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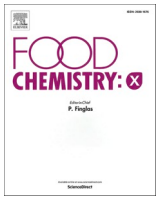

Mechanisms of cooking methods on flavor formation of Tibetan pork

Lujie Cheng [a,b,1], Xin Li [a,1], Yuting Tian [b], Qia Wang [a,b], Xiefei Li [a], Fengping An [b], Zhang Luo [c], Peng Shang [c,*], Zhendong Liu [c,*], Qun Huang [a,b,d,*]

[a] School of Public Health, Guizhou Province Engineering Research Center of Health Food Innovative Manufacturing, the Key Laboratory of Environmental Pollution Monitoring and Disease Control of Ministry of Education, Guizhou Medical University, Guiyang 550025, China
[b] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China
[c] College of Food Science, Tibet Agriculture and Animal Husbandry University, Linzhi, Tibet Autonomous Region 860000, China
[d] Institute for Egg Science and Technology, School of Food and Biological Engineering, Chengdu University, Chengdu 610106, China

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ABSTRACT

To obtain flavor-enriched Tibetan pork products, the impact of oxidation degree on the flavor of Tibetan pork with different cooking methods (microwaving, frying, boiling, and air frying) was evaluated using an E-nose, an E-tongue, GC–MS, and LC–MS. The level of oxidation was lower in M and F and higher in B and AF groups. Hexanal, pentanal, benzaldehyde, 1-octen-3-ol, and 3-hydroxy-2-butanone were identified as significant contributors to cooked samples. The volatile abundance of microwaved, fried, boiled, and air-fried pork was 1.61, 1.22, 1.47, and 1.69 times higher than raw, respectively. Leucine and threonine were detected to be the highest in the AF group, which were 1.30 and 3.60 times greater than RAW, respectively. In summary, oxidation of lipids and proteins caused by cooking treatments was the main source of flavor in cooked Tibetan pork. Air-frying treatment could greatly promote the production of flavor compounds and give unique flavor to Tibetan pork.

Introduction

The Tibetan pig is a breed that lives in high-altitude areas with extensive lean meat content, rich nutrients, and a unique flavor compared to commercial ordinary pork (Gu et al., 2019). As a local pig breed in China, Tibetan pigs feature a high level of intramuscular fat (Shang et al., 2019). Fats serve as precursors for volatile flavor substances, and their oxidation is an essential route for the synthesis of flavor compounds during processing. Unsaturated fatty acids are abundant in Tibetan pork and are easily oxidized and decomposed into aldehydes, ketones, alcohols, and other small molecules of volatile flavor compounds during treatment.

Meat products are frequently processed by cooking, which can not only eliminate pathogenic microorganisms from food and enhance edibility but also accelerate the oxidation process of fatty foods (Yu, Wang, Yin, Ge, & Liao, 2021). The oxidation caused by cooking plays an important role in the formation of flavors. Meanwhile, the overall flavor might be affected by variances in the level of oxidation. The study of Domínguez, Gómez, Fonseca, and Lorenzo (2014b) found that roasted foal meat with a higher degree of lipid oxidation presented richer flavor attributes. Similarly, Echegaray et al. (2020) discovered that the diminished lipid oxidation of fried pork resulted in a decreased concentration of flavor compounds compared to the roasted treatment. Although animal fats are crucial in the formation of flavor, the degradation of protein contributes to the flavor of meat and should not be underestimated. Normally, proteins are considered to be closely linked to the texture and flavor of food products. However, as breakdown products of proteins, amino acids greatly influence the flavor evaluation of food. It has been shown that a minor fraction of aldehydes is derived from protein degradation (Huang et al., 2022b).

There are various cooking methods, such as boiling, frying, air frying, and microwaving, that have a variety of heat transfer modes. The difference in thermal transfer routes is one of the main factors affecting the oxidation process of meat, which is also vital to the formation of food flavors (Penaranda et al., 2017). The lipids and proteins are altered as a result of the material exchange between the sample and the water in the

* Corresponding authors at: School of Public Health, Guizhou Province Engineering Research Center of Health Food Innovative Manufacturing, the Key Laboratory of Environmental Pollution Monitoring and Disease Control of Ministry of Education, Guizhou Medical University, Guiyang 550025, China (Q. Huang).
E-mail addresses: chenglujieclj@163.com (L. Cheng), lx813789123@163.com (X. Li), etingtian@hotmail.com (Y. Tian), Wangqia999@163.com (Q. Wang), xiefeili.edu@gmail.com (X. Li), pingfengan@163.com (F. An), luozhang1759@sohu.com (Z. Luo), nemoshpmh@126.com (P. Shang), liu304418091@126.com (Z. Liu), huangqunlaoshi@126.com (Q. Huang).
[1] authors contributed equally to this work.

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boiling treatment, which also impacts the flavor profile of the meat. Frying treatment utilizes hot oil as the heat transfer medium. By adding exogenous ingredients, the fatty acid composition of meat is altered and oxidized, presenting a pleasing texture and flavor of food. The function of air frying is to heat food evenly through the contact of hot air with the fat. In comparison to frying and deep frying, air frying utilizes substantially less oil. The circulation of hot air accelerates the oxidation process of food, giving it an excellent flavor. Meanwhile, the high temperature dehydrates the surface of the food quickly, creating a crusty layer to preserve the moisture inside the food (Liu et al., 2022b). Microwave treatment depends on the action of electromagnetic fields that make the water molecules inside the food crash to produce heat energy. Moreover, the singlet oxygen generated during heating possesses strong oxidizing activity and plays an active role in the oxidative degradation of lipids and proteins. However, microwaving works on the interior and exterior of foods simultaneously, which leads to greater culinary losses.

The objective of this study was to explore the formation of flavor in Tibetan pork and to obtain flavorful and unique cooked Tibetan pork. Therefore, Tibetan pork with different cooking treatments (boiling, frying, microwaving, and air frying) was used as the object of study. The effects of cooking on the quality of cooked pork were investigated by analyzing the thiobarbituric acid reactive substances (TBARS) value, carbonyl content and total sulfhydryl content. The electronic nose (E-nose) and electronic tongue (E-tongue) systems were applied to analyze the distinctions in cooked samples. Meanwhile, gas chromatography–mass spectrometry (GC–MS) was carried out to qualitatively assess the volatile flavor substances created by the cooking treatment of Tibetan pork. In addition, metabolomics techniques were combined to elucidate the mechanism of diverse cooking processes on the formation of flavor compounds in Tibetan pork. The results contribute to clarifying the mechanism of flavor formation in Tibetan pork during cooking and identifying cooking methods that boost the flavor composition of Tibetan pork, which may provide a theoretical basis for the further processing of Chinese indigenous pig breeds.

Materials and methods

Materials

The pork of Tibetan pigs was purchased from Jiuzhaigou, Tibetan region of Sichuan, China. Hind leg pork was selected for cooking treatment and storage in a −80℃ freezer. The pork was thawed in a refrigerator at 4 °C for 10 h before use, and then cut into 15 ± 2 mm slices.

Cooking processing

The sliced Tibetan pork was randomly and equally divided into 5 groups. The cooking processes were as follows:

Raw meat group (RAW group): raw meat was not processed. Microwave group (M group): Tibetan pork slices were placed in a ceramic dish and treated in a microwave oven at 400 W for 8 min, with turning every 1 min. Frying in oil group (F group): The pan was heated for 50 s. Oil was added to the pan at 5 mL/30 g (soybean oil/meat weight), heated for 30 s, the power was adjusted to 500 w, and the meat samples were fried for 7 min, with constant turning (every 20 s). Air-frying group (AF group): The air fryer was preheated at 200 °C for 5 min, and the sliced Tibetan pork was heated at 180 °C for 10 min, turning over halfway. Boiling group (B group): 2 L of distilled water was added to the pot, and when the water boiled, the power of the induction cooker was adjusted to 500 W. The Tibetan pork was added and the cooked starts for 30 min. All samples were brought to room temperature, churned with a meat grinder, vacuum-packed, and stored in a −80 °C freezer.

Oxidation indices

Carbonyl content

The myofibrillar protein (MP) concentration was diluted to 5 mg/mL with NaCl phosphate buffer (0.04 mol/L, pH 7.0) and the absorbance was determined (Multimode Microplate Reader, Bio Tek Synergy H1, Bio Tek Instruments, Inc, Vermont, USA) by the 2,4-dinitrophenylhydrazine (DNPH) method at 370 nm as described by Dong et al. (2023). The molar absorbance coefficient of 22,000 L/(mol·cm) was used to calculate the carbonyl content. The parameter was calculated with Formula (1) as follows:

$$Carbonyl\,(nmol/mg\,protein) = \frac{A_{370} \times 3000000}{0.5 \times \varepsilon \times C} \quad (1)$$

where "A_{370}" is the absorbance at 370 nm, "C" is the protein concentration (mg/mL), and "ε" is the molar absorbance coefficient.

Total sulfhydryl content

Total sulfhydryl content was extracted according to the method described by Huang et al. (2022a). The MP was diluted to 1 mg/mL with phosphate buffer (0.04 mol/L, pH 7.0) and the absorbance at 412 nm was assayed (Multimode Microplate Reader, Bio Tek Synergy H1, Bio Tek Instruments, Inc, Vermont, USA) by the 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) method. The total sulfhydryl content was calculated using a molar absorbance coefficient of 13,600 L/(mol·cm). The content of total sulfhydryl was calculated with Formula (2):

$$Total\,sulfhydryl\,(\mu mol/g\,protein) = \frac{A_{412} \times 10^6}{C \times 13600} \quad (2)$$

where "A_{412}" is the absorbance at 412 nm, and "C" is the protein concentration (mg/mL).

TBARS

TBARS was determined according to the method of Wang et al. (2022a). A Tibetan pork sample of 0.30 g was placed in a test tube and mixed with 3 mL of TBA solution and 17 mL of TCA-HCL solution by vortexing and boiling in a water bath for 30 min. After cooling, 5 mL of chloroform was added. Then, the solution was centrifuged (3000*g*, 10 min) (H1650R, Xiangyi Co., Ltd., Hunan, China). The absorbance value was measured at 532 nm (Multimode Microplate Reader, Bio Tek Synergy H1, Bio Tek Instruments, Inc., Vermont, USA) by taking the supernatant. The TBARS value was calculated with Formula (3):

$$TBARS\,value\,(mg/kg) = \frac{A_{532} \times 72.06}{m \times 115} \quad (3)$$

where "A_{532}" is the absorbance at 532 nm, "m" is the weight of the sample (g), "72.06" was the relative molecular mass of malondialdehyde (MDA), and "115" is the molar absorbance coefficient.

Volatile compound analysis

E-nose analysis

The flavor characteristics of Tibetan pork samples were analyzed according to the method of Li, Tu, Sha, Li, Li, and Huang (2022). The Tibetan pork samples (2.00 g) were placed in a 20 mL headspace bottle in a water bath at 60 °C for 10 min, and then brought to room temperature for determination. The E-nose (PEN3 Airsense, Schwerin, Germany) contains 10 metal oxide gas sensors: W1C (aromatic), W5S (nitrogen oxides), W3C (ammonia and aromatic), W6S (hydrogen), W5C (alkanes and aromatic), W1S (short-chain alkanes), W1W (inorganic sulfur), W2S (alcohols, ethers, aldehydes, and ketones), W2W (organic sulfur), and W3S (long-chain alkanes). The samples were was measured using an injection flow rate of 400 mL/min and an analytical sampling time of 120 s. Each sample was measured in triplicate for PCA plotting, and the average value was calculated for radar plotting.

Identification of volatile compounds

The volatile compounds of Tibetan pork samples were analyzed according to the method of Wang et al. (2022a). The sample (3.00 g) was placed into a headspace bottle and equilibrated in a water bath at 60 °C for 5 min. A 50/30 μm DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA) was inserted and sorbed for 40 min. Then, the fiber was immediately inserted into the injection port of the gas chromatograph (SHIMADZU GC–MS-QP2010Ultra, Shimadzu, Kyoto, Japan) and resolved at 230 °C for 5 min. Compounds were analyzed on a DB-WAX (30 m × 0.25 mm × 0.25 μm) capillary column in nonsplit mode. The heating up procedure was as follows: held at 40 °C for 5 min, raised to 50 °C at 10 °C /min and held for 2 min, raised to 120 °C at 4 °C /min and held for 2 min, raised to 230 °C at 12 °C /min and held for 5 min, and the total time of the procedure was 41.67 min. The ion source temperature was 230 °C, and the scanning range was 35–550 *m/z*.

Determination of nonvolatile flavor compounds

E-tongue analysis

The taste characteristics of Tibetan pork samples were analyzed using SA402B E-Tongue (Insent Company, Kanagawa, Japan) according to the method of Lee et al. (2019). Fifteen grams of meat sample was mixed with distilled water (150 mL). The mixed solution was filtered with four layers of gauze and then centrifuged at 4000 × *g* at 4 °C for 15 min. Finally, the supernatant was passed through qualitative filter paper at medium speeds (NEWSTAR, Hangzhou Specialty Paper Co., Zhejiang, China), which was used for the E-tongue analysis.

