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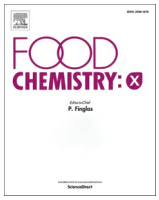

Aroma characterization of Sichuan and Cantonese sausages using electronic nose, gas chromatography–mass spectrometry, gas chromatography-olfactometry, odor activity values and metagenomic

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ABSTRACT

The interest of Chinese consumers in meat-free sausages has increased considerably due to their health benefits, but the aroma quality is far from reaching the traditional fermented meat sausages. This study evaluated the aroma characterization of Sichuan and Cantonese sausages using electronic nose (E-nose), gas chromatography–mass spectrometry (GC–MS), gas chromatography-olfactometry (GC-O), odor activity values (OAVs) and metagenomic. Ninety-eight volatile compounds were identified. Among them, 23 odorants were perceived, and their intensity differed in the two groups of sausages. There was a significant difference in the volatile compound profile between Sichuan and Cantonese cooked sausages. E-nose sensors could differentiate them through specific responses to these volatile compounds. Furthermore, there was a significant difference in microbial communities between Sichuan and Cantonese sausages. For aroma quality improvement of meat-free sausages, studies should focus on controlling the formation of aroma compounds by aroma precursors and using different microorganisms to produce diverse meat aromas. Our results provide a reference for the implementation of these strategies.

1. Introduction

China is one of the world's leading producers and consumers of fermented sausages. Sichuan and Cantonese sausages are the most popular fermented meat products in southwestern and southeastern China, respectively (Du & Ahn, 2006; Wang et al., 2021). The general processing procedures for Sichuan and Cantonese sausages are salting, enema, and drying. Their biggest difference is that the drying method for Cantonese sausages is oven-dried at 50 °C, while Sichuan sausages is air dried at room temperature, which resulted in their different aroma characteristics (Sun et al., 2010; Wang et al., 2021).

Aroma is a critical factor in determining the quality of sausages and consumer acceptance. In recent years, the interest of Chinese consumers in plant-based sausages has increased considerably due to the health benefits such as low sugar, low fat and low salt (Flores & Piornos, 2021; Yuan, Zhu, et al., 2022 and Yuan, Jiang, et al., 2022). Nevertheless, the aroma of meat-free sausages is far from reaching the level of traditional Chinese fermented meat sausages. Therefore, improving the aroma quality of meat-free sausages and elucidating the aroma profile of Chinese traditional fermented meat sausages is meaningful. Although hundreds of volatile compounds have been identified in Sichuan and Cantonese sausages (Du & Ahn, 2006; Yang, Li, et al., 2022; Yang, Zhong, et al., 2022), their key odorant profile remains to be identified. Moreover, although the aroma characteristics between Sichuan and Cantonese sausages differ significantly, their volatile compound profiles are very similar. Therefore, clarifying their flavor differences will help manufacturers distinguish and produce meat-free sausages of Sichuan and Cantonese styles.

Simultaneous distillation and extraction (SDE) is widely used to extract volatile compounds from cooked meat products due to its simple operation and high extraction efficiency (Wan et al., 2021; Zhang et al., 2019). Gas chromatography-mass spectroscopy (GC–MS), gas

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Table 1
Contents of volatile compounds in Sichuan and Cantonese sausages.

No.	LRI	Compounds	Contents (μg/kg) SS1 ~ SS4	CS1 ~ CS4	SS $_{average}$	CS $_{average}$
		Aldehydes				
1	811	Hexanal	98.3–152.2	78.6–110.5	122.1 ± 26.5 *	93.6 ± 13.2
2	850	(*E*)-2-hexenal	1.8–11.1	7.4–13.3	6.2 ± 4.7	11.5 ± 2.8 *
3	892	(*Z*)-4-heptenal	n.d.	0.4–1.8	n.d.	1.1 ± 0.7
4	894	Heptanal	16.3–62.3	36.2–122.2	43.3 ± 19.7	74.5 ± 38.0 *
5	901	(*E*, *E*)-2,4-hexadienal	0.1–6.5	1.9–3.7	3.1 ± 1.2	2.6 ± 0.8
6	949	(*E*)-2-heptenal	8.1–24.2	11.0–28.6	14.1 ± 7.7	20.8 ± 8.0 *
7	951	Benzaldehyde	3.0–8.2	4.7–19.2	6.2 ± 2.3	12.2 ± 6.4 *
8	970	2-Isopropylbutanal	4.5–8.4	n.d.	5.8 ± 1.8	n.d.
9	999	Octanal	3.2–15.5	15.5–19.8	9.5 ± 5.0	16.9 ± 2.0 *
10	1006	(*E*, *E*)-2,4-heptadienal	1.6–5.5	4.2–11.6	3.5 ± 1.9	8.0 ± 3.3 *
11	1038	Benzeneacetaldehyde	0.5–1.6	1.0–3.2	1.2 ± 0.7	1.9 ± 1.0
12	1040	(*E*, *Z*)-2,6-nonadienal	0.1–0.2	1.6–3.0	0.1 ± 0.1	2.3 ± 0.6
13	1053	(*E*)-2-octenal	12.3–20.2	9.4–23.5	16.3 ± 3.3	17.4 ± 6.1
14	1101	Nonanal	8.1–20.4	13.3–32.4	14.3 ± 6.0	23.5 ± 8.5 *
15	1116	β-Cyclocitral	1.8–3.5	3.6–4.2	2.1 ± 1.0	3.9 ± 0.3
16	1155	(*E*)-2-nonenal	3.3–7.5	8.6–12.4	4.6 ± 2.0	11.6 ± 2.6 *
17	1179	Ethyl-benzaldehyde	0.3–2.8	1.2–4.5	1.4 ± 1.2	2.9 ± 1.1
18	1202	Decanal	1.0–2.5	7.3–8.4	1.7 ± 0.7	7.8 ± 0.5 *
19	1209	(*E*, *E*)-2,4-nonadienal	2.8–6.4	4.8–8.0	4.2 ± 1.6	5.9 ± 1.5
20	1237	Citral	1.8–2.3	3.0–4.6	2.0 ± 0.2	3.9 ± 0.8
21	1258	(*E*)-2-decenal	52.0.3–21.3	28.0–45.6	10.9 ± 7.2	33.7 ± 8.3 *
22	1312	(*E*, *E*)-2,4-decadienal	36.0–55.4	80.0–114.2	42.4 ± 12.5	94.7 ± 14.5 *
23	1359	2-Undecenal	6.5–12.4	n.d. ~ 0.5	9.3 ± 2.8	n.d.
		Total			324.2 ± 28.9	450.9 ± 53.8 *
		Esters				
24	822	1-Butyl acetate	1.6–3.0	2.0–3.6	2.1 ± 0.6	2.5 ± 0.7
25	853	Ethyl-2-methylbutyrate	1.8–2.5	3.2–7.4	2.1 ± 0.5	5.7 ± 1.8
26	874	Isoamyl acetate	n.d.	1.7–8.2	n.d.	5.1 ± 2.8
27	883	Phenethyl acetate	6.8–38.5	23.8–34.5	21.7 ± 13.0	28.60 ± 4.4
28	908	Isobutyl butanoate	nd ~ 1.4	0.4–2.5	0.7 ± 0.6	1.5 ± 0.9
29	918	Methyl hexanoate	0.2–3.7	2.0–6.7	2.2 ± 1.5	3.8 ± 2.1
30	998	Ethyl hexyl	5.6–12.1	13.7–41.2	9.1 ± 3.0	26.6 ± 12.5 *
31	1049	Isobutyl angelate	3.4–8.4	11.4–20.6	5.5 ± 1.3	16.5 ± 5.2 *
32	1055	5-Octanolide	2.6–4.0	n.d.	3.7 ± 0.7	n.d.
33	1089	Methyl benzoate	0.5–1.6	1.0–3.8	1.1 ± 0.5	2.3 ± 1.2
34	1160	Benzyl acetate	1.7–5.3	7.4–22.3	3.5 ± 1.6	13.4 ± 6.3 *
35	1166	Benzoic ether	0.5–2.0	1.5–4.2	1.2 ± 0.7	3.3 ± 1.5
36	1089	Methyl salicylate	0.6–2.1	2.6–4.5	1.4 ± 0.7	3.3 ± 0.8
37	1194	Ethyl caprylate	1.8–5.8	22.6–54.2	3.9 ± 1.7	37.8 ± 15.1 *
38	1234	Hexyl-2-methylbutyrate	4.7–8.1	27.4–64.6	6.7 ± 1.5	44.9 ± 17.6 *
39	1253	Linalyl acetate	46.3–131.7	57.5–123.6	91.6 ± 35.1	96.1 ± 27.8
40	1282	Bornyl acetate	1.8–4.2	8.0–12.9	3.0 ± 1.0	9.9 ± 2.2
41	1321	Methyl decanoate	0.3–0.8	0.8–1.5	0.6 ± 0.1	1.2 ± 0.3
42	1341	Benzyl butyrate	0.0–0.5	1.3–2.6	0.3 ± 0.2	2.2 ± 1.0
43	1345	Terpinyl acetate	11.7–15.3	n.d.	15.2 ± 3.3 *	n.d.
44	1379	Geranyl acetate	5.2–8.6	5.7–9.5	6.7 ± 1.5	7.6 ± 1.9
45	1520	Methyl dodecanoate	0.2–0.8	0.5–5.6	0.5 ± 0.3	2.7 ± 1.1
46	1590	Ethyl laurate	n.d.	5.6–19.0	n.d.	11.4 ± 5.8 *
47	1917	Methyl tetradecanoate	n.d.	4.7–9.0	n.d.	6.8 ± 1.9 *
		Total			145.3 ± 29.2	261.5 ± 33.0 *
		Alcohols				
48	836	Isohexyl alcohol	8.6–17.6	2.7–5.4	13.3 ± 4.2 *	3.8 ± 1.2
49	854	*Z*-3-hexenol	1.5–2.7	1.2–2.5	2.1 ± 0.7	1.6 ± 0.6
50	866	1-Hexanol	20.6–41.2	n.d.	31.5 ± 8.5 *	n.d.
51	965	1-Heptanol	8.7–21.4	3.2–7.3	14.2 ± 5.8 *	4.7 ± 1.5
52	975	1-Octen-3-ol	4.8–9.5	4.6–6.2	6.6 ± 2.0	5.5 ± 0.7
53	1029	Benzyl alcohol	1.5–2.0	n.d.	1.7 ± 0.2	n.d.
		Total			69.4 ± 13.1 *	15.5 ± 3.0
		Terpenes				
54	925	β-thujene	2.7–4.2	18.0–32.5	3.6 ± 0.6	21.0 ± 8.0 *
55	939	Camphene	n.d.	3.4–8.3	n.d.	5.8 ± 2.1 *
56	967	Sabinene	1.2–3.5	0.8–2.3	4.5 ± 1.1	1.5 ± 0.6
57	969	β-Pinene	0.8–4.8	8.8–24.6	2.1 ± 1.9	13.8 ± 8.2 *
58	987	β-Myrcene	10.6–23.7	38.9–85.7	19.7 ± 6.5	60.8 ± 19.5 *
59	1012	α-Terpinene	1.8–4.6	9.5–21.8	3.8 ± 1.3	15.0 ± 5.8 *
60	1020	o-Cymene	48.3–68.4	7.5–16.3	59.2 ± 9.4 *	11.4 ± 4.1
61	1025	Limonene	96.2–134.2	188.3–148.2	115.2 ± 16.9	215.3 ± 26.6 *
62	1026	1,8-Cineole	60.3–85.0	25.2–45.8	71.3 ± 12.3 *	35.9 ± 8.4
63	1054	γ-Terpinene	3.2–9.1	8.6–24.8	6.4 ± 2.7	15.6 ± 7.7 *
64	1062	(Z)-β-terpineol	16.5–35.8	0.0–1.6	24.9 ± 8.4 *	0.5 ± 0.2
65	1068	Linalool oxide I	3.8–6.0	4.0–8.2	5.4 ± 1.6	6.3 ± 1.7
66	1084	Terpinolene	2.4–4.5	8.6–14.2	3.6 ± 1.0	11.2 ± 2.6 *

