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Changes in the characteristic volatile aromatic compounds in tuna cooking liquid during fermentation and deodorization by *Lactobacillus plantarum* RP26 and *Cyberlindnera fabianii* JGM9-1

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ABSTRACT

Tuna cooking liquid has unpleasant aroma. In our previous studies, *Cyberlindnera fabianii* JGM9-1 and *Lactobacillus plantarum* RP26 demonstrated the ability to degrade this unpleasant aroma. However, the mechanism of microbial deodorization remains unclear. In this study, tuna cooking liquid was fermented using JGM9-1 alone, RP26 alone, and a combination of both strains. Changes in volatile aromatic compounds during fermentation were analyzed using HS-SPME-GC/MS. The unpleasant aroma of tuna cooking liquid were nine characteristic aromatic compounds associated with fishy, stinky, and greasy aromas. Furthermore, we found that the fermentation of microbes removed these unpleasant aromatic compounds and replaced them with pleasant aromatic compounds that contributed to fruity, grassy, and floral aromas. Finally, we screened 21 strong pairwise correlations between the production and consumption of characteristic volatile aromatic compounds by RP26 and JGM9-1, through HCA, VIP, OAV and Spearman's pairwise correlation analysis. These results help to clarify the metabolic mechanisms of microbial deodorization in tuna cooking liquid.

1. Introduction

Tuna is an important pelagic fish species around the world. It is one of the three major nutritional fish, containing eight essential amino acids, polyunsaturated fatty acids such as EPA and DHA, as well as docosahexaenoic acid, eicosapentaenoic acid, and other nutrients and active compounds (Liu et al., 2020). Tuna has been processed into various kinds of processed products, such as canned tuna and instant fillets. As a result of processing, a large amount of tuna cooking liquid is produced. To best of our knowledge, the global output of tuna and tuna cooking liquid was approximately 5.5 million and 1.93 million tons, respectively (Erauskin-Extramiana et al., 2023). The composition of the cooking liquid is complex, consisting of proteins, amino acids, and polysaccharides (Sangkharak et al., 2021). However, tuna cooking liquid has few uses due to its unpleasant aroma. Currently, there is no effective method available to deodorize and reuse tuna cooking liquid. It is often simply discarded without treatment. This represents a significant waste of protein resources and is a source of environmental contamination (Marsh & Bugusu, 2007; Peinado, Koutsidis & Ames, 2016). Thus, it is necessary to reduce the unpleasant aroma of tuna cooking liquid to produce a high value-added product.

There are three methods commonly used to deodorize fish. The first method is physical deodorization, which reduces unpleasant aromas using physical techniques such as filtration or the application of activated carbon, chitosan, or zeolites (Güner, Yılmaz & Yüceer, 2019). However, physical deodorization does not completely eliminate

unpleasant aromas, and some aldehyde and fatty acid aromas remain (Marc et al., 2013). The second method is chemical deodorization, which reduces unpleasant aromas through the chemical reactions between chemicals and raw materials. For example, tea polyphenols and acetic acid are effective in reducing the contents of geosmin and 2-methylisoborneol in farm-raised catfish after harvest (Liu et al., 2017). The primary drawback of this method is that other unpleasant odors are easily produced through the reactions between chemicals and raw materials (Wentian et al., 2016). The third method is biological deodorization, which reduces unpleasant aromas through fermentation by microbes (Pirestani, Nasirpour, Keramat, & Desobry, 2017). Microbial fermentation can have a deodorization effect while also generating more desirable aromatic compounds, thus improving the overall aroma profiles of foods (Zhong et al., 2019). Unlike the physical and chemical methods, microbial deodorization could completely remove unpleasant aromas without requiring the addition of additives and chemicals that might produce other unpleasant odors. This method is often used in a variety of foods to eliminate the unpleasant odors carried by the ingredients themselves. For example, lactic acid bacteria have been found to be helpful in the improvement of the aromatic properties of mutton jerky (Zhou et al., 2022). In addition, yeast fermentation has been used to deodorize and improve the aroma of *Allomyrina dichotoma* larva (Kim et al., 2021). Some unpleasant aromatic compounds in kelp samples decreased after fermentation by *L. plantarum*, *P. pentosaceus*, and *S. cerevisiae* (Zhu et al., 2021). In sum, fermentation with lactic acid bacteria or yeasts can effectively reduce unpleasant aromas and infuse the product with a unique, pleasant fragrance.