Metabolite extraction

The extraction of metabolites was performed according to the method of Huang et al. (2022b). A 100 ± 2 mg Tibetan pork sample was weighed, placed in a 2 mL centrifuge tube, and ground (50 Hz, 60 s) with 1000 μL of tissue extract (75% 9:1 methanol:chloroform, 25% H_2O) and three steel beads. The process was repeated twice. The mixture was sonicated in an ice bath for 30 min and then centrifuged for 10 min (4 °C, 12,900 × *g*), and the supernatant was collected and dried. Two hundred microliters of 2-chloro-L-phenylalanine solution (4 ppm, 4 °C) was added to resolubilize the sample. The filtrate was added to the assay bottle for liquid chromatography–mass spectrometry (LC–MS) detection.

Metabolite identification

An ACQUITY UPLC® HSS T3 (2.1 × 150 mm, 1.8 μm) (Waters, Milford, MA, USA) column was used with a flow rate of 0.25 mL/min, a column temperature of 40 °C, and an injection volume of 2 μL. The mobile phases were 0.1% formic acid acetonitrile (C) and 0.1% formic acid water (D) for the positive ionization mode and acetonitrile (A) and 5 mM ammonium formate water (B) for the negative ionization mode.

The MS conditions were as follows: an electrospray ion source (ESI) was used with a positive ion spray voltage of 3.50 kV and a negative ion spray voltage of 2.50 kV, a sheath gas of 30 arb and an auxiliary gas of 10 arb. The capillary temperature was 325 °C, and a full scan was performed with a resolution of 70,000 and with a scanning range of 81–1000. Secondary cracking was performed using HCD with a collision voltage of 30 eV, and unnecessary MS/MS information was removed using dynamic exclusion.

The original data were imported into the metabolomics processing software ProgenesisQI (Waters Corporation, Milford, USA) for baseline filtering, peak identification, retention time correction, etc. Finally, information including retention time, quality-acquisition charge ratio, peak intensity, etc., was obtained. The software was used to identify characteristic peaks, match MS and MS/MS information to metabolic databases, and identify metabolites based on mass spectral match scores. All metabolites were obtained from the following databases: Metlin (https://metlin.scripps.edu/) and MoNA (https://mona.fiehnlab.ucdavis.edu//).

Data processing

At least three replicates of each experiment were performed. Differences between means were estimated using one-way ANOVA ($P < 0.05$), and the results are expressed as the mean ± standard deviation. Clustered heatmap analysis was performed using OmicStudio tools (https://www.omicstudio.cn/tool), and the cluster method was ward.D. Origin 2023 (OriginLab Co., Northampton, MA, USA), Gephi 0.10.1 (https://gephi.org), and SPSS 27.0 (SPSS Inc., Chicago, IL, USA) were used for correlation analysis (Pearson's correlation coefficient) among protein oxidation, fat oxidation, volatiles, and nonvolatiles. Simca 14.1 (Umetrics AB, Umea, Vasterbotten, Sweden) was used for PCA (the scaling type was UV) and OPLS-DA (the scaling type was Par) analyses to characterize the differences in odor and taste among Tibetan pork samples.

Results and discussion

Protein and lipid oxidation

Changes in carbonyl content, total sulfhydryl content, and TBARS values have been reported as signs of thermal oxidation of pork (Yin, Zhou, Pereira, Zhang, & Zhang, 2020). As seen from Fig. 1, the oxidation degree of Tibetan pork showed an overall rising tendency with increasing cooking intensity, indicating that heating could induce the decomposition of unsaturated fatty acids and amino acids, thus causing an elevated degree of oxidation. Moreover, the degree of oxidation of the cooked samples varied remarkably among the cooking treatments. A high level of oxidation was observed in air-fried treated and boiled treated samples, while a lower level of oxidation was noted in microwave and frying treatments.

Interestingly, the tendency of protein and fat oxidation was different in Groups B and AF groups. The AF group showed the highest degree of lipid oxidation, whereas its protein oxidation was lower than that of the B group. This could be due to the different reaction substrates of the air frying treatment and the boiling treatment. Air frying treatment heats food through the circulation of hot air, which can enhance the interaction between oxygen and food, thus encouraging lipid oxidation. However, boiling utilizes water as a medium to heat food, and the presence of water could promote the reaction of amino acids with the products of lipid oxidation in Tibetan pork, which contributed to the elevated level of protein oxidation (Wen, Hu, Zhang, Wang, Chen, & Kong, 2019).

Volatile compounds

E-nose

From Fig. S1, all the cooked samples responded strongly to W1W, W2W, W5S, W2S, and W1S, which meant that the samples contained higher levels of sulfides, pyrazines, terpenoids, aromatic compounds, alcohols, aldehydes, ketones, and short-chain alkanes. Meanwhile, the cooked pork groups presented greater amounts of aldehydes and alcohols in the GC–MS test. The greatest response values were detected in the AF and F groups. This result could be attributed to the Maillard reaction, which causes the formation of a large amount of heterocyclic compounds in Tibetan pork during air frying and frying treatments (Sun, Ji, Zhang, Zhang, Liu, & Song, 2022).

As shown in Fig. 2-a and Fig. 2-b, PC_1 and PC_2 explained 63.0% and 19.7% of the variation, respectively. The RAW group, which was located on the positive side of PC_2, was above the cooked Tibetan pork samples. In addition, the M, F, and B groups were clustered on the negative side of the PC_1 axis, whereas the AF group was distributed on the positive side of the PC_1 axis. This phenomenon demonstrated that the odor of air-fried treated pork was more distinctive from the other treatment groups. Meanwhile, the closer distances between the F and M groups indicated that the olfactory characteristics were similar in microwaved and fried Tibetan pork, which was in line with our GC–MS findings. The degree of

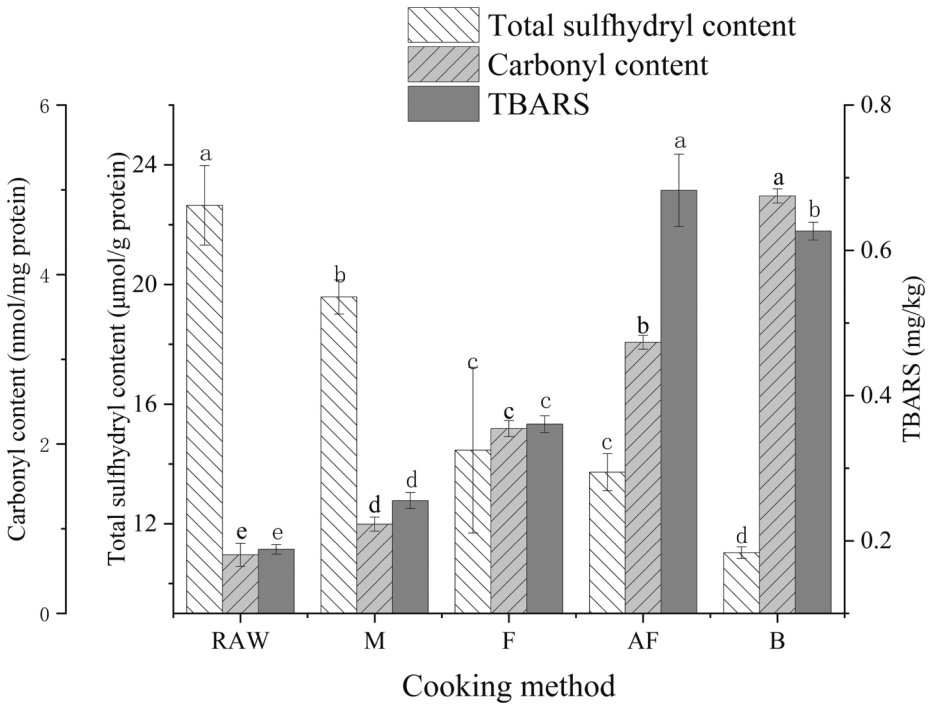

Fig. 1. Effect of cooking treatments on the content of TBARS, carbonyl and total sulfhydryl in Tibetan pork.

oxidation in the B group was comparable to that in the AF group, while the distance was relatively large, which could be explained by the variations in protein oxidation levels between them.

GC–Ms

A total of 93 compounds were identified, including 10 esters, 22 aldehydes, 16 alcohols, 8 ketones, 24 aliphatic hydrocarbons, 3 phenols, and 10 others (see Table 1). It was clearly seen that the types of compounds increased after cooking, suggesting that cooking treatment can promote the degradation of lipids and proteins and enrich the variety of volatile flavor compounds in Tibetan pork. Meanwhile, the highest percentage of aldehydes was discovered in all samples, which was in agreement with the findings of Han, Zhang, Fauconnier, and Mi (2020).

To further investigate the effect of different cooking methods on the volatile flavor of Tibetan pork, the OPLS-DA model was established to analyze the detected compounds (Fig. 2-c, 2-d). The total variance contributed by PC_1 and PC_2 was 86.5%, which may adequately reflect the majority of the characteristics of volatile odor among different cooking samples. The RAW group was distributed on the positive side of PC_1, and the cooking-treated samples gradually shifted to the negative side of PC_1. There was no area of overlap between the RAW group and the cooked samples, indicating that the cooking treatments changed the flavor of Tibetan pork. The F and M groups with lower degrees of oxidation were jointly located in the center of Fig. 2-c, suggesting that the fried and microwaved pork share some similarity in flavor. Groups AF and B with higher levels of oxidation were distributed on the negative side of PC1, indicating that air-fried and boiled pork exhibited noticeably different flavors from the other samples. In addition, Groups B and AF were located on both sides of PC2, which might be attributed to the differences in their protein and fat oxidation levels. The VIP values are shown in Fig. 2-d in accordance with the OPLS-DA model, which measured the peak intensity of each chemical component to characterize the extent of the variables' contribution. The higher the VIP value (VIP $>$ 1), the greater the amount of substances in the flavor of cooked Tibetan pork. A total of 19 volatile flavor compounds, including 8 aldehydes, 2 ketones, 4 alcohols, 4 aliphatic hydrocarbons, and 1 other compound, were identified that contributed substantially to the flavor of cooked Tibetan pork.

To visualize the effect of cooking treatments on the composition of volatile flavor substances in samples, a cluster heatmap analysis was performed on 93 volatile flavor compounds detected by GC–MS (Fig. 2-e). A total of 35, 57, 43, 53, and 61 volatile flavor components were detected in the samples of the RAW, M, F, B, and AF groups, respectively. This meant that the Tibetan pork generated new volatile flavor compounds after cooking. More importantly, the Tibetan pork after air frying presented the richest volatile flavor.

For aldehydes, new volatile flavor substances such as pentanal, benzaldehyde, 2-undecenal, and (2E,4E)-2,4-decadienal appeared after cooking. Except for (E,E)-2,4-dodecadienal, the concentration of other aldehydes increased prominently ($P < 0.05$) after cooking treatment and became the main aroma substances of cooked Tibetan pork. A total of 9 aldehydes were detected in the RAW group, 19 aldehydes in the M group, 13 in the F group, 18 in the B group, and 19 in the AF group. The linear aldehydes such as hexanal, nonanal, octanal, and 2-heptenal were mainly derived from the oxidative degradation of fatty acids, which have grassy and fruity aromas. These linear aldehydes played a significant role in the development of the overall flavor of cooked Tibetan pork because of their higher concentration and lower odor threshold. Apart from lipid oxidation, protein oxidation is also one of the main pathways of aldehyde production. For instance, benzaldehyde stems from the Strecker degradation of phenylalanine, which has a low odor threshold and can impart a nutty flavor to meat products (Delgado, González-Crespo, Cava, García-Parra, & Ramírez, 2010). In summary, the scent molecules from lipid and protein oxidation apparently contributed to the flavor of cooked pork. According to Fig. 2-e, the air frying treatment dramatically increased the quantity of aldehydes in Tibetan pork, which improved the overall flavor of Tibetan pork by providing more aroma compounds.

