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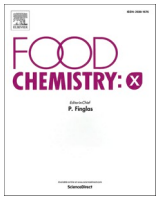

Characterization of key aroma-active compounds in different types of Douchi based on molecular sensory science approaches

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ABSTRACT

To attain the differences in the flavor profile of Douchi, the key aroma-active compounds of three types of Douchi were investigated. The "Sauce-like", "Smoky", "Nutty", "Roast", "Caramel", and "Flower" of Douchi were favored by customers. Further, a total of 179 volatile compounds were identified using HS-SPME-GC-MS, and 29 aroma compounds were detected using GC-O-MS. Based on the quantification, 9, 13, and 10 compounds were regarded as aroma-active compounds in Yangjiang Douchi (YJ), Pingjiang Douchi (PJ), and Liuyang Douchi (LY), respectively. Moreover, the mixture of these aroma-active compounds successfully simulated the main aromas of PJ, LY, and YJ. And omission experiments confirmed that guaiacol was the key aroma compound for LY, benzene acetaldehyde, dimethyl trisulfide, and 2-acetyl pyrrole were important for YJ, benzene acetaldehyde and 3,5-diethyl-2-methyl pyrazine notably contributed to key aroma of PJ.

Introduction

Douchi is a traditional Chinese seasoning, which is mainly made from black soybeans or soybeans produced by a fermentation process, including Yangjiang (YJ) Douchi, Liuyang (LY) Douchi, Pingjiang (PJ) Douchi, etc. Among them, both LY Douchi and YJ Douchi are the type of *Aspergillus*-type Douchi made from black soybeans, but *low salt* (<3%) fermentation environment of LY Douchi led to much shorter post fermentation than YJ Douchi with a higher amount of salt is added (>10 %) (Chen, Li, Liao, Qin, Jiang, & Liu, 2021; He, Huang, Liang, Wu, & Zhou, 2016; Zhang, Zeng, Wang, Song, & Suo, 2021). Differently, the PJ Douchi is a type of Mucor-type Douchi made from soybeans after fermenting for 1 year with high salt addition (Guo, Zhang, Long, Fu, & Ren, 2023). The variations in fermentation processes could cause differences in microbial metabolism and finally resulted in the inconsistent final flavors of different types of Douchi (He, Li, Hu, Zhang, Sun, & Qiu, 2019).

Aroma is one of the most important sensory properties reflecting the quality of traditional fermented seasoning and serves as a pivotal factor for consumer selection and purchase. At present, there are many studies on the flavors of Douchi. One hundred and thirty-one volatile compounds have been identified by Wang et al in different types of Douchi. Wherein height contents of acids, alcohols, and aldehydes were detected in *Mucor*-type Douchi, while *Aspergillus*-type Douchi contained more pyrazines and phenolic compounds (Wang, Mu, Liu, Bhandari, Saito, & Li, 2010). In our previous study (Chen et al., 2021), the changes in volatile compounds of Liuyang Douchi during fermentation were investigated by using headspace solid-phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) and headspace gas chromatography ion mobility spectrometry (HS-GC-IMS), and a total of 115 volatile compounds have been identified.

However, only a minority of the volatile compounds dominate the aroma of the food, namely the key aroma-active compounds (Dunkel, Steinhaus, Kotthoff, Nowak, Krautwurst, & Schieberle, 2014; Guichard, Ayed, Salles, Guichard, & Salles, 2023). It had been demonstrated that molecular sensory science, considered as a multidisciplinary and integrated technique for examining the sensory quality of food at the molecular level, was more accurate in the identification of key aroma-active compounds and the determination of the impact of compounds on the aroma characterization of samples. (Wang et al., 2021) analyzed Yongchuan Douchi. Although 49 volatile compounds were identified in Yongchuan Douchi, only 10 were finally recognized as key aroma-active compounds. Lu also found that only 11 aroma-active compounds among 43 volatile compounds contributed to the overall aroma of pomegranate

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juice (Lu, Zhang, Zhan, Wang, & Tian, 2023).

Although the aroma compounds of Douchi have been reported, there was no comparison of aroma-active compounds among different types of Douchi, as well as the correlation between aroma profile and consumer preferences. In present study, the Consumer-Acceptability Test with Aroma (CATA) was employed to determine consumer preferences for the aromas of the three distinct samples. And then, the qualitative and quantitative analysis of volatile compounds in three different types of Douchi were performed by using GC-O-MS and GC-MS. Additionally, aroma-active compounds were selected according to OAVs. As well as combining aroma recombination and omission, the key aroma-active compounds were further determined. The exploration of the key aroma-active compounds in three types of Douchi could provide new understanding for the compounds caused different aroma profiles of each Douchi, and further help to the flavor modulation and quality improvement of Douchi.

Materials and methods

Chemicals

To identify and quantify aroma compounds, the researchers procured the necessary standards from Macklin (Shanghai, China): benzaldehyde (>98.5 %, AR), benzene acetaldehyde (95 %), 3,7-dimethyl-2,6-octadienal (97 %), 2-phenyl-2-butenal (97 %), 6-octadien-3-ol (98 %), 3-hydroxy-2-methyl-4 h-pyran-4-one (99 %), phenylethyl alcohol (99 %), 1-octen-3-ol (98 %), gamma-nonanolactone (98 %), isopentyl formate (95 %), ethyl phenylacetate (99 %), phenethyl acetate (99 %), 1,4-dimethoxybenzene (99 %), acetic acid (98 %, AR), dl-3-methylvaleric acid (98 %), 2-methyl butyric acid (98 %), 4-methylvaleric acid (98 %), tetramethylpyrazine (98 %), 2-methylpyrazine (98 %), 2,5-dimethyl pyrazine (98 %), 2-acetyl pyrrole (99 %), guaiacol (>98 %), dimethyl trisulfide (98 %), 5-methyl-3-heptanone (≥95 %, GC). 3,5-diethyl-2-methylpyrazine (>97 %), 3,5-dimethyl-2-ethylpyrazine (>97 %) were obtained from Adamas (Shanghai, China). 2-isopropyl-5-methyl-2-hexenal (96 %), (2e)-5-methyl-2-phenyl-2-hexenal (95 %) were purchased from Aladdin (Shanghai, China).