In our previous experiments, we screened two microbial strains, *Lactobacillus plantarum* RP26 and *Cyberlindnera fabianii* JGM9-1, that can reduce the fishy aroma of tuna cooking liquid and produce a pleasant aroma. These strains are considered safe for use in the food industry and are recorded in the China Center of Industrial Culture Collection. However, the metabolic mechanisms of these microbes in the deodorization process remain unclear. In this study, *Lactobacillus plantarum* RP26 and *Cyberlindnera fabianii* JGM9-1 were applied individually and in combination to ferment tuna cooking liquid. Headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME-GC/MS) was used to analyze the dynamic changes in volatile aromatic compounds during the fermentation experiments. Changes in characteristic aromatic compounds in tuna cooking liquid during microbial fermentation and deodorization were revealed using hierarchical clustering algorithm (HCA) analysis, variable importance in projection (VIP) value analysis, and odor activity value (OAV) analysis of important compounds. Finally, Spearman's pairwise correlation determined the consumption and production of specific aromatic compounds by strains JGM9-1 and RP26. The results are helpful for clarifying the metabolic mechanism of different microorganisms in the deodorization of tuna cooking liquid, which will be useful for the further development of high-value products and promote the application of tuna cooking liquid products.

2. Materials and methods

2.1. Materials

Tuna cooking liquid was obtained from Fuzhou Hongdong Foods Co., Ltd. (Fuzhou, China), with a pH value of 6.67, total acid content of 0.24 g/100 g (recorded as lactic acid), soluble solid content of 3.70%, total sugar content of 0.09 g/100 g, crude polysaccharide content of 0.02 g/100 g, protein content of 2.84 g/100 g, and amino acid nitrogen content of 0.07 g/kg. The tuna cooking liquid solution was centrifuged at 8000 $\times$ *g* (8500 rpm in an R20A2 fixed angle rotor, Himac CR22N centrifuge, Germany Eppendorf Himac Co., Tokyo, Japan) at 4 °C for 10 min, filtered, and then stored at -20 ± 1 °C until further use.

Cyberlindnera fabianii JGM9-1 and *Lactobacillus plantarum* RP26 isolated from honeycombs in Fujian Province, China, were preserved in the China Center for Type Culture Collection under strain serial numbers "CCTCC NO. M 20221401" (JGM9-1) and "CCTCC NO. M 2019298" (RP26), respectively. RP26 strains were kept in our collection at -80 ± 1 °C in De Man, Rogosa, and Sharpe (MRS) medium (Solarbio, Ltd., Beijing, China) with 50% (v/v) glycerol. JGM9-1 strains were kept in our collection at -80 ± 1 °C in yeast peptone dextrose (YPD) medium (Solarbio, Ltd., Beijing, China) with 50% (v/v) glycerol.

2.2. Activation of microorganisms

Before application as a starter inoculum, RP26 strains were propagated on MRS medium plates containing 2.0% agar and incubated at 30 ± 1 °C for 24 h under anaerobic conditions to obtain pure colonies. The pure colonies were then inoculated in MRS medium and cultured at 30 ± 1 °C for 24 h. The bacterial colonies were counted using the flat colony counting method (Fujikawa & Tsubaki, 2019), and a minimum of 1.0×10^9 colony-forming units (CFUs)/mL was reached. RP26 strains were harvested after centrifugation at 8000 $\times$ *g* and 4 °C for 5 min, and the supernatant was discarded. JGM9-1 strains were propagated in YPD medium on plates containing 2.0% agar and incubated at 30 ± 1 °C for 24 h to obtain pure colonies. The pure colonies were inoculated in YPD medium and cultured at 30 ± 1 °C for 24 h, and a minimum of 1.0×10^8 CFUs/mL was reached. JGM9-1 strains were collected after centrifugation at 8000 $\times$ *g* at 4 °C for 5 min, and the supernatant was discarded.

2.3. Microbial fermentation and deodorization

Tuna cooking liquid was mixed with 20 g/L sugar and 3 g/L malic acid and then steam cooked to a core temperature of 100 °C for 10 min. After the mixture had cooled, the solution was transferred to 2 L glass flasks containing 1.5 kg of tuna cooking liquid. Using *Cyberlindnera fabianii* JGM9-1 inoculation (group J) and *Lactobacillus plantarum* RP26 inoculation (group R), multiple microorganism treatment groups (groups RJ1–RJ5) were inoculated, as shown in Appendix Table S1. Spontaneous fermentation with no starter was used as the control group (CK). Each flask was inoculated with the respective cultures, and each flask was capped with a silica gel plug and incubated at 30 ± 1 °C for 72 h. At the end of fermentation, samples (150 g) were collected from each flask. The contents of aromatic components were determined, and sensory evaluation was performed for each sample. Three independent fermentation experiments were performed using each sample.