(*continued on next page*)

Table 1 (*continued*)

No.	LRI	Compounds	Contents (μg/kg) SS1 ~ SS4	CS1 ~ CS4	SS $_{average}$	CS $_{average}$
67	1098	Linalool	76.4–124.3	13.2–23.6	102.4 ± 21.6 *	17.7 ± 5.2
68	1138	Camphor	18.0–32.4	37.2–51.4	25.2 ± 6.1	45.2 ± 4.2 *
69	1161	Borneol	1.6–4.5	5.8–9.3	2.9 ± 1.2	6.4 ± 2.2
70	1169	Menthol	4.2–6.8	8.7–13.6	5.5 ± 1.4	11.2 ± 2.0
71	1173	Terpinen-4-ol	46.5–78.6	5.6–20.8	65.8 ± 22.2 *	7.8 ± 4.1
72	1186	α-Terpineol	24.4–37.5	10.6–16.3	30.4 ± 6.3 *	12.9 ± 2.5
73	1225	Geraniol	3.8–5.0	n.d.	4.4 ± 0.6	n.d.
74	1281	Anethol	9.2–14.7	4.2–10.8	12.3 ± 3.4 *	6.7 ± 2.8
		Total			570.7 ± 35.9	518.2 ± 46.0
		Ketones				
75	884	2-Heptanone	1.0–3.2	0.8–3.5	2.1 ± 1.0	1.8 ± 0.9
76	973	1-Hepten-3-one	0.3–0.6	1.0–2.7	0.4 ± 0.1	1.8 ± 0.9
77	983	6-Methyl-5-hepten-2-one	7.6–10.4	9.7–14.8	8.9 ± 1.2	12.1 ± 2.6
78	1058	Artemesia	0.8–1.8	2.5–4.2	1.2 ± 0.4	3.3 ± 0.7
79	1060	Acetophenone	1.5–3.6	3.0–5.6	2.3 ± 0.9	3.9 ± 1.2
80	1136	Trans-3-nonen-2-one	0.3–1.2	2.0–3.0	0.8 ± 0.4	2.6 ± 0.4
		Total			15.6 ± 0.8	25.3 ± 2.1 *
		Sulfur-containing compounds				
81	912	Ethyl disulfide	5.2–10.7	9.5–17.4	7.9 ± 2.3	13.3 ± 3.8
		Total			7.9 ± 2.3	13.3 ± 3.8
		Acids				
82	812	2-Ethylbutanoic acid	1.0–2.3	7.5–9.0	1.8 ± 0.6	8.6 ± 0.7 *
83	1076	Butanoic acid	0.0–0.6	n.d.	< 1.0	n.d.
84	1363	Decanoic acid	0.1–1.0	n.d.	< 1.0	n.d.
85	1558	Dodecanoic acid	0.0–0.4	0.0–0.5	< 1.0	<1.0
		Total			2.4 ± 0.8	8.6 ± 0.7 *
		Phenols				
86	1074	*P*-cresol	0.0–0.3	n.d.	< 1.0	n.d.
87	1286	Isothymol	0.3–0.8	n.d.	< 1.0	n.d.
88	1353	Eugenol	0.7–1.0	0.2–0.5	< 1.0	< 1.0
89	1400	Methyleugenol	0.1–0.6	0.0–0.5	< 1.0	< 1.0
		Total			0.3 ± 0.1	0.7 ± 0.2
		Heterocycles				
90	829	2-Methylpyrazine	0.0–0.1	0.0–0.4	< 1.0	< 1.0
91	903	2,5-Dimethyl pyrazine	0.6–1.1	n.d. ~ 0.5	< 1.0	< 1.0
92	907	2-Ethylpyrazine	0.0–0.9	0.2–0.7	< 1.0	< 1.0
93	987	2-Pentylfuran	6.3–9.5	18.4–31.6	7.7 ± 1.4	24.2 ± 6.0 *
94	993	2-Ethyl-5-methyl-pyrazine	0.8–2.0	1.4–4.2	1.2 ± 0.5	2.7 ± 1.2
95	996	2-Ethyl-6-methylpyrazine	n.d.	0.5–2.0	n.d.	1.5 ± 0.7
96	1013	2-Acetylthiazole	0.0–0.2	0.0–1.0	< 1.0	< 1.0
97	1075	2,6-Diethylpyrazine	n.d.	0.1–0.3	n.d.	< 1.0
98	1144	2-Methyl-5-acetylfuran	n.d.	0.0–0.2	n.d.	< 1.0
		Total			8.2 ± 2.5	29.6 ± 7.9 *

