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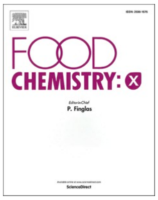

Characterization of key aroma compounds in Chinese smoked duck by SAFE-GC-O-MS and aroma-recombination experiments

Huan Liu [a,*], Jingyu Li [a], Nazimah Hamid [b], Junke Li [a], Xuemei Sun [a], Fang Wang [a], Dengyong Liu [c], Qianli Ma [b], Shuyang Sun [a], Hansheng Gong [a]

[a] *School of Food Engineering, Ludong University, Yantai Key Laboratory of Nanoscience and Technology for Prepared Food, Yantai Engineering Research Center of Green Food Processing and Quality Control, Yantai 264025, China*

[b] *Department of Food Science, Auckland University of Technology, Private Bag 92006, Auckland 1142, New Zealand*

[c] *College of Food Science and Technology, Bohai University, Jinzhou 121013, China*

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ABSTRACT

Smoked duck is a popular meat product in China. The aroma profile and key aroma compounds in smoked ducks were elucidated using solvent-assisted flavor evaporation-gas chromatography–olfactometry-mass spectrometry (SAFE-GC-O-MS), odor activity values (OAVs), aroma recombination and omission experiments, and sensory evaluation. The results indicated that the predominant aroma profiles of rice-, tea oil- and sugarcane-smoked ducks all contained strong smoky, roasty, fatty, meaty, and grassy aromas. A total of 31 aroma compounds were identified as important odorants by OAVs, including 8 aldehydes, 6 pyrazines, 5 phenols, and 2 sulfur compounds. The aroma recombination and omission experiments confirmed that 13 odorants were key aroma compounds in smoked ducks. Of these odorants, 2-methoxyphenol, 4-methylphenol, 5-ethyl-2,3-dimethylpyrazine, methional, 2-methyl-3-furanthiol, (*E, E*)-2,4-decadienal, 1-octen-3-ol, and anethole significantly contributed to the aroma profile of smoked duck flavor ($p < 0.01$).

1. Introduction

China is the world's largest producer and consumer of duck meat products, which is approximately 10 million tons, and accounting for about 70 % of the world production in recent years. Smoked poultry meat is popular in China, especially in southern China (Gasior et al., 2021). The most popular smoked ducks on the market mainly contains those smoked by tea, sugarcane, and rice with a history of 600 years. Previous studies have reported that the main aroma compounds of roasted and stewed duck meat products include aldehydes, alcohols, furans, and sulfur compounds such as hexanal, 1-octen-3-ol, 2-pentylfuran and dimethyl trisulfide (Li, Al-Dalali, Wang, Xu, & Zhou, 2022; Zhu et al., 2022). We only found one report about the aroma compounds of smoked duck, among which 67 odorants isolated and identified in smoked duck meat by SPME have found that alcohols, aldehydes, and phenols were the predominant odorants in smoked ducks (Jo, An, Arshad, & Kwona, 2018). The main differences of aroma profile of smoked chicken mainly were attributed to the contents of aldehydes, ketones, and phenols (Zhang, Chen, Liu, Xia, Wang, & Kong, 2022). However, research on the odorants responsible for the aroma profile of smoked duck meat are scarce, with respect to the confirmation of key aroma compounds.

SPME is an effective method to extract aroma compounds in samples although there exist some limitations due to the difficult quantitation and reproducibility (Murat, Gourrat, Jerosch, & Cayot, 2012). In depth study on key aroma compounds can be further carried out using the solvent assisted flavor evaporation (SAFE) in combination with gas chromatography–olfactometry-mass spectrometry (GC-O-MS) (Dach & Schieberle, 2021; Schmidberger & Schieberle, 2020). Off-flavor in samples can be contributed by hexanal, which is an indicator of oxidative rancidity (Heydanek & McGorrin, 1981). Other odorants like phenolics, aldehydes, ketones, sulfur compounds and pyrazines are responsible for the acceptable aroma of smoked duck meat (Jo et al., 2018). Therefore, the changes in concentrations and proportions of these compounds present in the meat should be investigated in detail.

Only a subset of certain odorants interacts with the olfactory receptors in the human nose to result in an aroma perception in our brain (Dunkel et al., 2014; Grosch, 2001; Schieberle & Hofmann, 2011).

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* Corresponding author at: School of Food Engineering, Ludong University, Yantai 264025, China.
E-mail address: sd_lh1990@126.com (H. Liu).

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Schieberle and Hofmann came up with the sensomics methodology that leverages advanced natural product analytics, human psychophysical techniques, and bioinformatics tools to identify and quantify key odorants in food and beverages, and to decipher their sensory impact on the overall aroma profile (Schieberle & Hofmann, 2011). This approach applies the sensory and GC–MS to evaluate the contribution of aroma compounds to the sensory qualities of samples. Subsequent aroma recombination and omission experiments conducted by combining flavor standards (OAVs > 1) in natural concentrations can yield a similar aroma profile with that of the sample itself that confirms the sensomics technology adopted (Dach & Schieberle, 2021).

To date, no comprehensive studies have been performed to characterize the key odorants in smoked ducks by employing the sensomics methodology. This study aimed to (i) first extract the aroma compounds in samples by employing the SAFE distillation, (ii) identify odorants using gas chromatography–olfactometry-mass spectrometry (GC-O-MS), (iii) quantitate the odorants using standard curves of authentic flavor standards, (iv) determine the importance of each odorant by means of GC-O and OAVs, and (v) confirm key odorants of smoked ducks by recombination and omission experiments. The outcome of this study will provide a comprehensive characterization of key odorants in smoked ducks using the sensomics methodology. The results may also have important implications for the food industry, as they provide insights into the factors that contribute to the unique aroma profiles of smoked meat products and can be used to guide the development of new products with specific sensory characteristics.