A total of 10 alcohols were detected in the RAW group. After the cooking treatment, the number of alcohol types dropped in Group F while rising in other groups, with the highest number in group AF, with 14 alcohols. Pentanol contributes to the fruity and balsamic aroma of the food (Dominguez, Gomez, Fonseca, & Lorenzo, 2014). Compared to the RAW group, the pentanol content was dramatically lower in the M and F groups, with no significant change in the B group, indicating that the cooking treatment caused a loss of pentanol. In contrast, the

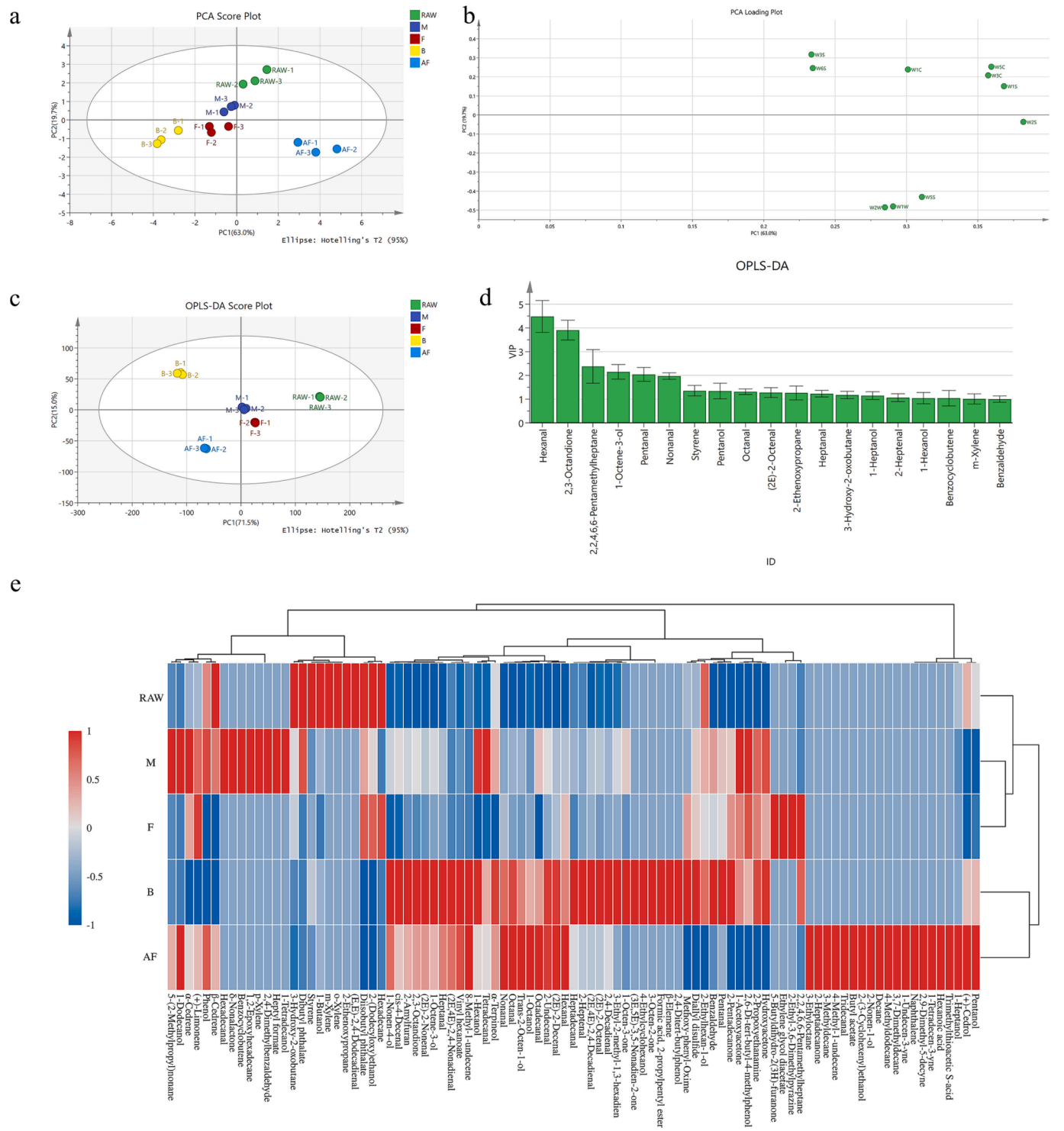

Fig. 2. Effect of cooking treatments on the odor composition of Tibetan pork. E-nose PCA score graph (a); E-nose loading graph (b); OPLS score graph of volatile flavor components (c); VIP value of volatile flavor components (d); heat map analysis of volatile flavor substance clustering (e).

concentration of pentanol was notably higher in the AF group, which could be explained by the air frying treatment enhancing the contact area of hot air with the lipids in the Tibetan pork, thus boosting the degradation rate of lipids to pentanol. 1-octen-3-ol is mainly produced by the oxidation of arachidonic acid and other unsaturated fatty acids, which can give meat products a mushroom odor (Zang et al., 2019). As shown in Fig. 3-e, 1-octen-3-ol was the most abundant alcohol compound, which contributed more to the flavor of cooked Tibetan pork because of its low odor threshold. The cooking treatments dramatically increased the concentration of 1-octen-3-ol. Similarly, we found that the volatile alcohols in Group F still showed a low richness. This phenomenon could be attributed to the increased temperature at the bottom of the pan during the frying process and the volatilization of the produced flavor substances into the environment. The result was consistent with a previous study by Rao, Meng, Li, Chen, Liu, and Zhang (2022) and Echegaray et al. (2020). In general, air frying increased the type and extent of alcohols in the pork samples, which benefited the overall flavor of Tibetan pork.

Table 1
Changes in volatile flavor substances during the cooking of Tibetan pork.

Number	Compound name	Formula	RAW	M	F	B	AF
1	Butyl acetate	$C_6H_{12}O_2$	–	–	–	–	20.59 ± 2.51
2	Heptyl formate	$C_8H_{16}O_2$	–	7.28 ± 0.48	–	–	–
3	Formic acid, 2-propylpentyl ester	$C_9H_{18}O_2$	–	–	–	22.49 ± 1.63	–
4	Vinyl hexanoate	$C_8H_{14}O_2$	–	22.19 ± 0.52^{c}	13.70 ± 2.21^{c}	160.36 ± 13.59^{a}	126.41 ± 2.66^{b}
5	2-(Dodecyloxy)ethanol	$C_{14}H_{30}O_2$	11.00 ± 1.66^{a}	5.34 ± 0.35^{c}	8.70 ± 1.41^{b}	–	–
6	Ethylene glycol diacetate	$C_6H_{10}O_4$	–	–	5.50 ± 0.54	–	–
7	Diisobutyl phthalate	$C_{16}H_{22}O_4$	31.05 ± 3.69^{a}	21.59 ± 2.82^{b}	27.81 ± 2.49^{a}	–	–
8	Dibutyl phthalate	$C_{16}H_{22}O_4$	52.30 ± 1.54^{a}	39.85 ± 5.58^{b}	–	–	–
9	δ-Nonalactone	$C_9H_{16}O_2$	–	8.82 ± 0.69	–	–	–
10	5-Butyldihydro-2(3H)-furanone	$C_8H_{14}O_2$	–	–	18.49 ± 3.57	–	–
11	Pentanal	$C_5H_{10}O$	–	858.50 ± 12.48^{b}	731.20 ± 26.94^{c}	1621.50 ± 12.87^{a}	532.53 ± 15.73^{d}
12	Hexanal	$C_6H_{12}O$	3526.85 ± 174.90^{d}	8158.39 ± 275.74^{c}	9601.06 ± 528.26^{b}	10697.44 ± 1516.15^{b}	12251.17 ± 132.59^{a}
13	Heptanal	$C_7H_{14}O$	73.58 ± 2.96^{e}	346.71 ± 28.57^{c}	279.87 ± 5.51^{d}	783.86 ± 32.90^{a}	572.72 ± 17.23^{b}
14	Octanal	$C_8H_{16}O$	8.37 ± 0.60^{e}	256.06 ± 7.31^{c}	217.72 ± 6.89^{d}	591.00 ± 28.54^{b}	676.15 ± 21.13^{a}
15	2-Heptenal	$C_7H_{12}O$	76.85 ± 12.68^{d}	110.45 ± 2.13^{c}	103.22 ± 4.27^{c}	409.45 ± 12.49^{a}	144.46 ± 5.86^{b}
16	Nonanal	$C_9H_{18}O$	126.99 ± 16.91^{e}	616.74 ± 32.53^{d}	714.04 ± 40.48^{c}	1275.88 ± 58.57^{b}	1610.58 ± 14.33^{a}
17	Benzaldehyde	C_7H_6O	–	184.91 ± 3.20^{b}	129.06 ± 9.91^{c}	310.14 ± 8.72^{a}	58.01 ± 3.42^{d}
18	(2E)-2-Octenal	$C_8H_{14}O$	100.65 ± 8.04^{d}	256.51 ± 12.82^{b}	175.66 ± 17.10^{c}	702.72 ± 14.54^{a}	263.21 ± 18.30^{b}
19	(2E)-2-Nonenal	$C_9H_{16}O$	20.38 ± 3.55^{e}	84.72 ± 3.70^{c}	58.50 ± 2.06^{d}	193.64 ± 15.48^{a}	126.69 ± 3.26^{b}
20	*cis*-4-Decenal	$C_{10}H_{18}O$	–	20.97 ± 2.52^{b}	–	55.25 ± 3.67^{a}	23.64 ± 1.37^{b}
21	(2E)-2-Decenal	$C_{10}H_{18}O$	–	41.51 ± 3.08^{b}	41.79 ± 4.50^{b}	82.82 ± 6.60^{a}	84.76 ± 2.81^{a}
22	2-Undecenal	$C_{11}H_{20}O$	–	26.05 ± 3.39^{b}	18.94 ± 3.74^{c}	42.53 ± 1.77^{a}	42.72 ± 1.55^{a}
23	2,4-Decadienal	$C_{10}H_{16}O$	20.33 ± 3.12^{d}	51.29 ± 1.85^{c}	44.81 ± 1.57^{c}	166.55 ± 5.36^{a}	68.63 ± 7.49^{b}
24	(2E,4E)-2,4-Nonadienal	$C_9H_{14}O$	–	–	–	76.44 ± 7.63^{a}	49.34 ± 1.19^{b}
25	(2E,4E)-2,4-Decadienal	$C_{10}H_{16}O$	–	36.30 ± 1.82^{b}	27.88 ± 1.52^{c}	129.21 ± 5.49^{b}	36.88 ± 5.28^{a}
26	(E,E)-2,4-Dodecadienal	$C_{12}H_{20}O$	12.57 ± 0.51	–	–	–	–
27	2,4-Dimethylbenzaldehyde	$C_9H_{10}O$	–	14.60 ± 2.97	–	–	–
28	Tridecanal	$C_{13}H_{26}O$	–	–	–	–	25.09 ± 0.66
29	Tetradecanal	$C_{14}H_{28}O$	–	37.82 ± 1.80^{a}	–	19.17 ± 0.52^{b}	15.07 ± 0.38^{c}
30	hexadecanal	$C_{16}H_{32}O$	–	13.41 ± 1.22	–	–	–
31	Heptadecanal	$C_{17}H_{34}O$	–	15.88 ± 5.05^{c}	–	95.19 ± 9.72^{a}	26.93 ± 3.81^{b}
32	Octadecanal	$C_{18}H_{36}O$	–	16.17 ± 1.46^{c}	–	19.53 ± 2.44^{b}	32.77 ± 1.95^{a}
33	1-Butanol	$C_4H_{10}O$	184.07 ± 14.29^{a}	34.81 ± 2.93^{b}	–	31.48 ± 3.72^{b}	31.64 ± 0.65^{b}
34	Pentanol	$C_5H_{12}O$	670.28 ± 7.90^{b}	543.00 ± 42.62^{c}	566.80 ± 20.65^{c}	698.30 ± 18.40^{b}	864.00 ± 36.12^{a}
35	1-Hexanol	$C_6H_{14}O$	96.02 ± 4.28^{c}	205.80 ± 2.42^{a}	78.57 ± 4.46^{d}	208.44 ± 4.00^{a}	152.77 ± 1.91^{b}
36	1-Octene-3-ol	$C_8H_{16}O$	324.89 ± 11.25^{e}	1309.03 ± 44.21^{c}	968.72 ± 23.37^{d}	2575.68 ± 62.54^{a}	1558.74 ± 20.01^{b}
37	2-Ethylhexan-1-ol	$C_8H_{18}O$	105.00 ± 11.70^{a}	89.41 ± 3.85^{b}	78.79 ± 6.04^{b}	101.43 ± 0.27^{a}	25.20 ± 0.39^{c}
38	4-Ethylcyclohexanol	$C_8H_{16}O$	–	–	–	63.40 ± 6.16	–
39	2-(3-Cyclohexenyl)ethanol	$C_8H_{14}O$	–	–	–	–	27.71 ± 0.29
40	1-Heptanol	$C_7H_{16}O$	40.58 ± 2.46^{b}	–	14.87 ± 0.68^{d}	25.86 ± 2.38^{c}	266.11 ± 7.32^{a}
41	1-Octanol	$C_8H_{18}O$	37.90 ± 1.95^{d}	68.04 ± 0.89^{c}	79.14 ± 2.28^{c}	149.32 ± 15.63^{b}	272.99 ± 5.26^{a}
42	Trans-2-Octen-1-ol	$C_8H_{16}O$	62.95 ± 2.72^{d}	153.26 ± 10.90^{c}	129.00 ± 13.05^{c}	264.80 ± 19.46^{b}	368.34 ± 1.16^{a}
43	2-Nonen-1-ol	$C_9H_{18}O$	–	–	–	–	61.02 ± 3.47
44	1-Nonen-4-ol	$C_9H_{18}O$	–	33.09 ± 3.90^{c}	–	85.09 ± 3.86^{a}	62.70 ± 2.29^{b}
45	α-Terpineol	$C_{10}H_{18}O$	12.96 ± 1.59^{c}	16.37 ± 0.92^{bc}	–	20.89 ± 3.99^{a}	17.21 ± 0.27^{b}
46	1-Dodecanol	$C_{12}H_{26}O$	–	15.49 ± 1.14^{a}	–	–	15.56 ± 1.67^{a}
47	1-Tetradecanol	$C_{14}H_{30}O$	–	13.34 ± 1.97	–	–	–
48	(+)-Cedrol	$C_{15}H_{26}O$	64.39 ± 4.00^{b}	32.52 ± 2.96^{c}	32.25 ± 1.26^{c}	63.24 ± 2.32^{b}	96.67 ± 10.77^{a}
49	3-Hydroxy-2-oxobutane	$C_4H_8O_2$	733.27 ± 10.92^{a}	317.92 ± 3.77^{b}	295.19 ± 11.80^{c}	157.87 ± 9.02^{d}	122.79 ± 2.26^{e}
50	Hydroxyacetone	$C_3H_6O_2$	–	66.96 ± 4.67^{a}	48.53 ± 5.84^{b}	71.09 ± 7.27^{a}	–
51	1-Octen-3-one	$C_8H_{14}O$	–	34.06 ± 0.66^{b}	–	104.07 ± 11.16^{a}	–
52	3-Octen-2-one	$C_8H_{14}O$	–	–	–	111.28 ± 4.85	–
53	2,3-Octandione	$C_8H_{14}O_2$	279.55 ± 22.58^{e}	3316.69 ± 83.49^{c}	1596.20 ± 92.39^{d}	6974.35 ± 351.65^{a}	4368.29 ± 130.66^{b}
54	2-Pentadecanone	$C_{15}H_{30}O$	–	11.71 ± 0.39^{c}	15.12 ± 2.64^{b}	24.45 ± 1.48^{a}	–
55	(3E,5E)-3,5-Nonadien-2-one	$C_9H_{14}O$	–	–	–	43.88 ± 0.94	–
56	2-Heptadecanone	$C_{17}H_{34}O$	–	–	–	–	10.33 ± 0.74
57	(+)-Limonene	$C_{10}H_{16}$	91.18 ± 12.75^{c}	166.45 ± 18.05^{a}	183.14 ± 15.01^{a}	–	133.33 ± 11.83^{b}
58	Decane	$C_{10}H_{22}$	–	–	–	–	95.92 ± 10.12
59	Hexadecane	$C_{16}H_{34}$	27.18 ± 1.18^{a}	–	21.75 ± 1.36^{b}	–	–
60	3-Ethyloctane	$C_{10}H_{22}$	–	–	–	–	39.80 ± 6.62
61	3-Methyldecane	$C_{11}H_{24}$	–	–	–	–	32.12 ± 6.58
62	4-Methyldodecane	$C_{13}H_{28}$	–	–	–	–	16.74 ± 0.28
63	5-(2-Methylpropyl)nonane	$C_{13}H_{28}$	–	27.79 ± 3.18^{a}	–	–	11.64 ± 0.19^{b}
64	2,2,4,6,6-Pentamethylheptane	$C_{12}H_{26}$	–	–	483.51 ± 39.09^{a}	339.62 ± 32.08^{b}	–
65	3,7-Dimethyldecane	$C_{12}H_{26}$	–	–	–	–	20.38 ± 0.82
66	3-Ethyl-2-methyl-1,3-hexadien	C_9H_{16}	–	80.76 ± 8.41^{b}	43.02 ± 2.05^{c}	264.23 ± 14.16^{a}	41.25 ± 2.01^{c}
67	4-Methyl-1-undecene	$C_{12}H_{24}$	–	–	–	–	44.42 ± 1.05
68	8-Methyl-1-undecene	$C_{12}H_{24}$	–	–	–	24.19 ± 1.34^{a}	23.83 ± 0.92^{a}
69	α-Cedrene	$C_{15}H_{24}$	71.89 ± 2.56^{bc}	98.91 ± 18.95^{a}	83.90 ± 5.12^{ab}	56.38 ± 3.45^{c}	79.62 ± 11.15^{ab}
70	β-Cedrene	$C_{15}H_{24}$	64.62 ± 3.34^{a}	46.90 ± 9.84^{b}	–	–	37.30 ± 2.79^{c}
71	β-Elemene	$C_{15}H_{24}$	–	–	–	80.23 ± 6.85	–
72	1-Undecen-3-yne	$C_{11}H_{18}$	–	–	–	–	22.52 ± 1.15
73	Naphthalene	$C_{10}H_8$	–	–	–	——	12.81 ± 2.78
74	2,9-Dimethyl-5-decyne	$C_{12}H_{22}$	–	–	–	–	14.70 ± 0.79