Sample

Douchi samples were collected from three manufacturers, numbered YJ, PJ, and LY, respectively. Different brands of Douchi have different production processes. Its main raw materials were black soybeans or soybeans, fermented through *Mucor* or *Aspergillus*. YJ came from Yangjiang, commonly fashioned from black soybeans, and fermented with *Aspergillus*. PJ is from Pingjiang and was made by fermenting soybeans with *Mucor*. Lastly, Liuyang Douchi short for LY, is made from black soybeans fermented with *Aspergillus*. The manufacturing process and material composition as for Fig. 1. The samples were all mature Douchi. Then the collected samples were transported back to the laboratory and stored at 4 °C before further analysis.

CATA (Check-All-That-Apply)

The CATA experiment was conducted following the methodology outlined by (Henrique, Deliza, & Rosenthal, 2015) with certain modifications. The sensory profiling study was conducted at Hunan Agricultural University in Changsha, China. A total of 100 volunteers, comprising 26 males and 74 females, were engaged in the study. Prior to the presentation of the Douchi samples to the participants, they were taken out of refrigeration and allowed to reach room temperature. Subsequently, the three samples were poured into 30 mL cups, each marked with a randomly assigned three-digit number, before being presented to the respondents.

The participants were instructed to evaluate the Douchi samples placed in front of them. Before smelling a new sample, participants were required to cleanse their olfactory senses with coffee. To prevent any potential confusion, the Douchi samples were assessed individually, with no opportunity for re-evaluation of a previous sample once the next sample had been initiated.

For the CATA questions, participants were instructed to select the attributes from a predetermined list of 10 terms that they perceived to be applicable to the test samples; no consideration of intensity was necessary. Following the selection of CATA attributes for each sample, a 9-point hedonic rating scale was utilized, with scores ranging from 1 to 9, denoting “dislike extremely” to “like extremely”, respectively.

Extraction of volatile compounds from Douchi by headspace solid-phase microextraction (HS-SPME)

The volatile compounds of Douchi were extracted using the headspace solid-phase microextraction (HS-SPME) which procedure described and modified by (Chen et al., 2021). Douchi (2 g), 2-methyl-3-heptanone (1.70 mg/mL, 20 μL, internal standard), and saturated NaCl (20 mL) solution were transferred to a 50 mL sample vial. The SPME fiber (50/30 μm DVB/CAR/PDMS, 57330-U polydimethylsiloxane extraction, Supelco, USA) was passed through the gasket and inserted into the vial and stirred using thermostatic magnetic stirrer (DF-101S, Gongyi Yuhua Instrument Co. LTD, China) at 70℃ for 40 min (30 rpm). Afterwards, the fiber was immediately injected into the GC-MS (7890B-5977A, Agilent, USA) at 250℃ in 5 min.

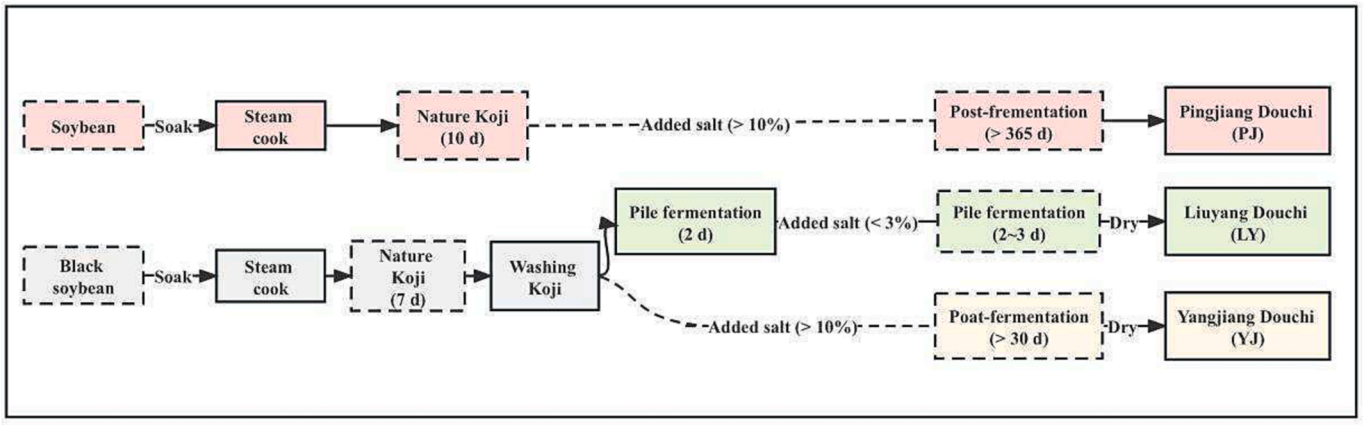

Fig. 1. The manufacturing process and material composition for the three types of Douchi.

Identification analysis of aroma compounds from Douchi by gas Chromatography-Olfactometry-mass spectrometry (GC-O-MS)

The detection of aroma compounds in Douchi was conducted using GC-O-MS (Agilent, USA). The GC-O-MS analysis was modified based on the method of (Liu, Wang, Zhang, Shen, Pan, & Hui, 2019). The samples were analyzed on a DB-5 capillary column (30 mm × 0.25 mm × 0.25 μm, Agilent, USA). Helium was used as the carrier gas with a flow rate of 1.6 mL/min. The oven temperature was increased from 40 °C for 2 min, then ramped up to 200 °C at a rate of 5 °C/min for 6 min, and finally increased to 250 °C at a rate of 15 °C/min for 3 min. Simultaneously, moist air was introduced into the suction port at a speed of 45 mL/min to rapidly remove odorants washed off from the suction port. Three trained panelists were selected for the GC-O-MS analysis. A five-point intensity scale ranging from "0″ to "4" was used to evaluate the aroma intensities, where "0" represented no aroma, "1" represented weak, recognizable aroma, "2" represented moderate aroma, "3" represented clear, but not strong aroma; and "4" represented strong aroma. Each panelist conducted the experiments in triplicate for each sample.