2. Materials and methods

2.1. Samples collection and grouping

Three popular types of smoked duck meat were purchased from local commercial factories that included tea oil smoked duck, sugarcane smoked duck, and rice smoked duck. The breast skin and breast muscle of smoked ducks were minced under ice conditions using a QSJ-B02X5 chopper (Bear Electric Co., Ltd., Guangdong, China). All samples were wrapped with nylon/polyethylene, frozen, and stored at −80 °C for less than a week until the analysis.

The C_7-C_{40}, C_6-C_{25} *n*-alkanes (97 %, external standard) were utilized for identification analysis, which was applied from O2si Smart Solutions (Shanghai, China). An internal standard (2-methyl-3-heptanone, 99 %) was used to verify reproducibility and linear retention indices (LRI), which was purchased from Dr. Ehrenstorfer (Beijing, China). The standards were obtained from Sigma-Aldrich (Shanghai, China) included: 2,3-butanedione (97 %), 2,3-pentanedione (97 %), 2-heptanone (99 %), pentanal (98 %), hexanal (98 %), heptanal (97 %), octanal (99 %), nonanal (99.5 %), benzaldehyde (99.5 %), eucalyptol (99 %), 1-octen-3-ol (98 %), 2-pentylfuran (98 %), 2-furfural (99 %), 2-furanmethanol (98 %), 2-methylpyrazine (99 %), 2-ethenylpyrazine (97 %), 2,6-dimethylpyrazine (98 %), methional (97 %), anethole (99 %), 2-methoxyphenol (99 %), 2-methylphenol (99 %), 4-methylphenol (99 %), and 3-methylphenol (99 %). Moreover, 2-ethyl-3,5-dimethylpyrazine (99 %), 2-ethyl-5-methylpyrazine (98 %), and (*E*, *E*)-2,4-decadienal (98 %) were both purchased from Macklin (Beijing, China). 5-Ethyl-2,3-dimethylpyrazine (98 %), (*E*)-2-heptenal (97 %), 3-ethylphenol (99 %), and 1-methylnaphthalene (98 %) were purchased from Aladdin (Beijing, China), TCI (Beijing, China), CATO (Beijing, China), and Dr. Ehrenstorfer (Beijing, China), respectively.

2.2. Isolation of aroma compounds by solvent-assisted flavor evaporation (SAFE)

A total of 50 g samples were extracted with dichloromethane (50 mL) for 3 h at a room temperature using an IKA KS 260 oscillator. Then 2-methyl-3-heptanone (2 μg/μL) was added into the mixture as an internal standard to achieve a concentration of 1000 ng/g. The filter residue was re-extracted with dichloromethane three times. Subsequently, the obtained organic compounds were subjected to high vacuum distillation by using the SAFE technique (Jonas & Schieberle, 2021). The distillate was further dried using anhydrous sodium sulfate for 12 h at −20 °C. Subsequently, a Vigreux column (50 × 1 cm inner diameter) was applied to concentrate the extracts to 5 mL. Thereafter, the extracts were concentrated to 200 μL under a gentle flow of nitrogen.

2.3. Gas chromatography–olfactometry-mass spectrometry (GC-O-MS) analysis

The aroma compounds in smoked ducks were analyzed by a Thermo Scientific™ TRACE™ 1310 gas chromatography equipped with TSQ 9000 mass spectrometer (Thermo Scientific, Bremen, Germany) and an olfactory port (OP275 Pro II, GL Sciences Inc., Japan). The polar DB-Wax and non-polar TG-5SILMS columns (both 30 m × 0.25 mm i.d., 0.25 μm film thickness) were utilized to separate the odorants. For the DB-Wax column, the initial temperature was 40 °C for 3 min, heated to 70 °C at 2 °C/min, then to 130 °C at 3 °C/min, ramped to 230 °C at 10 °C/min, and held at the final temperature for 10 min. For the TG-5SILMS column, the initial temperature was 40 °C for 2 min, ramped to 50 °C at 4 °C/min and held for 2 min, then increased to 250 °C at 6 °C/min and kept for 10 min. The sample injection was conducted in the splitless mode, among which the aroma compounds were split at a ratio of 1:1 (v: v) into the mass spectrometer (MS) and olfactometer at 230 °C. The MS transfer line temperature and ion source temperature were set at 240 °Cand 260 °C, respectively. Ultrahigh-purity helium (purity: 99.99 %) was applied at a flow rate of 1.5 mL/min as the carrier gas. The electron impact (EI) mass spectra were set at 70 eV ionization energy (m/z: 40–500).

2.4. Identification and quantitation analysis

Identification Analysis. The aroma compounds were identified using the mass spectrometry library (MS), odor qualities (O), linear retention indices (LRI), and authentic flavor standards (S). Briefly, the aroma compounds were first identified by comparing the recorded retention time and the data in the mass spectrometry library (NIST 2020). LRI was calculated from the retention time of *n*-alkanes by linear interpolation. The aroma compounds were further identified using GC-O from 3 trained panelists. Furthermore, the authentic flavor standards were detected in consistent with the GC-O-MS program of the sample. The aroma compounds were verified by comparing the retention time of the flavor standards to the sample.

Quantitation Analysis. The aroma compounds were quantitated by GC–MS equipped with a DB-Wax and TG-5SILMS capillary column. The aroma compounds from GC-O and OAVs ≥ 1 were accurately quantitated by the standard curve method in SIM mode (Yang et al., 2022). The ions obtained are presented in Table 1. The mixed authentic standard compounds were dissolved in dichloromethane, and further diluted to various concentrations. Subsequently, the 2-methyl-3-heptanone (2 μg/μL) was spiked into each standard solution as an internal standard, which did not co-elute with all odorants. The calibration equations were constructed using the ratios of concentrations and their ion peak area ratios.

2.5. OAVs (odor activity values) analysis

Preliminary elucidation of important aroma compounds OAVs were obtained by the calculating ratio of concentrations and their medium odor thresholds (Schieberle, 1995). The aroma compound with higher OAV might play a greater role on the general aroma profile of smoked duck meat.