(*continued on next page*)

Table 1 (*continued*)

Number	Compound name	Formula	RAW	M	F	B	AF
75	1-Tetradecen-3-yne	$C_{14}H_{24}$	–	–	–	–	27.99 ± 0.54
76	Styrene	C_8H_8	453.09 ± 18.59^a	–	35.39 ± 8.31^c	91.15 ± 7.75^b	–
77	Benzocyclobutene	C_8H_8	–	74.63 ± 4.23	–	–	–
78	o-Xylene	C_8H_{10}	64.14 ± 6.50	–	–	–	–
79	m-Xylene	C_8H_{10}	298.65 ± 30.08	–	–	–	–
80	p-Xylene	C_8H_{10}	–	54.73 ± 1.52	–	–	–
81	Phenol	C_6H_6O	10.99 ± 2.80^a	13.72 ± 1.74^a	–	–	13.11 ± 0.37^a
82	2,4-Ditert-butylphenol	$C_{14}H_{22}O$	–	–	–	7.03 ± 0.78	–
83	2,6-Di-*tert*-butyl-4-methylphenol	$C_{15}H_{24}O$	–	18.11 ± 0.59^a	16.62 ± 1.82^a	11.89 ± 1.45^b	–
84	2-Propoxyethanamine	$C_5H_{13}NO$	–	133.81 ± 5.76^{ab}	122.47 ± 10.02^b	143.73 ± 12.60^a	–
85	1-Acetoxyacetone	$C_5H_8O_3$	–	37.00 ± 2.47^a	28.54 ± 2.19^b	28.05 ± 1.35^b	–
86	Diallyl disulfide	$C_6H_{10}S_2$	50.45 ± 4.08^c	70.31 ± 11.16^b	70.16 ± 6.37^b	129.01 ± 12.21^a	–
87	2-Ethenoxypropane	$C_5H_{10}O$	430.48 ± 33.80	–	–	–	–
88	Methoxy-phenyl-Oxime	$C_8H_9NO_2$	145.42 ± 13.11^b	136.81 ± 12.34^{bc}	172.74 ± 12.06^b	220.70 ± 40.43^a	100.98 ± 7.29^c
89	1,2-Epoxyhexadecane	$C_{16}H_{32}O$	–	17.08 ± 0.76	–	–	–
90	2-Amylfuran	$C_9H_{14}O$	–	86.32 ± 3.83^c	42.91 ± 7.24^d	211.63 ± 6.53^a	116.43 ± 8.24^b
91	2-Ethyl-3,6-Dimethylpyrazine	$C_8H_{12}N_2$	–	–	6.79 ± 0.25^a	–	3.16 ± 0.14^b
92	Trimethylthioacetic S-acid	$C_5H_{10}OS$	–	–	–	–	24.88 ± 0.64
93	Hexanoic acid	$C_6H_{12}O_2$	–	–	–	–	46.08 ± 2.84

Note: $P < 0.05$.

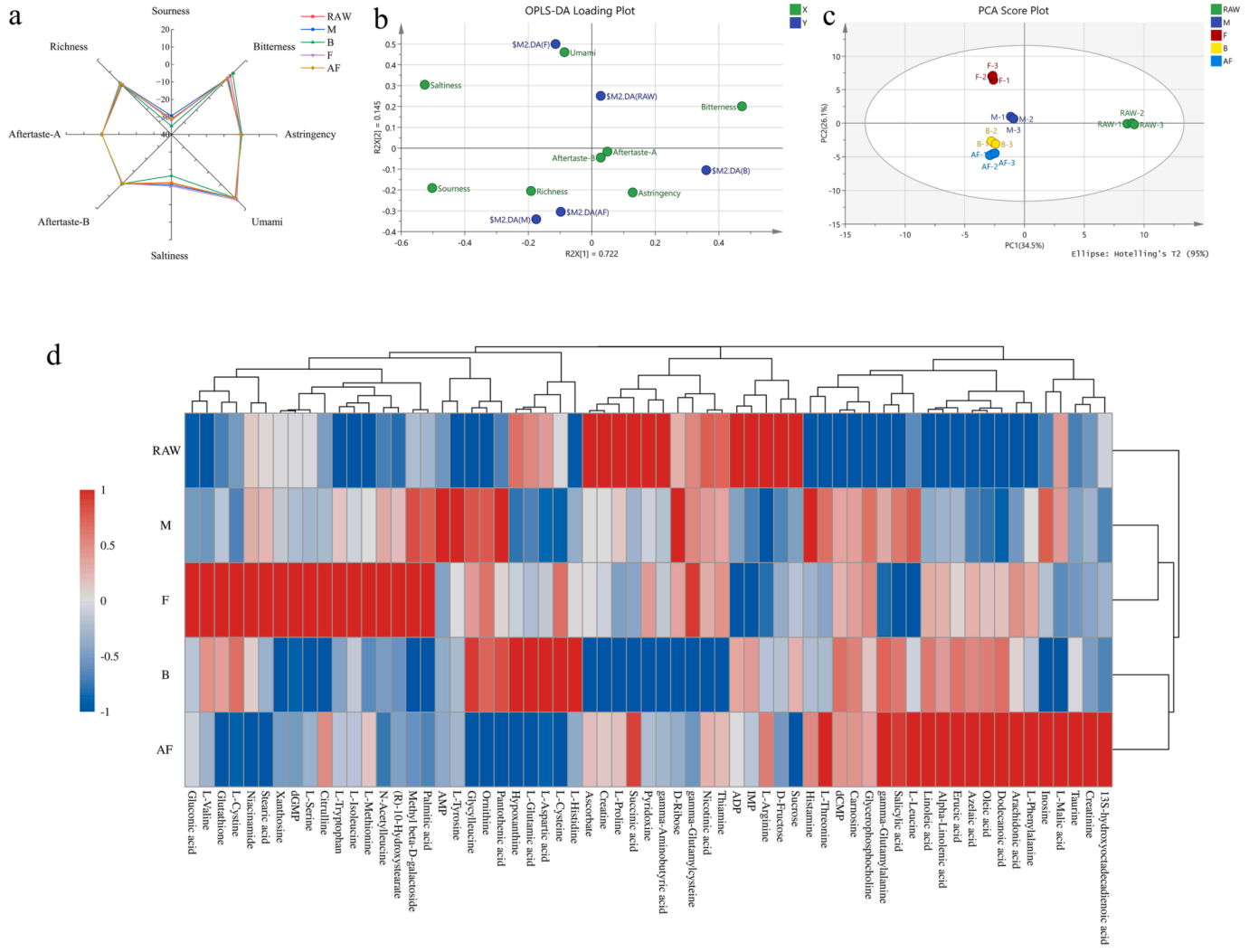

Fig. 3. Effect of cooking methods on the taste components of Tibetan pork.E-tongue radar plot (a); E-tongue OPLS-DA plot (b); PCA score of differential metabolites (c); Cluster heat map analysis of differential metabolites (d).

Ketones are derived from the degradation of amino acids and the oxidation of fatty acids. Ketones can also be involved in the Maillard reaction as flavor precursor substances (Liu, Shen, Xiao, Jiang, & Shi, 2022a). 2,3-Octanedione was present in substantially greater concentrations in the sample than other ketones, which can give food a fruity aroma (Zhang et al., 2019). Elmore et al. (2005) detected high levels of

2,3-octanedione in grilled lamb fed a forage with a high content of unsaturated fatty acids, suggesting that 2,3-octanedione was mainly derived from the degradation of unsaturated fatty acids. 3-Hydroxy-2-butanone, a common methyl ketone found in meat, is generated from β-keto acids formed from triglycerides and has a fruity and buttery flavor (Dominguez et al., 2014), giving meat products a pleasant fatty flavor. In this manuscript, the content of 3-hydroxy-2-butanone was reduced remarkably after cooking treatment, with the least amount in the AF group and a gradually increasing amount augment in the B, F, and M groups in that order. This implied that the concentration of 3-hydroxy-2-butanone showed a tendency to decline with increasing cooking treatment temperature or time, which was in agreement with the findings of Dominguez et al. (2014). This phenomenon is probably due to its involvement in the Maillard reaction as a precursor substance.

In addition, 2-pentylfuran was not identified as an important flavor compound in the results, although it was found several times in cooked meat. According to these reports, 2-pentylfuran may come from a Maillard reaction or Strecker degradation, and its fruity and grassy odor could play an important role in the overall flavor of cooked Tibetan pork. (Wang, Song, Zhang, Tang, & Yu, 2016). The higher content of 2-pentylfuran in the B group could result from the fact that the long cooking time facilitated the reaction of sugar compounds with amino acids to produce furan compounds. Furthermore, 2-ethyl-3,6-dimethylpyrazine and trimethylthioacetic acid were discovered in groups AF and F, which implied that high temperatures produced additional heterocyclic molecules. This result also agreed with the response values of the WIW, W2W, and W5S sensors in the E-nose analysis.

Nonvolatile compounds

E-tongue

As shown in Fig. 3-b, PC_1 and PC_2 explained 72.2% and 14.5% of the variation, respectively. The contribution of the first principal component was much larger than that of the second principal component, so the variations between various samples were mainly reflected in PC_1. In the PC_1, sourness, saltiness, and bitterness tastes contributed more to the taste of all groups of samples. According to Fig. 3-a, compared to the raw meat, the salty response value was the lowest in boiled Tibetan pork, the sour response value was considerably increased and decreased in microwaved and boiled samples, respectively. This might be a result of the interaction of amino acids with acids and inorganic salts in Tibetan pork after cooking treatment (Lee et al., 2019). Besides, in contrast to raw meat, the bitter response value was notably decreased and increased in air-fried and boiled Tibetan pork, respectively. The findings revealed that different cooking treatments affected the concentration of taste-presenting substances such as amino acids and nucleotides in fresh pork, providing cooked Tibetan pork with varied flavor characteristics. Above all, the air frying process decreased the bitterness and astringency of the Tibetan pork, which improved its palatability.