Quantitation analysis of aroma compounds from Douchi by gas chromatography-mass spectrometry (GC-MS)

The GC-MS (7890B-5977A, Agilent, USA) setup program was the same as that used for GC-O-MS and analysis was performed as described in (Liu et al., 2019) with slight modifications. The samples were analyzed on a DB-5 ms capillary column (30 mm × 0.25 mm × 0.25 μm, Agilent, USA). Twenty-eight mixed aroma compounds standards of varying concentrations were prepared and then diluted into six gradients. Six gradient aroma mixed standards (ranging in concentration from 0.0036 mg/kg to 3.6 mg/kg) were added respectively to the artificial odorless matrix containing 2-methyl-3-heptanone (internal standard, 1.70 mg/mL) and analyzed using gas chromatography-selected-ion monitoring (GC-SIM) for these aroma mixed standards. Notably, all calibration curves exhibited strong linear correlations, with R^2 values exceeding 0.99 (Table 1), as well as the recovery ranged from 71.46 % to 125.83 %. The method of making artificial odorless matrix was as follows: the artificial odorless matrix, composed of dichloromethane and *n*-hexane added to Douchi at a ratio of 2:1:1 (w/w/w), was treated to enhance the extraction process. The mixture was shaken for 12 h, and the organic solvent was filtered. This operation was repeated four times to ensure thorough extraction of the aroma. Subsequently, the samples were stored in a −80 °C refrigerator (DW-HL218, Melng, Anhui, China) and then subjected to vacuum freeze drying at −40 °C for 48 h in a vacuum freeze dryer (SCIENTZ-10 N, SCIZNTZ, Ningbo, China).

Meanwhile, aroma compounds in three types Douchi species were detected in SIM mode. The quantification of aroma compounds in three tpyes of Douchi relied on the calibration curves.

Odor Activity value (OAVs)

The Odor Activity Values were calculated by comparing the concentrations (C) of aroma-active compounds with their sensory thresholds (T), which were obtained from the literature. The formula is referred to (Zhang et al., 2022) as follows:

$$OAV = \frac{C}{T}$$

Sensory analysis

Panel training

The sensory profiling study was conducted at the Laboratory of Hunan Agricultural University in Changsha, China. The panel comprised of 3 males and 6 females, aged between 22 and 27 years, who were selected from students majoring in Food Science and Technology using the national standard method (GB/T 16291).

Aroma recombination and omission experiments

Aroma Recombination method described by (Wang et al., 2021) was utilized for Douchi. The aroma recombination model was performed by mixing all quantitated key aroma-active compounds (OAVs > 1) by their

Table 1
Aroma-active compounds with OAVs > 1 in three types of Douchi.

Compounds	Threshold (mg/kg)[a]	OAVs			calibration eq [b]	R^2
		LY	PJ	YJ		
Acetic acid	99	<1	<1	<1	y = 0.1043x + 0.050	0.99
Isopentyl formate	-[c]	–	–	–	y = 0.1203x + 1.7633	0.994
2-Methylpyrazine	30	<1	<1	<1	y = 0.1397x + 0.0018	0.993
DL-3-Methylvaleric acid	0.046	4	6	5	y = 0.1999x + 0.0116	0.998
2-Methyl butyric acid	5.8	<1	<1	<1	y = 0.0518x + 0,0012	0.997
2,5-Dimethyl pyrazine	1.75	<1	<1	<1	y = 0.2767x + 0.0111	0.992
4-Methylvaleric acid	0.041	8	32	6	y = 0.4975–0.001	0.991
5-Methyl-3-heptanone	0.81	<1	<1	<1	y = 0.5012x-3.473	0.995
Benzaldehyde	0.75089	<1	2	<1	y = 1.405x + 0.2134	0.994
Dimethyl trisulfide	0.0001	377	1183	81	y = 0.8858x + 0.0104	0.993
1-Octen-3-ol	0.0015	59	398	124	y = 0.7863x + 0.0061	0.992
Benzeneacetaldehyde	0.0063	30	290	244	y = 0.6230x + 0.453208	0.996
2-Acetyl pyrrole	1	<1	2	1	y = 0.1247x + 0.0054	0.99
3,5-Dimethyl-2-ethylpyrazine	-[c]	–	–	–	y = 0.5020x + 0.0019	0.998
Tetramethylpyrazine	2.52502	<1	<1	<1	y = 0.3160x + 0.0023	0.9929
Guaiacol	0.00084	2186	1130	430	y = 0.5280x + 0.0060	0.995
6-Octadien-3-ol	0.00022	135	130	97	y = 0.3971x + 0.0144	0.991
Phenylethyl Alcohol	0.69	1	3	<1	y = 3552x + 0.0106	0.991
3-Hydroxy-2-methyl-4H-pyran-4-one	0.56423	<1	<1	<1	y = 0.0247x + 0.0051	0.998
2-Isopropyl-5-methyl-2-hexenal	1.24	<1	<1	<1	y = 0.1329x + 0.0023	0.99
3,5-diethyl-2-methylpyrazine	0.0075	18	3	<1	y = 2.7747x + 0.0054	0.997
1,4-Dimethoxybenzene	–	–	–	–	y = 5.0524x + 0.0247	0.994
3,7-Dimethyl-2,6-octadienal	0.04	<1	<1	<1	0.2453x + 0.0064	0.993
Ethyl phenylacetate	0.15555	<1	4	<1	y = 5.5175x + 0.0298	0.99
Phenethyl acetate	0.24959	<1	<1	<1	y = 4.4276x + 0.0136	0.993
2-Phenyl-2-butenal	7.38	<1	<1	<1	y = 0.0789x + 0.0052	0.992
gamma-Nonanolactone	0.79	<1	<1	<1	y = 1.9592x + 0.0110	0.991
(2E)-5-Methyl-2-phenyl-2-hexenal	0.05	2	15	19	y = 4.2395x + 0.0124	0.991

a: Odor thresholds in water taken from the literature. b: Equations of standard curves. c: The odor threshold was not queried from the literature.

natural concentrations in an artificial odorless matrix.

