Food Chemistry: X 25 (2025) 102002

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Tracing the change of the volatile compounds of soy sauce at different fermentation times by PTR-TOF-MS, *E*-nose and GC–MS

Qixin Kan [a,1], Longbipei Cao [b,1], Liping He [a], Peipei Wang [a], Guangdie Deng [a], Jun Li [a,c], Jiangyan Fu [c], Qingrong Huang [b], Chi-Tang Ho [b], Yunqi Li [d], Chunhui Xie [d], Yong Cao [a,*], Linfeng Wen [a,*]

[a] Guangdong Provincial Key Laboratory of Nutraceuticals and Functional Foods, College of Food Sciences, South China Agricultural University, Guangzhou, Guangdong 510642, China

[b] Department of Food Science, Rutgers University, New Brunswick, NJ 08901, USA

[c] Guangdong Meiweixian Flavoring Foods Co., Ltd., Zhongshan 528437, China

[d] Department of Polymer Materials and Engineering, College of Materials and Metallurgy, Guizhou University, Guiyang 550025, China

ARTICLE INFO

Keywords:
Soy sauce
VOC
PTR-TOF-MS
Machine learning

ABSTRACT

Proton-transfer reaction time-of-flight mass spectrometry (PTR-TOF-MS), combined with electronic nose (*E*-nose), was first used to track the change of volatile organic compounds (VOCs) in soy sauce in this study. The results showed that 163 VOCs with different mass numbers were identified. Based on the differences in VOCs, the entire fermentation cycle was divided into four stages (0D and 15D; 30D-75D; 90D; 105D—120D). The key stage of aroma formation was on day 90, when the VOC content reached its peak. It was clear that the key feature differential component was nonanal and phenethyl acetate by partial least squares discriminant analysis model, which showed significant correlation with the fermentation time. Moreover, the Quadratic Discriminant Analysis and Linear Discriminant Analysis models can accurately predict the fermentation cycle of soy sauce. The results of study demonstrated the potential of PTR-TOF-MS for comprehensive online monitoring and distinguishing of VOCs change during soy sauce fermentation.

1. Introduction

Soy sauce is an ancient and traditional flavoring condiment. It is mostly generated from soybean, wheat, salt and water following their natural fermentation with *Aspergillus*, halophilic lactic acid bacteria, salt-tolerant yeast and other microorganisms (Devanthi & Gkatzionis, 2019; Ito & Matsuyama, 2021). Chinese soy sauce was first recorded 2200 years ago during the Western Han Dynasty (Diez-Simon, Eichelsheim, Mumm, & Hall, 2020). As Buddhist etiquette gained popularity, it expanded to Japan, Korea, and Southeast Asian countries (Lioe, Selamat, & Yasuda, 2010). Currently, soy sauce is an indispensable component on the dining tables of East Asian countries.

In general, there are two types of soy sauce fermentation in actual production: high-salt liquid-state fermentation and low-salt solid-state fermentation in China (Wang et al., 2023). Typically, it takes 4 to 6 months to produce traditional high-salt liquid-state fermentation soy sauce, which goes through three stages of fermentation: culturing mold, fermentation, and refining (Devanthi & Gkatzionis, 2019). Throughout the entire fermentation process, the difference of raw materials used, the duration of the fermentation process, the temperature, the composition of the microbial flora, proteases and other factors might affect the flavor and quality of soy sauce (Chen et al., 2023; Liu, Yang, Liu, Lu, & Wu, 2023). The substrates and fermentation processes used in the production of Japanese and Chinese soy sauces were key determinants of their distinct flavor profiles (Diez-Simon et al., 2020). Japanese soy sauces generally contain a higher wheat content than Chinese soy sauces, leading to the conversion of wheat into additional carbohydrates during fermentation. These alterations in carbohydrate composition significantly influenced the final aroma and taste characteristics of the soy sauce (Wang et al., 2019). Furthermore, Japanese soy sauces were typically fermented under controlled temperatures, whereas Chinese soy sauces undergo open-air fermentation. Previous research had

* Corresponding authors at: College of Food Science, South China Agricultural University, Guangzhou 510642, China.
E-mail addresses: caoyong2181@scau.edu.cn (Y. Cao), wenlinfeng123@126.com (L. Wen).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.fochx.2024.102002
Received 5 September 2024; Received in revised form 30 October 2024; Accepted 12 November 2024
Available online 14 November 2024

highlighted that controlled fermentation can enhance the sensory attributes of winter-produced soy sauces, providing insights into the molecular underpinnings of these improvements (Liu, Feng, Zhao, & Huang, 2023).

In contrast to the soy sauce production in Japan, traditional Chinese high-salt liquid-state soy sauce undergoes fermentation under variable conditions, including open environments, fluctuating temperatures, and disturbances from external microorganisms (Devanthi et al., 2019). In contrast, the fermentation process of Japanese soy sauce has developed a stable control system, whereas the traditional fermentation process of Chinese soy sauce remains traditional process (Murooka & Yamshita, 2008). Due to the lengthy fermentation process, the complexity of soy sauce flavor made it difficult for traditional flavor analysis techniques to meet the needs of large scale production testing. However, there is still a lack of objective and quick detection measure in actual production. At present, even in relatively modern factories, soy sauce fermentation in China is mainly judged by the subjective experience of the sensory experts, leading to confusion in quality control and affects the consistency of the final product quality (Wei et al., 2013). In China, the prevailing approach to evaluating the quality of soy sauce fermentation adheres to the GB 2717–2018 standard. The assessment predominantly focuses on sensory attributes, including color, taste, and aroma, in conjunction with the determination of amino acid nitrogen content. In the production involving tens of thousands of large fermenters, the problem of determining a fast, objective, and accurate method to meet the needs of large-scale industrial production and soy sauce product control is a practical one.

With the progression of artificial intelligence technology and the innovation of biomimetic materials, a new generation of intelligent sensory systems has come to the fore. These systems, which include the electronic nose, electronic tongue, electronic hand, and electronic eye, are characterized by the seamless integration of advanced sensing hardware and machine learning techniques as their central framework, thereby evolving incrementally within the domain (Loutfi, Coradeschi, Mani, Shankar, & Rayappan, 2015; Peris & Escuder-Gilabert, 2009). Intelligent senses systems and machine learning techniques both play important role in the process of food production, processing, quality control and storage (Lin, Ma, Wang, & Sun, 2022; Lu, Hu, Hu, Li, & Tian, 2022). In recent decades, electronic nose has been fully applied in the field of food flavor, including fermentation, fruits and vegetables, beverages, meat, condiments and other food research hotspots. The rapid, non-destructive measurement method and low sensor cost of *E*-nose have attracted a lot of research attention (Feng, Wang, Wang, Huang, & Kan, 2022; Ghasemi-Varnamkhasti et al., 2011; Song et al., 2023). The application of electronic noses (*E*-noses) in soy sauce analysis has shown promising potential. However, initial studies, such as those by Aishima, have highlighted the challenges of correlating e-nose responses to sensory attributes due to sensor limitations (Aishima, 2004). The electronic nose has relatively few applications in the actual industry. Currently most research is applied to the analysis of differences among various soy sauce varieties (Gao et al., 2017). Recent research has demonstrated the effectiveness of e-noses in distinguishing different types of soy sauce and identifying key volatile compounds (Li et al., 2024; Wang, Liang, Zhang, Wu, & Liu, 2023). E-nose analysis, combined with techniques like GC-IMS, GC–MS, and PLS-DA, provided valuable insights into the complex aroma profiles of soy sauce and offers a rapid, non-destructive alternative to traditional sensory evaluation methods (Li et al., 2024). However, further development of more specific and diverse sensors is crucial for enhancing the discriminative power and reliability of e-noses in soy sauce analysis.

As a special analytical method of VOCs, PTR-MS is mainly used to study the emission, distribution and chemical evolution of VOCs in the atmosphere (Yuan et al., 2017). Different from traditional chromatographic analysis, organic matter (VOC) is ionized to form VOC-H^+ (protonated compounds), and then monitored by mass spectrometry. It is also a rapid, direct and highly sensitive method for non-invasive online monitoring of VOCs (Yuan et al., 2017). At present, PTR-MS has been preliminarily applied to the analysis of VOCs in different fields of food science: first, it is used to explore the flavor differences of different spices, fruit, milk, tea and other foods, for non-destructive rapid identification and determination (Ciesa et al., 2015; Silvis, Luning, Klose, Jansen, & van Ruth, 2019); second, it is used to investigate the relationship between dynamic release of aroma components and sensory evaluation (Muñoz-González, Canon, Feron, Guichard, & Pozo-Bayón, 2019; Romano et al., 2022); third, it is used to track the influence of microbial metabolism on VOCs during the fermentation process (Berbegal et al., 2020). Compared with traditional GC–MS and GC-IMS, this technology opens the door for rapid monitoring of complex VOCs with high sensitivity and selectivity and providing possibilities for online measurements.