2.6. Aroma profile analysis

The informed consent for aroma profile analysis from panelists was

Table 1
Identification analysis of aroma compounds in tea oil-, sugarcane-, and rice-smoked ducks.

Compounds	odor descriptions [a]	LRI [b]		Identification [d]
		DB-Wax	TG-5SILMS	
2,3-butanedione	butter	973	601	MS, LRI, O, S
pentanal	green	975	n.d. [c]	MS, LRI, O, S
2,3-pentanedione	creamy, buttery	1054	n.d.	MS, LRI, O, S
hexanal	green, grass	1078	800	MS, LRI, O, S
2-heptanone	floral	1178	n.d.	MS, LRI, O, S
heptanal	green	1181	900	MS, LRI, O, S
eucalyptol	herbal	1204	1035	MS, LRI, O, S
2-pentylfuran	green	1230	n.d.	MS, LRI, O, S
2-methylpyrazine	roasted	1262	823	MS, LRI, O, S
octanal	green	1286	989	MS, LRI, O, S
(*E*)-2-heptenal	fatty	1319	n.d.	MS, LRI, O, S
2,6-dimethylpyrazine	roasted, meaty	1325	912	MS, LRI, O, S
2-methyl-3-furanthiol	meaty	1327	844	MS, LRI, O, S
2-ethyl-5-methylpyrazine	roasted	1389	n.d.	MS, LRI, O, S
nonanal	green	1391	1104	MS, LRI, O, S
2-ethenylpyrazine	roasted	1432	n.d.	MS, LRI, O, S
5-ethyl-2,3-dimethylpyrazine	roasted	1444	n.d.	MS, LRI, O, S
methional	cooked potato	1448	907	MS, LRI, O, S
1-octen-3-ol	mushroom	1453	981	MS, LRI, O, S
2-furfural	baked bread	1458	837	MS, LRI, O, S
benzaldehyde	nutty, cherry	1514	964	MS, LRI, O, S
2-furanmethanol	burnt	1665	869	MS, LRI, O, S
(*E, E*)-2,4-decadienal	fatty	1807	1319	MS, LRI, O, S
anethole	sweet, anise	1828	n.d.	MS, LRI, O, S
1-methylnaphthalene	medicinal	1850	1302	MS, LRI, O, S
2-methoxyphenol	smoky	1861	1060	MS, LRI, O, S
2-methylphenol	medical, smoky	2005	1073	MS, LRI, O, S
4-methylphenol	medical, smoky	2086	1093	MS, LRI, O, S
3-methylphenol	medical, smoky	2093	n.d.	MS, LRI, O, S
3-ethylphenol	burnt	2180	n.d.	MS, LRI, O, S
2-ethyl-3,5-dimethylpyrazine	burnt, roasted	n.d.	1077	MS, LRI, O, S

[a] Odor attributes obtained by GC-O.

[b] The linear retention indices calculated with *n*-alkanes ($C_7 - C_{40}$) on the DB-Wax and TG-5SILMS columns.

[c] n.d., not detected.

[d] MS, mass spectrometry library; O, odor qualities; LRI, linear retention indices; S, authentic flavor standards.

obtained in the study. All panelists were selected and trained according to the guidelines of ISO 4121:2003 and GB/T 29604–2013. The results of sensory evaluation from different parts (skin and meat) of three types of smoked ducks indicated that the skin of sugarcane smoked duck presented the richest aroma and the best overall acceptability. Therefore, the skin of sugarcane smoked duck was selected to determination of aroma profile through aroma recombination and omission experiments. The sensory evaluation was conducted at a 25 °C laboratory that met ISO 8589 standard. Twenty-five panelists attended three weekly sensory training sessions to recognize aroma. References were used to define aroma attributes of samples agreed during vocabulary development in training sessions. The reference standards used included 2-methoxyphenol (smoky note), 2-ethyl-3,5-dimethylpyrazine (roasty note), (*E, E*)-2,4-decadienal (fatty note), 2-methyl-3-furanthiol (meaty note), anethole (sweet note), and hexanal (grassy note). The chemical standards were presented in aqueous solutions at a concentration of 50 times above the aroma threshold. The aroma profile of samples was determined by rating each odorant using a 7-point scale (in steps of 0.5) from 0 (not perceivable) to 3 (strongly perceivable). The average score of each aroma attribute obtained from the trained panelists was presented in a spider diagram.

2.7. *Aroma recombination and omission experiments*

The key aroma compounds in smoked ducks were further validated using the recombination and omission models, using odorants with OAVs ≥ 1. Prior to the experiment, the odorless matrix was prepared. A mixture of diethyl ether and pentane (diethyl ether-to-*n*-pentane ratio of 2: 1, w: w) was added to smoked duck, shaken for 8 h, and then filtered using filter paper. The smoked duck was deodorized, and extraction was repeated three times by using the organic solvents until no aroma compounds were observed by GC-O-MS. The odorless matrix consisted the odorless smoked duck and ultrapure water. The recombination model was prepared by adding 31 flavor standards with the same concentration as smoked ducks (recombination model 1) into the odorless matrix and. Subsequently, a series of mixed models were established by omitting one odorant from the 31 odorants (recombination model 2). Recombination model 3 was then constructed using the odorless matrix and key aroma compounds. The difference in overall aroma profile between omission experiments and smoked duck aroma was compared using sensory evaluation (Liu et al., 2019; Xu et al., 2022).

2.8. *Statistical analysis*

The statistical data of smoked ducks were performed by SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Fisher's least significant difference test and Duncan's multiple range test were utilized for pairwise comparisons of samples ($p < 0.05$). The data were exhibited as mean ± standard deviation.