Omission experiments were used for Douchi, as described by (Liu et al., 2019). Thirty-two models were constructed by omitting only one odorant from the recombination model for each of the three samples. The sensory evaluation was performed by triangle test between the aroma-removed model and the fully reconstituted model.

Statistical analysis

The data were subjected to statistical analysis using Microsoft Excel 2016. The results were presented as mean ± standard deviation, based on triplicate measurements. Additionally, radar charts and Flavor Wheel Construction were generated using Microsoft Excel 2016. Heatmap was executed through online tools (https://www.chiplot.online/). And stacked bar graph was drawn by DataGraph 5.0. Analysis of CATA data using XLSTAT 2023. SPSS 26.0 software was used for significance analysis. A difference of $P < 0.05$ was considered significant.

Results and discussions

Cata analysis of three different types Douchi

As illustrated in Fig. 2A, with the ranking of consumer preference for the three varieties of Douchi as follows: LY > PJ > YJ. However, the results indicated that there were no significant differences among the three samples, possibly due to the diverse nature of consumer preferences. Further, the ordinate in the Fig. 2B represents the difference in average consumer preference when the descriptor is present or absent, while the abscissa represents the frequency of descriptor usage. Visibly, "Sour", "Beany flavor", "Sulfur" and "Alcohol" were the primary driving factors in reducing consumer preference for Douchi. The "Sauce-like", "Smoky", "Nutty", "Roast", "Caramel", and "Flower" were positive driving forces in consumer preference.

A higher quality of examination was obtained, as 100 % of the total inertia was explained by F1 and F2 in Fig. 2C. And the Fig. 2C shows a contrast between the contexts that products (PJ, LY, YJ) and aroma attributes ("Sauce-like", "Smoky", "Caramel", "Flower", "Nutty", "Sulfur", "Sour", "Alcohol", "Roast", "Beany flavor"). In detail, the expression of sensory characteristics associated with "Smoky", "Caramel", and "Sauce-like" of LY were the primary positive driving factors for consumers preferences, and aroma like "Roast", "Nutty", "Flower" and "Sauce-like" in YJ were considered to be the important factor for consumer selection. Although the aroma like "Sauce-like" and "Flower" of PJ is also favorited, its "Sour" and "Alcohol" aroma might led to nonacceptance of consumers.

Volatile compounds in three different types Douchi

As shown in Fig. 2D, a total of 179 compounds were detected by GC-MS, 102 compounds in PJ, 90 compounds in YJ, and 88 compounds in LY. However, only 28 common compounds were found. As (Wang, Yin, Cheng, & Li, 2012) reported that different production processes or fermentation environments could affected the composition of volatile compounds of Douchi. Volatile compounds were classified into 10 different chemical classes (Fig. 2E and F), in which including hydrocarbons (44), esters (33), and aldehydes (21) were the predominant volatile compounds in Douchi, followed by acids (12), pyrazines (12), ketones (11), alcohols (10), phenols (8), ethers (5), and other compounds (23). From Fig. 2F PJ had various elevated levels of total volatile content (118.86 mg/kg), and then LY (72.73 mg/kg), while the YJ had the lowest level (62.46 mg/kg). Since fermentation time is an essential factor to ensure the flavor of fermented foods' final products, the long fermentation time of PJ Douchi might contribute to the formation of volatile content. Furthermore, the contents of aldehydes, acids, hydrocarbons, and ethers were higher in Douchi. Specifically, acids were more elevated in LY, it might be due to the factor that the low salt environment was conducive to bacterial metabolism to produce additional acid (Chun, Kim, Jeong, & Jeon, 2020). Whereas the aldehyde content was the main substance in PJ and YJ, it is speculated that the differences may be related to more salt addition in them. Research showed that salt-

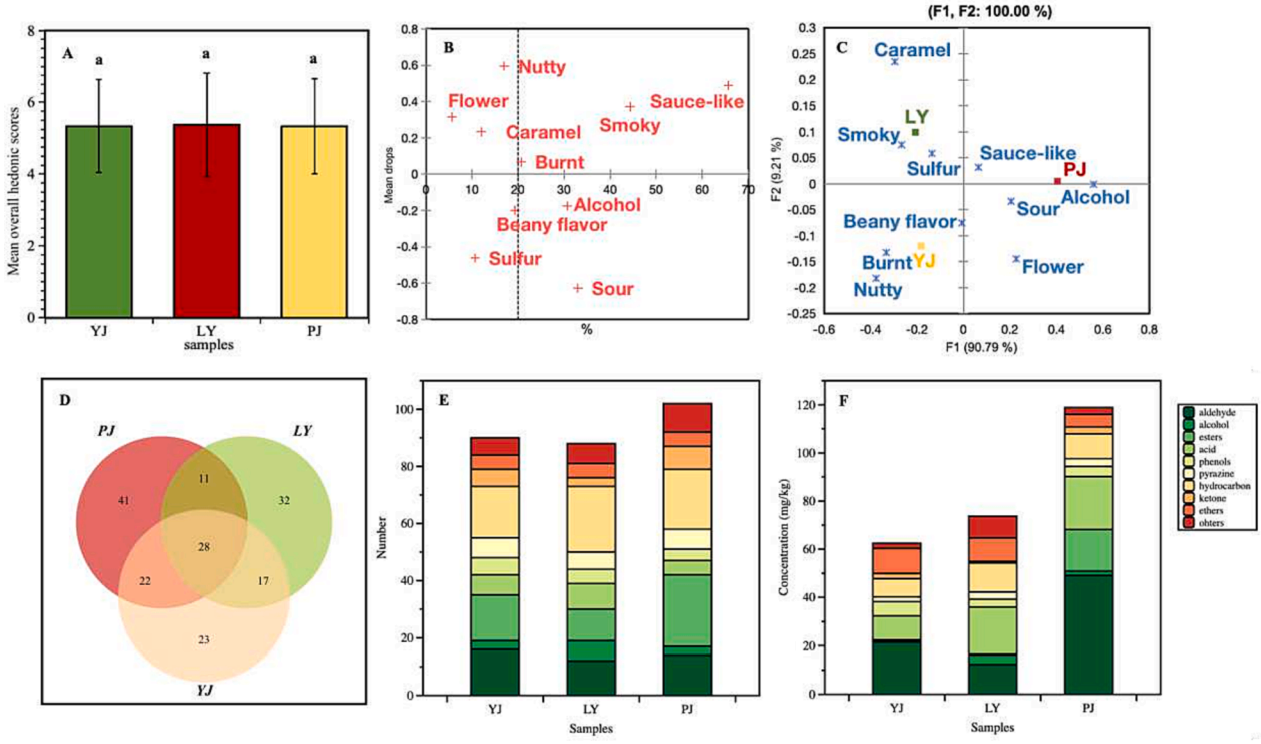

Fig. 2. Bar chart of 9-point hedonic (A), Penalty analysis (B), Catatis (C), Venn graph of volatile compounds (D), Stacked bar graph of volatile compounds Number (E), and Concentration (F) in the three types of Douchi.