LRI: linear retention index wea calculated on InertCap-purWax capillary column. SS1 ~ SS4: the content ranges of volatile compounds in Sichuan sausage samples (SS1, SS2, SS3, SS4). CS1 ~ CS4: the content ranges of volatile compounds in Cantonese samples (CS1, CS2, CS3, CS4). SS $_{average}$: average content level of volatile compounds in Sichuan sausages. CS $_{average}$: average content level of volatile compounds in Cantonese sausages. n.d.: not detected. *: significant differences between varieties of sausages ($P < 0.05$).

chromatography-olfactometry (GC-O), odor activity values (OAVs) and electronic nose (E-nose) are potent detection analytical tools in distinguishing the flavor profile, flavor evaluation, odorant detection, and aroma characteristics analysis of sausages (Yin et al., 2021; Zhang et al., 2023). Microbial fermentation significantly impacted the flavor of sausages (Flores & Piornos, 2021). Recently, high-throughput sequencing technology has received increasing attention due to its precise characterization ability of microbial communities in sausages (Wang et al., 2018; Yang, Li, et al., 2022; Yang, Zhong, et al., 2022). Therefore, to gain a deeper understanding of the flavor characteristics of Sichuan and Cantonese cooked sausages, the aroma extraction was prepared by the SDE method, the volatile compounds and odorants were identified using GC–MS, GC-O and OAVs, the aroma profile was analyzed using E-nose, and the microorganism was determined by high-throughput sequencing. The results would contribute to improving the aroma quality of meat-free sausages and developing unique flavor products in Sichuan and Cantonese styles.

2. Materials and methods

2.1. Chemicals

Standards of compounds with numbers 1–28, 30, 34–40, 58–68, 84–93 and 95 in Table 1 were purchased from J&K Scientific Ltd. (Beijing, China). Standards of compounds with numbers 29, 31–33, 41–57, 39–83, 94, 96–98 in Table 1 were from ANPEL Laboratory Technologies Inc. (Shanghai, China). The n-alkanes (C9-C27) and ethyl caprate (99.5 %) were purchased from Sigma-Aldrich Corporation (Shanghai, China). Methylene chloride and anhydrous sodium sulfate were from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China).

2.2. Sausage samples

Four batches of Sichuan sausage samples (SS1 ~ SS4) were selected from local markets in Chengdu, and four batches of Cantonese sausages (CS1 ~ CS4) were purchased from Guangdong, based on length (20 ± 2 cm), diameter (2.5 ± 0.2 cm), raw material (pork from pig's hind legs only) and popularity.

2.3. E-nose analysis

The sausages' aroma profile was analyzed using a SuperNose electronic nose system (ISENSO Co., France) with ten metal oxide sensors (Sensor 1: sensitive to alkanes, Sensor 2: sensitive to alcohols, aldehydes, and short-chain alkanes, Sensor3: sensitive to ozone, Sensor 4: sensitive to sulfur-containing organics, Sensor 5: sensitive to nitrogen oxides, Sensor 6: sensitive to phenylketones, alcohols and aldehydes, aromatics, Sensor 7: sensitive to ketones and alcohols, Sensor 8: sensitive to short-chain alkanes, Sensor 9: sensitive to organic solvents, and Sensor 10: sensitive to hydrogen). The detection was performed using the method described by Min et al. (2023). Briefly, the sausages (150 g) were cooked with one liter of boiling water for 40 min and then cooled to room temperature (25 ± 1 °C). Five g of the sausage samples were sealed into a sampling apparatus and equilibrated for 20 min. Subsequently, the headspace volatiles were pumped into the sensor chamber at a 400 ml/min rate for measurement. The detection time was 60 s, and the interval for data collection was 1 s.

2.4. Simultaneous distillation extraction (SDE) of volatile compounds

The SDE method used to extract volatiles in sausages has been described previously (Chen et al., 2020). Five g of sausage samples and 30 ml of dichloromethane were used as raw materials and solvents for volatile compound extraction, respectively. After the sausage was distilled in a flask containing 300 ml of distilled water for 40 min, the chilled aroma extract solvent was concentrated to 1.0 ml by a gentle nitrogen stream.

2.5. GC–MS analysis

A GC2010plus-TQ8040MS/MS (Shimadzu Technologies, Tokyo, Japan) was used to analyze the aroma samples. Separating volatile compounds was done in an InertCap-purWax (30 m × 0.25mmi.d. × 0.25 μm film thickness, Shimadzu Technologies, Tokyo, Japan). The heating program of the oven used the method described by Chen et al. (2019): held at 40 °C for 5 min, raised to 220 °C at a rate of 3 °C/min, and held for 5 min. The mass range was 40–400 *m/z* in full scan mode. The linear retention index (RI) was calculated using an n-alkanes series (C6-C27). The identification analysis of volatile compounds was performed using Mass spectrum, RI and authentic compounds.

The volatiles were quantified in a Shimadzu 2010 plus GC system (Shimadzu, Tokyo, Japan) with a flame ionization detector (FID). The operating conditions were the same as the GC–MS method. The concentration of volatile compounds was calculated by the internal standard method, with the internal standard being ethyl caprate (3.0 μg/ml in methylene chloride). The response factor of each standard to the internal standard was detected using the method reported by Zhang et al. (2015).

2.6. GC-O and OAVs analysis

The experimental procedures used in this study were approved by the Management Committee of Shaanxi Provincial Key Laboratory of Bioresources (Approval No. 2023-12). All sensory panelists have read and signed the consent form before participating in this study. All experiments were conducted strictly following the Shaanxi University of Technology regulations and the guidelines proposed in the Helsinki Declaration.

The odorants were identified using a Shimadzu GC2010plus-FID coupled with a Shimadzu OPV277 olfactometry system. The GC column type and temperature program were the same as described above. The FID and sniffing port temperatures were 250 °C and 170 °C, respectively, and the split ratio was 1:1. Twenty experienced panelists (ten males and ten females aged 22–24) were recruited to perceive the odor attribute during GC-O analysis. Before the formal experiment, they had been trained for one month using the reference aqueous solutions of compounds in Table 1. The sensory evaluators were asked to record the perceived odor characteristics and intensity. The intensity ranges from 0 (none) to 5 (strong). Each of the assessors conducted the experiment in triplicate. The concentration of identified odorants was further quantified by an external calibration method. The OAV of aroma compounds was the concentration ratio to their odor threshold.

2.7. High throughput sequencing

E.Z.N.ATM Mag-Bind Soil DNA Kit (OMEGA, USA) was used to extract the microbial DNA of the sausage samples. The concentration and purification of extracted DNA were assayed using Nanodrop 2000 (Thermo Fisher Scientific, USA), and 1.5 % agarose gel electrophoresis was used to determine the quality of DNA. Finally, the extracted DNA was quantified using the Qubit® 4.0 DNA HS assay kit (ThermoFisher, USA), which followed the manufacturer's instructions.

Primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) were used to amplify the ITS1-ITS2 region of the ITS sequence. The bacterial-specific primers 341F (5′-CCTAYGGGRBGCASCAG-3′) and 805R (5′-GACTACHVGGGTATC-TAATCC -3′) were used to amplify the V3 ~ V4 region of the 16S rRNA gene. The PCR reactions were carried out in 30 μl reactions with 15 μl of 2 × Hieff® Robust PCR Master Mix (Yeasen, Shanghai, China), 1 μl primers, and approximately 20 ng template DNA. A total of two step-PCR amplifications were performed. In the first step of PCR amplification, 1 μl of bar-PCR primer F and 1 μl of Primer R were used as primers. The PCR step consisted of initial denaturation at 94 °C for 3 min, followed by 5 cycles of denaturation for 30 s at 94 °C, annealing at 45 °C for 20s and elongation at 65 °C for 30s, and 20 cycles of denaturation at 20 s at 94 °C, annealing at 55 °C for 20s and elongation at 72 °C for 30s. The final extension at 72 °C for another 5 min. For the second step of PCR amplification, the Illumina bridge PCR compatible primers were introduced, and 1 μl of bar-PCR primer F and 1 μl of index-PCR primer R were used. The reaction conditions were as below: denaturation at 95 °C for 3 min, followed by 5 cycles of denaturation for 20 s at 94 °C, annealing at 55 °C for 20s and elongation at 72 °C for 30 s, and the final extension at 72 °C for 5 min. Then, the quality of PCR products was detected by 2 % agarose gel electrophoresis and quantified using the Fluorescence quantitative analyzer Qubit® 4.0 (ThermoFisher, USA). At last, the final PCR products were sequenced on an Illumina Miseq sequencing platform at Sangon Biotech Co., Ltd. (Shanghai, China). The final sequencing results were used for bioinformatics analysis.