3. Results and discussion

3.1. *Aroma compounds in smoked duck were accurately identified and quantitated by various ways*

Identification analysis. To identify the aroma compounds accountable for the complete flavor profile, the volatiles were extracted using dichloromethane and non-volatile constituents removed using the SAFE technique. To confirm the successful isolation of odorants, a single drop of the distillate was placed on a filter paper. Upon analysis, the overall aroma was found to be characteristic of smoked ducks, providing preliminary evidence that the isolation process was successful. Table 1 displays a comprehensive list of 31 aroma compounds that are accurately identified using MS, LRIs, and odor qualities, which are then compared to data from previous reports. To ensure the accuracy of the identification process, each aroma compound was analyzed using two separate columns - the polar DB-Wax and non-polar TG-5SILMS columns. This was done by comparing the compound with an authentic flavor standard (S), providing additional confirmation of the compound's identity. Aldehydes, pyrazines, and phenols accounted for more than 60 % of the total aroma compounds, suggesting that these odorants might predominantly contribute to the aroma profile of samples. This result was contrary to the previous study, which indicated that alcohols comprised the largest number of detected compounds (Jo et al., 2018). Most aroma compounds were identified in the samples for the first time that included 2-methoxyphenol, 3-ethylphenol, 2-ethyl-3,5-dimethylpyrazine, 2-methyl-3-furanthiol and others. Kosowska and co-workers reported that these above odorants were the main odorants in the loin smoked by beech and alder wood chips (Kosowska, Majcher, Jelen, & Fortuna, 2018). The five phenolic compounds with smoky notes perceptible in smoked duck, included 2-methoxyphenol, 2-methylphenol, 3-methylphenol, 4-methylphenol, and 3-ethylphenol. Similarly, 4-methylphenol, 3-methylphenol, and 2-methylphenol were associated with smoky aroma in smoked dry-cured hams (Marusic Radovcic, Vidacek, Janci, & Medic, 2016).

Quantitation analysis. In addition to identifying 31 aroma compounds, this study also presented a novel contribution by introducing calibration equations and quantitation results for key aroma compounds in smoked ducks. A total of 31 odorants were selected for the quantitation by the standard curve of authentic flavor standards ($R^2 > 0.99$) as shown in Table 2. Table 3 displays a wide range of concentrations, spanning 10^5 units. Notably, the compound 2-furfural exhibited the highest concentration by far, with a value of 10714.71 ng/g. In contrast, the compound 2-methyl-3-furanthiol had the lowest concentration, with a value of only 1.46 ng/g. 2-Furfural was the most abundant aroma compound in sugar-smoked chicken, which might be generated from the pyrolysis of glucose (Zhang, Chen, Liu, Xia, Wang, & Kong, 2022). The odorants that were also present at high concentrations included anethole (5242.87 ng/g), 2-furanmethanol (4255.31 ng/g), 4-methylphenol (4072.96 ng/g), (*E, E*)-2,4-decadienal (2620.16 ng/g), 2-methoxyphenol (1066.55 ng/g), and hexanal (988.14 ng/g). This suggested that the aroma profile of smoked ducks was associated with typical smoky, roasty, fatty, and sweet notes. Meanwhile, some odorants only appeared in trace amounts (<9 ng/g), namely, 1-methylnaphthalene (4.23 ng/g), 2-pentylfuran (4.70 ng/g), 2-ethyl-5-methylpyrazine (6.13 ng/g), and 2-methylpyrazine (8.23 ng/g). These findings are consistent with the results of a previous study (Kosowska et al., 2018), which observed a prevalence of smoky and fatty aroma compounds and relatively low concentrations of pyrazines and sulfurs. Specifically, compounds such as 2-methoxyphenol, hexanal, and 2-methyl-3-furanthiol were found to be present in low concentrations. The sugarcane smoked ducks were found to have a distinct aroma profile, with higher concentrations of anethole, 2-methoxyphenol, and 3-ethylphenol significantly more abundant compared to tea oil and rice smoked duck. This indicated that these compounds might play a key role in differentiating the aroma of sugarcane smoked ducks from other smoked duck varieties. The concentrations of phenols of duck skins were generally higher ($p < 0.05$) than duck meat.

3.2. Aldehydes, alcohols, phenols, and pyrazines were predominant aroma compounds in smoked duck based on OAVs and GC-O analysis

The importance of aroma compounds in smoked ducks depends on not only the concentrations but also their OAVs and sensory intensity (GC-O). The 31 odorants were analyzed by GC-O using trained panelists (Table 1). Fig. 1 shows that 29 out of the 31 identified odorants may play a significant role in the overall aroma profile of smoked ducks as these odorants had odor activity values (OAVs) greater than or equal to 1. The highest OAVs were found for the following odorants: fatty (*E, E*)-2,4-decadienal (970.43), followed by grassy 1-octen-3-ol (912.01), cooked methional (599.51), meaty 2-methyl-3-furanthiol (464.29), smoky 2-methoxyphenol (353.85), grassy hexanal (213.83), and sweet anethole (113.98). This result was consistent with a previous study, which reported 2-methoxyphenol, 2-methyl-3-furanthiol, and methional having higher OAVs in smoked loins (Kosowska et al., 2018). Meanwhile, the OAVs of 2-heptanone, 2,6-dimethylpyrazine, and 2-ethyl-5-methylpyrazine were lower than 1. This result suggested that the contribution of an odorant could not be determined by the OAVs only, because matrix effects and aroma release should be taken into account as well (Yang et al., 2022). While certain aroma compounds were known to contribute specific notes to the overall aroma profile, it was important to note that the unique and distinctive aroma of this flavor could not be attributed to a single compound (Fricke & Schieberle, 2020). The typical meaty aroma was generally ascribed to 2-methyl-3-furanthiol. However, this aroma might be synergistically generated from other odorants like 5-ethyl-2,3-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, and 2,6-dimethylpyrazine (Gasior et al., 2021).