tolerant yeast or collaborative fermentation of multi strains could effectively raise the content of aldehyde in soy sauce (Gao et al., 2023).

Aroma compounds in three different types Douchi

GC-O-MS analysis was conducted to gain a deeper understanding of the contribution of each individual volatile compound to the overall aroma. The aroma compounds were identified and characterized by comparing the mass spectra data with the NIST20 database and comparing odor descriptions to reference standards. Twenty-nine compounds were detected (Fig. 3A) which include 6 aldehydes, 4 acids, 4 pyrazines, 4 alcohols, 4 esters, 2 heterocycles, 1 ether, 1 ketone, 1 sulfur-containing compound, and 1 unknown compound with RI values of 894. In YJ, PJ and LY Douchi, 17, 24 and 25 aroma compounds were observed, respectively. These compounds are mainly "Roast", "Fresh", and "Flower" aromas.

During soybean fermentation, the decarboxylation and deamination reactions of the corresponding amino acids were typically involved in the generation of most aldehydes. Furthermore, Millard's reaction during the heating process resulted in the production of some aldehydes (Diez-Simon, Eichelsheim, Mumm, & Hall, 2020). In this study, a total of six aldehydes were identified among three samples. 2-Isopropyl-5-methyl-2-hexenal (Odor intensities range in the sample, 3.0–3.7) emerged as the predominant aroma compound among the saturated aldehydes identified in all samples. This compound exhibited aromas reminiscent of "Smoky", "Medicine", and "Wood", and was consistently observed as one of the major key aroma compounds in fermented soybean food products (Li, Dong, Jeon, So, Zhao, & Baek, 2019). Benzene acetaldehyde, which was detected in the present study with an intensity ranging from 2.0 to 2.7, was found to possess a "Flower" aroma. This compound was also identified as one of the aroma compounds in Yongchuan Douchi, as reported in a previous study (Wang et al., 2021). Additionally, 2-phenyl-2-butenal (2.0–3.0), gamma-nonanolactone (1.7–2.0), and (2E)-5-, ethyl-2-phenyl-2-hexenal (1.7–2.0) were only detected in PJ and LY samples. Among them, 2-phenyl-2-butenal (floral aroma) was the key aroma-active compound in vinegar (Al-Dalali, Zheng, Sun, Chen, Wang, & Wang, 2020) while gamma-nonanolactone was identified in fermented soybean products (Zhao, Feng, Hadiatullah, Zheng, & Yao, 2021). A smaller number of aldehydes were identified in YJ Douchi. Similar reports have been made in previous studies (Guo, Zhang, Long, Fu, & Ren, 2023).

For alcohols, 6-octadien-3-ol with intensity 4, which had a burnt and wood aroma, could be detected in all the Douchi samples. Its high intensity indicated a noteworthy contribution to Douchi favor. Additionally, 1-octene-3-ol with a "Mushroom" aroma only existed in YJ and PJ, which was reported as a common flavor compound in fermented soybean products and might have been produced from the metabolism of unsaturated fatty acids by microorganisms (Lee, Kim, & Kim, 2019). Phenylethyl alcohol ("Flower", intensity 2–4) was also detected in PJ and LY samples. Usually, phenylethyl alcohol could be produced through the benzene acetaldehyde/phenethylamine route, as well as the pathway of *trans*-cinnamic acid and phenyl lactate (Gao et al., 2021).

Pyrazines were found to have been present in the fermentation of soybean products and were derived from two pathways, involving the reaction of carbonyl compounds formed by lipid oxidation with amino groups (Diez-Simon et al., 2020). A total of 5 pyrazines were identified, including tetramethyl pyrazine, 2-methyl pyrazine, 2,5-dimethyl pyrazine, 3,5-diethyl-2-methyl pyrazine, and 3,5-dimethyl-2-ethyl pyrazine. Wherein 2,5-dimethyl pyrazine ("Roasted nut") might be the major aroma-active compounds according to their high odor intensity (2.3–4), and the obvious difference of 2,5-dimethylpyrazine in samples was found, indicating that it could be the discriminating aroma of Douchi. Due to the different pyrazines production capabilities of the different strains (Yin et al., 2018), 3,5-dimethyl-2-ethylpyrazine was not detected in YJ while 3,5-diethyl-2-methylpyrazine and tetramethyl pyrazine just existed in LY Douchi. Meanwhile, the discrepancy may be related to the production processes in the content of water and salt, or raw materials, or the combination of these factors (Que, Jin, Huang, Zhou, & Wu, 2023; Wang et al., 2012).

Acids were produced by two pathways, microbial-induced oxidation as well as the hydrolysis of esters. They were also considered to be the most essential precursor compounds for the synthesis of esters, aldehydes, alcohols, and other compounds (Fan, Liu, Qiao, Zhang, Leng, & Chen, 2019). Both dl-3-methylvaleric acid and 4-methylvaleric acid were identified in the three samples, similar results were reported by (Zhao, Cao, Cai, Su, & Feng, 2016). Besides, 2-methyl butyric acid ("Popcorn", 2.7–3) had a high aroma intensity in the PJ and LY samples. A strong positive correlation between 2-methyl butyric acid and soluble protein levels has been reported (Yan & Dong, 2019).