Table 1
Sensors used in PEN3 electronic nose and main applications.

No.	Sensor name	Performance parameter
1	W1C	Aromatic
2	W5S	Broadrange
3	W3C	Aromatic
4	W6C	Hydrogen
5	W5C	Arom-aliph
6	W1S	Broad-methane
7	W1W	Sulfur-organic
8	W2S	Broad-alcohol
9	W2W	Sulph-chlor
10	W3S	Methane-aliph

In this study, PTR-TOF-MS and electronic nose were first used to jointly monitor the change law of VOCs in soy sauce over time. The study took full advantage of the non-destructive detection, fast and accurate sampling capabilities of PTR-TOF-MS to elucidate the main differential metabolites of volatile metabolites in different fermentation stages of soy sauce. Additionally, this study constructed classification and prediction models for soy sauce with different fermentation levels using various machine learning methods. This study also aimed to propose a new method to monitor the fermentation process of soy sauce and track the quality of soy sauce products by PTR-TOF-MS.

2. Materials and methods

2.1. Materials

All samples of soy sauce were provided by Guangdong Meiweixian Flavoring Foods Co., Ltd. (Guangdong, China). The samples of soy sauce were collected from different fermentation stages of soy sauce from three big production fermenters about 100 t. The initial fermentation time was set to day 0, with subsequent sampling points at days 15, 30, 45, 60, 75, 90, 105, and 120. Samples were gathered and kept at −20 °C for further analysis.

2.2. Reagents

Analytical standards, including C7-C30 normal alkanes (O2SI, American). Sodium chloride, methanol, ethanol, acetonitrile, formaldehyde, and acetaldehyde (analytical grade) were purchased from Tianjin Chemical Reagents Company (Tianjin, China). The VOC standards used in the study were all chromatographic grade and were all purchased from Macklin (Shanghai, China): linalool (98 %), 2-methylbutanal (98 %), nonanal (>97 %), benzaldehyde (99.5 %), guaiacol (95 %), octanal (99 %), ethyl acetate (≥99.7 %), isoamyl acetate (≥99.5 %), acetic acid (≥99.9 %), 1-octen-3-ol (98 %), *p*-cymene (99 %), 2-ethyl-3-methylpyrazine (99 %), HEMF (96 %).

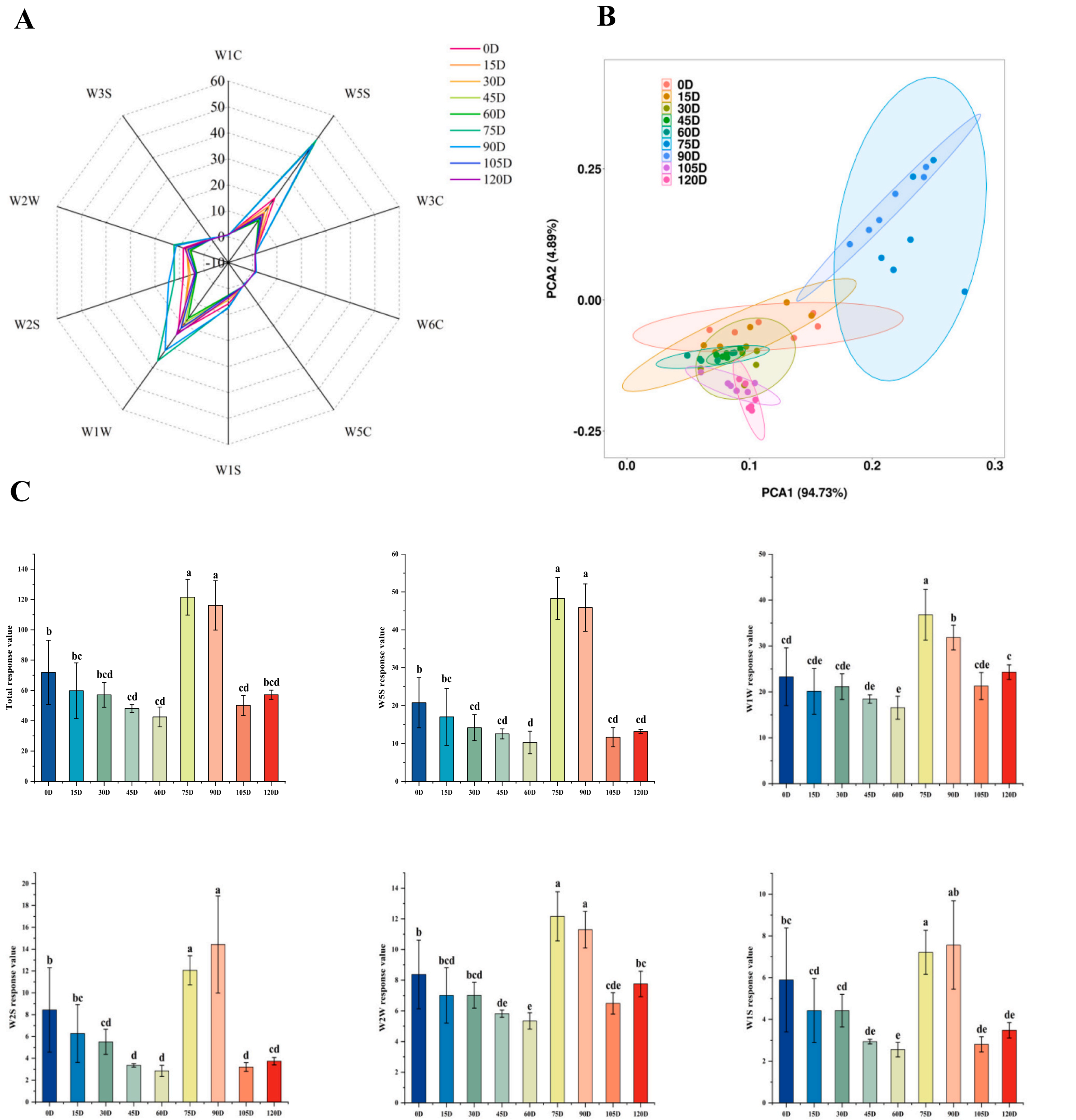

Fig. 1. Radar diagram of the volatile flavor of soy sauce in different fermentation periods (A), the classification of soy sauce subjected under different fermentation days by principal component analysis (PCA) (B), Changing law of total response value and characteristic probe response value (C).
Note: Different letters were considered statistically significant differences among groups according to Duncan's multiple range test ($p < 0.05$). Number of experimental repetitions ($n = 6$).

2.3. Analysis of the volatile odors of soy sauce using electronic nose

An electronic nose PEN3 was used for the aroma of analyses (Airsense Analytics GmbH, Schwerin, Germany), and Table 1 listed all sensors of PEN3 and major application. With a few modest alterations, a previously published approach was used to explore the analytical method (Gao et al., 2017). A 40 mL headspace bottle was filled with a 2 mL sample of soy sauce and then closed. The vials were heated to 45 °C using a thermostatic bath, and the samples were injected one by one after 15 min of headspace generation. 120 s were spent flushing after 150 s of measurement. The smooth response signals from each of the 10 sensors response points, recorded over 100 s using the electronic nose, were used for analysis.