The phenolic compounds that originated from polyphenols in plant cell wall, included ferulic acid and 2-methoxyphenol. These compounds

Table 2
Authentic standards, scanned ions, concentrations of standard solutions, and calibration equations of aroma compounds by SAFE combined with GC–MS in the selected ion monitoring (SIM) mode.

aroma compounds	ions (m/z) [a]	calibration equations [b]	R^2	ranges of concentration for provided linearity (mg/L) [c]
2,3-butanedione	43, 57, 86	$y = 1.9625x + 0.0626$	0.9999	9.90 ~ 4950
pentanal	43, 44, 58	$y = 2.5684x - 0.017$	0.9995	3.95 ~ 395
2,3-pentanedione	43, 44, 57	$y = 1.9377x + 0.0028$	0.9999	4.95 ~ 495
hexanal	41, 44, 56	$y = 0.6399x + 0.1385$	0.9998	4 ~ 4000
2-ethyl-3,5-dimethylpyrazine	56, 135, 136	$y = 0.3121x + 0.0042$	0.9981	0.49 ~ 48.50
2-heptanone	43, 58, 71	$y = 0.5344x - 0.002$	0.9984	0.82 ~ 82
heptanal	43, 44, 70	$y = 1.2082x + 0.0066$	0.9994	0.85 ~ 85
eucalyptol	43, 71, 81	$y = 0.8797x - 0.0021$	0.9999	4.60 ~ 460
2-pentylfuran	81, 82, 138	$y = 0.3725x + 0.0011$	0.9984	0.89 ~ 89
2-methylpyrazine	53, 67, 94	$y = 0.727x - 0.0421$	0.9978	5.15 ~ 515
octanal	41, 43, 44	$y = 0.9386x - 0.0025$	0.9950	0.82 ~ 82
(*E*)-2-heptenal	41, 55, 83	$y = 0.9352x + 0.0004$	0.9915	0.83 ~ 83
2,6-dimethylpyrazine	41, 42, 108	$y = 0.4137x - 0.0028$	0.9981	0.99 ~ 99
2-methyl-3-furanthiol	85, 113, 114	$y = 2.6216x + 0.0018$	0.9990	0.58 ~ 58.50
2-ethyl-5-methylpyrazine	56, 121, 122	$y = 0.9493x - 0.0069$	0.9972	0.98 ~ 98
nonanal	41, 56, 57	$y = 0.9702x + 0.002$	0.9996	4.15 ~ 415
2-ethenylpyrazine	52, 79, 106	$y = 0.6457x - 0.0007$	0.9937	1 ~ 100
5-ethyl-2,3-dimethylpyrazine	42, 135, 136	$y = 0.552x + 0.0033$	0.9999	0.97 ~ 97
methional	47, 48, 104	$y = 3.3893x + 0.0191$	0.9914	1.04 ~ 104
1-octen-3-ol	43, 57, 72	$y = 1.0335x - 0.009$	0.9998	4.20 ~ 840
2-furfural	67, 95, 96	$y = 0.9305x + 0.0513$	0.9998	11.60 ~ 5800
benzaldehyde	77, 105, 106	$y = 0.4939x - 0.005$	0.9950	1.04 ~ 104
2-furanmethanol	81, 97, 98	$y = 0.6567x + 0.7279$	0.9914	11.30 ~ 5650
(*E, E*)-2,4-decadienal	41, 76, 81	$y = 2.8027x + 0.3056$	0.9946	9 ~ 4500
anethole	117, 147, 148	$y = 0.4322x + 0.4991$	0.9994	9.90 ~ 19800
1-methylnaphthalene	115, 141, 142	$y = 0.2887x - 0.0012$	0.9988	1 ~ 100
2-methoxyphenol	81, 109, 124	$y = 0.6715x - 0.011$	0.9998	5.65 ~ 1130
2-methylphenol	79, 107, 108	$y = 0.6302x + 0.1171$	0.9996	11.30 ~ 5650
4-methylphenol	77, 107, 108	$y = 0.4729x + 0.5475$	0.9921	10.40 ~ 5200
3-methylphenol	79, 107, 108	$y = 0.7559x - 0.0242$	0.9999	6.55 ~ 1310
3-ethylphenol	77, 107, 122	$y = 0.4321x - 0.0204$	0.9999	5 ~ 1000

[a] Selected ions (m/z) used in quantitation analysis.

[b] x is the peak area relative to that of the internal standard (2-methyl-3-heptanone), and y is the concentration (ng/g) in the samples relative to that of the internal standard.

[c] Concentrations of the standard solutions prepared in dichloromethane.

Table 3
Quantitation analysis of aroma compounds in tea oil-, sugarcane-, and rice-smoked ducks.