2.4. Sensory analysis

The sensory evaluation was conducted following the method of Wang's method (Wang et al., 2020) with a few modifications. Thirty panelists (23–52 years old, 15 men and 15 women) were trained to specify aroma attributes over 4 weeks. Finally, 20 panelists (10 men and 10 women) were selected according to their sensitive olfaction, and six attributes were evaluated: fishy, stinky, greasy, grassy, floral, and fruity aromas (Poisson & Schieberle, 2008). The sensory evaluation criteria employed volatile aromatic compounds as follows (Shi et al., 2021): fishy compound, 200 mg/L of 1-octen-3-ol; stinky compound, 200 mg/L of trimethylamine; greasy compound, 200 mg/L of 2-nonanone; grassy compound, 200 mg/L of 2-dodecenal; fruity compound, 200 mg/L of ethyl hexanoate; and floral compound, 200 mg/L of phenethyl alcohol. Each of the above volatile aromatic compounds had an intensity score of 5.0. Samples were coded with random numbers. The aroma attribute intensities were scored from 0 to 5, in which 0 indicates no aroma detection, 1 indicates a very faint aroma, 3 indicates a moderate aroma intensity, and 5 indicates the highest aroma intensity.

2.5. Analysis of volatile aromatic compounds

The volatile aromatic compounds in the fermented tuna cooking liquid were analyzed using HS-SPME-GC/MS (Hu et al., 2019) with

minor modifications. A GCMS-QP2020NX system (Shimadzu QP2010 Ultra, Shimadzu, Tokyo, Japan) was employed. Accurately weighed fermented tuna cooking liquid was transferred to the sample vials and equilibrated at 60 °C for 10 min. An SPME fiber equipped with a manual injection handle (Agilent Technologies Corp., Santa Clara, CA, USA) was immersed in the headspace for 30 min at 60 °C. A DB-5 ms capillary (30 m × 0.25 mm i.d., 0.25 μm; Restek, Bellefonte, PA, USA) was used for separation. The GC injector was held at 250 °C for 5 min, and the carrier gas was helium (purity, 99.99%), with a flow rate of 1.0 mL/min. The temperature program was as follows: 35 °C for 2 min, followed by an increase at 3 °C/min to 90 °C for 1 min, then an increase of 5 °C/min to 140 °C for 1 min, and a final increase of 8 °C/min to 250 °C. The temperature was held at 250 °C for 5 min. The transfer line temperature between the GC and the MS systems was 250 °C, and the ion source temperature was 200 °C. The MS was run in electron ionization (EI) mode and scanned over a mass acquisition range of 35–550 m/z at 0.2 s intervals. Volatile aromatic substances were identified by matching them with the reference mass spectra of the NIST library, Wiley library and flavors and fragrances library from Shimadzu Corporation, as well as with the retention index data found in public domain databases, and related references (Zhou et al., 2016).

For each extraction, 4-methyl-2-pentanol (5 μg/μL) was used as an internal standard (I.S.). Ten microliters of the standard solution was added to a sample of 3 g homogenized tuna cooking liquid before headspace analysis (Wu, Tao & Gu, 2014). Then, the peak area of each volatile compound was calculated relative to the I.S. concentration. The relative content (μg/g) of volatile compounds in a sample was calculated as follows:

$$Relative\ concentration = \frac{Peak\ area\ of\ unknown\ compound}{Peak\ area\ of\ IS} \times \frac{50\ ug\ of\ IS}{3\ g\ of\ sample} \tag{1}$$

2.6. Odor activity value

OAVs were used to determine and measure the contribution of individual volatile compounds to the overall aroma spectrum. The OAVs of the individual volatile compounds were calculated using Liu's method (Liu et al., 2022):

$$OVAi = \frac{Ci}{Ti} \tag{2}$$

where i is a compound in one sample; Ci is the concentration of the compound i in a sample (μg/g); and Ti is its odor threshold concentration. Compounds with OAV > 1 were considered key volatile aromatic compounds.