2.8. Statistical analysis

Eight independent batches of Sichuan (four batches) and Cantonese (four batches) sausages were used, and each determination was repeated three times. Data was expressed as mean ± standard deviation. The difference in volatile compound profile between Sichuan and Cantonese sausages was evaluated by the principal component analysis (PCA) using Originpro 2021 (OriginLab, Northampton, MA) and one-way analysis of variance (ANOVA) using SPSS statistics 19.0 software (SPSS Inc., Chicago, IL, USA) with a significant difference at $P < 0.05$, respectively. The relationships between the E-nose responses and the volatile compounds in the two groups of sausages were investigated by correlation heatmap using Originpro 2021 (OriginLab, Northampton, MA) and partial least squares regression (PLSR) using Unscrambler version 9.7 (CAMO ASA, Oslo, Norway), respectively. R software (version 3.4.3, Auckland, New Zealand) was used to analyze the alpha diversity index for 16S rRNA sequencing.

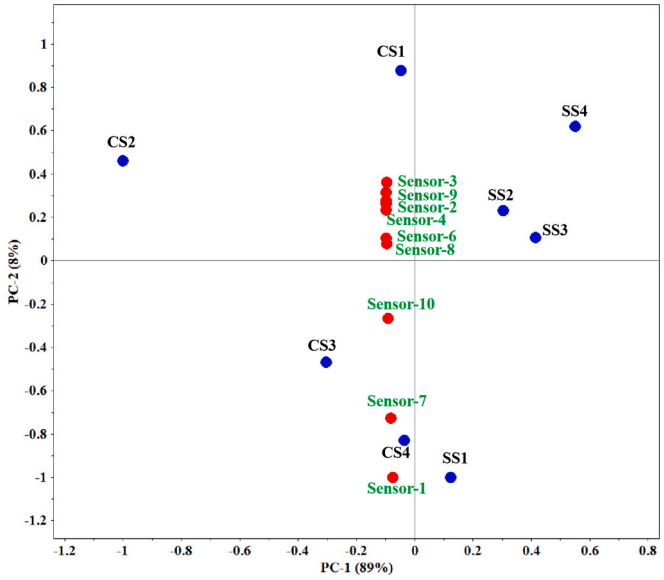

Fig. 1. Principal component analysis loading plot of electronic nose responses in Sichuan (SS1 ~ SS4) and Cantonese (CS1 ~ CS4) sausages.

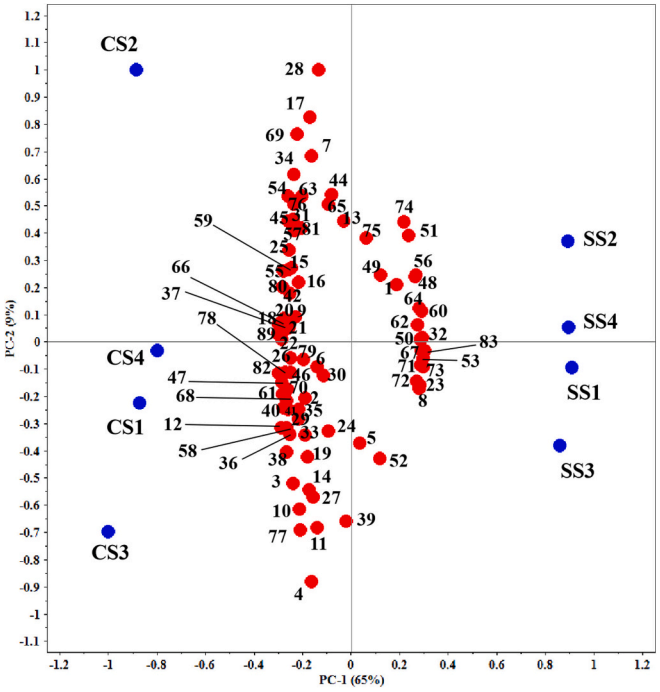

Fig. 2. Principal component analysis loading plot of volatile compounds in Sichuan (SS1 ~ SS4) and Cantonese (CS1 ~ CS4) sausages. Numbers correspond to Table 1.

3. Results and discussion

3.1. Aroma profile of Sichuan and Cantonese sausages

The E-nose results of Sichuan and Cantonese sausages are presented in Fig. 1. The first two PCs contributed 96.2 % of the total variance, indicating that the PCA model was successfully constructed. The Sichuan sausage samples (SS1 ~ SS4) were clustered in the right quadrant and positively correlated with PC1, while the Cantonese sausage samples (CS1 ~ CS4) were located on the left quadrant and negatively correlated with PC1, demonstrating that the aroma profile of the two types of sausages was significantly different. Moreover, Sensor 1 (sensitive to alkanes) and Sensor7 (sensitive to ketones and alcohols) contributed more to the CS4 and SS1 samples attributed to the closer distance, indicating that these sausages had a higher abundance of these compounds. Similarly, CS3 likely had a higher abundance of aromatic compounds due to its proximity to sensor 10 (sensitive to hydrogen). In addition, the Cantonese sausages (CS1 ~ CS4) and Sensor 1 ~ Sensor 10 were clustered in the left quadrant of PC1, suggesting that the Cantonese sausages contain higher abundances of volatile compounds than Sichuan sausages. Chinese-style sausages with different oxidation degrees were distinguished using a similar method (Gu et al., 2017). Moreover, the high levels of ketone and alcohol in Chinese dry fermented sausages also strongly responded to the Sensors sensitive to ketones and alcohols (Chen et al., 2021). These results indicated that there was a significant difference in the aroma profile between Sichuan and Cantonese sausages.

3.2. Volatile compounds profile of Sichuan and Cantonese sausages

As shown in Table 1, a total of 98 volatile components were identified in Sichuan and Cantonese sausages, which were composed of terpenes (49.9 % and 39.2 % in Sichuan and Cantonese sausages respectively), aldehydes (28.3 % and 34.1 % respectively), esters (12.7 % and 19.8 % respectively), alcohols (6.1 % and 1.2 % respectively), ketones (1.4 % and 1.9 % respectively), sulfur-containing compounds (0.7 % and 1.0 % respectively), acids (0.2 % and 0.6 % respectively), phenols (0.1 % and 0.1 % respectively) and heterocycles (0.7 % and 2.2 % respectively).

Terpenes were the most abundant volatile compounds, and 43 terpenes were detected. The contents of β-thujene, camphene, β-pinene, β-myrcene, α-terpinene, limonene, γ-terpinene, terpinolene, and camphor in the Cantonese sausages were significantly higher than those in the Sichuan sausages ($P<0.05$). In contrast, the contents of o-cymene, 1,8-cineole, (*Z*)-β-terpineol, linalool, terpinen-4-ol, α-terpineol and anethol in the Sichuan sausages were higher ($P<0.05$). Most of the terpenes in sausages were considered to originate from the spices (Petričević et al., 2018), such as *Citri Reticulatae Pericarpium*, fennel and bay leaves (Díaz-Maroto et al., 2002; Li et al., 2023; Petričević et al., 2018; Zeller & Rychlik, 2006). These spices were commonly used as 'flavor enhancers' to provide robust, spicy, and floral notes for Chinese fermented meat sausages. Aldehydes were the second most abundant group of volatile compounds. The contents of (*E*)-2-hexenal, heptanal, (*E*)-2-heptenal, benzaldehyde, octanal, (*E*, *E*)-2,4-heptadienal, nonanal, (*E*)-2-nonenal, decanal, (*E*)-2-decenal, and (*E*, *E*)-2,4-decadienal in the Cantonese sausages were higher than those in the Sichuan sausages ($P<0.05$). In contrast, a higher level of hexanal was presented in the Sichuan sausages ($P<0.05$). Aldehydes were an essential source of the oily smells of fermented meat sausages and mainly derived from the oxidation of unsaturated fatty acids during sausage drying, such as autoxidation, photo-oxidation, thermal oxidation, and lipoxygenase-mediated lipid oxidation (Ahmed et al., 2016; Johnson & Decker, 2015). Esters were the products of carboxylic acids and alcohol esterification, which were suggested as essential contributors to the fruity and sweet aromas in sausages (Kohno et al., 2019). In this study, the contents of ethyl hexyl, isobutyl angelate, benzyl acetate, ethyl caprylate, hexyl-2-methylbutyrate, ethyl laurate and methyl tetradecanoate in the Cantonese sausages were higher ($P<0.05$) than those in the Sichuan sausages. Lipid oxidation, caused by lipoxygenase, is the main pathway for generating alcohols (Dragoev, 2024). A total of six alcohols were detected. Among them, the contents of isohexyl alcohol, 1-hexanol, and 1-heptanol in Sichuan sausages were higher ($P<0.05$) than those in Cantonese sausages. The thermal degradation of lipids played a vital role in the formation of ketones, and their content was closely related to the degree of lipid oxidation (Pham et al., 2008; Shahidi & Oh, 2020). A total of six ketones were identified in the Sichuan and Cantonese sausages. Among them, 6-methyl-5-hepten-2-one exhibited relatively high