aroma compounds		concentrations (ng/g) [a]		
		tea oil smoked duck	sugarcane smoked duck	rice smoked duck
2,3-butanedione	skin	1052.54 ± 95.46^{aA}	90.66 ± 3.48^{cB}	1689.25 ± 62.27^{bA}
	meat	385.70 ± 70.77^{bB}	133.18 ± 17.39^{cA}	625.76 ± 12.32^{aB}
pentanal	skin	429.20 ± 47.99^{a}	89.64 ± 20.50^{bA}	0^{c}
	meat	485.61 ± 108.58^{a}	0^{bB}	0^{b}
2,3-pentanedione	skin	387.02 ± 38.18^{bA}	23.29 ± 4.75^{c}	514.39 ± 34.66^{aA}
	meat	143.85 ± 31.70^{bB}	19.47 ± 5.10^{c}	241.15 ± 4.05^{aB}
hexanal	skin	962.25 ± 80.01^{a}	158.95 ± 1.95^{cB}	270.32 ± 11.55^{bB}
	meat	988.14 ± 183.04^{a}	202.33 ± 4.17^{cA}	354.35 ± 3.53^{bA}
2-ethyl-3,5-dimethylpyrazine	skin	11.68 ± 0.62^{A}	10.69 ± 1.65^{A}	13.07 ± 1.43^{A}
	meat	0^{B}	0^{B}	0^{B}
2-heptanone	skin	59.67 ± 6.27^{aA}	0^{b}	0^{b}
	meat	0^{B}	0	0
heptanal	skin	99.61 ± 9.78^{aA}	0^{c}	56.73 ± 5.50^{bA}
	meat	45.43 ± 10.34^{aB}	0^{c}	21.74 ± 2.74^{bB}
eucalyptol	skin	0^{b}	307.08 ± 23.66^{aA}	0^{b}
	meat	0^{b}	67.81 ± 4.54^{aB}	0^{b}
2-pentylfuran	skin	106.19 ± 9.84^{aA}	4.70 ± 0.11^{bA}	0^{c}
	meat	30.08 ± 7.61^{aB}	0^{bB}	0^{b}
2-methylpyrazine	skin	93.86 ± 13.30^{b}	11.55 ± 4.54^{c}	433.90 ± 43.58^{a}
	meat	97.96 ± 34.77^{b}	8.23 ± 3.08^{c}	373.30 ± 6.34^{a}
octanal	skin	60.53 ± 5.65^{aA}	0^{c}	28.32 ± 5.39^{bA}
	meat	17.44 ± 4.76^{aB}	0^{b}	0^{bB}
(*E*)-2-heptenal	skin	51.45 ± 5.25^{bA}	16.73 ± 2.14^{cA}	64.97 ± 4.53^{aA}
	meat	17.41 ± 4.58^{aB}	0^{bB}	0^{bB}
2,6-dimethylpyrazine	skin	0^{b}	22.59 ± 6.20^{a}	0^{bB}
	meat	0^{c}	20.96 ± 0.64^{a}	14.29 ± 0.34^{bA}
2-methyl-3-furanthiol	skin	2.11 ± 0.21^{bA}	2.94 ± 0.17^{aA}	2.79 ± 0.41^{a}
	meat	1.46 ± 0.04^{cB}	2.03 ± 0.03^{bB}	3.25 ± 0.02^{a}
2-ethyl-5-methylpyrazine	skin	10.57 ± 3.61^{aA}	6.13 ± 0.78^{bA}	0^{c}
	meat	0^{B}	0^{B}	0
nonanal	skin	223.07 ± 20.93^{aA}	48.09 ± 2.75^{cA}	140.97 ± 16.46^{bA}
	meat	91.57 ± 28.27^{aB}	16.95 ± 0.68^{bB}	28.30 ± 2.02^{bB}
2-ethenylpyrazine	skin	9.32 ± 0.40^{A}	9.70 ± 0.35^{A}	7.71 ± 4.70^{A}
	meat	0^{B}	0^{B}	0^{B}
5-ethyl-2,3-dimethylpyrazine	skin	0^{B}	0	0^{B}
	meat	9.45 ± 2.87^{aA}	0^{b}	15.97 ± 0.55^{aA}
methional	skin	54.55 ± 6.70^{bB}	54.18 ± 1.56^{bB}	98.23 ± 4.32^{aB}
	meat	83.02 ± 14.21^{bA}	75.20 ± 1.32^{bA}	119.90 ± 5.00^{aA}
1-octen-3-ol	skin	912.01 ± 88.44^{aA}	27.36 ± 2.48^{bA}	0^{cB}
	meat	845.62 ± 225.35^{aB}	18.32 ± 1.14^{cB}	97.47 ± 7.37^{bA}
2-furfural	skin	4707.83 ± 399.30^{b}	1664.82 ± 133.21^{c}	10714.71 ± 745.26^{aA}
	meat	3908.51 ± 870.07^{b}	1693.68 ± 86.01^{c}	8906.42 ± 151.65^{aB}
benzaldehyde	skin	110.63 ± 16.52^{a}	0^{b}	0^{b}
	meat	72.20 ± 20.75^{a}	0^{b}	0^{b}
2-furanmethanol	skin	1853.72 ± 92.34^{b}	2010.15 ± 96.16^{b}	4255.31 ± 293.21^{aA}
	meat	1935.27 ± 268.07^{b}	2123.14 ± 78.93^{b}	3103.30 ± 24.17^{aB}
(*E, E*)-2,4-decadienal	skin	2620.16 ± 175.69^{aA}	0^{b}	2357.91 ± 270.91^{aA}
	meat	0^{B}	0	0^{B}
anethole	skin	0^{b}	5242.87 ± 215.76^{aA}	0^{b}
	meat	0^{b}	3256.67 ± 83.28^{aB}	0^{b}
1-methylnaphthalene	skin	16.85 ± 1.59^{aA}	1.10 ± 0.18^{bA}	14.50 ± 2.19^{aA}
	meat	4.23 ± 3.04^{aB}	0^{bB}	0^{bB}
2-methoxyphenol	skin	12.05 ± 3.94^{cA}	1061.55 ± 58.72^{aA}	690.10 ± 76.67^{bA}
	meat	0^{cB}	950.21 ± 33.35^{aB}	216.15 ± 12.52^{bB}
2-methylphenol	skin	482.94 ± 27.19^{bA}	0^{cB}	1735.90 ± 142.20^{aA}
	meat	250.04 ± 32.86^{bB}	402.01 ± 7.51^{aA}	439.54 ± 17.04^{aB}
4-methylphenol	skin	1326.79 ± 63.97^{bA}	1527.45 ± 41.11^{bA}	4072.96 ± 325.09^{aA}
	meat	840.10 ± 73.85^{bB}	1259.80 ± 20.41^{aB}	1227.70 ± 42.24^{aB}
3-methylphenol	skin	290.17 ± 29.21^{cA}	531.07 ± 21.37^{bA}	1531.21 ± 154.85^{aA}
	meat	0^{bB}	0^{bB}	259.68 ± 17.85^{aB}
3-ethylphenol	skin	107.87 ± 9.30^{cA}	1444.76 ± 37.83^{aA}	694.95 ± 86.92^{bA}
	meat	0^{bB}	901.39 ± 20.52^{aB}	0^{bB}

[a] means ± standard deviation ($n = 3$). Data in the same row with different superscripts (a, b and c) are significantly different at a level of $p < 0.05$. Data in the same column with different superscripts (A and B) are significantly different at a level of $p < 0.05$.