2.4. Analysis of the volatile organic compounds in soy sauce using PTR-TOF-MS

All samples of soy sauce were used to volatile compounds tentative

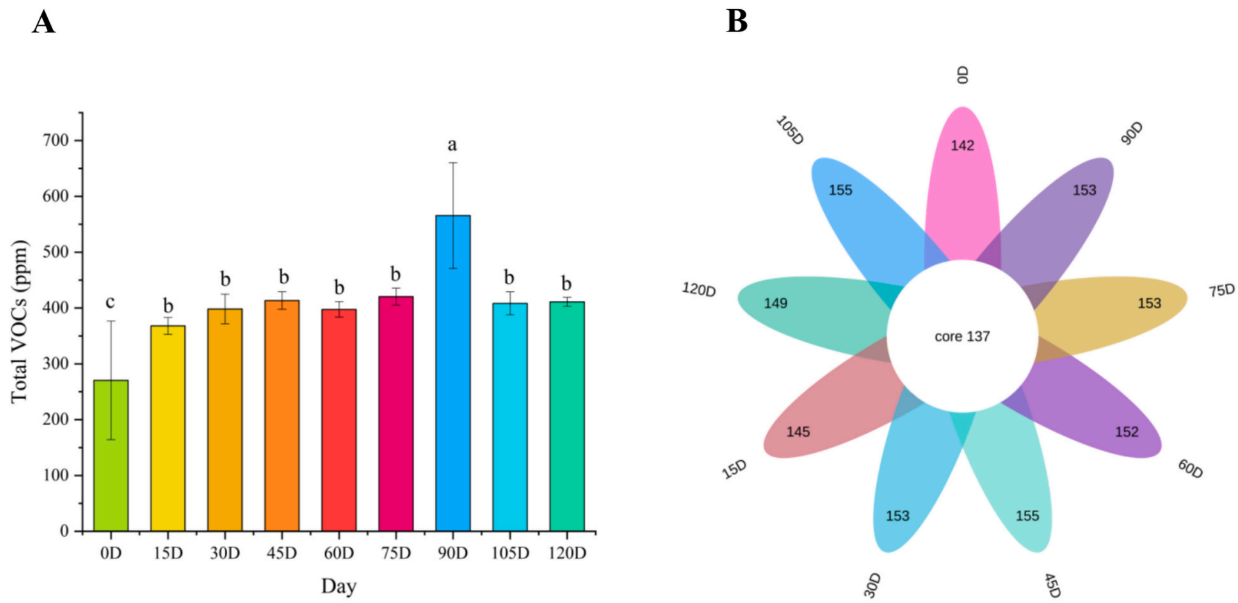

Fig. 2. The change trend of total volatile organic compounds content in soy sauce at different stages (A), the change trend of total volatile organic compounds species in soy sauce at different stages (B), Analysis of VOCs average clustering in soy sauce at different fermentation stages (C).

identification with a PTR-TOF-MS 8000 system (Ionicon GmbH, Innsbruck, Austria). The experimental methods were revised appropriately according to the literature (Berbegal et al., 2020). The preparation of soy sauce and instrument parameters all were optimized. A 6 g sample of soy sauce was placed in the sample bottle, to whi ch 1 g NaCl was added, and the mixture was incubated in 50 °C water for 5 min, and then opening the nitrogen valve and regulating pressure relief valve to stabilize at 0.2 MPa. The chamber ionization conditions were kept as follows: The ion source temperature was 40 °C, drift temperature 80 °C, drift voltage 450 V and drift pressure 2.30 mb, E/N value of 102 Td, inlet sample flow rate of 100 sscm, and 300 cycles were collected.

2.5. Analysis of the volatile organic compounds in soy sauce using GC–MS

Mixed samples were extracted and analyzed according to the method described by (Wang et al., 2023), GC–MS analysis was performed with a slight modification using an HP 6890 series gas chromatograph coupled with an Agilent 5973 N series mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA). The capillary column was a DB-WAX column(60 m length × 0.25 mm i.d. × 0.25 μm; Agilent 122–7062). The oven temperature was initially maintained at 45 °C for 2 min, then increased to 120 °C at a rate of 4 °C/min and held for 2 min, and finally heated to 210 °C at 7 °C/min and held for 10 min. Helium (>99.999 %) was used as the carrier gas at a flow rate of 1 mL/ min. The inlet temperature was maintained at 250 °C. In this study, the analysis of HS-SPME method used a 1:1 split flow mode. The mass spectrometer conditions were set as follows: injector temperature 280 °C, ion source temperature 230 °C, quadrupole temperature 150 °C, scanning range 35–500 *m/z*, and ionization voltage 70 eV. Volatile components of interest were identified by comparing their retention indices (RI) and mass spectra.

2.6. Statistical analysis

All data were expressed as mean ± standard deviation with Microsoft Excel version 2022. Statistical analysis was performed using one-way analysis of variance (ANOVA) with Duncan's test by SPSS Statistics software (version 26). A p-value below 0.05 was considered significant, and a p-value below 0.01 was considered extremely significant. The R Programming Language (version 4.0.3) was used to apply to the analysis of principal components analysis (PCA) (stats package, version 3.5.0), partial least squares discriminant analysis model (PLS-DA) (metaX package, version 2.0.0) and cluster heatmap (stats package, version 3.5.0).

2.7. Machine learning

All machine-learning models were analyzed using Python 3.10 and computed on a Windows 10 64-bit system. Ten different machine learning algorithms were used to distinguish and predict soy sauce samples at different fermentation stages in this study. In research, first of all, the volatile organic compounds served as a predictor, different fermentation periods act as output variables. Then the original dataset was split into training and testing subsets (80 % and 20 %, respectively) of randomly selected records, and the training hyperparameters were optimized and manually tested. Finally, 10 fold cross difference verification was used to prevent over fitting and selection bias problems.

3. Results and discussion

3.1. The analysis of the volatile compounds of soy sauce using electronic nose

In the past few decades, many fields have been reported to use electronic nose applications. Only a few online studies have focused on the electronic nose to be used in the actual production stage of soy sauce fermentation. As shown in Fig. 1A, during the whole fermentation cycle of soy sauce, the *E*-nose response of aroma components in soy sauce was mainly concentrated in W5S, W1W, W2W, W2S and W1S sensors. Based on the characteristics of sensors in Table 1A, it was speculated that soy sauce may contain high content of sulfide; nitrogen oxides; organic sulfur compounds; alcohols; aldehyde compounds and methyl compounds. These results were consistent with previous reports (Gao et al., 2017). The total E-nose response to volatile aroma components across different fermentation periods showed a trend of initial decrease, followed by an increase and then a decrease again, with significant sensor response values at different fermentation stages. The important turning

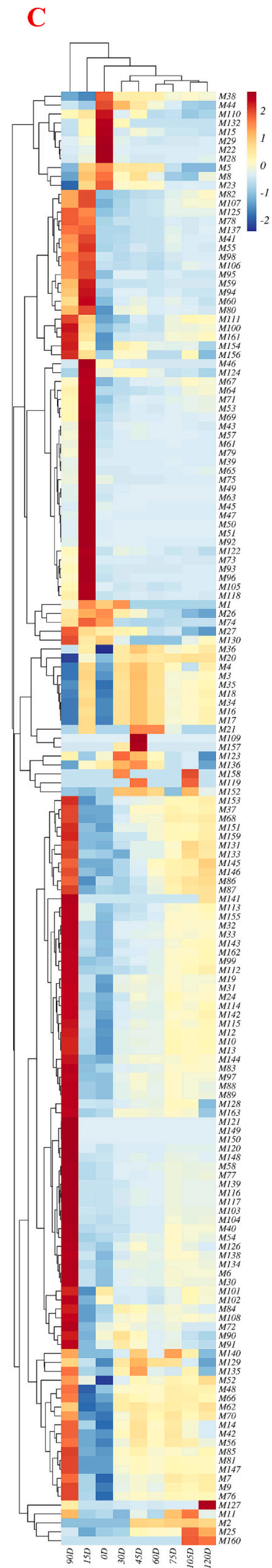

Fig. 2. (*continued*).

point was on day 75 and day 90, when the aroma composition of soy sauce reached its peak, as shown in Fig. 1C.

Principal Component Analysis (PCA) is a statistical technique used to reduce the dimensionality of data while retaining as much of the variability as possible. In the context of soy sauce analysis, PCA can help identify the most significant volatile compounds that contribute to the unique aroma profiles of different soy sauce products (Wang et al., 2019). To differentiate them, the analysis of PCA principal component showed that soy sauce in different fermentation stages can be divided into two categories. Samples from day 75 and day 90 of fermentation were grouped into one category, while the remaining samples were grouped into another category, as depicted in Fig. 1B. The percentage of variance of the first principal component was 94.73 %, the second one was 4.89 %, and the overall score of the 2D principal component was 99.62 %. Additionally, the findings indicated a degree of similarity among soy sauce samples across varying fermentation periods. On the PC1 axis, samples from days 75D and 90D exhibited positive distribution, while samples from days 45D, 60D, 105D and 120D showed negative distribution. On the PC2 axis, there was a strong overlap among the samples, with some confidence intervals overlapping. The results indicated that 75D—90D was the key node for significant change during the soy sauce fermentation cycle. The electronic nose, a biomimetic instrument, can exhibit certain variations in the detection of flavor components (Aishima, 2004). The changes in clearly indicated the overall trend of flavor development in Cantonese fermented soy sauce throughout the fermentation process.