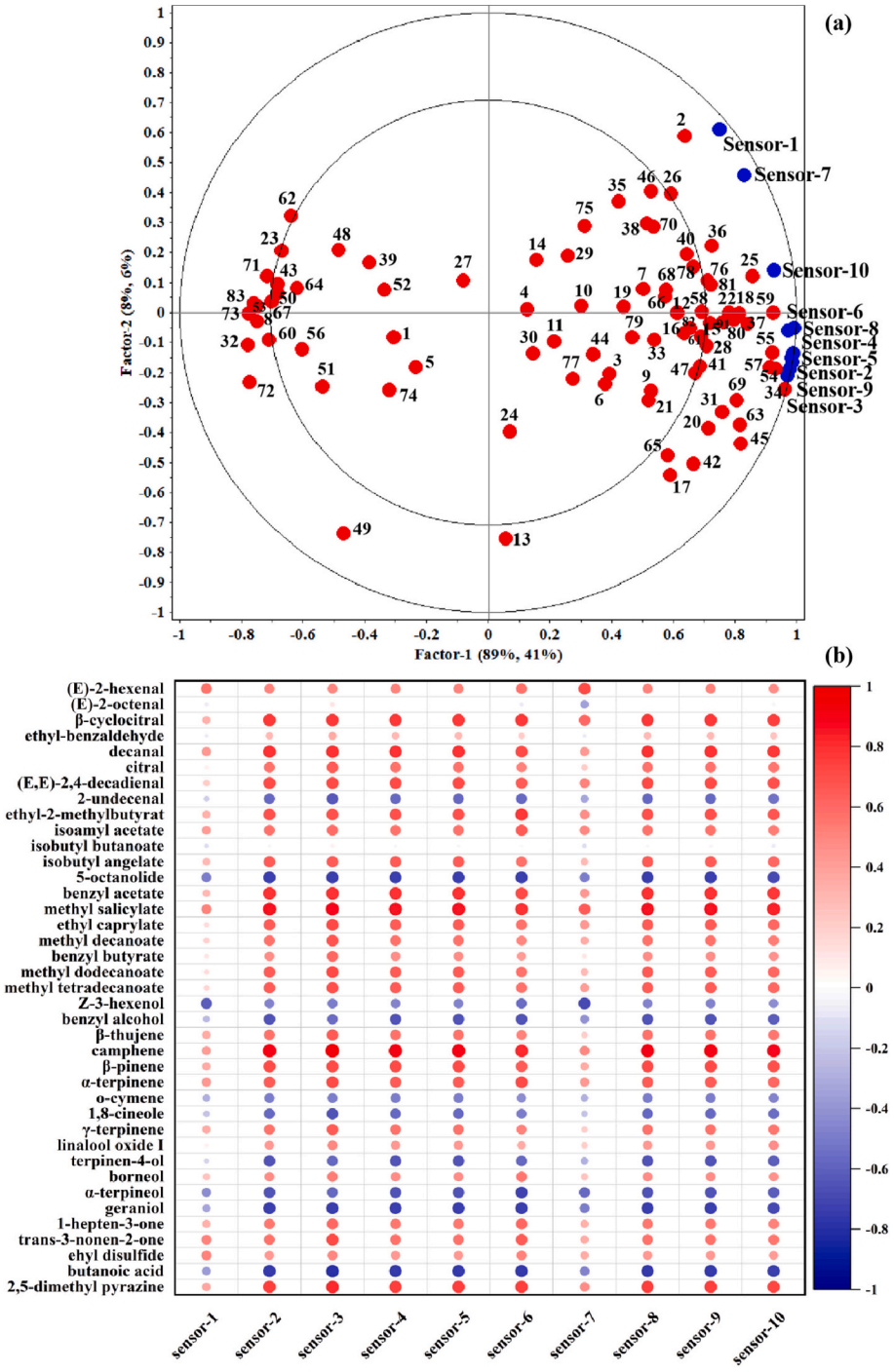

Fig. 3. The partial least square regression loading plot (a) and correlation heatmap (b) between E-nose sensor responses and volatile compounds in Sichuan and Cantonese sausages. Numbers correspond to Table 1.

contents in both types of sausages. Ethyl disulfide with garlic and onion-like odor notes was the only sulfur-containing compound detected in this study, which derived from Strecker degradation of methionine (Ho et al., 2015) and might be playing a major role in the formation of sausage flavor due to its low threshold (0.02 ppb in water). Compared with Sichuan sausages, total ketones in Cantonese sausages were higher ($P<0.05$). Similar results were also reported in E-nose analysis. In addition, numerous acids, phenols and heterocycles with low content were also detected in Sichuan and Cantonese sausages (Table 1). Although the content of these compounds is low, they might still have a critical impact on the sausage flavor through synergistic and additive effects (Zhu et al., 2016).

The PCA was executed to elucidate further the difference in volatile profile between Sichuan and Cantonese sausages. As shown in Fig. 2, 74 % of the total variance contribution rate was obtained, demonstrating good discrimination efficiency. The Sichuan sausage samples were correlated with anethol, o-cymene, 1-heptanol, sabinene, hexanal, iso-hexyl alcohol, 1-hexanol, (*Z*)-β-terpineol, *Z*-3-hexenol, 5-octanolide, 1,8-cineole, geraniol, terpinen-4-ol, 2-isopropylbutanal, 2-undecenal, linalool, benzyl alcohol, 1-octen-3-ol, (*E*, *E*)-2,4-hexadienal, terpinyl acetate and α-terpineol. The Cantonese sausage samples were correlated with the remaining volatile compounds. It could be seen that there is a

Table 2
Odorants in Sichuan and Cantonese sausages.

LRI	Compounds	OT (ppb)	$OAV_{average}$		Odor $intensity_{average}$		Odor attributes
			SS	CS	SS	CS	
811	Hexanal	20	6.1 ± 1.3	4.7 ± 0.7	weak	weak	grassy, greasy
853	Ethyl-2-methylbutyrate	0.01	210 ± 46.9	572.5 ± 180.6	strong	strong	fruity, green
874	Isoamyl acetate	3.0	n.d.	1.7 ± 0.9	n.d. / n.d.	weak	fruity
883	Phenethyl acetate	20	1.1 ± 0.7	1.4 ± 0.2	weak	weak	honey, floral
892	(*Z*)-4-heptenal	0.06	n.d.	17.9 ± 12.1	n.d. / n.d.	medium	grassy, greasy
894	Heptanal	5	8.7 ± 3.9	14.9 ± 7.6	weak	medium	fatty, greasy
901	(*E*, *E*)-2,4-hexadienal	1.6	1.9 ± 1.8	1.6 ± 0.5	weak	weak	sweet, spicy
908	Isobutyl butanoate	1.0	0.7 ± 0.6	1.5 ± 0.9	n.d. / n.d.	weak	sweet, fruity
912	Ethyl disulfide	0.02	395 ± 113.8	663.8 ± 191.1	strong	strong	onion, garlic
949	(E)-2-heptenal	0.5	28.3 ± 15.3	41.6 ± 16.0	weak	medium	fatty, spicy
975	1-Octen-3-ol	2	3.3 ± 1.0	2.7 ± 0.3	weak	weak	soil, mushroom
987	β-Myrcene	16	1.2 ± 0.4	3.8 ± 1.2	weak	weak	pepper, spicy
998	Ethyl hexyl	8	1.1 ± 0.4	3.3 ± 1.6	weak	weak	sweet, fruity
999	Octanal	0.6	15.9 ± 8.4	28.2 ± 3.1	weak	weak	fatty, citrusy
1025	Limonene	200	0.6 ± 0.1	1.1 ± 0.1	n.d. / n.d.	weak	citrusy
1026	1,8-Cineole	10	7.3 ± 1.2	3.6 ± 0.8	weak	n.d. / n.d.	herbal, spices
1040	(*E*, *Z*)-2,6-nonadienal	0.03	3.3 ± 2.7	75.0 ± 20.0	weak	medium	cucumber like
1068	Linalool oxide I	6	0.9 ± 0.1	1.0 ± 0.3	n.d. / n.d.	weak	floral
1098	Linalool	0.6	170.6 ± 36.0	24.1 ± 13.2	medium	weak	floral
1101	Nonanal	3.5	4.1 ± 1.7	6.7 ± 2.4	weak	weak	grassy, fatty
1155	(*E*)-2-nonenal	0.4	11.6 ± 4.9	29.1 ± 6.4	medium	medium	grassy, fatty
1202	Decanal	5	0.3 ± 0.1	1.6 ± 0.1	n.d. / n.d.	weak	fatty
1209	(*E*, *E*)-2,4-nonadienal	0.06	69.2 ± 26.1	98.3 ± 24.4	medium	strong	fatty, greasy
1312	(*E*, *E*)-2,4-decadienal	0.16	264.8 ± 55.1	592.0 ± 88.0	strong	strong	fatty, greasy