might also be generated from the microbial fermentation of lignin or diterpenes and the microbial metabolism of tyrosine (Belitz, Grosch, & Schieberle, 2009; Kosowska et al., 2018; Pu et al., 2020). Phenolic compounds were also present in the stomachs of poultry, that were ether grass-or grain-fed (Gasior et al., 2021). Phenolic compounds, such as guaiacol, maltol, and phenol, identified in Beijing roasted duck were believed to be produced through the burning of wood during roasting and the microbial composition of the duck's digestive tract (Liu et al., 2019). In addition, another class of important compounds detected in smoked ducks included pyrazines could be produced from the Maillard reaction. The condensation of α-aminoketones (e.g. glyoxal, methylglyoxal, ethylglyoxal) via α-diones from Maillard reaction generated pyrazines like 2-methylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 5-ethyl-2,3-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine (Scalone, Lamichhane, Cucu, De Kimpe, & De Meulenaer, 2019). The aldol-type condensation and cleavage of glucose played a crucial

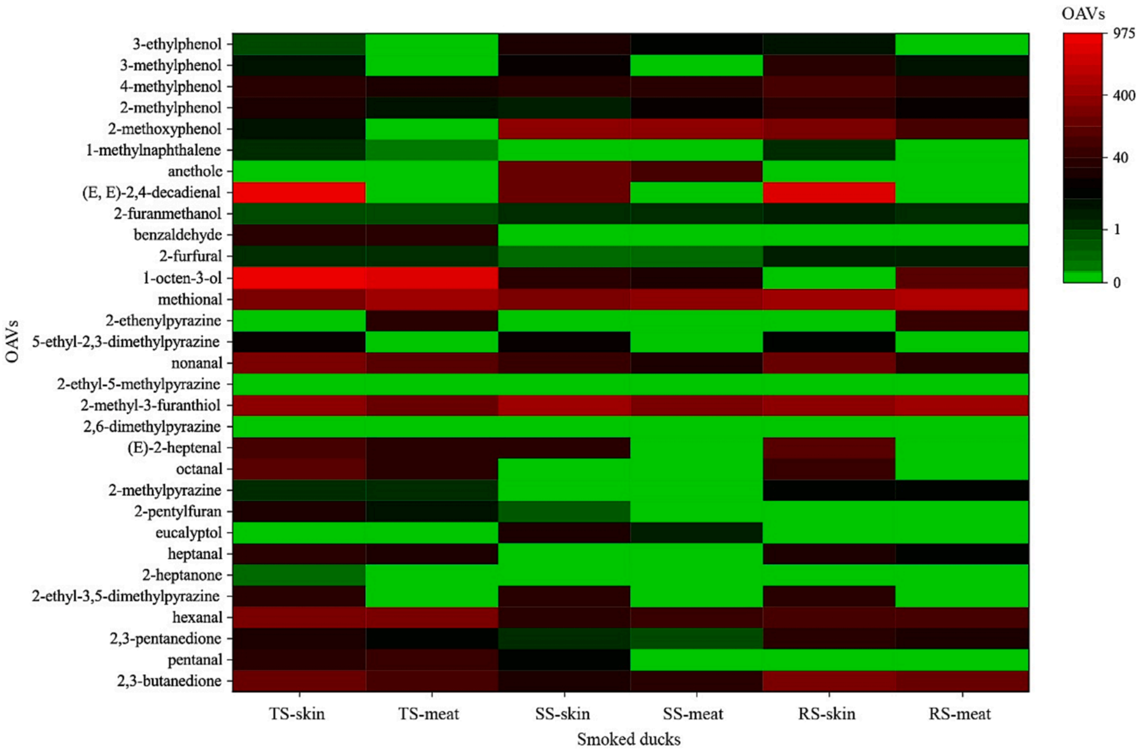

Fig. 1. Heat map depicting odour active values (OAVs) of TS, tea oil smoked duck; SS, sugarcane smoked duck; and RS, rice smoked duck.

role on the synthesis of these pyrazines in Maillard reaction between glucose and glycine (Zhang et al., 2022). The 2-methyl-3-furanthiol was produced upon the Maillard reaction between cysteine and glucose/ribose or upon the thiamine thermal degradation (Liu, Wang, Zhang, Shen, Hui, & Ma, 2020; Thomas, Mercier, Tournayre, Martin, & Berdague, 2014).

The formation of methional might be attributed to the thermal degradation of methionine (Yu & Ho, 1995). The phospholipids with unsaturated fatty acids might also play a key role in the generation of fatty aldehydes and alcohols rather than triacylglycerols (Dannenberger, Lorenz, Nuernberg, Scollan, Ender, & Nuernberg, 2006; Liu et al., 2022). The n-9 polyunsaturated fatty acids (PUFA), including oleic acids, might produce nonanal by pyrolysis and decomposition reaction (Tanimoto, Kitabayashi, Fukusima, Sugiyama, & Hashimoto, 2015). (*E, E*)-2,4-decadienal (fatty note) and hexanal (grassy note) might be produced from the n-6 PUFA, such as linoleic acid and arachidonic acid (Blank, Lin, Vera, Welti, & Fay, 2001; Liu, Wang, Hui, Fang, & Zhang, 2021). Hexanal, nonanal and (*E, E*)-2,4-decadienal might also be produced from the pyrolysis reaction of n-3 PUFA like α-linolenic acid (Elmore, Mottram, Enser, & Wood, 1999; Zhang et al., 2019). The secondary hydroperoxides degradation of fatty acids was crucial in the 1-octen-3-ol generation (Yang, Zhang, Wang, Pan, Sun, & Cao, 2017). Building on the above analysis, it was reasonable to speculate that certain aroma compounds such as 2-methoxyphenol, cooked methional, 2-methyl-3-furanthiol, (*E, E*)-2,4-decadienal, 1-octen-3-ol, and anethole may be particularly important, given their higher odor activity values (OAVs) and perceived odor intensity. Further research was needed to confirm the role of these compounds in the overall aroma profile of smoked ducks.