3.2. The analysis of VOCs in soy sauces by PTR-TOF-MS

3.2.1. Identification of volatile components

In contrast to analytical techniques such as gas chromatography–mass spectrometry (GC–MS) and gas chromatography-ion mobility spectrometry (GC-IMS), the PTR-TOF-MS method lacks a separation system, leading to detected data that may coincide with isomeric substances. Initial identification of each mass peak detected by PTR-TOF-MS relies on an in-house chemical standard library, compounds detected by GC–MS analysis, reported literatures, or standard compounds.

Fig. 2A showed the change trend of total VOCs content in soy sauce at different stages. The concentration of volatile compounds in soy sauce varied significantly during different fermentation periods. From the overall change in VOCs content, it can be observed that the aroma content increased as the fermentation time progresses. The VOCs content in the 90D soy sauce sample reached the highest level at 565.58 ± 94.69 ppbv, while the 0D soy sauce sample had the lowest VOCs content at 270.43 ± 106.12 ppbv. A total of 163 mass peaks were identified through careful analysis of the raw data from soy sauce samples, in which 137 VOCs were identified from different fermentation periods in Fig. 2B. The distribution of mass numbers is between 14.01415 and 279.02203, Among them, the mass numbers 19.02842 (NI), 21.02137 (ethylene), 29.0452 (NI), 33.03982 (methanol), 37.04147 (NI), 47.06427 (ethanol), 47.64104 (NI), 61.04382 (acetic acid), 65.07336 (hydrated ethanol) were the main mass numbers (concentration>10,000 ppbv). At the same time, the concentrations of most VOCs showed significant differences across different fermentation cycles ($p<0.05$), especially in the whole fermentation process, 127 mass numbers reached the highest in 90 days. The results of ANOVA of soy sauce at different fermentation stages are shown in Supplementary documentation 3. Through the actual sampling analysis and detection, the detection method of PTR-TOF-MS was closer to the process of human sniffing, which did not require high temperature separation of GC and did not have the problems of thermal decomposition and oxidation of substances (Pozo-Bayón, Santos, Martín-Álvarez, & Reineccius, 2009). Among the detected substances, those with mass numbers between 14 and 33 were frequently found. It remained to be further confirmed whether these components were characteristic of the odor.

3.2.2. Variations in volatile components

In this study, the characteristic products of VOCs were tentatively identified and analyzed in combination with GC–MS and by referencing previous studies. As shown in Supplementary Material 3, 163 VOCs were detected, including acids, alcohols, esters, aldehydes, furans, ketones, phenols, pyrazines, sulfur compounds, and other components. To better analyze the change patterns of VOCs during fermentation, this paper illustrated the change trends of different types of VOCs.

Alcohols, as the main product of soy and wheat metabolism, the main alcohols detected in soy sauce were methanol, ethanol, 2-methyl-1-propanol, 2-methylbutanol, 2,3-butanediol, 2-furanmethanol, benzyl alcohol, hexylene glycol, 1-octen-3-ol, 3-octanol, 1,8-octanediol, linalool and linalool oxide. In most reports, the setting of GC–MS detection method often ignored the molecular weight of less than 33, thus ignoring the presence of methanol components. As the key component of soy sauce, ethanol was the main product of sugar metabolism in the whole fermentation process of soy sauce, which mainly presents a significant alcohol flavor (Wang et al., 2023). Benzyl alcohol, 2-methyl-1-propanol, 2,3-butanediol, 1-octen-3-ol, linalool, linalool oxide and other VOCs have also been reported in other literature (Feng et al., 2022). 2-methyl-1-propanol showed a faint bitter taste, 2,3-butanediol presented a fruity flavor (Gao et al., 2020); benzyl alcohol presents an almond flavor (Zheng et al., 2024); 1-octen-3-ol presents a mushroom flavor, which produced in lipid oxidation in the fermentation process (Feng et al., 2014); linalool and oxidation of linalool presents the pungent odor, which were produced in the metabolism of terpenes (Wang et al., 2023). Most alcohol compounds, except for methanol and 2,3-butanediol, reached their highest content on day 90, which was indeed a critical period for the production of fermented flavors. Similar to other literature reports, the production of ethanol mainly originated from the metabolism of sugars in the substrate. Some alcohols were also produced through the conversion of aldehydes (Chen et al., 2024). In the process of high-salt dilute soy sauce fermentation, 90 days represented the peak point for the accumulation of soy sauce fermentation odor. Over time, the soy sauce underwent further decomposition, and the alcohols were gradually converted into acids or CO_2.

Esters, the characteristic esters components including methyl acetate, ethyl acetate, γ-hexalactone, butylacetate, hexyl acetate, isoamyl lactate, isoamyl acetate, ethyl benzoate, methyl octanoate and phenethyl acetate were detected in this study. Most esters had a distinct fruity flavor and sweetness (Feng et al., 2015; Wang et al., 2023). The key stage of its production was in the late stage of fermentation, which was the most prominent in the 90D fermentation stage. Major free acids and alcohols were esterified by microbial metabolism to produce various esters (Diez-Simon et al., 2020). Moreover, they were mainly produced by long-chain fatty acids after long -term constant fermentation in the presence of fungal lipases. This process played a key role in masking the flavor characteristics of soy sauce (Diez-Simon et al., 2020).

Aldehydes, formaldehyde, acetaldehyde, 2-butenal, 2-methylpropanal, 2-pentenal, 2-methybutanal, 2-furfural, hexanal, benzaldehyde, 2,4-heptadienal, 5-methyl-2-furfural, 2-heptenal, benzeneacetaldehyde, cinnamaldehyde, 2-nonenal, nonanal, hotrienol and decanal were all found during soy sauce fermentation, and some components were first reported from PTR-TOF-MS (Feng et al., 2014). Similar to the variation pattern of other components, the accumulation of most aldehyde components peaked at 90D, including the characteristic flavor components benzaldehyde (burnt sugar, caramel-like), benzeneacetaldehyde (honey-like) and nonanal (green, fatty) (Feng et al., 2014). These short, branched aldehydes can be produced by amino acids through the Maillard reaction or microbial catabolism (Diez-Simon et al., 2020).

Phenols, smoky flavor was the metabolic characteristic of soy sauce; the characteristic components represented by phenol and guaiacol were detected in soy sauce (Feng et al., 2017). The key stage of production was also in the later stage of fermentation, and among them, 90D fermentation stage was the most significant. Phenols were mainly produced through the metabolic pathway of ferulic acid in yeast or related precursor components (Huang et al., 2022).

Ketones and Furans, ketones and furans, as special categories of soy sauce flavor, mainly showed caramel-like aroma. 4-Hydroxy-2-methyl-5-ethyl-3(2*H*)furanone (HEMF), as the most key characteristic aroma ingredients, was sweet, creamy and sauce flavor (Wang et al., 2023).