LRI: linear retention index calculated on InertCap-Wax capillary column. Compounds: the compounds were identified by GC–MS, standard and odor perception. OT: the odor threshold data were taken from Gemert (2011). $OAV_{average}$: the odor activity values were the average value of each group of sausages. SS: Sichuan sausages. CS: Cantonese sausages. Odor $intensity_{average}$: the odor intensity was the average value of each group of sausages. Odor attributes: the odor attributes were perceived by GC-O. n.d.: not detected.

significant difference in the volatile compound profile between Sichuan and Cantonese sausages. Different from the volatile compounds profile of Sichuan and Cantonese sausages, in the meat-free sausages made in China, the volatile compounds were mainly composed of alcohols and acids (Yuan, Zhu, et al., 2022). Moreover, some compounds with high content in Sichuan and Cantonese sausages were lower or absent in meat-free sausages, such as heptanal, (E)-2-heptenal, octanal and (E)-2-octenal (Yuan, Jiang, et al., 2022). Those distinct aroma compounds might significantly influence the formation of meat aroma in meat-free sausages.

3.3. Correlation between the E-nose and GC–MS

Partial least squares regression (PLSR) was used to analyze the correlation between the E-nose variables (x) and volatile compounds variables (y). As shown in Fig. 3a, 97 % of the explained cross validation variance was obtained by factor-1 and factor-2, indicating that the two factors could explain the overall information of the samples. A total of 39 volatile compounds were located between small ellipse ($R^2 = 0.5$) and large ellipse ($R^2 = 1.0$), indicating that the electronic nose sensors were sensitive to these compounds (Yin et al., 2021). As present in Fig. 3b, further analysis based on the correlation heatmap shows that sensors −2 ~ −6, −8 ~ −10 presented highly correlation to β-cyclocitral, decanal, benzyl acetate, methyl salicylate and camphene, indicating a high sensitivity between them. Moreover, a moderate correlation was found between sensors −2 ~ −6, −8 ~ −10 and (E)-2-hexenal, citral, ethyl-2-methylbutyrate, isobutyl angelate, ethyl caprylate, methyl dodecanoate, methyl tetradecanoate, β-thujene, β-pinene and α-terpinene. In contrast, there was a weak correlation between sensors- 1, −7 and these compounds, indicating they had weak sensitivity to them. These results suggested that the E-nose sensors could differentiate the unique flavors of Sichuan and Cantonese sausages by specific reactions to these volatile compounds.

3.4. Odorants in Sichuan and Cantonese sausages

The OAVs and GC-O analysis were performed further to analyze the key odorants in Sichuan and Cantonese sausages. As summarized in Table 2, Figs. 4a and b, 24 odor activity compounds were detected in the two groups of sausages by OAV analysis. Among them, the highest OAVs compounds were dominated by ethyl-2-methylbutyrate (210 and 572.5 in Sichuan and Cantonese sausages, respectively), ethyl disulfide (395 and 663.8, respectively), (*E*)-2-heptenal (541.6 in Cantonese sausages), linalool (170.6 in Sichuan sausages) and (*E*, *E*)-2,4-decadienal (264.8 and 592, respectively), followed by (*E*, *E*)-2,4-nonadienal (69.2 and 98.3, respectively), (*E*, *Z*)-2,6-nonadienal (75 in Cantonese sausages), (*E*)-2-nonenal (11.6 and 29.1, respectively), octanal (15.9 and 28.2, respectively), heptanal (14.9 in Cantonese sausages), and (*Z*)-4-heptenal (17.9 in Cantonese sausages).

GC-O analysis showed that the compounds with high OAVs exhibited strong or medium odor intensity. Among them, ethyl-2-methylbutyrate (odor intensity = 4.3 ± 0.5 and 5.0 ± 0.0 in Sichuan and Cantonese sausages, respectively; fruity), ethyl disulfide (4.4 ± 0.5 and 5.0 ± 0.0, respectively; onion-like) and (*E*, E)-2,4-decadienal (4.5 ± 0.5 and 5.0 ± 0.0, respectively; greasy) exhibited strong odor intensity in Sichuan and Cantonese sausages. (*E*, *E*)-2,4-nonadienal with greasy aroma also presented strong intensity (4.0 ± 0.7) in Cantonese sausages but was medium (3.0 ± 0.7) in Sichuan sausages. (*E*)-2-nonenal with fatty aroma had medium intensity (2.6 ± 0.5 and 2.7 ± 0.5, respectively) in Sichuan and Cantonese sausages. Linalool with floral aroma presented medium intensity (3.7 ± 0.5) in Sichuan sausages, while it was weak in Cantonese sausages (2.0 ± 0.7). (*E*, *Z*)-2,6-nonadienal with cucumber-like aroma, heptanal (3.0 ± 0.7), (Z)-4-heptenal (3.2 ± 0.6) and (*E*)-2-heptenal (3.0 ± 0.5) with fatty and greasy aromas presented medium odor intensity in Cantonese sausages, and were weak in Sichuan sausages.

Similar results were also reported in other sausages. For example, linalool has been reported to exhibit high OAVs in fermented German sausages (Olivares et al., 2015). The high OAVs and intensity of heptanal, (*E*, *E*)-2,4-decadienal, 1-octen-3-ol, nonanal, (*E*)-2-heptenal,