2.7. Statistical analysis

Biological replicates were performed in triplicate for each sample group. One-way ANOVA was performed with Duncan's multiple testing using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). The metabolomics were measured and analyzed as the reference method (Chen et al., 2022; Li et al., 2022). The changes in volatile aromatic compounds were predicted using HCA and VIP value analyses in SIMCA 14.1 (Umetrics AB, Umea, Sweden). Spearman's pairwise correlation (r) was calculated in R (ver. 3.5.3), and correlations were considered for those with $|r| > 0.5$ and $p < 0.05$ (Liang et al., 2023). Stacked bar charts and histograms were generated in Origin 2022 (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Sensory profile evaluation of tuna cooking liquid with microbial fermentation and deodorization

The sensory profiles of the fermented tuna cooking liquid and the control group were plotted on radar charts for data visualization. As shown in Fig. 1, the fishy, stinky, greasy, grassy, floral, and fruity aromas were the main sensory attributes of the fermented tuna cooking liquid. The overall aroma profile was better in the treatment groups than in the control. During fermentation, different treatment groups generated variations in distinct sensory characteristics and dramatically transformed the sensory profile of the tuna cooking liquid. Among the six sensory modalities evaluated by the participants, the different ratios of lactic acid bacteria to yeast in the fermented tuna cooking liquid resulted in the perception of different categories of aroma by the participants. Fermentation with RP26 strains alone resulted in a decrease in greasy and stinky aromas and the production of a grassy aroma. However, this treatment failed to reduce the fishy aroma. Fermentation with JGM9-1 strains alone removed fishy and stinky aromas and simultaneously produced notable floral and fruity aromas. However, there was still a greasy aroma. The combination of JGM9-1 and RP26 was better than single-strain fermentation. Furthermore, when the proportion of JGM9-1 increased and that of RP26 decreased, fishy and stinky aromas in the fermentation liquid decreased, and more floral and fruity aromas were produced. When the proportion of RP26 increased and that of JGM9-1 decreased, greasy and stinky aromas in the fermentation liquid decreased, and more grassy aromas were produced. The results showed that groups RJ4 and RJ5 were the best aroma treatment, and the optimal inoculation ratio of lactic acid bacteria to yeast was 1:1 or 5:1. At this ratio, the fermentation liquid had no obvious unpleasant aroma and could produce unique grassy, floral, and fruity aromas.

3.2. Change in volatile aromatic compounds of tuna cooking liquid during microbial fermentation and deodorization

The concentrations of the individual compounds were estimated, and a description of the aroma of each compound (Giri et al., 2010; Liang
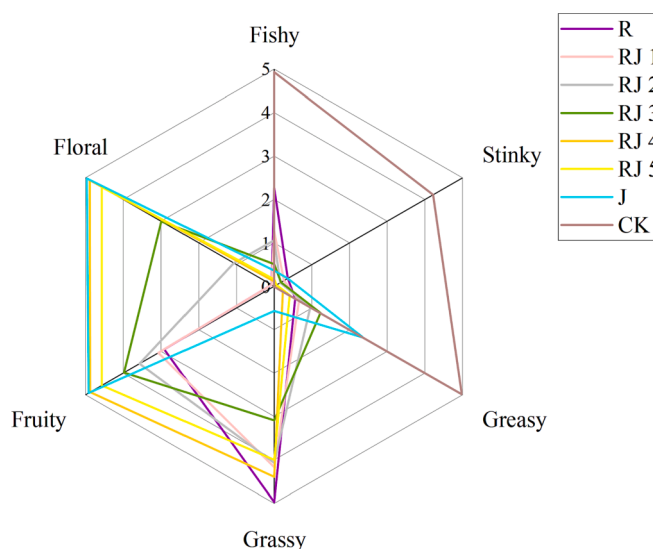
Fig. 1. The sensory profiles of the fermented tuna cooking liquid and the control group plotted on radar charts. Note: CK: Tuna cooking liquid; group R: RP26; group J: JGM9-1; group RJ1: Multiple strains of RP26 and JGM9-1 at a ratio of 100:1; group RJ2: Multiple strains of RP26 and JGM9-1 at a ratio of 20:1; group RJ3: Multiple strains of RP26 and JGM9-1 at a ratio of 10:1; group RJ4: Multiple strains of RP26 and JGM9-1 at a ratio of 5:1; group RJ5: Multiple strains of RP26 and JGM9-1 at a ratio of 1:1.