Metabolic variation analysis

Nonvolatile flavor compounds largely affect the taste of foods and participate in several metabolic pathways as flavor precursors to generate volatile flavor substances. The differential metabolites in Tibetan pork samples (VIP $>$ 1, $P < 0.05$) were identified based on the projected importance of variables (VIP) in the OPLS-DA model, and a total of 68 differential metabolites were detected (Table 2), including amino acids, organic acids, sugars, lipids, nucleotides and their derivatives, etc. To more visually analyze the differences in metabolites of Tibetan pork caused by cooking, cluster heatmap analysis and PCA were performed utilizing the relative concentrations of 68 differential metabolites, and the results are shown in Fig. 3-c and Fig. 3-d. It was clear from Fig. 3-c that the RAW group was distributed on the positive side of PC_1 and that the cooking-treated samples were located on the negative side of PC_1, indicating that the cooking treatment significantly changed the distribution of nonvolatile compounds in Tibetan pork. Additionally, the lower oxidized F and M groups were located on the positive axis of PC_2, while the higher oxidized B and AF groups were distributed on the negative axis of PC_2. This implied that protein and lipid oxidation were significant in the distribution of Tibetan pork metabolites. In addition, the B and AF groups were close to each other, meaning that their metabolite distributions were relatively similar. This could be because protein oxidation interacts with lipid oxidation during boiling and air frying treatments, resulting in a more similar concentration of their fatty acids, amino acids, and other metabolites.

Pork fat contains a large number of phospholipids and glycerides, which are broken down into free fatty acids during cooking, and then the fatty acids could be further oxidatively degraded into small molecule flavor compounds such as aldehydes, ketones, and alcohols (Huang et al., 2022b). In this research, 10 free fatty acids were identified, including four saturated fatty acids, two monounsaturated fatty acids, and four polyunsaturated fatty acids (Fig. 3-d). Monounsaturated fatty acids are cardioprotective, and α-linolenic acid, as an ω-3 fatty acid, can prevent cardiovascular disease by reducing LDL levels and the ratio of cholesterol and triglycerides to HDL (Alencar & Kiefer, 2023). Aaslyng and Schäfer (2007) found that adding α-linolenic acid can make pork taste like fried fish or fish liver oil. Meanwhile, α-linolenic acid acts as a flavor precursor substance and can be oxidized to aldehydes, alcohols, ketones, and other volatile substances in the cooking process, thus enriching the flavor of Tibetan pork. As the precursor substance of hexanal (Chang, Wang, Chen, Zhang, & Sun, 2021), linoleic acid showed a key impact on the flavor of cooked Tibetan pork. It was observed that the linoleic acid content was substantially higher in the cooked groups than in the RAW group. Among them, the content of linoleic acid in the AF and B groups showed a greater degree of increase, which could be explained by the longer boiling time or the higher air frying treatment temperature, leading to a large amount of fat hydrolysis in Tibetan pork. Additionally, along with the increase in cooking time, the intramuscular fat of Tibetan pork would also generate more free fatty acids. The linoleic acid concentration in the F group was second only to that in the AF and B groups, which might be due to the addition of soybean during the frying process, which contains a large amount of linoleic acid. The lowest linoleic acid content was found in the M group. The possibility was that microwave cooking resulted in a massive loss of water from the pork, which inhibited the lipolysis process and reduced the production of free fatty acids. Meanwhile, the single linear state of oxygen generated during microwave treatment could also rapidly trigger the oxidation of fatty acids, thus generating compounds such as aldehydes, leading to a further reduction in the linoleic acid concentration in the M group. These results were also consistent with the changes in hexanal concentration in our previous experiments. As an ω-6 fatty acid, arachidonic acid plays an important role in preserving human health. Simultaneously, it serves as a flavor precursor substance in processed meat products, participating in the creation of hexanal and 1-octen-3-ol (Bassam, Noleto-Dias, & Farag, 2022). Compared to the raw meat, the content of arachidonic acid was increased to different degrees in all four cooked Tibetan pork groups, with the lowest concentration in the M group. This might be due to the evaporation of water during the microwave treatment, which led to a loss of fatty acids. At the same time, the concentration of arachidonic acid in the B group was slightly higher than that in the M group, which could be attributed to a portion of the fatty acids being dissolved during boiling treatment. In addition, the content of stearic and palmitic acids showed greater changes after cooking, with a decline in the B and AF groups and an increase in the F and M groups. As a common saturated fatty acid in pork, stearic and palmitic acids are mainly found in neutral lipids and can be interconverted with unsaturated fatty acids, which may also explain the variation in their concentration between cooking methods.

Free amino acids are important taste-presenting substances that affect the umami, bitterness, sourness, sweetness, and saltiness of foods. Aspartic acid and glutamic acid play an important role in the umami of foods. The content of umami amino acids in cooked Tibetan pork

Table 2
Changes in differential metabolites during the cooking of Tibetan pork.

Name	Formula	Retention time(s)	Specific charge	RAW	B	M	F	AF	p value	VIP	Mode
Arachidonic acid	$C_{20}H_{32}O_2$	959.3	303.233	12784.42 ± 1163.47^{e}	33075.45 ± 3604.57^{c}	24379.57 ± 4546.43^{d}	39050.09 ± 2851.29^{b}	51027.86 ± 1893.08^{a}	3.40E−07	2.322	neg
Alpha-Linolenic acid	$C_{18}H_{30}O_2$	855.1	279.232	621.51 ± 27.87^{d}	809.57 ± 8.37^{b}	738.13 ± 17.22^{c}	796.46 ± 9.91^{b}	877.86 ± 12.40^{a}	5.24E−08	2.163	pos
L-Phenylalanine	$C_9H_{11}NO_2$	306.3	164.072	190.23 ± 15.21^{e}	3182.92 ± 132.41^{d}	3626.96 ± 84.17^{c}	4376.79 ± 293.00^{b}	6604.90 ± 261.77^{a}	3.13E−11	2.036	neg
ADP	$C_{10}H_{15}N_5O_{10}P_2$	87.4	426.02	59.26 ± 1.76^{a}	50.38 ± 2.78^{b}	43.60 ± 2.23^{c}	36.16 ± 1.99^{d}	47.57 ± 2.26^{bc}	3.00E−06	2.009	neg
Niacinamide	$C_6H_6N_2O$	126.3	123.056	21559.09 ± 2048.59^{a}	21407.47 ± 1964.72^{a}	22074.13 ± 2689.80^{a}	24663.91 ± 1567.67^{a}	15830.86 ± 2798.29^{b}	9.36E−03	1.933	pos
Linoleic acid	$C_{18}H_{32}O_2$	865.7	281.248	886.82 ± 66.76^{e}	1698.11 ± 9.61^{b}	1322.93 ± 35.97^{d}	1578.00 ± 73.98^{c}	1850.42 ± 31.52^{a}	3.41E−09	1.928	pos
L-Valine	$C_5H_{11}NO_2$	111.1	116.072	555.37 ± 8.52^{d}	789.87 ± 7.60^{b}	637.06 ± 11.55^{c}	943.91 ± 55.26^{a}	674.18 ± 13.01^{c}	5.61E−08	1.921	neg
Oleic acid	$C_{18}H_{34}O_2$	812.4	282.279	21581.50 ± 1071.52^{e}	33968.48 ± 1516.54^{b}	24590.27 ± 1803.05^{d}	31214.97 ± 1132.75^{c}	38291.03 ± 1339.56^{a}	2.59E−07	1.897	pos
AMP	$C_{10}H_{14}N_5O_7P$	124.7	348.069	1577.84 ± 51.59^{c}	3708.86 ± 92.31^{b}	31442.02 ± 1180.71^{a}	2917.12 ± 202.15^{b}	3561.53 ± 37.18^{b}	4.20E−14	1.893	pos
L-Aspartic acid	$C_4H_7NO_4$	88.6	134.045	192.09 ± 16.82^{b}	220.43 ± 12.67^{a}	157.56 ± 5.38^{c}	177.09 ± 3.41^{b}	156.10 ± 2.15^{c}	6.10E−05	1.862	pos
L-Glutamic acid	$C_5H_9NO_4$	98.7	128.036	142.98 ± 4.54^{b}	174.60 ± 11.63^{a}	97.77 ± 3.48^{d}	114.45 ± 10.44^{c}	84.80 ± 5.68^{d}	4.55E−07	1.826	neg
L-Cysteine	$C_3H_7NO_2S$	527.9	122.06	75.10 ± 5.90^{c}	130.12 ± 2.62^{a}	37.39 ± 3.58^{d}	104.58 ± 3.96^{b}	32.13 ± 3.84^{d}	1.48E−02	1.8	pos
Succinic acid	$C_4H_6O_4$	74.2	116.984	12.97 ± 0.77^{a}	6.65 ± 0.26^{c}	9.28 ± 0.27^{b}	8.94 ± 0.06^{b}	12.44 ± 1.14^{a}	1.00E−06	1.799	neg
IMP	$C_{10}H_{13}N_4O_8P$	140.9	349.055	1316.75 ± 42.42^{a}	1102.05 ± 45.23^{b}	874.30 ± 9.37^{d}	803.38 ± 8.96^{e}	983.41 ± 27.92^{c}	1.50E−08	1.77	pos
L-Serine	$C_3H_7NO_3$	86.9	104.036	325.15 ± 4.79^{b}	211.36 ± 1.03^{d}	293.90 ± 6.36^{c}	522.27 ± 10.97^{a}	286.85 ± 1.38^{c}	3.79E−13	1.723	neg
Stearic acid	$C_{18}H_{36}O_2$	914.6	284.269	1858.81 ± 69.22^{bc}	1652.39 ± 161.74^{c}	1960.17 ± 87.84^{b}	2562.98 ± 150.72^{a}	1133.03 ± 87.05^{d}	6.81E−07	1.708	neg
L-Leucine	$C_6H_{13}NO_2$	224.1	132.102	39997.20 ± 1319.87^{c}	43759.75 ± 2415.45^{b}	49327.28 ± 1725.49^{a}	36047.61 ± 1123.67^{d}	51858.55 ± 476.67^{a}	1.00E−06	1.696	pos
Thiamine	$C_{12}H_{17}N_4OS$	115.9	265.111	503.02 ± 2.43^{a}	67.86 ± 0.61^{d}	440.46 ± 18.01^{b}	458.04 ± 10.89^{b}	414.94 ± 10.68^{c}	1.53E−12	1.694	pos
L-Tyrosine	$C_9H_{11}NO_3$	125.8	180.067	1366.05 ± 79.27^{c}	1949.52 ± 113.05^{b}	3328.16 ± 161.34^{a}	2140.75 ± 291.22^{b}	1936.79 ± 64.94^{b}	6.76E−07	1.663	neg
Pantothenic acid	$C_9H_{17}NO_5$	359.2	220.118	4564.20 ± 150.69^{c}	7483.28 ± 356.55^{a}	7870.12 ± 132.74^{a}	6019.79 ± 731.10^{b}	3372.49 ± 85.28^{d}	1.79E−07	1.658	pos
Glycylleucine	$C_8H_{16}N_2O_3$	352.5	189.123	58.42 ± 2.56^{c}	86.95 ± 3.11^{a}	85.33 ± 3.00^{a}	79.22 ± 3.75^{b}	57.58 ± 0.29^{c}	1.53E−07	1.645	pos
L-Cystine	$C_6H_{12}N_2O_4S_2$	84.7	239.017	36.93 ± 3.59^{c}	91.55 ± 15.27^{b}	27.94 ± 0.31 ^{cd}	124.73 ± 10.70^{a}	17.84 ± 1.73^{d}	9.47E−08	1.623	neg
L-Proline	$C_5H_9NO_2$	390.4	116.071	222.06 ± 8.83^{a}	139.50 ± 1.96^{d}	183.27 ± 2.58^{b}	164.90 ± 11.74^{c}	184.10 ± 14.43^{b}	1.20E−05	1.612	pos
L-Isoleucine	$C_6H_{13}NO_2$	153.9	131.072	6.28 ± 0.07^{d}	9.32 ± 0.10^{bc}	9.49 ± 0.11^{b}	14.33 ± 0.26^{a}	9.21 ± 0.14^{c}	3.15E−13	1.607	neg
Palmitic acid	$C_{16}H_{32}O_2$	848.5	255.235	542.14 ± 22.09^{c}	282.38 ± 19.11^{e}	871.95 ± 47.75^{b}	1034.33 ± 79.10^{a}	425.59 ± 58.25^{d}	2.56E−08	1.586	neg
dCMP	$C_9H_{14}N_3O_7P$	474.6	307.206	18.58 ± 0.98^{c}	43.34 ± 0.68^{a}	40.11 ± 3.72^{ab}	39.01 ± 1.57^{b}	40.84 ± 1.41^{ab}	1.78E−07	1.584	pos
D-Ribose	$C_5H_{10}O_5$	332.5	151.062	101.41 ± 11.98^{ab}	78.87 ± 5.10^{c}	112.93 ± 9.52^{a}	102.28 ± 8.40^{ab}	93.26 ± 6.14^{bc}	7.53E−03	1.571	pos
Glutathione	$C_{10}H_{17}N_3O_6S$	181.6	307.083	1093.14 ± 13.61 ^{cd}	1519.16 ± 222.59^{b}	1302.33 ± 193.97^{bc}	1957.72 ± 102.35^{a}	965.76 ± 43.98^{d}	5.00E−05	1.55	pos
L-Arginine	$C_6H_{14}N_4O_2$	84.6	175.119	4084.79 ± 262.60^{a}	3521.36 ± 179.97^{bc}	3221.15 ± 323.51^{c}	3294.80 ± 314.06^{c}	3792.43 ± 134.10^{ab}	1.01E−02	1.55	pos
L-Threonine	$C_4H_9NO_3$	102.9	120.066	96.30 ± 2.32^{d}	194.79 ± 7.49^{c}	288.42 ± 6.08^{b}	180.90 ± 6.84^{c}	345.93 ± 18.97^{a}	3.11E−10	1.534	pos
L-Histidine	$C_6H_9N_3O_2$	102.8	156.077	811.95 ± 71.56^{d}	1598.95 ± 75.52^{a}	990.00 ± 43.38^{c}	1111.60 ± 47.47^{b}	983.64 ± 44.89^{c}	1.59E−07	1.514	pos
Taurine	$C_2H_7NO_3S$	73.5	125.985	1017.18 ± 77.91^{c}	1294.66 ± 54.67^{b}	1115.89 ± 48.70^{c}	1087.97 ± 49.74^{c}	2023.75 ± 183.76^{a}	1.00E−06	1.508	pos
Methyl beta-D-galactoside	$C_7H_{14}O_6$	808.3	194.083	68.24 ± 3.52^{b}	28.84 ± 2.76^{c}	111.33 ± 16.33^{a}	124.13 ± 18.51^{a}	54.98 ± 6.15^{b}	7.00E−06	1.472	neg
gamma-Glutamylalanine	$C_7H_{11}N_2O_5R$	148.4	219.098	42.81 ± 2.13^{e}	96.48 ± 2.33^{b}	85.34 ± 3.63^{c}	53.46 ± 0.69^{d}	105.24 ± 9.59^{a}	6.10E−08	1.465	pos
L-Tryptophan	$C_{11}H_{12}N_2O_2$	367.9	203.083	304.05 ± 51.46^{e}	1358.18 ± 100.09^{d}	2289.39 ± 128.59^{b}	4509.95 ± 187.17^{a}	1759.45 ± 112.91^{c}	2.39E−11	1.465	neg
Histamine	$C_5H_9N_3$	75.7	112.087	950.05 ± 10.01^{c}	965.44 ± 8.69^{bc}	987.36 ± 16.31^{ab}	964.25 ± 3.12^{bc}	975.92 ± 9.97^{a}	1.49E−02	1.465	pos