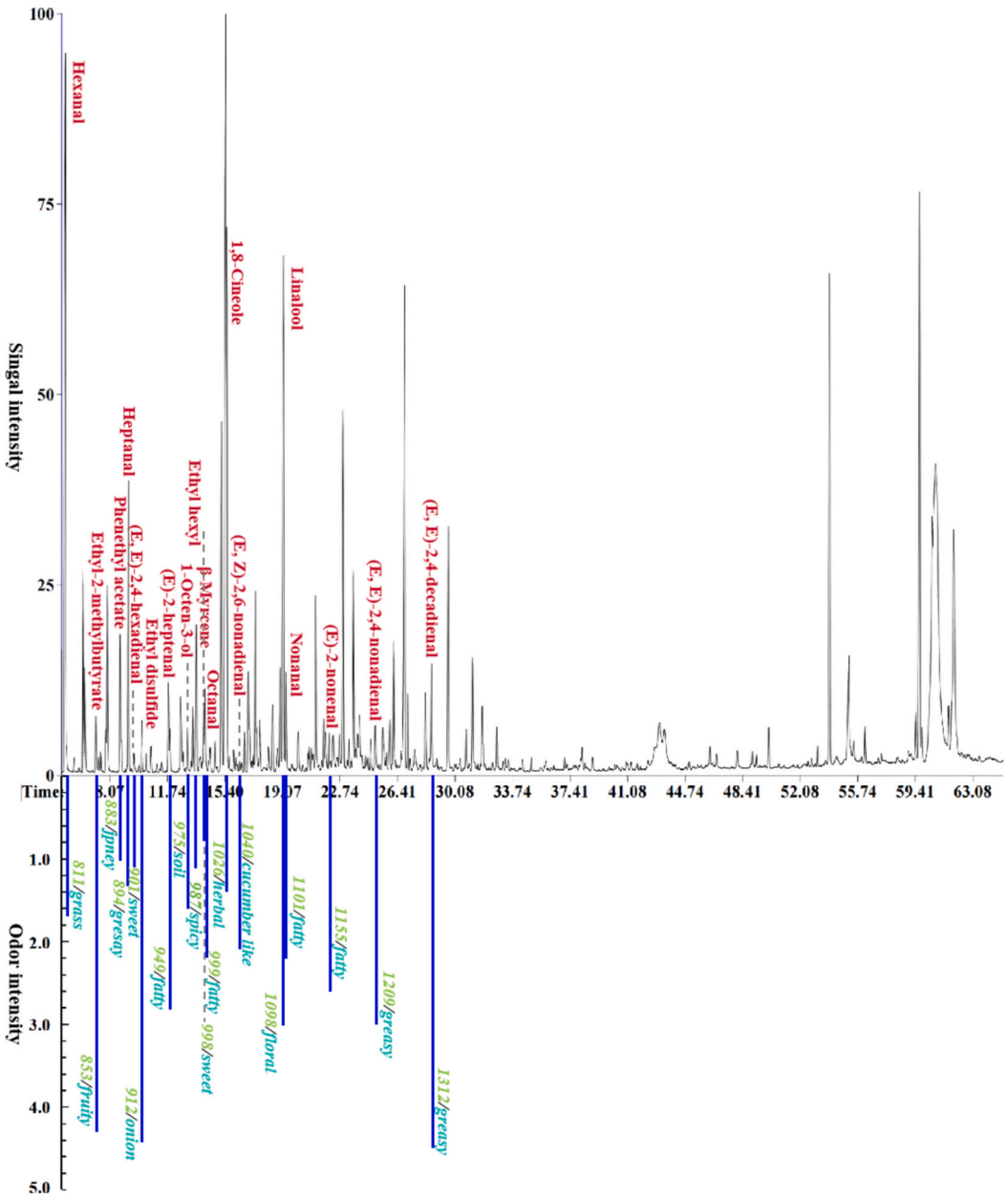

Fig. 4. (a) Odor fingerprints of Sichuan sausages. Numbers corresponded to in Table 2. Odor intensity is the average of samples.
(b) Odor fingerprints of Cantonese sausages. Numbers corresponded to in Table 2. Odor intensity is the average of samples.

hexanal and ethyl disulfide were found in Chinese smoked duck, traditional Hunan smoke-cured pork leg and Turkish heat-treated sausages (Liu et al., 2023; Ozkara et al., 2019; Pu et al., 2020). The high OAVs of nonanal, octanal, hexanal, linalool, 1,8-cineole, limonene, heptanal and 1-octen-3-ol were detected in Chinese dry fermented sausages (Zhou et al., 2021). Ethyl disulfide, (*E*)-2-heptenal, nonanal, (*E*)-2-nonenal, decanal, (*E*, *E*)-2,4-nonadienal and (*E*, *E*)-2,4-decadienal were important sources for the meaty aroma of Sichuan and Cantonese sausages. However, their content was low or absent in the meat-free sausages (Yuan, Jiang, et al., 2022; Yuan, Zhu, et al., 2022). Therefore, increasing the content of these compounds in meat-free sausages is expected to improve the aroma quality.

3.5. Microbial profile in Sichuan and Cantonese sausages

The microbial communities of Sichuan and Cantonese sausages were analyzed using high-throughput sequencing. As presented in Table 3, the Good's coverage was 99 % for the two groups of sausage samples, indicating that most of the microbial phylotypes were detected. Shannon, Ace, Simpson, and Chao indices are commonly used indicators to examine microbial communities' species abundance and diversity (Song et al., 2022). It could be seen that although their values varied in each sample, there is no significant difference between Sichuan and Cantonese sausages.

The relative abundance of microbial community proportions in phylum and genus levels in Sichuan and Cantonese sausages are summarized in Fig. 5. The dominant bacterial phylum for Sichuan sausages was *Proteobacteria,* with an abundance range from 63.7 % to 82.4 %, followed by *Firmicutes*, accounting for 15.7 %–27.9 % of the total bacterium. The high abundance of *Streptophyta* genus was detected at the bacterial genus level, comprising about 64.3 %–86.2 %, followed by *Staphylococcus* with an abundance range from 13.3 %–27.5 %.

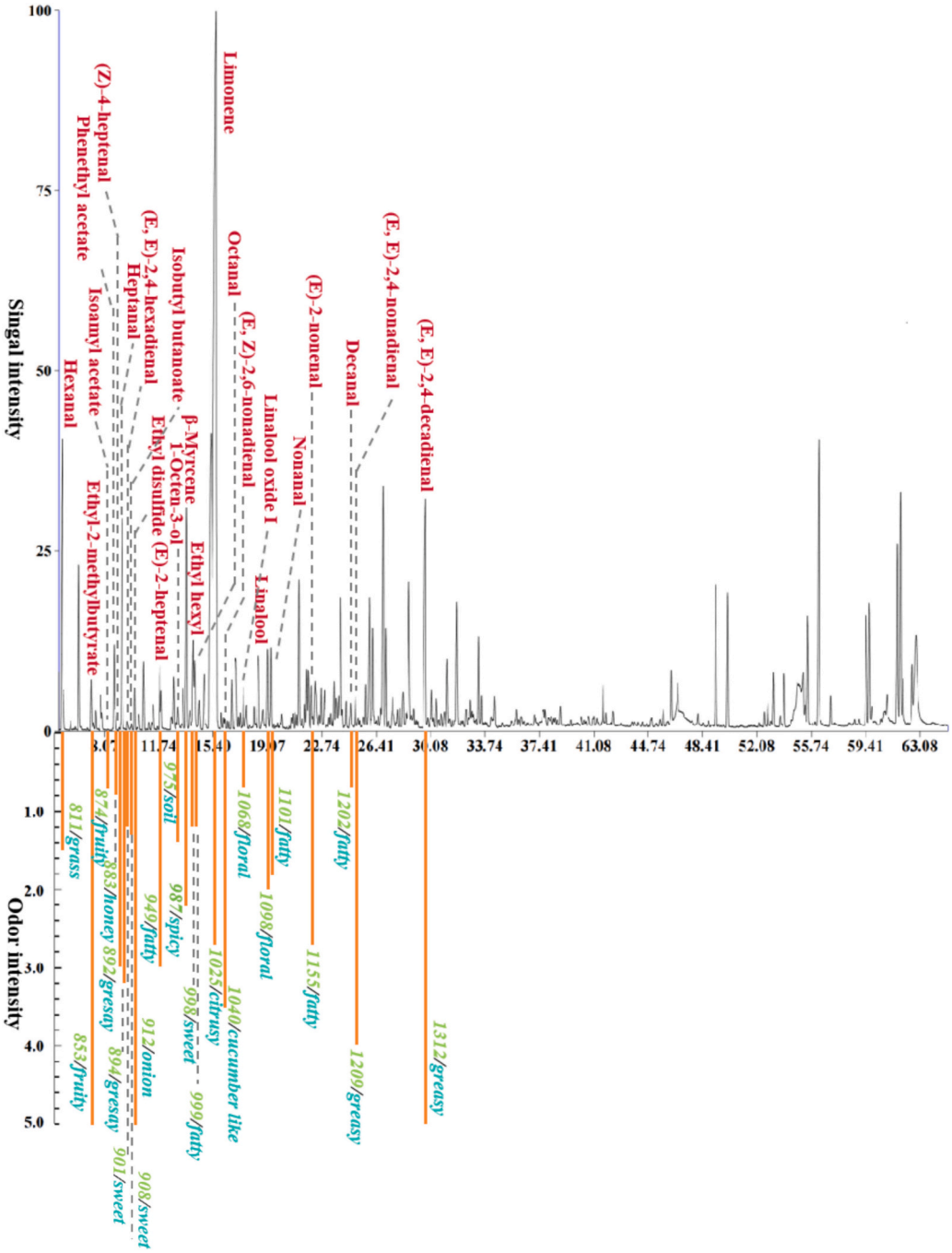

Fig. 4. (*continued*).

Table 3
Alpha diversity index for 16S rRNA sequencing of Sichuan (SS) and Cantonese (CS) sausages.

Groups	Bacteria		Fungi	
Samples	SS	CS	SS	CS
Number	41,408 ± 6340	29,001 ± 5486	37,314 ± 5979	49,444 ± 75
OTUs	142 ± 16	149 ± 27	374 ± 82	235.8 ± 75.2
Shannon	1.9 ± 0.5	2.5 ± 0.3	2.4 ± 0.2	2.4 ± 0.5
Chao	139.7 ± 15.7	153.6 ± 15.6	395.0 ± 117.4	194.2 ± 58.6
Ace	144.2 ± 13.2	156.2 ± 11.4	420.3 ± 115.6	190.8 ± 53.2
Simpson	0.6 ± 0.2	0.6 ± 0.1	0.5 ± 0.2	0.2 ± 0.1
Coverage	0.99 ± 0.0	0.99 ± 0.0	0.99 ± 0.0	0.99 ± 0.0

Ascomycota was the main fungal phyla in all Sichuan sausage samples, ranging from 93.7 % to 78.5 %. *Penicillium* and *Debaryomyces* were the dominant fungal genus, covering 47.0 %–62.4 % and 5.4 %–10.4 %, respectively. Similar results have also been reported in other Sichuan fermented meat products. For example, *Proteobacteria*, *Firmicutes* and *Staphylococcus* were identified as the dominant microbial communities of Sichuan bacon and air-dried bacon (Song et al., 2022; Wang et al., 2018; Wang et al., 2021).