et al., 2023; Moreira et al., 2013; Wu et al., 2014; Zhu et al., 2021) is presented in Table 1. A total of 108 volatile aromatic compounds were identified and categorized into seven classes, namely 22 aldehydes, 20 alcohols, 13 ketones, 26 hydrocarbons, 17 esters, 5 acids, and 5 other compounds. For a clearer indication of the contribution of each class of volatile aromatic compounds to the entire aroma profiles of the samples, the proportion of each group of compounds to the classes, numbers, and concentrations is presented in Fig. 2, Appendix Fig. S2, and Appendix Fig. S3, as a stacked bar chart, a heatmap, and a histogram, respectively. In total, 59 volatile aromatic compounds were detected in the control (CK group), which mainly consisted of hydrocarbons and aldehydes at concentrations of 267.48 ± 4.13 µ/g and 135.93 ± 1.01 µ/g, respectively. Hydrocarbons typically have high odor thresholds and have less effect on the overall aroma characteristics. Although hydrocarbons have little effect on the aroma, some are important precursors of other volatile aromatic compounds in fermentation, such as ketones, alcohols, and esters (Zou et al., 2018). Aldehydes have low odor thresholds and generally significantly influence the overall aroma characteristics (Zhou et al., 2016).

After the RP26 single-strain fermentation (group R), 66 volatile aromatic compounds were detected. Aldehydes and ketones with grassy aromas were primarily identified, with contents of 62.77 ± 0.76 µg/g and 157.27 ± 1.55 µg/g, respectively; these concentrations were 390.23 ± 12.55% and 15.67 ± 2.72% higher than that of the control group, respectively. Through fermentation using JGM9-1 (group J), a total of 65 volatile aromatic compounds were detected, and an increase in alcohols and esters with floral and fruity aromas was identified; their contents were 174.29 ± 2.73 µg/g and 192.68 ± 3.11 µg/g, which were 930.08 ± 32.42% and 4017.09 ± 57.15% higher than that of the control, respectively. Fermentation with different proportions of RP26 and JGM9-1 strains resulted in the metabolism of hydrocarbon and aldehyde compounds and the production of numerous ketones, alcohols, esters, and aldehydes. In the dominant leading group RP26 (groups RJ1, RJ2, and RJ3), 80 types of volatile aromatic compounds were detected, mainly including ketones, alcohols, and aldehydes. For groups RJ4 and RJ5, in which JGM9-1 was the predominant strain, a total of 68 types of volatile aromatic compounds were detected, mainly including ketones, alcohols, and esters.

3.3. Changes in characteristic aromatic compounds in tuna cooking liquid during microbial fermentation and deodorization

The normalized dataset of the fermented tuna cooking liquid samples and the control was imported for processing. HCA analysis depends on volatile aromatic compound intergroup differences and similarities between compounds in the samples fermented by different microbes and the similarities between compounds. As shown in Fig. 3, in the HCA model, the samples were divided into five classes at a relative distance of 200. Groups (RJ 1), (RJ 2), and (RJ 3) were in the same class, named class RJ, with lactic acid bacteria RP26 as the dominant bacteria. Groups (RJ 4) and (RJ 5) were in the same class—class JR—in which JGM9-1 was the dominant microorganism. In contrast, groups R, J, and CK were in separate classes.

VIP values were used to measure the effect of volatile aromatic compounds on the overall aroma profile of the treatment groups. OAV analysis takes into account the concentration and odor threshold of aromatic compounds, indicating that aromatic compounds are of key significance to the overall aroma profile of the sample (Moreira et al., 2010; Liang et al., 2023; Wu et al., 2014; Zhou et al., 2016; Zhu et al., 2021). Compounds with VIP values of >1.0 and OAV values of >1.0 were determined to have a significant effect on the essential aromatic characteristics of the fermented tuna cooking liquid; the results are shown in Appendix Fig. S4 and Appendix Table S2. Changes in the characteristic aromatic compounds of five main classes in tuna cooking liquid were determined.

There were nine characteristic aromatic compounds in the first class (CK group), which were associated with fishy, stinky, and greasy aromas. Specifically, 1-octen-3-ol (3.18), hexanal (3.62), and decanal (6.35) contributed to fishy aromas (the values in parentheses are OVA values). 2-Nonanone (26.10) contributed to the greasy aroma. Trimethylamine (6.62) and dimethyltrisulfide (3.41) contributed to stinky aromas. The results showed that the unpleasant aromatic components of tuna cooking liquid were mainly 1-octen-3-ol, hexanal, decanal, 2-nonanone, trimethylamine, and dimethyltrisulfide. Furthermore, the CK group contained some pleasant aromatic compounds, such as 2-ethylfuran (11.03) and 2-pentylfuran (14.91).