Furthermore, guaiacol (intensity = 4), the aroma of "Smoke" and

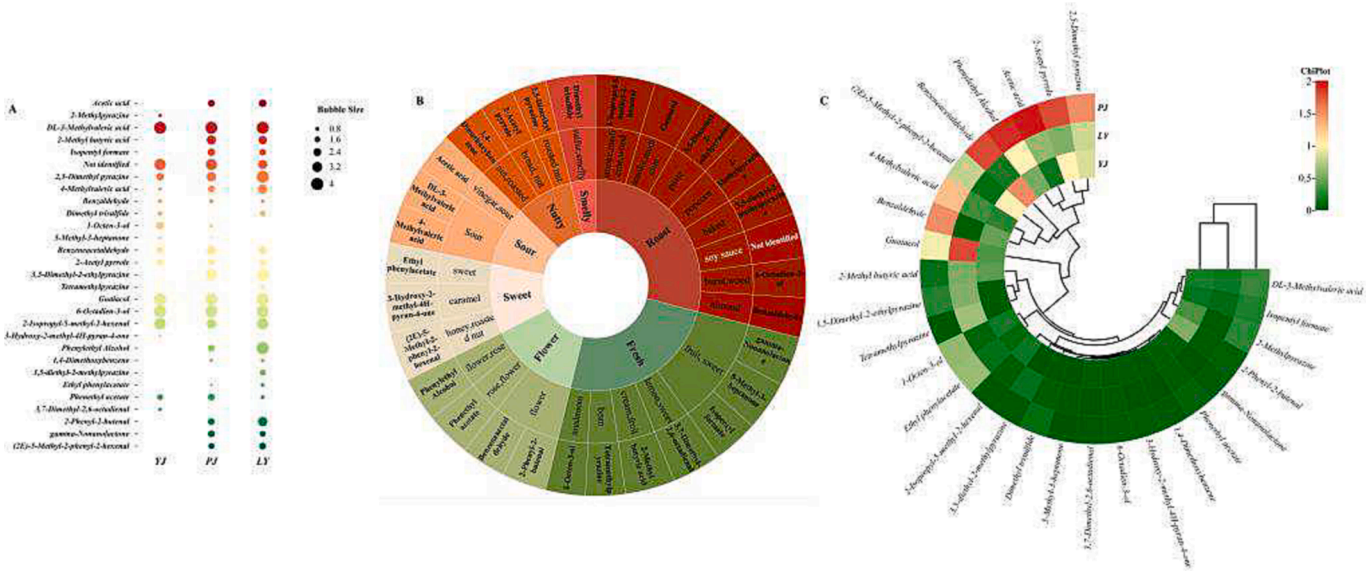

Fig. 3. Heatmap of the aroma active compounds in three types of Douchi by GC-O-MS (A); Flavor Wheel Construction of aroma active compounds in three types of Douchi by GC-O-MS (B); Heatmap of aroma compounds in three types of Douchi. Color coding is graded from green to red, with relative strength increasing from low (green) to high (red) (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

"Medicine", as well as the unidentified compounds emitting "Sauce" aroma (3–3.7), had strong aroma intensity in all samples, indicating their elevated contribution to aroma of Douchi. Although 2-acetyl pyrrole ("Bread", "Nut"), 1,4-dimethoxybenzene ("Nut", "Roasted"), dimethyl trisulfide ("Sulfur", "Smelly"), and 5-methyl-3-heptanone ("Fruit", "Sweet") simply showed a lower aroma intensity in Douchi, it also might play an influential role in the harmony and complementation of Douchi flavor (Chen, Xu, Wu, Xu & Pan, 2011).

Aroma-active compounds identified by OAVs in three different types Douchi

The food aroma was correlated with the Odor Activity Values (OAVs) of the volatile compounds. Generally, compounds with OAVs > 1 made a more substantial impact on the aroma of Douchi. The concentrations and OAVs of the volatile compounds, as determined by GC-MS, are presented in Fig. 3C and Table 1.

Obviously, difference could be intuitively seen in the volatile flavor substances content of the three samples according to a heatmap (Fig. 3C). Specifically, samples of LY and YJ Douchi had a lower remarkably average overall concentration of total compounds (8.15 mg/kg, 8.52 mg/kg) than those from Douchi produced in the PJ (16.52 mg/kg). This could be also seen from the cluster analysis that the aroma-active compounds of LY and YJ were more similar in composition (Fig. 3C). It might be due to similar dominant fermentation microbe (*Aspergillus*) and raw material of YJ and LY Douchi. Specifically, 9, 13 and 10 aroma compounds with OAVs > 1 were calculated in YJ, PJ and LY Douchi, respectively (Table 1). The aroma-active compounds were categorized into "Sour", "Flower", "Nutty", "Smelly", "Roast", and "Fresh" types according to their aroma characteristics except for the OAVs of compounds with "Roast" aroma (benzaldehyde, 3,5-diethyl-2-methylpyrazine, guaiacol, 6-octadien-3-ol) compounds in LY were 84.68 % and 343.64 % higher than those in PJ and YJ, other OAVs of aroma compounds in PJ was higher than that in LY and YJ. These differences in OAVs of aroma compounds would inevitably lead to aroma discrepancies among the three types of Douchi.