For Cantonese sausages, the high abundance bacterial composition consist of *Firmicutes* (68.4 %–80.8 %) and *Proteobacteria* (13.2 %–18.0 %) at phylum level, and *Lactococcus* (37.5 %–53.5 %), *Macrococcus* (8.7 %–14.2 %), *Weissella* (6.8 %–10.7 %), *Streptophyta* (4.2 %–9.4 %) and *Acinetobacter* (5.4 %–7.5 %) at genus level.

Two dominant fungal phyla were found, including *Ascomycota* (41.9

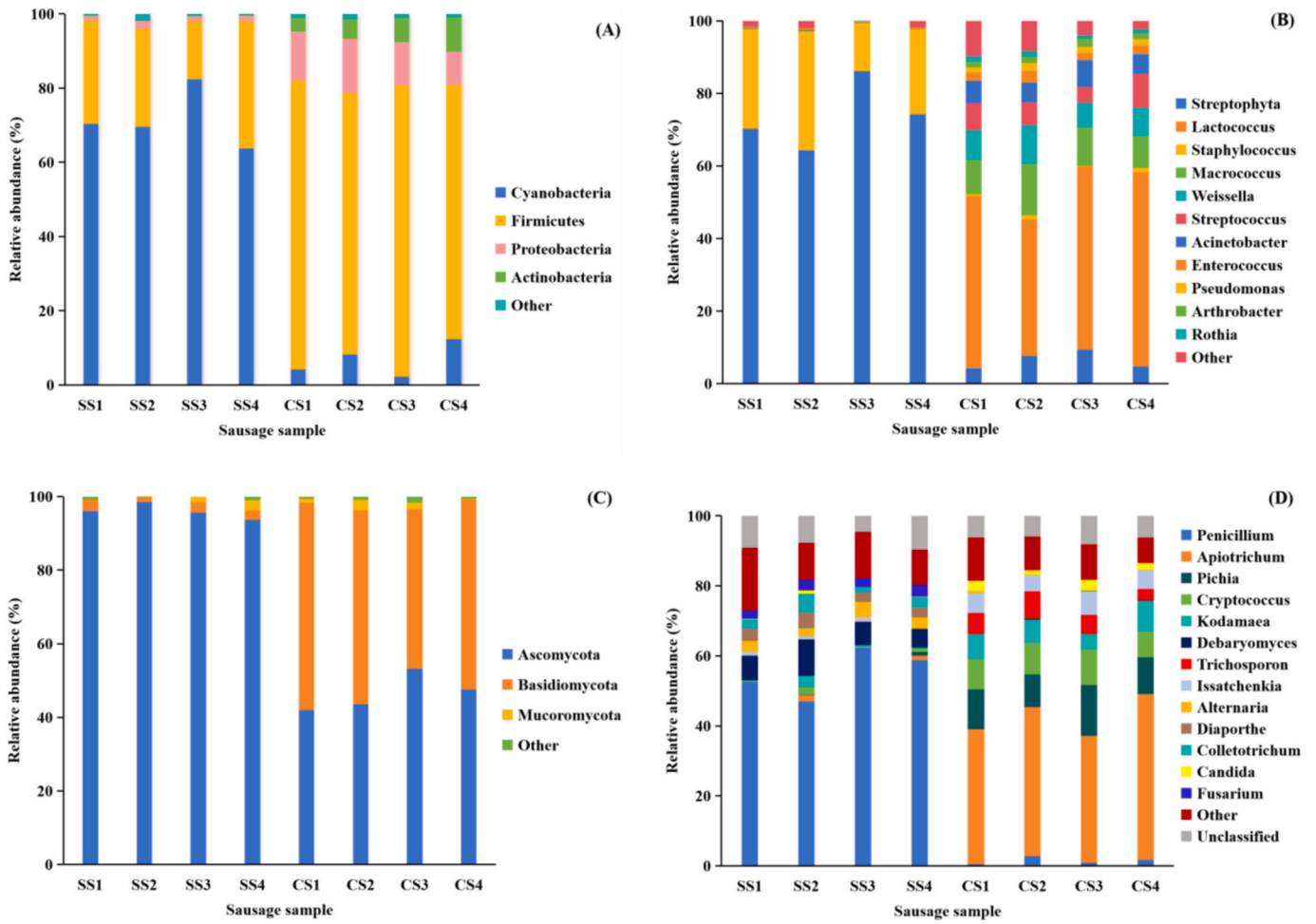

Fig. 5. Microbial relative abundance at phyla (A and C for bacterium and fungus, respectively) and genus (B and D for bacterium and fungus, respectively) levels in Sichuan (SS1 ~ SS4) and Cantonese (CS1 ~ CS4) sausages.

%–53.2 %) and *Basidiomycota* (43.4 %–52.7 %). *Apiotrichum* (36.2 %–47.3 %) presented the highest content at the fungal genus level, followed by *Pichia* (9.4 %–14.6 %), *Cryptococcus* (7.3 %–10.2 %), *Kodamaea* (4.4 %–8.8 %), *Trichosporon* (3.3 %–7.8 %) and *Issatchenkia* (4.5 % ~ 6.5 %). Similar results were also reported by Wang et al. (2021).

It could be seen that the abundance of *Proteobacteria*, *Streptophyta*, *Ascomycota*, *Staphylococcus*, *Penicillium*, *Debaryomyces*, *Alternaria*, *Diaporthe*, *Colletotrichum* and *Fusarium* in Sichuan sausages was higher ($P<0.05$) than those in Cantonese sausages. In comparison, the abundance of *Firmicutes*, *Actinobacteria*, *Lactococcus*, *Macrococcus*, *Weissella*, *Streptococcus*, *Acinetobacter*, *Enterococcus*, *Pseudomonas*, *Arthrobacter*, *Basidiomycota*, *Apiotrichum*, *Pichia*, *Cryptococcus*, *Trichosporon*, and *Issatchenkia* in Cantonese sausages was higher ($P<0.05$) than those in Sichuan sausages.

Microorganisms are the key to the flavor and quality of fermented sausages and are significantly influenced by processing techniques and the environment (Chen et al., 2016). For example, meat products containing fermentation broth cultures of lactic acid bacteria and coagulase-negative Staphylococcus had better flavors (Lu et al., 2015). *Candida* could produce branched-chain amino acids (BCAAs) through the Ehrlich pathway, producing more flavor compounds (Meftah et al., 2019). The high-fat lipolytic activity of *Debaryomyces* might positively contribute to improving the flavor of meat products (Mendoza et al., 2014). Our results suggested that the difference in microorganisms might be one of the important reasons for the unique aroma formation of Sichuan and Cantonese sausages. Thus, using different microorganisms to produce a fermented meaty aroma might be one of the effective strategies to improve the aroma quality of meat-free sausages.

4. Conclusions

There was a significant difference in the volatile compound profile between Sichuan and Cantonese cooked sausages. E-nose sensors could differentiate the unique flavors of Sichuan and Cantonese sausages through specific responses to these volatile compounds. The important odorants included ethyl-2-methylbutyrate, ethyl disulfide, (*E*, *E*)-2,4-nonadienal and (*E*, *E*)-2,4-decadienal. They presented different intensities in the two groups of sausages. Furthermore, there was a significant difference in microbial communities between Sichuan and Cantonese sausages. The main reason meat-free sausages cannot reproduce the aroma of traditional fermented sausages is related to the aroma compounds, mainly attributed to the differences in aroma precursors and microorganisms between them. Therefore, studies should focus on controlling the formation of aroma compounds by aroma precursors and using different microorganisms to produce diverse meat aromas. Our results provide a reference for the implementation of these strategies.

CRediT authorship contribution statement

Xiaohua Chen: Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Fei Yan:** Formal analysis. **Dong Qu:** Supervision, Resources. **Tian Wan:** Investigation. **Linjie Xi:** Investigation. **Ching Yuan Hu:** Writing – review & editing, Conceptualization.

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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