3.3. Key aroma compounds in smoked duck were confirmed by aroma recombination and omission experiments

While OAVs and gas chromatography–olfactometry (GC-O) were useful tools for identifying key aroma compounds in smoked ducks, to truly assess the contribution of each odorant to the overall aroma profile of smoked ducks, aroma recombination and omission experiments were conducted. GC-O results revealed that the skin of sugarcane smoked ducks had the most complex and intense aroma profile in terms of smoky, roasty, meaty, fatty, grassy, and sweet aromas, making it an ideal candidate for these experiments. The recombination model 1 contained all odorants detected by GC-O in the concentrations that existed in samples (Table 3). The overall similarity of this model containing the skin of smoked ducks was found to be 2.82 on a scale from 0 to 3, This demonstrated that the deodorized sample was an ideal matrix for sensory evaluation.

The recombination model 3 comprised 13 aroma compounds that might significantly influence the aroma profile of smoked ducks in recombination model 2, namely, 2-methoxyphenol, 4-methylphenol, 3-methylphenol, 3-ethylphenol, 5-ethyl-2,3-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, methional, 2-methyl-3-furanthiol, anethole, hexanal, nonanal, 1-octen-3-ol, and (*E, E*)-2,4-decadienal. The aroma profile of recombination model 3 was found to closely mimic the overall aroma impression (score of 2.72), indicating that the 13 odorants included in this model were likely to be key contributors to the aroma profile of smoked ducks (Fig. 2). It had been shown that only a small fraction of the volatile compounds present in foods were primarily responsible for the overall aroma profile (Xiao, Chen, Niu, & Zhu, 2021). A total of 8 odorants, including 2-methoxyphenol, 4-methylphenol, 5-ethyl-2,3-dimethylpyrazine, methional, 2-methyl-3-furanthiol, (*E, E*)-2,4-decadienal, 1-octen-3-ol, and anethole, significantly contributed to the aroma profile of smoked ducks ($p < 0.01$) that might predominantly attribute to smoky, roasty, fatty, meaty, sweet, and grassy aromas. The smoky attribute was predominantly ascribed to 2-methoxyphenol and 4-methylphenol. Meanwhile, among the alkyl and methoxy-phenolic compounds determined in smoked ducks, 3-ethylphenol were also recognized in smoked duck samples. The 4-methylphenol, 2-methoxyphenol, and 2-methoxyphenol were confirmed as the key odorants responsible for smoky flavor in smoked pork loin (Kosowska et al., 2018; Varlet, Knockaert, Prost, & Serot, 2006). The pyrazines, including 2-ethyl-3,5-dimethylpyrazine and 5-ethyl-2,3-dimethylpyrazine, were considered as key aroma compounds that contribute to roasty odor in

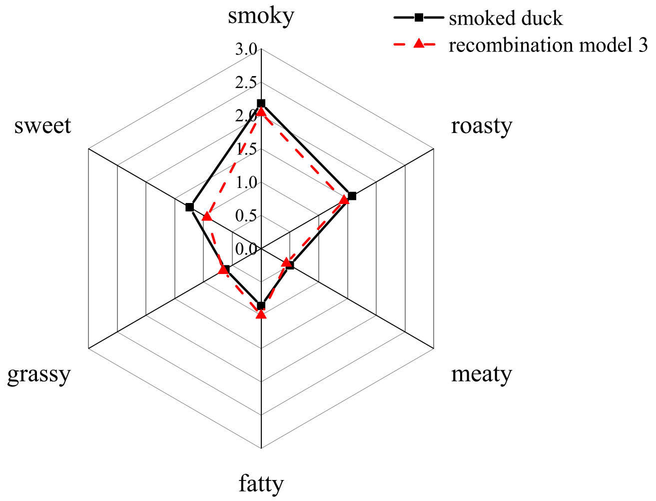

Fig. 2. Aroma profiles of smoked ducks using recombination model 3.

roasted pork, goose and peas (Bi et al., 2020; Gasior et al., 2021; Liu et al., 2023). Meanwhile, methional, 2-methyl-3-furanthiol, hexanal, nonanal, 1-octen-3-ol, and (*E, E*)-2,4-decadienal were also confirmed as key aroma compounds in meat products, including duck, goose, chicken, and mutton (Fan et al., 2018; Liu, Hui, Fang, Li, Wang, & Zhang, 2021; Liu et al., 2019).

4. Conclusion

The aldehydes, pyrazines, and phenols were found to be the major contributors to the aroma profile of smoked duck. Several aroma compounds were identified in smoked ducks for the first time, including 2-methoxyphenol, 3-ethylphenol, 2-ethyl-3,5-dimethylpyrazine, and 2-methyl-3-furanthiol. Five phenolic compounds with smoky notes were perceptible in smoked ducks. A total of 13 key odorants were found to closely mimic the overall aroma impression of smoked ducks by the aroma recombination and omission experiments. These findings confirm that sensomics is a valuable tool for identifying and assessing the relative importance of individual aroma compounds in complex food matrices. For meat industries, we can achieve the better aroma profile of smoked duck by developing new technologies to regulate the key aroma compounds. In the future study, we will focus on the formation mechanism of pyrazines and phenols in smoked duck.

CRediT authorship contribution statement

Huan Liu: Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Funding acquisition. **Jingyu Li:** Writing – review & editing. **Nazimah Hamid:** Writing – review & editing. **Junke Li:** Investigation, Validation, Visualization. **Xuemei Sun:** Investigation, Data curation, Validation. **Fang Wang:** Validation, Data curation, Visualization. **Dengyong Liu:** Writing – review & editing. **Qianli Ma:** Data curation, Writing – review & editing. **Shuyang Sun:** Writing – review & editing. **Hansheng Gong:** Conceptualization, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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