(*continued on next page*)

Table 2 (*continued*)

Name	Formula	Retention time(s)	Specific charge	RAW	B	M	F	AF	p value	VIP	Mode
Hypoxanthine	$C_5H_4N_4O$	125.8	135.032	8802.64 ± 122.20^{b}	9641.95 ± 226.79^{a}	6838.95 ± 72.86^{d}	7760.81 ± 221.63^{c}	6442.36 ± 116.94^{e}	1.77E−09	1.45	neg
Creatine	$C_4H_9N_3O_2$	97.4	130.063	4763.86 ± 168.68^{a}	2033.83 ± 101.19^{c}	3422.86 ± 137.84^{b}	3347.42 ± 96.47^{b}	3572.18 ± 142.25^{b}	4.88E−09	1.445	neg
N-Acetylleucine	$C_8H_{15}NO_3$	301.6	173.079	108.81 ± 11.23^{c}	120.88 ± 14.77^{bc}	165.94 ± 9.63^{b}	238.21 ± 51.61^{a}	106.23 ± 18.19^{c}	4.66E−04	1.442	pos
Nicotinic acid	$C_6H_5NO_2$	85.2	124.087	382.62 ± 15.15^{a}	64.46 ± 2.38^{c}	336.60 ± 28.59^{b}	322.98 ± 16.18^{b}	321.46 ± 16.31^{b}	6.80E−09	1.313	pos
L-Methionine	$C_5H_{11}NO_2S$	125.8	148.044	530.90 ± 0.98^{c}	547.69 ± 7.39^{c}	571.14 ± 4.37^{b}	624.93 ± 21.44^{a}	575.90 ± 10.27^{b}	1.60E−05	1.301	neg
gamma-Glutamylcysteine	$C_8H_{14}N_2O_5S$	91	248.961	277.00 ± 19.48^{a}	104.96 ± 77.92^{c}	276.68 ± 5.21^{a}	309.98 ± 35.03^{a}	183.73 ± 11.73^{b}	4.64E−04	1.267	neg
(R)-10-Hydroxystearate	$C_{18}H_{36}O_3$	903.3	283.263	2420.54 ± 53.09^{d}	2642.20 ± 56.70 ^{cd}	3306.42 ± 72.15^{b}	4584.77 ± 237.70^{a}	2721.07 ± 172.02^{c}	2.29E−08	1.258	pos
Creatinine	$C_4H_7N_3O$	104.2	114.066	12404.43 ± 1391.16^{d}	14062.71 ± 459.39^{c}	19540.26 ± 1110.88^{a}	15954.58 ± 127.41^{b}	20512.06 ± 629.68^{a}	3.77E−01	1.247	pos
dGMP	$C_{10}H_{14}N_5O_7P$	111.7	346.057	607.38 ± 45.68^{b}	312.20 ± 50.23^{c}	539.82 ± 33.69^{b}	1188.15 ± 175.48^{a}	444.88 ± 12.91^{bc}	2.00E−06	1.239	neg
Carnosine	$C_9H_{14}N_4O_3$	84.7	227.114	21134.62 ± 402.95^{b}	30690.72 ± 1815.04^{a}	30005.15 ± 1634.11^{a}	30018.39 ± 489.76^{a}	30213.92 ± 1824.57^{a}	3.00E−05	1.239	pos
L-Malic acid	$C_4H_6O_5$	125.5	133.015	96.11 ± 10.50^{b}	63.15 ± 1.63^{d}	95.80 ± 9.73^{b}	76.65 ± 1.18^{c}	110.11 ± 7.59^{a}	1.11E−04	1.215	neg
D-Fructose	$C_6H_{12}O_6$	82	179.057	1040.60 ± 52.58^{a}	593.77 ± 11.67^{b}	492.45 ± 20.11^{d}	557.26 ± 14.26^{bc}	522.86 ± 20.89 ^{cd}	1.94E−09	1.213	neg
Glycerophosphocholine	$C_8H_{21}NO_6P$	902	258.11	1604.01 ± 145.65^{d}	22514.44 ± 756.48^{c}	27977.27 ± 374.20^{a}	26747.29 ± 1067.97^{a}	24726.90 ± 986.16^{b}	6.05E−12	1.206	pos
Ornithine	$C_5H_{12}N_2O_2$	77.6	133.097	105.78 ± 4.78^{b}	142.13 ± 13.87^{a}	142.68 ± 4.24^{a}	139.78 ± 3.79^{a}	101.61 ± 7.97^{b}	7.60E−05	1.205	pos
Sucrose	$C_{12}H_{22}O_{11}$	743	343.295	310.48 ± 11.88^{a}	198.09 ± 11.39^{b}	133.80 ± 2.74^{d}	159.64 ± 1.09^{c}	75.00 ± 4.90^{e}	7.06E−11	1.19	pos
Inosine	$C_{10}H_{12}N_4O_5$	176.1	269.088	5752.00 ± 120.61^{d}	5080.47 ± 244.23^{e}	8054.60 ± 89.17^{b}	6538.87 ± 219.64^{c}	8824.10 ± 202.17^{a}	1.21E−09	1.172	pos
Pyridoxine	$C_8H_{11}NO_3$	97.8	170.033	1220.81 ± 67.53^{a}	805.21 ± 8.42^{d}	1009.85 ± 15.71^{c}	1087.88 ± 2.79^{b}	990.28 ± 24.30^{c}	4.70E−07	1.153	pos
Citrulline	$C_6H_{13}N_3O_3$	91.6	176.103	282.90 ± 49.36^{c}	34.57 ± 6.39^{d}	364.49 ± 43.44^{c}	974.08 ± 86.00^{a}	638.06 ± 71.65^{b}	2.58E−08	1.141	pos
Ascorbate	$C_6H_8O_6$	154.1	159.028	1285.26 ± 70.47^{a}	1044.03 ± 20.17^{c}	1175.88 ± 23.89^{b}	1174.35 ± 38.16^{b}	1191.08 ± 53.90^{b}	1.21E−03	1.119	pos
Azelaic acid	$C_9H_{16}O_4$	125.8	187.098	70.51 ± 7.68^{d}	157.02 ± 11.99^{b}	98.22 ± 3.25^{c}	145.00 ± 10.48^{b}	186.38 ± 1.54^{a}	5.09E−08	1.103	neg
Dodecanoic acid	$C_{12}H_{24}O_2$	989.4	199.975	489.04 ± 18.37^{c}	602.91 ± 13.16^{ab}	504.62 ± 10.39^{c}	571.18 ± 13.77^{b}	632.98 ± 32.76^{a}	1.40E−05	1.097	pos
Erucic acid	$C_{22}H_{42}O_2$	819.7	338.209	90.51 ± 2.10^{e}	237.68 ± 21.63^{b}	173.98 ± 5.20^{d}	197.72 ± 3.96^{c}	268.01 ± 15.88^{a}	8.25E−08	1.091	neg
gamma-Aminobutyric acid	$C_4H_9NO_2$	131	104.107	284.82 ± 24.71^{a}	126.63 ± 10.66^{c}	175.98 ± 9.04^{b}	179.17 ± 18.06^{b}	170.32 ± 15.80^{b}	6.00E−06	1.086	pos
Salicylic acid	$C_7H_6O_3$	586.8	139.112	4.09 ± 0.75^{c}	26.45 ± 2.35^{b}	26.63 ± 0.62^{b}	2.66 ± 0.21^{c}	30.88 ± 1.42^{a}	1.54E−10	1.08	pos
Gluconic acid	$C_6H_{12}O_7$	81.5	195.052	769.90 ± 59.86^{b}	1803.74 ± 80.05^{b}	1346.99 ± 293.43^{b}	4301.41 ± 1998.51^{a}	1948.67 ± 531.74^{b}	8.77E−03	1.058	neg
Xanthosine	$C_{10}H_{12}N_4O_6$	123.5	283.068	26.22 ± 2.03^{b}	11.65 ± 0.95^{d}	24.37 ± 1.73^{b}	50.75 ± 4.16^{a}	19.42 ± 1.91^{c}	2.97E−08	1.018	neg
13S-hydroxyoctadecadienoic acid	$C_{18}H_{32}O_3$	871.9	279.232	468.54 ± 13.00^{b}	456.97 ± 9.49^{b}	457.04 ± 24.57^{b}	463.71 ± 12.58^{b}	503.26 ± 11.82^{a}	2.02E−02	1.017	pos

Note: $P < 0.05$.

samples changed evidently. In contrast to the RAW group, the concentrations of aspartic acid and glutamic acid rose in the B group and fell in the other groups, suggesting that some amino acids are lost by cooking at higher temperatures. The levels of aspartic acid and glutamic acid were lowest in the AF group and sequentially higher in the F, M, and B groups, which differed from the results of the umami sensor in the E-tongue analysis. This may result from the fact that E-tongue measures the overall flavor of the samples, while the umami taste of Tibetan pork was not exclusively contributed by aspartic acid and glutamic acid. It is well known that nucleotides as well as synergistic effects between different taste substances can also affect the umami taste of cooked Tibetan pork. A total of three sulfur-containing amino acids, methionine, cysteine, and cystine, were detected in Tibetan pork samples. Among them, methionine and cysteine were important precursors for the formation of volatile sulfides, which were reduced by sugar compounds during cooking treatments and then engaged in a complex series of reactions to create sulfides (Cerny, 2015). In addition, alanine and cysteine can take part in the Strecker reaction to produce pyrazines substituted by ethyl groups, which usually possess a lower threshold (Parker, 2015) and play an active role in the flavor formation of meat products. Li et al. (2021) discovered that the content of leucine and threonine was heightened by heat treatment, in which air frying treatment obviously facilitated the creation of partial amino acids in tilapia fillets. This agrees with what we discovered. According to the research, the amount of essential amino acids such as leucine and threonine showed an increase in the cooking process, most significantly in air frying. This demonstrated that heat treatment boosted the content of nutrients in Tibetan pork, especially air frying treatment. Taurine has been reported to lack a taste of its own but

could enhance the flavor of meat through an unknown mechanism (Suzuki et al., 2017). The levels of taurine in the cooked groups were boosted compared to those in the RAW group, and according to the E-tongue results, the richness of the cooked samples was also increased. Therefore, the assumption was made that the contribution of taurine to umami might be reflected in the aftertaste of umami, which is the richness of the sample flavor. As seen in Fig. 3-d, the proline and arginine content of meat diminished after cooking treatment. This might be because proline and arginine were converted to carbonyl compounds under the effects of heat (Wen et al., 2019), which resulted in a decline in the content of proline and arginine and an increase in carbonyl compounds, which is also consistent with the findings of carbonyl content findings (Fig. 1). Moreover, the derivatives of amino acids are also essential taste-presenting ingredients. It has been established that heating processes result in creatine loss (Yu et al., 2021). This might be because creatine was converted to creatinine during cooking treatment, thus diminishing its concentration (Wang, Qiao, Ding, Zhang, Sun, & Chen, 2018). In this study, cooking treatments markedly reduced the levels of creatine, while creatinine levels increased. Although they provided food with a strong flavor and boosted the flavor richness of meat products, creatine and creatinine may also strengthen the bitterness of food (Mora et al., 2008).

