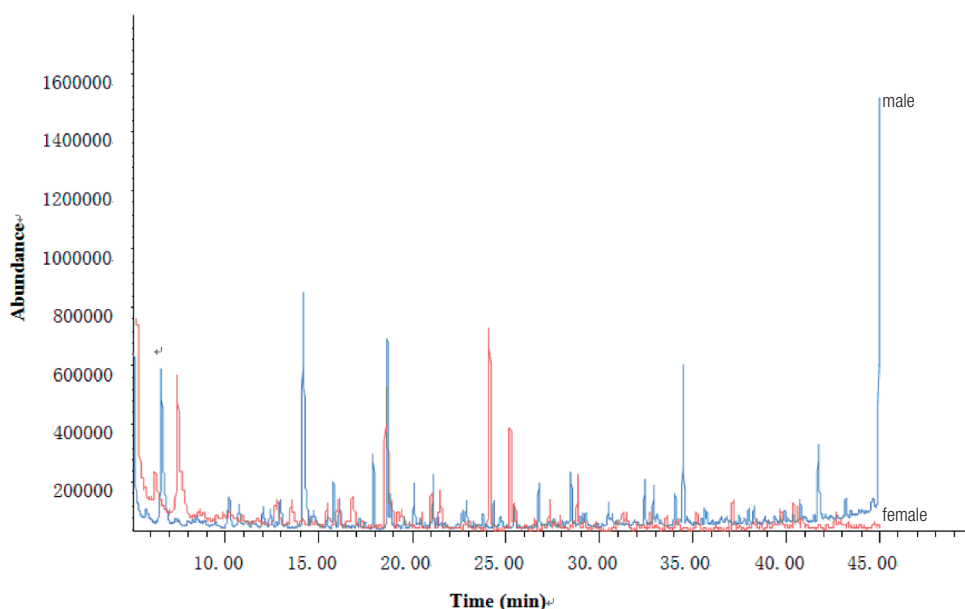


Figure 1: Total ion chromatograms (TIC) of male (blue) and female (red) rabbit meat extracted by SDE-GC-MS (for the peak identification, see Table 1).

Difference in odour between male and female rabbits

The volatile compounds of male and female rabbits were identified by GC-MS with SDE (Figure 1). Results show that all 7 key odorants were detected in both males and females, and hexanal and nonanal were the first and second highest in concentration in both males and females. However, the contents of the volatile compounds between males and females differ significantly (Table 2). The concentrations of both volatile compounds and key odorants were significantly higher in male than in female rabbit meat, which could explain why male rabbit meat had stronger flavour than female rabbit meat. Thus, gender is an effective factor in the flavour of rabbit meat. The influence of gender on flavour is related to genetic control of hormonal metabolism and the composition and metabolism of lipids. On the contrary, gender has been found to have a minor effect on meat sensory attributes, including flavour (Hoffman *et al.*, 2007). Despite the influence of gender on meat flavour, it does not significantly affect rabbit meat odour. Nonetheless, the influence of gender on flavour of Hyla rabbit meat needs further research.

Key odorant of rabbit meat

OAV was calculated by dividing the content by the odour threshold. When the OAV of a volatile compound was higher than 1, the compound can be regarded as a potential key odorant (Ayseii *et al.*, 2014). Aldehydes such as hexanal, heptanal, (Z)-2-heptenal, octanal, decanal, and 2-undecenal are mainly produced by lipid oxidation, especially the oxidation of polyunsaturated or saturated fatty acid (Shi *et al.*, 2013). Rabbit meat is richer in polyunsaturated fatty

Table 2: Odor differences between male and female rabbit meat.

Contents	Female (µg/kg)	Male (µg/kg)
All volatile compounds	157.00±0.90*	162.00±0.50*
Seven key odorants	23.75±0.03*	35.85±0.05*

*Significantly difference between female and male at α=0.01.