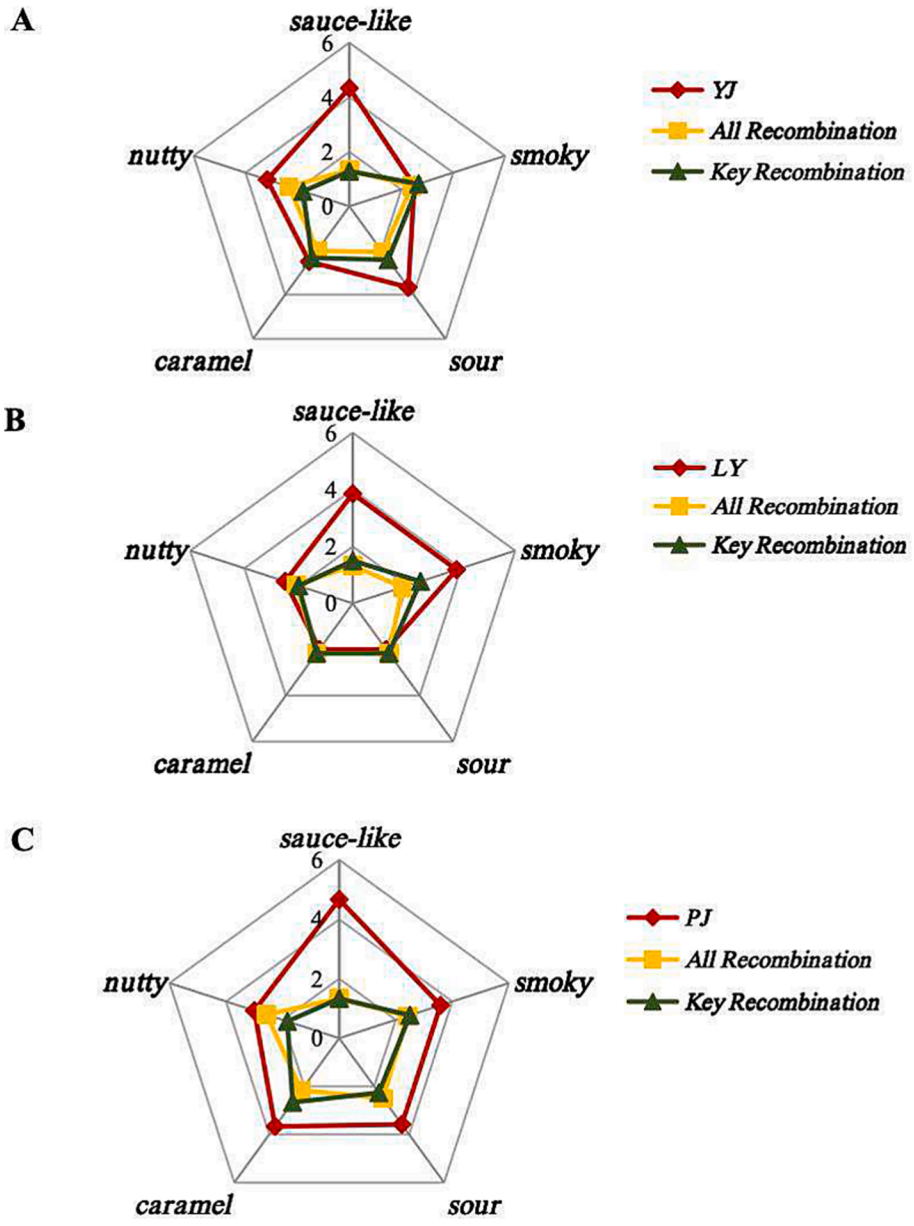

Fig. 4. Aroma recombination modeling in three types of Douchi (A, B, C), including original aroma, all aroma-active recombination models as well as key aroma-active recombination models.

Some volatile compounds with high OAVs, such as dimethyl trisulfide and 1-octen-3-ol with high OAVs exhibited relatively low intensities in GC-O-MS, while some volatile compounds (i.e., 2,5-dimethyl pyrazine) had obvious aroma intensities but low OAVs similar results were found by (Bi, Xu, Luo, Lao, Pang, & Shen, 2020; Zhu & Xiao, 2018). It might be due to the factor that the aroma propagation conditions, and medium cannot be entirely unified, as well as the deviation caused by smelling by different sensory evaluators might result in OAVs and aroma intensity are inconsistent (Zhu & Xiao, 2018).

Aroma recombination analysis

To further validate the contribution of high OAVs compounds to the overall aroma of the three samples, aroma recombination experiments were conducted. The model of recombination experiment was prepared from odorless-matrix, saturated NaCl, and standards of aroma compounds with OAVs > 1. The model was evaluated by nine trained panelists. Having considered the frequency of descriptor usage and their driving factors for consumers, as well as referencing the work of (Wang et al., 2021), ultimately, we opted to conduct aroma recombination experiments using "Smoky", "Sauce-like", "Caramel", "Nutty", and "Sour" aroma characteristics.

As illustrated in Fig. 4, YJ, PJ and LY and their respective recombination models had slightly different intensities of the various aroma characteristics (Potential key aroma-active compounds or all aroma compounds), especially for the "Sauce-like" aroma. It might be related to the unidentified compounds with soy sauce aroma (Fig. 3B). The compounds with like "Sauce" aroma in Yongchuan Douchi was also unidentified (Wang et al., 2021). Additionally, the "Sauce-like" aroma may be related to the combined influence of multiple compounds including pyrazines, sulfides, furans, phenolic, acid, and ester.

In YJ, the aroma of "Caramel", "Smoky" and "Nutty" was simulated fine. As well as, "Caramel", "Sour", "Smoky", and "Nutty" aromas simulate nicely in LY, indicating that those aroma-active compounds (e.g., 6-octadien-3-ol, benzene acetaldehyde et) could characterize the aroma profiles of YJ and LY. Notably, the simulation results of the PJ model were poor, it indicated that some low-intensity aroma-active compounds that might contribute to the overall aroma of PJ were ignored in GC-O-MS identification. Moreover, when aroma compounds interact together might also affect the olfactory of sensory panelists (Zhang, Gao, Xia, Jiang, Liu, & Xu, 2022).

Notably, YJ Douchi has a heavier aroma of "Sour" and "Nutty". Similarly, Guo Weidan et al. (2023) observed a higher concentration of lactic acid in Yangjiang Douchi than in Liuyang Douchi and Yongchuan Douchi. Whereas LY Douchi was more prominent in the "Smoky" aroma, it might result from the higher level of guaiacol. Specially, PJ Douchi has excellent performance in all aromas, which was consistent with the results of GC-MS and may be related to its much longer fermentation time (above 1 year).

Omission analysis

To investigate the important contribution of this aroma compound to Douchi, omission experiments were performed. According to Table 2, individual compounds were omitted in three samples, and a total of 32 omitted models were prepared. The omitted models were compared to a recombinant model containing all compounds with OAVs > 1.