Nucleotides are another critical important taste-presenting substance in cooked meat products. For example, IMP can synergize with glutamic acid and aspartic acid to enhance the umami of meat products, while AMP can reduce bitterness and impart excellent salty and sweet flavors to foods (Huang et al., 2022b). AMP is degraded from ADP and can further transform into IMP, which is the precursor substance of inosine and hypoxanthine. According to Fig. 3-d, the content of AMP was increased in all the cooking treatment groups compared to the RAW group, while the content of IMP decreased, but their contents were in a certain dynamic equilibrium. Moreover, the contents of inosine and hypoxanthine were significantly increased and decreased in the AF group, respectively, and significantly diminished and augmented in the B group, respectively. It could be inferred that the products were scarcely dependent on the cooking method in the reaction stage from ADP to IMP during ATP degradation. Conversely, in the advanced stages of the reaction, that is, the generation of inosine and hypoxanthine, the products were strongly impacted by the mode of cooking. Hypoxanthine apparently contributed to the formation of bitterness in meat products (Wang et al., 2022a). According to the results, the concentration of hypoxanthine was the lowest in the AF group, rising sequentially in the M, F, RAW, and B groups, which was in agreement with the variation in response values of the bitterness sensor in the E-tongue analysis.

Although only a small amount of organic acids was detected, their presence might affect the flavor of the pork after cooking to a certain extent. Succinic acid is an important taste-presenting substance among organic acids, and its sodium salt can interact with monosodium glutamate to improve the umami taste of foods (Wang et al., 2018). In Fig. 3-d, it can be seen that the content of succinic acid dropped in the cooked Tibetan pork compared to the RAW group, suggesting that the cooking treatment would cause some loss of organic acids.

In addition, several other compounds were identified, including peptides, glycans, and cholines. Among them, glutathione and glycerophosphocholine have been identified as marker metabolites in chicken. Moreover, it has been reported that glutathione possesses antioxidant properties and reduces harmful chemical components in food (Wang, Wang, Zhang, Chen, Yang, & Xu, 2022b). Glycerophosphocholine was also identified as a crucial indicator for distinguishing raw beef from that of inferior quality (Jeong et al., 2020). With the cooking treatment, the content of fructose decreased, demonstrating that it was involved as a precursor substance in the Maillard reaction to generate volatile flavor compounds. Furthermore, a substance was detected to make an important contribution to flavor, namely, thiamine, whose level decreased notably after cooking. On the one hand, this could be due to its thermal instability and water solubility, resulting in its loss during cooking. On the other hand, some of its degradation products could be involved in the Maillard reaction to generate volatile components, thus diminishing the levels of thiamine in the meat.

Correlation analysis

To observe the relationship between lipids, protein oxidation, and flavor more visually, correlation network diagrams between the oxidation index, metabolites, and volatile flavor substances were constructed, as shown in Fig. 4-a and 4-b. A total of 59 positive and 34 negative correlations were identified between volatile flavor substances and TBARS, 28 positive and 65 negative correlations with total sulfhydryl content, and 64 positive and 29 negative correlations with carbonyl content. Similarly, there were 27 positive and 36 negative correlations between metabolites and TBARS, 36 positive and 27 negative correlations with total sulfhydryl content, and 26 positive and 37 negative correlations with carbonyl content.

The results showed that TBARS positively correlated with the majority of aldehydes, alcohols, and ketones, as well as with free fatty acids such as linoleic acid, α-linolenic acid, oleic acid, and arachidonic acid. In Fig. 4-c, linoleic acid, α-linolenic acid, oleic acid, and arachidonic acid were strongly correlated with volatile flavor substances such as hexanal, octanal, nonanal, pentanol, and 3-hydroxy-2-butanone. Lipid oxidation is a critical pathway for the development of flavor, and it is a chain of radical reactions. The phospholipids and triglycerides in fat are decomposed into fatty acids by heat, and unsaturated fatty acids generate hydroperoxides under the action of radicals and reactive oxygen species, which are further decomposed into alkyl radicals and hydroxyl peroxide radicals. As the reaction proceeds, the different radicals react with each other to eventually generate more stable aldehydes, alcohols, ketones, and other volatile flavor compounds. Hexanal is an oxidation product of linoleic and arachidonic acids and can give a grassy and apple aroma to meat products (Merlo et al., 2021). Similarly, 1-octen-3-ol, as a representative compound among alcohols, also derives from the degradation of arachidonic acid and can impart a mushroom odor. However, in the correlation analysis of Fig. 4-c, arachidonic acid did not show a significant correlation with 1-octen-3-ol. This might be because arachidonic acid is involved in the production of not only hexanal and 1-octen-3-ol but also other aldehydes or alcohols during the cooking process. Arachidonic acid can act as an intermediate in the fatty acid reaction process (Wang et al., 2022c). Meanwhile, 1-octen-3-ol could also originate from the oxidation of linear aldehydes (Liu et al., 2022a). All of these reasons might lead to a poor correlation between arachidonic acid and 1-octen-3-ol data.

In addition to lipid oxidation, the oxidation of protein and the Maillard reaction between amino acids and reducing sugars also affect flavor to some extent. Carbonyl was mainly derived from the oxidation of more unstable amino acids, such as arginine and proline, and Fig. 4-b shows that the carbonyl content was negatively correlated with proline and arginine. During the cooking treatment, amino acids were susceptible to attack by radicals to form amino radicals, generating amino ions in the presence of metal ions. The amino ions again generated compounds with carbonyl groups in the side chain through hydration reactions, which eventually led to an increase in the carbonyl content. The pattern of the association between the total sulfhydryl content and the amino acids was entirely opposite to the correlation between the carbonyl concentration and the amino acids. This could be attributed to the fact that sulfhydryls were oxidized into disulfide bonds during the heat breakdown of proteins, resulting in a drop in the total sulfhydryl content. Therefore, there was a negative correlation between the total sulfhydryl and carbonyl content. In addition, 2-pentylfuran was positively correlated with aspartic acid and glutamic acid and negatively correlated with tryptophan and methionine. 2-Pentylfuran also presented a significant correlation with palmitic acid and glycerophosphocholine. The results indicated that 2-pentylfuran could be generated not only by protein degradation reactions but also by the

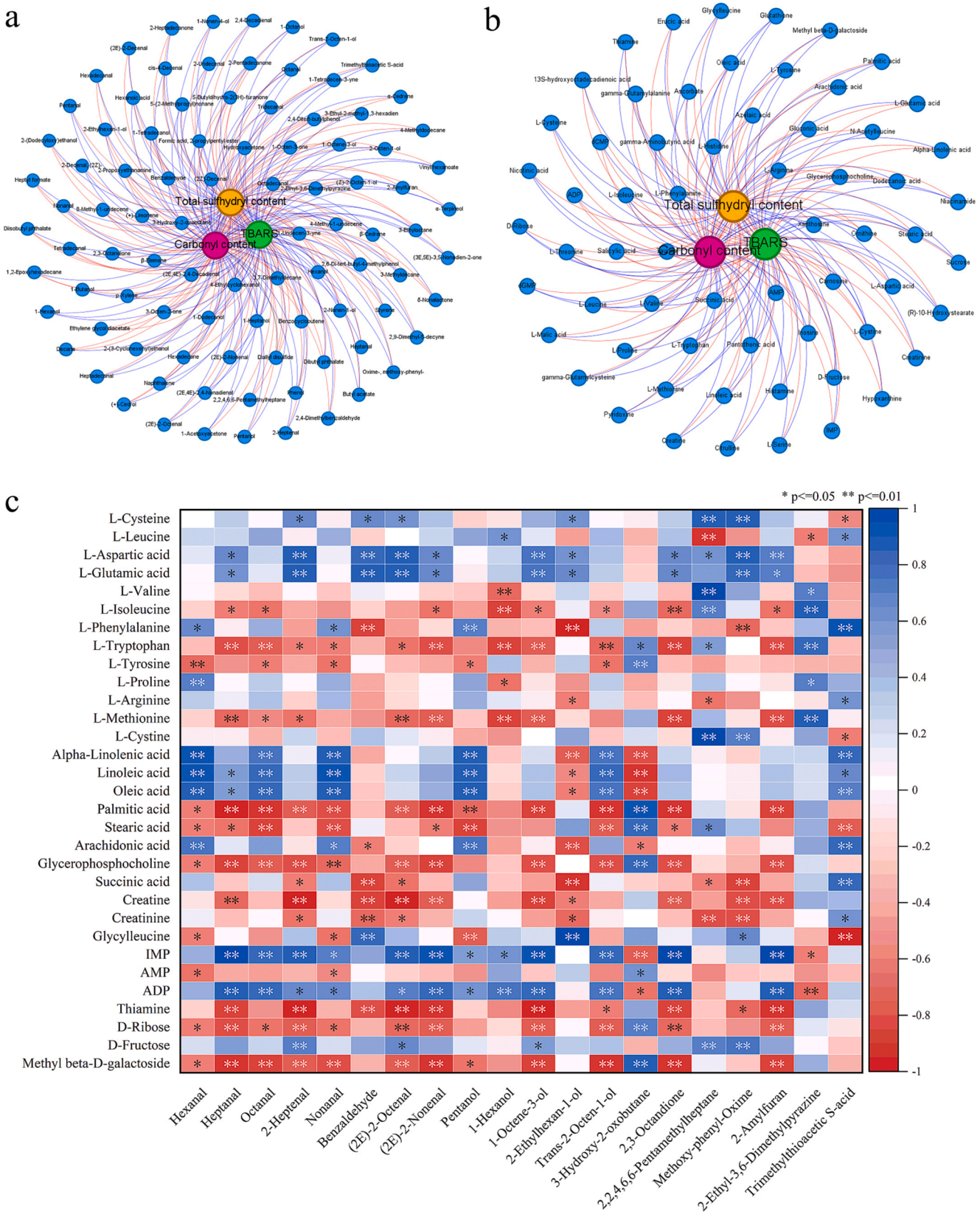

Fig. 4. Correlation of oxidation indicators with volatile flavor substances and differential metabolites in Tibetan spiced pigs during cooking. Network diagram of correlation between oxidation indicators and volatile flavor substances (a); network diagram of correlation between oxidation indicators and differential metabolites (b); blue line: positive correlation, red line: negative correlation; heat map of correlation between key volatiles and differential metabolites (c). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oxidation of fatty acids. 2-Ethyl-3,6-dimethylpyrazine showed a strong correlation with various amino acids such as valine and isoleucine, and it could be speculated that these amino acids were involved in the Maillard reaction to generate pyrazines. In addition, trimethylthioacetic acid was negatively correlated with cysteine and cystine and positively correlated with leucine and phenylalanine, as well as showing some correlation with a fraction of fatty acids. This phenomenon demonstrated that trimethylthioacetic acid might be generated by the reaction of the products of the Maillard reaction with the products of fatty acid oxidation.

Benzaldehyde has been reported to be derived mainly from the Strecker degradation of phenylalanine and to give food a pleasant nutty flavor (Wen et al., 2019). In Fig. 4-c, it was found that benzaldehyde showed a strong correlation with phenylalanine and glutamic acid and a poor correlation with fatty acids. All of these phenomena indicated that the production of benzaldehyde was mainly contributed by some amino acids during the maturation of Tibetan pork, and the degradation of fatty acids affected it mildly, which was in line with the findings of Wang et al. (2016). Fructose and galactosides were mostly negatively correlated with volatile flavor substances. Glycogenic substances in Tibetan pork were thermally decomposed during the cooking process to create monosaccharide substances such as fructose, glucose, and galactose. Then, these monosaccharides could be utilized as flavor precursors to participate in the Maillard reaction and generate volatile flavor substances, thus affecting the overall flavor of cooked Tibetan pork.

Conclusion

In this study, volatile and nonvolatile flavor substances were identified by E-nose, E-tongue, GC–MS, and LC–MS techniques for Tibetan pork with different cooking treatments (boiling, frying, microwaving, and air frying). The results demonstrated that the oxidation of lipids and proteins was greater in the AF and B groups and lower in the F and M groups, while both were far higher than in the RAW group. The boost in the level of oxidation favored the enrichment of the type and content of volatile compounds in Tibetan pork. The principal component analysis of the E-nose and E-tongue was combined with GC–MS and LC–MS to efficiently differentiate Tibetan pork cooked in various ways. The thermally induced reaction of Tibetan pork in the AF group was expedited by the hot air circulation mode of operation, leading to enrichment of the type and content of aroma substances and creating a unique flavor profile. The differential metabolites of cooked flavor in Tibetan pork were mainly fatty acids, nucleotides, organic acids, and amino acids, which were the critical precursors responsible for the flavor variation in Tibetan pork. The strong connection of fatty acids and amino acids with the majority of volatile components implied that lipid and protein oxidation was a major factor determining the flavor of Tibetan pork. Generally, varied cooking techniques provided distinctive flavors for Tibetan pork. The richest flavor compounds could be generated in Tibetan pork by air frying. The findings of this study could be useful as a theoretical foundation for deep-processing pork. Nevertheless, several of the cooking methods in this study utilized higher temperatures, and whether they produced harmful substances during the heating process should be investigated further.