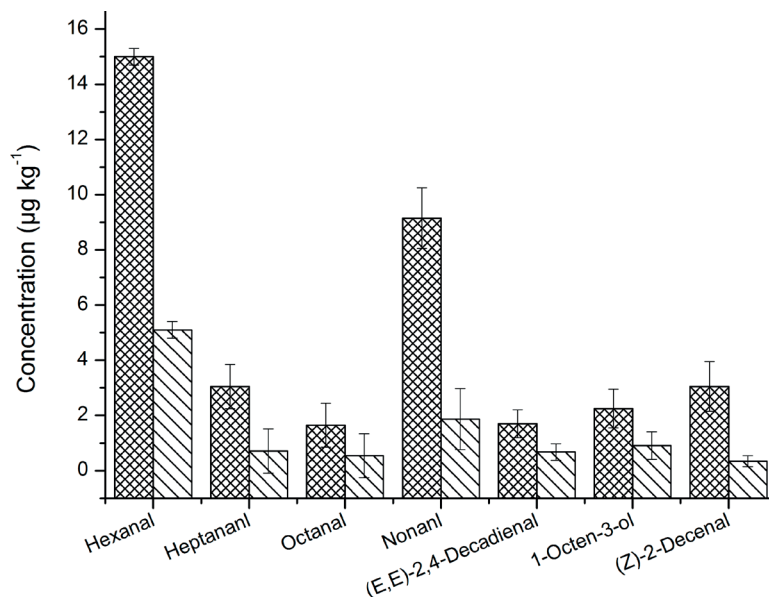


Figure 2: The concentration of the key odorants of male (▨) and female (▧) rabbit meat.

acid than other meat species (Dalle Zotte and Szendrő, 2011). As a consequence, volatile aldehydes generated from the oxidation reaction and thermal degradation of fatty acids may be responsible for the flavour of rabbit meat, which is considerably different from other meats (Rødbotten *et al.*, 2004). Aniseed and liver odour have been considered negative sensory traits of rabbit meat, and only aniseed flavour is regarded as a positive trait (Ariño *et al.*, 2007). Hexanal, heptanal, octanal, nonanal, (E, E)-2, 4-decadienal, 1-octen-3-ol, and (Z)-2-decenal had OAV of higher than 1, which indicated that they were the dominant odorants in rabbit meat (Table 3). Hexanal was an auto-oxidation product of special importance, because it is used as an indicator of oxidative deterioration of foods (Shahidi and Pegg, 1994; Pastorelli *et al.*, 2006). The odour of octanal is defined as solvent, lemon, and bitter, and octanal has been found in fruits and several fermented foods. In addition, octanal can reduce and block the citral response. Nonanal has also been found in some fermented foods, and the aroma quality has been described as oily and bitter (Takakura *et al.*, 2014). (E, E)-2, 4-Decadienal has a fatty aroma and is usually generated from the oxidation of linoleic and arachidonic acid (Madruga *et al.*, 2009). The characteristic flavour of 2-decenal is described as tallow and orange. 2-Decenal contributes tallowy, fatty and pungent flavour to the overall odorant in rabbit meat, and has the second highest OAV. 1-Octen-3-ol is an unsaturated alcohol derived from linoleic acid oxidation, and is regarded a key odorant because of its low odour threshold (1 µg/kg) (Song *et al.*, 2014). 1-Octen-3-ol may originate from oxidation of linoleic acid (C18:2), and is also the natural component of clover, which is generally consumed by rabbit (Nóbrega

Table 3: The key odorants determined by OAV in rabbit meat.

Compound	Odor threshold ^a (µg/kg)	Odor active value	Odor description
Hexanal	4.5	3.3	Green
Heptanal	3	1	Fatty
Octanal	0.7	2.4	Solvent, lemon, bitter
Nonanal	1.0	9.1	Green, oil
(E, E)-2, 4-Decadienal	0.07	24.30	Aldehyde, rancid
1-Octen-3-ol	1.0	2.2	Toasted, mushroom, metallic
(Z)-2-Decenal	0.3	10.2	Poultry, orange

^a Odor threshold values were taken from the literature (Chen *et al.*, 2009; Selli and Cayhan, 2009).

et al., 2007). The “boiled meaty flavour” of beef extract has been attributed to 1-octen-3-ol (Takakura *et al.*, 2014). Figure 2 shows that (E, E)-2, 4-decadienal had the greatest contribution to the overall aroma of rabbit meat with the highest OAV, which is 2 times greater than that of (Z)-2-decenal. Although heptanal had the lowest OAV, it contributed to the flavour and overall aroma of rabbit meat, and may have interacted with other volatile compounds.

CONCLUSIONS

This study investigated the volatile compounds of rabbit meat by GC-MS in combination with SDE. The dominant flavour compounds of rabbit meat were hexanal, heptanal, octanal, nonanal, (E, E)-2, 4-decadienal, 1-octen-3-ol, and (Z)-2-decenal. (E, E)-2, 4-Decadienal showed the highest OAV, thus contributing to the overall flavour. Furthermore, the concentration of key odorants in male rabbit meat was higher than that in female rabbit meat, which may explain the relatively stronger odour of male rabbit meat.

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