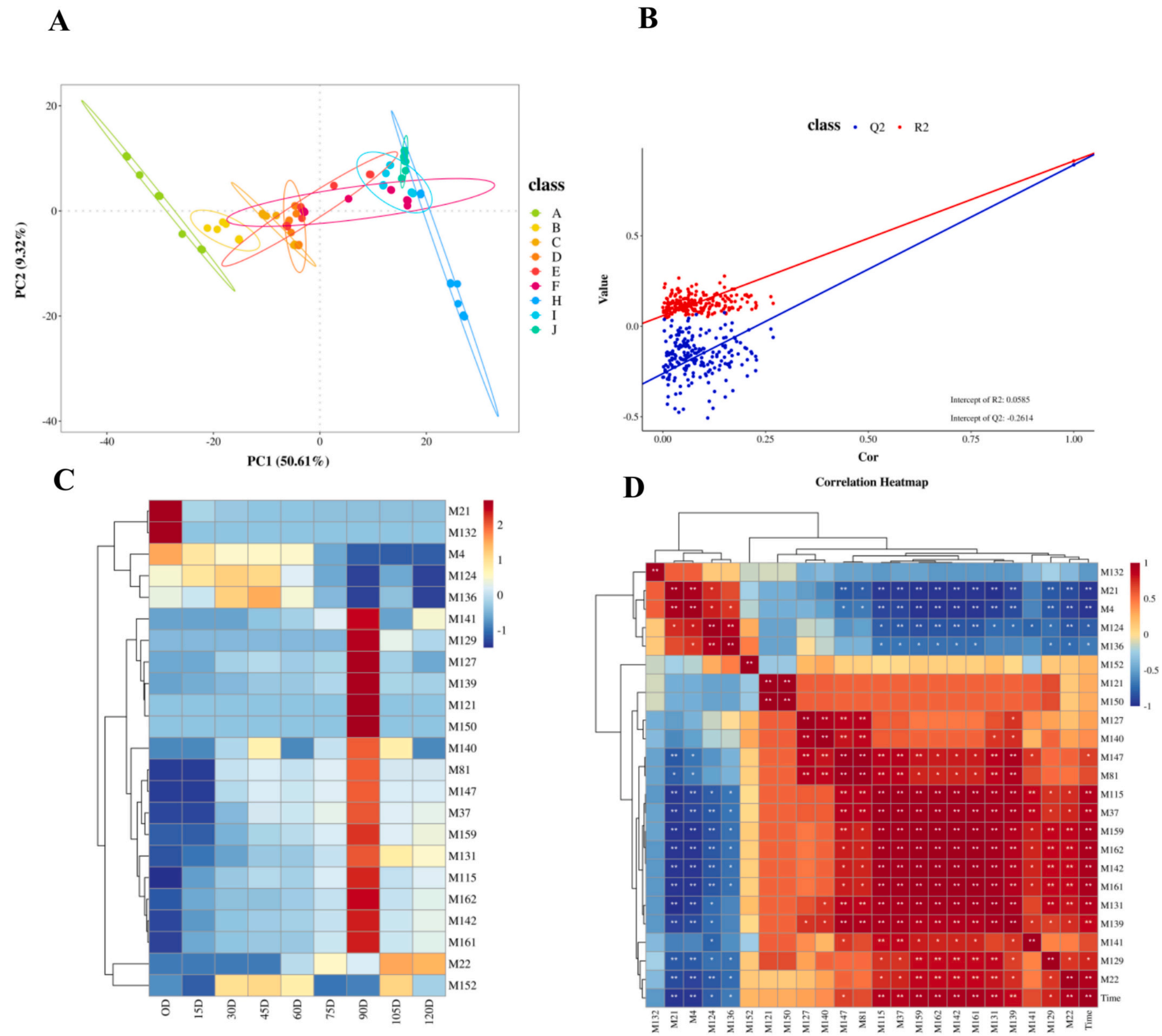

Fig. 3. The dynamic change pattern of volatile organic compounds in soy sauce production by PLS-DA. PLS-DA scatter score plot of VOCs composed of soy sauces in different fermentation periods (A), Cross-validation of PLS-DA model by 200 permutations (B), heat map visualization of the dynamic changes of 23 key differential volatile organic compounds (VIP>1, p<0.05)during fermentation production of soy sauce (C), heatmap of spearman association between characteristic differential metabolites and fermentation time (D).

Note: A– 0D; B– 15D; C– 30D; D– 45D; E– 60D; F– 75D; G– 90D; H– 105D; I– 120D. Number of experimental repetitions (n = 9).

HEMF mainly related to carbohydrate metabolism (Huang et al., 2022).

3.2.3. Differential metabolites and association analysis in different fermentation periods

The formation of flavor in the fermentation process of soy sauce primarily involves the metabolism of the bacteria and the metabolism of the substrate, which including sugars, proteins, fats and the Maillard reaction (Devanthi et al., 2019; Diez-Simon et al., 2020). Based on the results of hierarchical clustering and the rules of the fermentation cycle, the changes rules of volatile organic compounds in the whole fermentation process can be categorized into 0D and 15D (first stage), 30D, 45D, 60D and 75D (second stage), 90D (third stage), and 105 and 120D (fourth stage) in Fig. 2C. The same trend was also demonstrated in our previous study (Wen et al., 2024).

To further visualize the variation pattern of the flavor composition of soy sauce during fermentation, the change pattern of soy sauce VOCs during the whole fermentation process was obtained from the PLS-DA score chart (PC1 = 50.61 %, PC2 = 9.32 %, Fig. 3A) and cross-verified by 200 permutations (Fig. 3B). During the fermentation process of soy sauce, 90D was the time period where the most significant changes occurred, and most of the samples were distributed from right to left as the fermentation time increased. The major differential metabolites in the overall fermentation of soy sauce were M21 (*M/Z*: 37.50939), M22 (M/Z: 38.02142), M4 (M/Z: 17.02437, H_3N^+), M37 (M/Z: 54.0375), M159 (M/Z: 219.05649), M124 (M/Z: 144.09857, nonanal, $C_9H_{19}O^+$), M131 (M/Z: 153.01645), M81 (M/Z: 97.9817), M139 (M/Z: 163.0479), M147 (M/Z: 175.06451), M136 (M/Z: 158.11375), M141 (M/Z: 165.02162, phenethyl acetate, $C_{10}H_{13}O_2^+$), M129 (M/Z: 150.04909), M142 (M/Z: 167.00722), M127 (M/Z: 148.04855), M115 (M/Z: 134.04477, 3-methyl indole, $C_9H_{10}N^+$), M132

(M/Z: 153.04408, hotrienol, $C_9H_{13}O_2^+$), M162 (M/Z: 247.16843), M161 (M/Z: 233.17813), M152 (M/Z: 185.05197), M121 (M/Z: 140.05847), M150 (M/Z: 181.03024), M140 (M/Z: 164.95738) judged by VIP value >1 and $p < 0.05$. Of these, only M124 (M/Z: 144.09857, nonanal, $C_9H_{19}O^+$), M141 (M/Z: 165.02162, phenethyl acetate, $C_{10}H_{13}O_2^+$), M115 (M/Z: 134.04477, 3-methyl indole, $C_9H_{10}N^+$) and M132 (M/Z: 153.04408, hotrienol, $C_9H_{13}O_2^+$) were accurately identified. Moreover, based on the characteristic metabolites described in Fig. 2 (D), spearman correlation analysis showed that M21 (M/Z: 37.50939), M4 (M/Z: 17.02437, H_3N^+) showed a very significant negative correlation ($p < 0.01$), and M124 (M/Z: 144.09857, nonanal, $C_9H_{19}O^+$), M136 (M/Z: 158.11375) showed a significant negative correlation ($p < 0.05$). Additionally, M115 (M/Z: 134.04477, 3-methyl indole, $C_9H_{10}N^+$), M37 (M/Z: 54.0375), M159 (M/Z: 219.05649), M162(M/Z: 247.16843), M142 (M/Z: 167.00722), M161 (M/Z: 233.17813), M131 (M/Z: 153.01645), M139 (M/Z: 163.0479), M22 (M/Z: 38.02142) showed a very significant positive correlation ($p < 0.01$), and M129 as well as M47 showed a significant positive correlation ($p < 0.05$). The correlation analysis indicated that the overall volatile flavor compounds in soy sauce change as fermentation time progresses. Despite some gaps in substance identification, the mass numbers can still serve as a basis for assessing the fermentation process. According to earlier studies, the Maillard process and fat metabolism were the primary areas where nonanal alterations occurred during heating. The formation of nonanal was significantly impacted by variations in linoleic acid, linolenic acid, and arachidonic acid in particular (Liu, Wang, Zhang, Shen, Hui, & Ma, 2020), and the oxidative lysis of unsaturated fatty acids was one of the key sources of nonanal (Liu, Zhao, Zeng, & Xu, 2024).

Overall, the fermentation of soy sauce was a balanced process involving the microflora and the substrate, as previously studied (Chen et al., 2023), 0D—15D served as the initial stage of soy sauce fermentation. Following the initial 15D stage, the fermentation period from 30D to 75D represented the middle stage, during which volatile compound accumulation and the emergence of representative flavor components were observed. The volatile aroma composition peaked at 90D, marking a key point in the fermentation process. After 90D, the content of volatile organic compounds began to decline. OPLS-DA was effective in filtering out irrelevant variables and screening for reliable markers of differences between groups (Chen et al., 2024). Pairwise analysis, as shown in Supplementary Documentation 1, revealed that when comparing the fermentation stages of 15D and 30D, all R2Y and Q2 values were greater than 0.95 according to the OPLS-DA results, indicating the model's feasibility. Cross-validation confirmed that the OPLS-DA model was not overfit, suggesting it was very good. Significantly regulated metabolites were further screened based on a combination of VIP values greater than 1 and p values less than 0.05.