CRediT authorship contribution statement

Lujie Cheng: Investigation, Formal analysis, Visualization, Writing – original draft. **Xin Li:** Methodology, Formal analysis, Writing – review & editing. **Yuting Tian:** Writing – review & editing. **Qia Wang:** Investigation, Formal analysis, Data curation. **Xiefei Li:** Validation, Writing – review & editing. **Fengping An:** Conceptualization, Writing – review & editing. **Zhang Luo:** Validation, Writing – review & editing. **Peng Shang:** Funding acquisition, Conceptualization. **Zhendong Liu:** Funding acquisition, Supervision. **Qun Huang:** 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

The data that has been used is confidential.

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Ethical guidelines

Ethics approval was not required for this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100873.

References

Aaslyng, M. D., & Schäfer, A. (2007). The effect of free fatty acids on the odour of pork investigated by sensory profiling and GC-O-MS. *European Food Research and Technology, 226*(5), 937–948. https://doi.org/10.1007/s00217-007-0647-3

Alencar, S. A. D. S., & Kiefer, C. (2023). Dietary lipid sources on the fatty acid profile of swine fat: A review. *Ciência Rural, 53*(3). https://doi.org/10.1590/0103-8478cr20210554

Bassam, S. M., Noleto-Dias, C., & Farag, M. A. (2022). Dissecting grilled red and white meat flavor: Its characteristics, production mechanisms, influencing factors and chemical hazards. *Food Chemistry, 371*, Article 131139. https://doi.org/10.1016/j.foodchem.2021.131139

Cerny, C. (2015). The role of sulfur chemistry in thermal generation of aroma. In *Flavour development, analysis and perception in food and beverages* (pp. 187–210). Woodhead Publishing Series in Food Science, Technology and Nutrition.

Chang, Y., Wang, S., Chen, H., Zhang, N., & Sun, J. (2021). Characterization of the key aroma compounds in pork broth by sensory-directed flavor analysis. *Journal of Food Science, 86*(11), 4932–4945. https://doi.org/10.1111/1750-3841.15937

Delgado, F. J., González-Crespo, J., Cava, R., García-Parra, J., & Ramírez, R. (2010). Characterisation by SPME–GC–MS of the volatile profile of a Spanish soft cheese P.D.O. Torta del Casar during ripening. *Food Chemistry, 118*(1), 182–189. https://doi.org/10.1016/j.foodchem.2009.04.081

Dominguez, R., Gomez, M., Fonseca, S., & Lorenzo, J. M. (2014a). Effect of different cooking methods on lipid oxidation and formation of volatile compounds in foal meat. *Meat Science, 97*(2), 223–230. https://doi.org/10.1016/j.meatsci.2014.01.023

Dominguez, R., Gomez, M., Fonseca, S., & Lorenzo, J. M. (2014b). Influence of thermal treatment on formation of volatile compounds, cooking loss and lipid oxidation in foal meat. *LWT - Food Science and Technology, 58*(2), 439–445. https://doi.org/10.1016/j.lwt.2014.04.006

Dong, K., Guan, Y., Wang, Q., Huang, Y., An, F., Zeng, Q., … Huang, Q. (2023). Non-destructive prediction of yak meat freshness indicator by hyperspectral techniques in the oxidation process. *Food Chemistry: X, 17*. https://doi.org/10.1016/j.fochx.2022.100541

Echegaray, N., Paterio, M., Dominguez, R., Purrinos, L., Bermudez, R., Carballo, J., & Lorenzo, J. M. (2020). Effects of different cooking methods and of the inclusion of chestnut (*Castanea sativa Miller*) in the finishing diet of Celta pig breed on the physicochemical parameters and volatile profile of Longissimus thoracis et lumborum muscle. *Food Research International, 137*, Article 109407. https://doi.org/10.1016/j.foodres.2020.109407

Elmore, J. S., Cooper, S. L., Enser, M., Mottram, D. S., Sinclair, L. A., Wilkinson, R. G., & Wood, J. D. (2005). Dietary manipulation of fatty acid composition in lamb meat and its effect on the volatile aroma compounds of grilled lamb. *Meat Science, 69*(2), 233–242. https://doi.org/10.1016/j.meatsci.2004.07.002

Gu, X., Gao, Y., Luo, Z., Yang, L., Chi, F., Xiao, J., … Geng, F. (2019). In-depth mapping of the proteome of Tibetan pig tenderloin (*longissimus dorsi*) using offline high-pH reversed-phase fractionation and LC-MS/MS. *Journal of Food Biochemistry, 43*(11), 13015. https://doi.org/10.1111/jfbc.13015

Han, D., Zhang, C., Fauconnier, M. L., & Mi, S. (2020). Characterization and differentiation of boiled pork from Tibetan, Sanmenxia and Duroc x (Landrac x

Yorkshire) pigs by volatiles profiling and chemometrics analysis. *Food Research International, 130*, Article 108910. https://doi.org/10.1016/j.foodres.2019.108910

Huang, Q., Dong, K., Wang, Q., Huang, X., Wang, G., An, F., … Luo, P. (2022b). Changes in volatile flavor of yak meat during oxidation based on multi-omics. *Food Chemistry, 371*, Article 131103. https://doi.org/10.1016/j.foodchem.2021.131103

Huang, X., Sun, L., Dong, K., Wang, G. Z., Luo, P., Tang, D. B., & Huang, Q. (2022a). Mulberry fruit powder enhanced the antioxidant capacity and gel properties of hammered minced beef: Oxidation degree, rheological, and structure. *LWT - Food Science and Technology, 154*, Article 112648. https://doi.org/10.1016/j.lwt.2021.112648

Jeong, J. Y., Kim, M., Ji, S. Y., Baek, Y. C., Lee, S., Oh, Y. K., … Lee, H. J. (2020). Metabolomics Analysis of the Beef Samples with Different Meat Qualities and Tastes. *Food Science of Animal Resources, 40*(6), 924–937. https://doi.org/10.5851/kosfa.2020.e59

Lee, H. J., Choe, J., Kim, M., Kim, H. C., Yoon, J. W., Oh, S. W., & Jo, C. (2019). Role of moisture evaporation in the taste attributes of dry- and wet-aged beef determined by chemical and electronic tongue analyses. *Meat Science, 151*, 82–88. https://doi.org/10.1016/j.meatsci.2019.02.001

Li, R., Sun, Z., Zhao, Y., Li, L., Yang, X., Cen, J., … Wang, Y. (2021). Application of UHPLC-Q-TOF-MS/MS metabolomics approach to investigate the taste and nutrition changes in tilapia fillets treated with different thermal processing methods. *Food Chemistry, 356*, Article 129737. https://doi.org/10.1016/j.foodchem.2021.129737

Li, X., Tu, Z., Sha, X., Li, Z., Li, J., & Huang, M. (2022). Effect of coating on flavor metabolism of fish under different storage temperatures. *Food Chemistry: X, 13*, Article 100256. https://doi.org/10.1016/j.fochx.2022.100256

Liu, J., Shen, S., Xiao, N., Jiang, Q., & Shi, W. (2022a). Effect of glycation on physicochemical properties and volatile flavor characteristics of silver carp mince. *Food Chemistry, 386*, Article 132741. https://doi.org/10.1016/j.foodchem.2022.132741

Liu, L., Huang, P., Xie, W., Wang, J., Li, Y., Wang, H., … Zhao, Y. (2022b). Effect of air fryer frying temperature on the quality attributes of sturgeon steak and comparison of its performance with traditional deep fat frying. *Food Science & Nutrition, 10*(2), 342–353. https://doi.org/10.1002/fsn3.2472

Merlo, T. C., Lorenzo, J. M., Saldana, E., Patinho, I., Oliveira, A. C., Menegali, B. S., … Contreras-Castillo, C. J. (2021). Relationship between volatile organic compounds, free amino acids, and sensory profile of smoked bacon. *Meat Science, 181*, Article 108596. https://doi.org/10.1016/j.meatsci.2021.108596

Mora, L., Sentandreu, M.Á., & Toldrá, F. (2008). Effect of Cooking Conditions on Creatinine Formation in Cooked Ham. *Journal of Agricultural and Food Chemistry, 56*(23), 11279–11284. https://doi.org/10.1021/jf801953t

Parker, J. K. (2015). Thermal generation or aroma. In *Flavour development, analysis and perception in food and beverages* (pp. 151–185). Woodhead Publishing Series in Food Science, Technology and Nutrition.

Penaranda, I., Garrido, M. D., Egea, M., Diaz, P., Alvarez, D., Oliver, M. A., & Linares, M. B. (2017). Sensory perception of meat from entire male pigs processed by different heating methods. *Meat Science, 134*, 98–102. https://doi.org/10.1016/j.meatsci.2017.07.021

Rao, J., Meng, F., Li, Y., Chen, W., Liu, D., & Zhang, J. (2022). Effect of cooking methods on the edible, nutritive qualities and volatile flavor compounds of rabbit meat. *Journal of the Science of Food and Agriculture, 102*(10), 4218–4228. https://doi.org/10.1002/jsfa.11773

Shang, P., Li, W., Liu, G., Zhang, J., Li, M., Wu, L., … Chamba, Y. (2019). Identification of lncRNAs and genes responsible for fatness and fatty acid composition traits between the Tibetan and Yorkshire pigs. *International Journal of Genomics, 2019*, 5070975. https://doi.org/10.1155/2019/5070975

Sun, W., Ji, H., Zhang, D., Zhang, Z., Liu, S., & Song, W. (2022). Evaluation of aroma characteristics of dried shrimp (*Litopenaeus vannamei*) prepared by five different procedures. *Foods, 11*, 3532. https://doi.org/10.3390/foods11213532

Suzuki, K., Shioura, H., Yokota, S., Katoh, K., Roh, S. G., Iida, F., … Yamada, S. (2017). Search for an index for the taste of Japanese Black cattle beef by panel testing and chemical composition analysis. *Animal Science Journal, 88*(3), 421–432. https://doi.org/10.1111/asj.12663

Wang, K., Wang, X., Zhang, L., Chen, A., Yang, S., & Xu, Z. (2022b). Identification of novel biomarkers in chilled and frozen chicken using metabolomics profiling and its application. *Food Chemistry, 393*. https://doi.org/10.1016/j.foodchem.2022.133334

Wang, L., Qiao, K., Ding, Q., Zhang, Y., Sun, B., & Chen, H. (2018). Effects of two cooking methods on the taste components of Sanhuang chicken and Black-bone silky fowl meat. *Journal of Food Processing and Preservation, 42*(11). https://doi.org/10.1111/jfpp.13772

Wang, Q., Dong, K., Wu, Y., An, F., Luo, Z., Huang, Q., & Wei, S. (2022c). Exploring the formation mechanism of off-flavor of irradiated yak meat based on metabolomics. *Food Chemistry: X, 16*, Article 100494. https://doi.org/10.1016/j.fochx.2022.100494

Wang, Q., Li, X., Xue, B., Wu, Y., Song, H., Luo, Z., … Huang, Q. (2022a). Low-salt fermentation improves flavor and quality of sour meat: Microbiology and metabolomics. *LWT - Food Science and Technology, 171*. https://doi.org/10.1016/j.lwt.2022.114157

Wang, Y., Song, H., Zhang, Y., Tang, J., & Yu, D. (2016). Determination of aroma compounds in pork broth produced by different processing methods. *Flavour and Fragrance Journal, 31*(4), 319–328. https://doi.org/10.1002/ffj.3320

Wen, R., Hu, Y., Zhang, L., Wang, Y., Chen, Q., & Kong, B. (2019). Effect of NaCl substitutes on lipid and protein oxidation and flavor development of Harbin dry sausage. *Meat Science, 156*, 33–43. https://doi.org/10.1016/j.meatsci.2019.05.011

Yin, Y., Zhou, L., Pereira, J., Zhang, J., & Zhang, W. (2020). Insights into digestibility and peptide profiling of beef muscle proteins with different cooking methods. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/acs.jafc.0c04054

Yu, Y., Wang, G., Yin, X., Ge, C., & Liao, G. (2021). Effects of different cooking methods on free fatty acid profile, water-soluble compounds and flavor compounds in Chinese Piao chicken meat. *Food Research International, 149*, Article 110696. https://doi.org/10.1016/j.foodres.2021.110696

Zang, M., Wang, L., Zhang, Z., Zhang, K., Li, D., Li, X., … Chen, H. (2019). Changes in flavour compound profiles of precooked pork after reheating (warmed-over flavour) using gas chromatography–olfactometry–mass spectrometry with chromatographic feature extraction. *International Journal of Food Science & Technology, 55*(3), 978–987. https://doi.org/10.1111/ijfs.14306

Zhang, J., Cao, J., Pei, Z., Wei, P., Xiang, D., Cao, X., … Li, C. (2019). Volatile flavour components and the mechanisms underlying their production in golden pompano (*Trachinotus blochii*) fillets subjected to different drying methods: A comparative study using an electronic nose, an electronic tongue and SDE-GC-MS. *Food Research International, 123*, 217–225. https://doi.org/10.1016/j.foodres.2019.04.069