As presented in Table 2, dimethyl trisulfide, benzene acetaldehyde, and 3,5-diethyl-2-methylpyrazine contributed to the overall aroma of PJ. It was also discovered by Zhang et al. that the "Barbecue" flavor was contributed to by 2-ethyl-3,5-dimethylpyrazine, which was produced by the condensation of carbonyl compounds produced by Strecker's degradation of amino acids or by reaction with Maillard (Zhang, Cao, Pei, Wei, Xiang, & Cao, 2019). In particular, as far as the present results are concerned, the aroma of 2-ethyl-3,5-dimethylpyrazine stands out as a crucial differentiating key aroma-active compound that distinguishes PJ from other Douchi. Ineffably, this result was not consistent with the CATA result. The CATA may be more aligned with the sensory perceptions of consumers, but it presents challenges in assessing the intensity of aroma characteristics (Alexi et al., 2018). Dimethyl trisulfide may be derived from methionine, which was abundant in soybeans, by enzymatic or non-enzymatic degradation (Qin & Ding, 2007). Similarly, phenylacetaldehyde has been identified as the critical aromatic compound responsible for the "Honey-like" odor in soy sauce, which was formed in the Ehrlich pathway (Zhao, Ding, Hadiatullah, Li, Wang, & Yao, 2020).

A significant difference ($P \leq 0.01$) was observed when benzene acetaldehyde was omitted, indicating its crucial role in the aroma of YJ. Additionally, the omission of dimethyl trisulfide and 2-acetyl pyrrole resulted in a significant difference ($P \leq 0.05$), suggesting their contribution to the "Sulfur", "Smelly", "Bread", "Nutty", and "Floral notes" in the aroma of YJ. This finding was consistent with the results of CATA analysis (Fig. 1C). Furthermore, 2-acetyl pyrrole serves as the cornerstone for defining rice quality (Sansenya et al., 2018). Sulfur-containing compounds widely exist in many kinds of food, generally low content of sulfur-containing compounds produces good aroma effects on foods, whereas at high content there was adverse aroma formation. Their distinctive nature contributed to the unique aromas of PJ and YJ.

In the case of LY Douchi, the omission of guaiacol led to a significant difference ($P \leq 0.05$), highlighting the significant contribution of "Smoky", "Medicine" to the aroma of LY. As reported previously, *Aspergillus* can secret laccase and release phenolic compounds to stimulate cell wall degradation and result in the decarboxylation of lignin-related phenolic components, which contribute to the formation of

Table 2
Omission Experiments from the recombinant model.

Aroma-active compounds omitted from the recombinant model	YJ		LY		PJ	
	N [a]	Significance [b]	N	Significance	N	Significance
DL-3-Methylvaleric acid	5		3		5	
4-Methylvaleric acid	5		1		3	
Benzaldehyde	/[c]		/[c]		1	
Dimethyl trisulfide	6	*	3		6	*
1-Octen-3-ol	5		3		5	
Benzeneacetaldehyde	8	***	5		7	**
2-Acetyl pyrrole	6	*	/[c]		5	
Guaiacol	4		7	**	5	
6-Octadien-3-ol	2		3		3	
Phenylethyl Alcohol	/[c]		3		5	
3,5-diethyl-2-methylpyrazine	/[c]		2		6	*
Ethyl phenylacetate	/[c]		/[c]		4	
(2E)-5-Methyl-2-phenyl-2-hexenal	4		5		5	

a: Number of correct judgments. b: Significance: "*", significant ($P > 0.05$); "**", highly significant ($P \leq 0.05$); "***", very highly significant ($P \leq 0.01$). c: Compound was not present in the model.

phenols (Chung, Hui, & Cheng, 2002). Guaiacol not only serves as a key aroma-active compound in LY, identically, but also the clearest aroma that distinguishes LY from other Douchi, and similar results were obtained in the CATA analysis (Fig. 1C).

Nevertheless, some aroma-active compounds with OAVs > 1 did not show significant differences, like dimethyl trisulfide in LY, and ethyl palmitate in PJ and YJ. High OAVs were not always associated with high significance due to aroma omission experiments. Ethyl propionate, ethyl 2-methyl butyrate, and ethyl 2-methyl propionate, which had high OAVs but no significant effect on the aroma of citrus (Xiao, Wu, Niu, Wu, Zhu, & Zhou, 2017). Several studies had demonstrated that the masking, antagonistic, or synergistic effects between different aroma compounds, as well as structural differences between the odorless matrix and the original sample, may be led to different results for OAVs and omission experiments (Xu, Xu, Jia, Feng, Huang, & Ho, 2019; Zhang et al., 2022).

Conclusion

In summary, there were notably differences in aromas profile of different types of Douchi. Besides "Sauce-like" was positive driving forces in consumer preference for three types of douchi, the "Smoky" for LY, "Nutty" and "Roast" for YJ,"Flower" for PJ were also favored by consumers. Further, a total of 179 volatile compounds were identified by GC-MS, followed by 29 aroma compounds were characterized by GC-O-MS. However, there were just 9, 13, and 10 aroma-active compounds with OAVs > 1 found in YJ, PJ, and LY, respectively. Among them, guaiacol ("Smoky" and "Medicine" aromas) was proved to be the key aroma-active compound in LY, benzene acetaldehyde ("Flower" aroma), dimethyl trisulfide ("Sulfur" and "Smelly" aromas), and 2-acetyl pyrrole ("Nutty" aroma) were considered to contribute to the aroma of YJ, and benzene acetaldehyde ("Flower" aroma) and 3,5-diethyl-2-methyl pyrazine ("Roast" aroma) contributed to the overall aroma of PJ. Moreover, one unknown aroma compound (RI values of 894) presenting the aroma of soy sauce has not been characterized and still needs to be obtained and evaluated in future work.

Ethical approval

Informed consent was obtained from all the participants prior to the enrollment of this sensory analysis panel training.

CRediT authorship contribution statement

Ziqian Wu: Writing – review & editing, Writing – original draft, Data curation. **Jin Chao:** Supervision, Resources. **Hui Tang:** Resources. **Tengxia Liu:** Supervision. **Liwen Jiang:** Funding acquisition, Resources, Supervision. **Yang Liu:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, 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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