By comparing the differences in 15D and 30D soy sauce, 79 differential metabolites were identified, Among the top 10 key feature components, the following were mainly included: M80 (*M/Z*: 97.03568, furfural, $C_5H_5O_2^+$), M25 (M/Z: 42.02576, acetonitrile, $C_2H_4N^+$), M44 (M/Z: 61.04382, acetonitrile, $C_2H_5O_2^+$), M38 (M/Z: 55.03636, cyclobutene, $C_4H_7^+$), M145 (M/Z: 173.05788, decanoic acid, $C_{10}H_{21}O_2^+$), M88 (M/Z: 105.03505, styrene, $C_8H_9^+$), M68 (M/Z: 85.05382, 2-pentenal, $C_5H_8OH^+$), M107 (M/Z: 125.03712, guaiacol, $C_7H_9O_2^+$), M69 (M/Z: 86.05477, 2-pyrrolidione, $C_4H_8ON^+$) and M126 (M/Z: 147.03683, 1,8-octanediol, $C_9H_7O_2^+$) (VIP > 1). In the early fermentation period, the main differential metabolites were aldehydes, alcohols, and acids. Based on the changing pattern of the microflora reported earlier, the initial phase of fermentation, spanning the first 15–30 days, was primarily dominated by *A. oryzae* and *T. halophilus*. This phase mainly involved the initial degradation of protein, fat, and sugar macromolecular components (Wen et al., 2024). During the 15D—30D period, the citrate cycle (TCA cycle) was also a key metabolic activity. The main metabolic pathways included sugar metabolism, butyrate metabolism, starch and sucrose metabolism, the interconversion of pentose and glucuronide, and the metabolism of glyoxylate and dicarboxylic acids (Chen et al., 2024). Among these, furfural, 2-pentenal, formaldehyde, acetic acid, decanoic acid and ethanol were all the key characteristics of sugar metabolism production. Additionally, the change of M135 (decanal) mainly related to Maillard reaction and fat metabolism (Zhang et al., 2021).

Comparing the differences between the 75D and 90D soy sauce samples, respectively, 111 differential metabolites were identified. In the top 10 key feature components, the following were included: M63 (*M/Z*: 80.03668, pyridine, $C_5H_6N^+$), M42 (M/Z: 59.04867, acetone, $C_3H_7O^+$), M80 (M/Z: 97.03568, furfural, $C_5H_5O_2^+$), M14 (M/Z: 31.01595, formaldehyde, CH_3O^+), M64 (M/Z: 81.02326, pyrazine, $C_4H_5N_2^+$), M62 (M/Z: 79.03574, benzene, $C_6H_7^+$), M145 (M/Z: 173.05788, decanoic acid, $C_{10}H_{21}O_2^+$), M50 (M/Z: 67.04706, cyclopentadiene, $C_5H_7^+$) as potential differential volatile organic compounds (VIP > 1). As the fermentation process advanced to the mid-phase, between days 60 and 90, the dominant microorganisms shifted to include Halophilic tetrachococcus, Fussinia, Enterica as the main dominant bacteria. In the 90D sample, Xanthomonas and Rudsinia yeasts were highlighted, mainly involved in the metabolism of amino acids, biosynthesis and metabolism of sugars, lipid metabolism, and metabolism of terpenoids and polyketide compounds (Wen et al., 2024). Compared with the early stage of fermentation, the middle fermentation key nodes showed more significant differential metabolic components, such as pyridine, acetone, furfural, pyrazine, cyclopentadiene, formaldehyde, and decanoic acid, with relatively reduced metabolic differences. These compounds are mainly formed through the Maillard reaction. At the same time, pyrazines have a relatively high odor threshold, which may have a certain effect on the sensory characteristics of soy sauce (Diez-Simon et al., 2020). Differential metabolites also react differently and exhibit distinct characteristics from the early fermentation state. Differences in metabolites can guide production to judge the progress of fermentation, which is beneficial for controlling the fermentation process.

Comparing the differences in 75D and 90D soy sauce, 107 differential metabolites were identified. In the top 10 key feature components, the following were included: M141 (M/Z: 165.02162, phenethyl acetate, $C_{10}H_{13}O_2^+$), M42 (M/Z: 59.04867, acetone, $C_3H_7O^+$), M14 (M/Z: 31.01595, formaldehyde, CH_3O^+), M126 (M/Z: 147.03683, 1,8-octanediol, $C_9H_7O_2^+$), M50 (M/Z: 67.04706, cyclopentadiene, $C_5H_7^+$), M80 (M/Z: 97.03568, furfural, $C_5H_5O_2^+$), M88 (M/Z: 105.03505, styrene, $C_8H_9^+$), M54 (M/Z: 71.07084, 2-butenal, $C_4H_7O^+$), M90 (M/Z: 107.02673, benzaldehyde, $C_7H_6OH^+$), M66 (M/Z: 83.05901, methylfuran, $C_5H_7O^+$) as potential differential VOCs (VIP > 1). Finally, during the late fermentation period, from day 90 to day 105, the entire microbial community tended to stabilize (Wen et al., 2024). The composition of volatile organic compounds decreased slowly, and after the accumulation of alcohol and acid components in the early stage, phenethyl acetate became prominent in the late stage. Its production is mainly related to the lipid metabolism of yeast, and its change pattern is conducive to judging the progress of fermentation (Devanthi & Gkatzionis, 2019).

The analysis of PLS-DA and OPLS-DA can reveal that nonanal and phenethyl acetate were significant differential components, which served as potential characteristic metabolites for judging the key nodes of soy sauce fermentation.

3.3. Prediction of soy sauce in different fermentation processes by machine learning

As a technology of the new era, machine learning is a data-driven method for pattern recognition, aiming to identify discriminative or generative models from a given dataset and to leverage the statistical associations between features (Lin et al., 2022). Due to PTR-TOF-MS data were rich in complex features, making it an ideal candidate for analysis using machine learning.

In this article, 10 different machine learning methods were used to classify and predict the fermentation stages of soy sauce, these include

Table 2
Performance parameters obtained from the confusion matrix.

Model	Accuracy	AUC	Recall	Precision	F1 score	Kappa	MCC	TT (Sec)
Quadratic Discriminant Analysis	1	1	1	1	1	1	1	1.937
Linear Discriminant Analysis	1	1	1	1	1	1	1	2.035
Extra Trees Classifier	0.9983	1	0.9983	0.9985	0.9983	0.9981	0.9981	2.006
Random Forest Classifier	0.9966	1	0.9966	0.997	0.9966	0.9962	0.9962	2.043
Logistic Regression	0.9932	0.9996	0.9932	0.9941	0.9932	0.9924	0.9925	2.129
Naive Bayes	0.9846	1	0.9846	0.9875	0.9843	0.9826	0.9831	1.87
Ridge Classifier	0.983	0	0.983	0.9848	0.9825	0.9808	0.9811	1.885
Gradient Boosting Classifier	0.9812	0.9999	0.9812	0.9838	0.9809	0.9788	0.9792	2.269
Decision Tree Classifier	0.9745	0.9856	0.9745	0.9782	0.9745	0.9712	0.9718	2.003
K Neighbors Classifier	0.9608	0.9976	0.9608	0.9642	0.9602	0.9559	0.9564	1.941

the presence of a Quadratic Discriminant Analysis (QDA), Linear Discriminant Analysis (LDA), Extra Trees Classifier, Random Forest Classifier, Logistic Regression, Naive Bayes, Ridge Classifier, Gradient Boosting Classifier, Decision Tree Classifier, K Neighbors Classifier. The whole analysis process was shown as follows: To distinguish soy sauce from different fermentation periods, PTR-TOF-MS data were first randomly split into ttraining and testing sets in an 8:2 ratio, and define the detected volatile organic compounds as the variable X, Different fermentation time points are for the label Y. All of the algorithms were implemented in python. Additionally, the training parameters were optimized and tested manually. The parameters of the top five models with the highest accuracy were provided in Supplementary Document 2.

The evaluation of different models based on 8 key parameters included accuracy, area under the curve (AUC), precision, recall, F1 score, kappa, MCC, TT (Sec). By comparing the performance of the 10 algorithms, the accuracy results were presented in Table 2. Using the optimal parameters, the accuracy of the dataset constructed from PTR-TOF-MS data can exceed 96 %, and the AUC can exceed 98 %. In particular, the identification accuracy of QDA and LDA reached 100 %, which was higher than that of the other models. These results indicated that the PTR-TOF-MS data had high discriminative performance in classifying soy sauce samples from different fermentation periods. Machine learning has a better accuracy, compared with the PCA classification.

Overall, the combination of PTR-TOF-MS data acquisition and machine learning differentiation methods has the potential to create a rapid and intelligent control system for soy sauce production, which could be beneficial for stabilizing soy sauce fermentation and ensuring product quality.

4. Conclusion

In this paper, the monitoring characteristics of an electronic nose and PTR-TOF-MS during the soy sauce fermentation process were compared in detail. The metabolic change patterns of volatile organic compounds (VOCs) throughout the soy sauce fermentation process were analyzed comprehensively and objectively. The overall monitoring patterns by the electronic nose and PTR-TOF-MS were basically the same, and the overall fermentation process of soy sauce can be divided into four stages: D0-D15, D30-D75, D90, and D105-D120. Stage D90 was identified as the key node for the production of fermented flavor. The detection of VOCs in soy sauce by PTR-TOF-MS identified 163 mass numbers, including acids, alcohols, esters, aldehydes, furans, ketones, phenols, pyrazines, sulfur compounds, and other components. Although there were some gaps in the identification of these components, the analysis enriched the methods for identifying volatile organic compounds.

In the early stage of fermentation, the main sources of the flavor profile compounds were presumed to be sugar metabolism, butyric acid metabolism, starch and sucrose metabolism, and the interconversion of pentose and glucuronic acid, which are the main metabolic pathways. The key flavor components generated were aldehydes, alcohols, and acids. In the middle stage of fermentation, amino acid degradation and sugar metabolism in soy sauce tended to stabilize, and the main differences came from the pyrazine, pyridine, and ketone compounds produced by the Maillard reaction. The content of these volatile organic compounds also reached its peak. Finally, in the later stages of fermentation, the composition of volatile organic compounds showed a slow decline. As sugar and amino acid metabolism tended to stabilize, the microorganisms may produce more esterases, forming characteristic components such as fruity and sweet flavors. This also reduced the alcoholic and acidic flavors in the soy sauce.

Additionally, PTR-TOF-MS was more suitable for on-line monitoring during soy sauce fermentation compared to other monitoring methods in terms of accuracy. The accuracy of the direct injection system and on-line analysis can effectively assist production staff in determining the status of soy sauce fermentation within a short period of time. Data modeling, as a key monitoring module, was still in need of collecting a larger dataset and integrating it with deep learning algorithms to develop a more accurate and rapid prediction model. Of course, since there are still some limitations in the identification capabilities of PTR-TOF-MS, we plan to use additional GC–MS, GC-IMS, and GC-O methods to further elaborate on the soy sauce flavor profile in subsequent studies.

CRediT authorship contribution statement

Qixin Kan: Writing – original draft, Software, Methodology, Formal analysis. **Longbipei Cao:** Software, Methodology, Data curation. **Liping He:** Writing – review & editing, Supervision, Conceptualization. **Peipei Wang:** Software, Methodology, Data curation. **Guangdie Deng:** Software, Methodology, Data curation. **Jun Li:** Visualization, Supervision, Investigation. **Jiangyan Fu:** Visualization, Supervision, Investigation. **Qingrong Huang:** Conceptualization. **Chi-Tang Ho:** Conceptualization. **Yunqi Li:** Conceptualization. **Chunhui Xie:** Conceptualization. **Yong Cao:** Conceptualization. **Linfeng Wen:** Writing – review & editing, Methodology, Data curation.

Declaration of competing interest

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

Acknowledgments

This work was financially supported by Study on the Formation Mechanism of Flavor Metabolites and Intelligent Manufacturing in Cantonese Soy Sauce (CXTD2020006), Program for Guangdong Introducing Innovative and En-trepreneurial Teams (2019ZT08N291), and Guangdong Provincial Key Laboratory of Nutraceuticals and Functional Foods (2018B030322010).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.fochx.2024.102002.

Data availability

Data will be made available on request.

References

Aishima, T. (2004). Correlating sensory attributes to gas chromatography–mass spectrometry profiles and e-nose responses using partial least squares regression analysis. *Journal of Chromatography A, 1054*(1–2), 39–46. https://doi.org/10.1016/j.chroma.2004.08.056

Berbegal, C., Khomenko, I., Russo, P., Spano, G., Fragasso, M., Biasioli, F., & Capozzi, V. (2020). PTR-ToF-MS for the online monitoring of alcoholic fermentation in wine: Assessment of VOCs variability associated with different combinations of Saccharomyces/non-Saccharomyces as a case-study. *Fermentation, 6*(2), 55. https://doi.org/10.3390/fermentation6020055

Chen, C., Hou, S., Wu, C., Cao, Y., Tong, X., & Chen, Y. (2023). Improving protein utilization and fermentation quality of soy sauce by adding protease. *Journal of Food Composition and Analysis, 121*, Article 105399. https://doi.org/10.1016/j.jfca.2023.105399

Chen, C., Wen, L. F., Yang, L. X., Li, J., Kan, Q. X., Xu, T., … Cao, Y. (2023). Metagenomic and metaproteomic analyses of microbial amino acid metabolism during Cantonese soy sauce fermentation. *Frontiers in Nutrition, 10*, 1271648. https://doi.org/10.3389/fnut.2023.1271648

Chen, X., Meng, F., Tian, H., Chen, A., Wang, X., & Li, Y. (2024). Dynamic changes in metabolomics and flavoromics during industrial scale fermentation of Chinese traditional soy sauce. *Food Bioscience, 59*, Article 103940. https://doi.org/10.1016/j.fbio.2024.103940

Ciesa, F., Höller, I., Guerra, W., Berger, J., Dalla Via, J., & Oberhuber, M. (2015). Chemodiversity in the fingerprint analysis of volatile organic compounds (VOCs) of 35 old and 7 modern apple cultivars determined by proton-transfer-reaction mass spectrometry (PTR-MS) in two different seasons. *Chemistry & Biodiversity, 12*(5), 800–812. https://doi.org/10.1002/cbdv.201400384

Devanthi, P. V. P., & Gkatzionis, K. (2019). Soy sauce fermentation: Microorganisms, aroma formation, and process modification. *Food Research International, 120*, 364–374. https://doi.org/10.1016/j.foodres.2019.03.010

Diez-Simon, C., Eichelsheim, C., Mumm, R., & Hall, R. D. (2020). Chemical and sensory characteristics of soy sauce: A review. *Journal of Agricultural and Food Chemistry, 68*(42), 11612–11630. https://doi.org/10.1021/acs.jafc.0c04274

Feng, X., Wang, H., Wang, Z., Huang, P., & Kan, J. (2022). Discrimination and characterization of the volatile organic compounds in eight kinds of huajiao with geographical indication of China using electronic nose. *HS-GC-IMS and HS-SPME-GC-MS. Food Chemistry, 375*, Article 131671. https://doi.org/10.1016/j.foodchem.2021.131671

Feng, Y., Cai, Y., Su, G., Zhao, H., Wang, C., & Zhao, M. (2014). Evaluation of aroma differences between high-salt liquid-state fermentation and low-salt solid-state fermentation soy sauces from China. *Food Chemistry, 145*, 126–134. https://doi.org/10.1016/j.foodchem.2013.07.072

Feng, Y., Su, G., Sun-Waterhouse, D., Cai, Y., Zhao, H., Cui, C., & Zhao, M. (2017). Optimization of headspace solid-phase Micro-extraction (HS-SPME) for analyzing soy sauce aroma compounds via coupling with direct GC-Olfactometry (D-GC-O) and gas chromatography-mass spectrometry (GC-MS). *Food Analytical Methods, 10*(3), 713–726. https://doi.org/10.1007/s12161-016-0612-5

Feng, Y., Su, G., Zhao, H., Cai, Y., Cui, C., Sun-Waterhouse, D., & Zhao, M. (2015). Characterisation of aroma profiles of commercial soy sauce by odour activity value and omission test. *Food Chemistry, 167*, 220–228. https://doi.org/10.1016/j.foodchem.2014.06.057

Gao, L., Liu, T., An, X., Zhang, J., Ma, X., & Cui, J. (2017). Analysis of volatile flavor compounds influencing Chinese-type soy sauces using GC–MS combined with HS-SPME and discrimination with electronic nose. *Journal of Food Science and Technology, 54*(1), 130–143. https://doi.org/10.1007/s13197-016-2444-0

Gao, X., Liu, E., Zhang, J., Yang, L., Huang, Q., Chen, S., Ma, H., Ho, C., & Liao, L. (2020). Accelerating aroma formation of raw soy sauce using low intensity sonication. *Food Chemistry, 329*, Article 127118. https://doi.org/10.1016/j.foodchem.2020.127118

Ghasemi-Varnamkhasti, M., Mohtasebi, S. S., Rodriguez-Mendez, M. L., Lozano, J., Razavi, S. H., & Ahmadi, H. (2011). Potential application of electronic nose technology in brewery. *Trends in Food Science & Technology, 22*(4), 165–174. https://doi.org/10.1016/j.tifs.2010.12.005

Huang, Y., Wan, J., Wang, Z., Sun, M., Feng, T., Ho, C., & Song, S. (2022). Variation of volatile compounds and corresponding aroma profiles in Chinese steamed bread by various yeast species fermented at different times. *Journal of Agricultural and Food Chemistry, 70*(12), 3795–3806. https://doi.org/10.1021/acs.jafc.2c00550

Ito, K., & Matsuyama, A. (2021). Koji molds for Japanese soy sauce brewing: Characteristics and key enzymes. *Journal of Fungi, 7*(8), 658. https://doi.org/10.3390/jof7080658

Li, A., Feng, X., Yang, G., Peng, X., Du, M., Song, J., & Kan, J. (2024). Impact of aroma-enhancing microorganisms on aroma attributes of industrial Douchi: An integrated analysis using E-nose, GC-IMS, GC–MS, and descriptive sensory evaluation. *Food Research International, 182*, Article 114181. https://doi.org/10.1016/j.foodres.2024.114181

Lin, Y., Ma, J., Wang, Q., & Sun, D. (2022). Applications of machine learning techniques for enhancing nondestructive food quality and safety detection. *Critical Reviews in Food Science and Nutrition, 63*(12), 1649–1669. https://doi.org/10.1080/10408398.2022.2131725

Lioe, H. N., Selamat, J., & Yasuda, M. (2010). Soy sauce and its umami taste: A link from the past to current situation. *Journal of Food Science, 75*(3), R71–R76. https://doi.org/10.1111/j.1750-3841.2010.01529.x

Liu, H., Yang, S., Liu, J., Lu, J., & Wu, D. (2023). Effect of salt concentration on Chinese soy sauce fermentation and characteristics. *Food Bioscience, 53*, Article 102825. https://doi.org/10.1016/j.fbio.2023.102825

Liu, M., Feng, Y., Zhao, M., & Huang, M. (2023). Decoding the molecular basis for temperature control by metabolomics to improve the taste quality of soy sauce fermented in winter. *Food Bioscience, 53*, Article 102889. https://doi.org/10.1016/j.fbio.2023.102889

Loutfi, A., Coradeschi, S., Mani, G. K., Shankar, P., & Rayappan, J. B. B. (2015). Electronic noses for food quality: A review. *Journal of Food Engineering, 144*, 103–111. https://doi.org/10.1016/j.jfoodeng.2014.07.019

Lu, L., Hu, Z., Hu, X., Li, D., & Tian, S. (2022). Electronic tongue and electronic nose for food quality and safety. *Food Research International, 162*, Article 112214. https://doi.org/10.1016/j.foodres.2022.112214

Muñoz-González, C., Canon, F., Feron, G., Guichard, E., & Pozo-Bayón, M. (2019). Assessment wine aroma persistence by using an in vivo PTR-ToF-MS approach and its relationship with salivary parameters. *Molecules, 24*(7), 1277. https://doi.org/10.3390/molecules24071277

Murooka, Y., & Yamshita, M. (2008). Traditional healthful fermented products of Japan. *Journal of Industrial Microbiology & Biotechnology, 35*(8), 791–798. https://doi.org/10.1007/s10295-008-0362-5

Peris, M., & Escuder-Gilabert, L. (2009). A 21st century technique for food control: Electronic noses. *Analytica Chimica Acta, 638*(1), 1–15. https://doi.org/10.1016/j.aca.2009.02.009

Pozo-Bayón, M.Á., Santos, M., Martín-Álvarez, P. J., & Reineccius, G. (2009). Influence of carbonation on aroma release from liquid systems using an artificial throat and a proton transfer reaction-mass spectrometric technique (PTR-MS). *Flavour and Fragrance Journal, 24*(5), 226–233. https://doi.org/10.1002/ffj.1934

Romano, A., Cappellin, L., Bogialli, S., Pastore, P., Navarini, L., & Biasioli, F. (2022). Monitoring in vitro and in vivo aroma release of espresso coffees with proton-transfer-reaction time-of-flight mass spectrometry. *Applied Sciences, 12*(20), 10272. https://doi.org/10.3390/app122010272

Silvis, I. C. J., Luning, P. A., Klose, N., Jansen, M., & van Ruth, S. M. (2019). Similarities and differences of the volatile profiles of six spices explored by proton transfer reaction mass spectrometry. *Food Chemistry, 271*, 318–327. https://doi.org/10.1016/j.foodchem.2018.07.021

Song, F., Xiang, H., Li, Z., Li, J., Li, L., & Fang Song, C. (2023). Monitoring the baking quality of Tieguanyin via electronic nose combined with GC–MS. *Food Research International, 165*, Article 112513. https://doi.org/10.1016/j.foodres.2023.112513

Wang, P., Kan, Q., Yang, L., Huang, W., Wen, L., Fu, J., Liu, Z., Lan, Y., Huang, Q., Ho, C., & Cao, Y. (2023). Characterization of the key aroma compounds in soy sauce by gas chromatography-mass spectrometry-olfactometry, headspace-gas chromatography-ion mobility spectrometry, odor activity value, and aroma recombination and omission analysis. *Food Chemistry, 419*, Article 135995. https://doi.org/10.1016/j.foodchem.2023.135995

Wang, R., Liang, M., Zhang, Z., Wu, Y., & Liu, Y. (2023). Comparison of flavor profile relationship of soy sauce under different storage conditions. *Foods, 12*(14), 2707. https://doi.org/10.3390/foods12142707

Wang, S., Tamura, T., Kyouno, N., Liu, X., Zhang, H., Akiyama, Y., & Chen, J. Y. (2019). Effect of volatile compounds on the quality of Japanese fermented soy sauce. *Lwt, 111*, 594–601. https://doi.org/10.1016/j.lwt.2019.05.050

Wei, Q., Wang, H., Lv, Z., Hu, G., Li, Y., Liu, Y., Wang, Y., & Lu, F. (2013). Search for potential molecular indices for the fermentation progress of soy sauce through dynamic changes of volatile compounds. *Food Research International, 53*(1), 189–194. https://doi.org/10.1016/j.foodres.2013.04.001

Wen, L., Lei, J., Yang, L., Kan, Q., Wang, P., Li, J., Chen, C., He, L., Fu, J., Ho, C., Huang, Q., & Cao., Y.. (2024). Metagenomics and untargeted metabolomics analyses to unravel the formation mechanism of characteristic metabolites in Cantonese soy sauce during different fermentation stages. *Food Research International, 181*, Article 114116. https://doi.org/10.1016/j.foodres.2024.114116

Yuan, B., Koss, A. R., Warneke, C., Coggon, M., Sekimoto, K., & de Gouw, J. A. (2017). Proton-transfer-reaction mass spectrometry: Applications in atmospheric sciences. *Chemical Reviews, 117*(21), 13187–13229. https://doi.org/10.1021/acs.chemrev.7b00325

Zhang, Z., Zang, M., Zhang, K., Wang, S., Li, D., & Li, X. (2021). Effects of phospholipids and reheating treatment on volatile compounds in phospholipid-xylose-cysteine reaction systems. *Food Research International, 139*, Article 109918. https://doi.org/10.1016/j.foodres.2020.109918

Zheng, Y., Li, Y., Pan, L., Guan, M., Yuan, X., Li, S., Ren, D., Gu, Y., Liang, M., & Yi, L. (2024). Aroma and taste analysis of pickled tea from spontaneous and yeast-enhanced fermentation by mass spectrometry and sensory evaluation. *Food Chemistry, 442*, Article 138472. https://doi.org/10.1016/j.foodchem.2024.138472