There were five characteristic aromatic compounds in the second class, which consisted of group R. These compounds included E-2-dodecenal (2.01), which contributed to the grassy aroma, and 2-butyl-2-octenal (1.52) and 2-undecanone (5.32), which contributed to the fruity aroma. Furthermore, the meaty aromas of 2-ethylfuran (21.47) and 2-pentylfuran (15.61) was detected.

There were seven characteristic aromatic compounds in the third class, which consisted of class RJ (RJ1, RJ2, and RJ3). These compounds included phenethyl alcohol (4.64) and ethyl phenylacetate (3.70), which contributed to the floral aroma and 2-undecanone (7.05), and ethyl pyruvate (7.45), and 2-butyl-2-octenal (1.07), which contributed to the fruity aroma. In addition, the meaty aromas of 2-ethylfuran (10.37) and 2-pentylfuran (6.60) was detected.

There were 12 characteristic aromatic compounds in the fourth class, which was composed of class JR (RJ4 and RJ5). These compounds included phenethyl alcohol (8.94), ethyl myristate (1.50), and ethyl phenylacetate (5.07), which contributed to the floral aroma; ethyl caprylate (10.54), 1-octanol (7.60), ethyl propanoate (1.53), ethyl acetate (2.30), 2-undecanone (1.08), and 2-butyl-2-octenal (1.47), which contributed to the fruity aroma; and E-2-dodecenal (2.18), which contributed to the grassy aroma. In addition, the meaty aromas of 2-ethylfuran (1.47) and 2-pentylfuran (2.38) was detected.

There were 11 characteristic aromatic compounds in the fifth class (group J). These compounds included phenethyl alcohol (11.85), ethyl myristate (1.08), and ethyl phenylacetate, (5.57) which contributed to floral aromas, as well as ethyl caprylate (6.37), 1-octanol (2.74), ethyl propanoate (1.76), ethyl acetate (2.86), ethyl pyruvate (24.74), ethyl laurate (1.27), and 3-methyl-1-butanol (1.28), which contributed to fruity aromas. In addition, the meaty aromas of 2-ethylfuran (1.50) and 2-pentylfuran (3.23) was detected.

3.4. Characteristic volatile aromatic compounds produced and consumed by RP26 and JGM9-1

The Spearman's pairwise correlations between microbes and volatile aromatic compounds are shown in Appendix Fig. S5, where $|r| > 0.5$ and $P < 0.05$ represent a robust correlation. Twenty-five volatile aromatic compounds were strongly negatively correlated with *Lactobacillus plantarum* RP26 strains, and nineteen volatile aromatic compounds were strongly positively correlated with *Lactobacillus plantarum* RP26 strains. Thirty-eight volatile aromatic compounds were strongly negatively correlated with *Cyberlindnera fabianii* JGM9-1 strains, and 30 volatile aromatic compounds were strongly positively correlated with *Cyberlindnera fabianii* JGM9-1 strains.

Through HCA analysis, VIP value analysis, OAV analysis, Spearman's pairwise correlation, and reviewing the aroma descriptions of the related compounds in Table 1, we clarified the characteristic volatile aromatic compounds that were produced and consumed by RP26 and JGM9-1. Using Spearman's correlation analysis, we screened 21 strong pairwise correlations with a VIP value of >1.0, an OAV value of >1.0, and $|r| > 0.5$. The correlation network visualization analysis is shown in Fig. 4. Six characteristic aromatic compounds were strongly correlated with *Lactobacillus plantarum* RP26. The characteristic aromatic compounds consumed by RP26 through fermentation were 2-nonanone (greasy aroma), trimethylamine (stinky aroma), and dimethyltrisulfide (stinky aroma). Furthermore, the characteristic aromatic compounds

Table 1
Changes in volatile aromatic compounds during fermentation of tuna cooking liquid.

Retention time	Retention index	Volatile aromatic compound	Classes	Estimated concentration ($\mu g \cdot g^{-1}$)								Aroma description
				R	RJ 1	RJ 2	RJ 3	RJ 4	RJ 5	J	CK	
2.288	369	Ethanol	Alcohols	–	5.92 ± 0.06[e]	23.8 ± 1.06[d]	34.07 ± 1.26[b]	31.40 ± 1.09[c]	35.31 ± 1.13[b]	42.53 ± 2.11[a]	2.17 ± 0.02[f]	Irritant
2.984	697	3-Methyl-1-butanol	Alcohols	–	–	1.56 ± 0.03[d]	2.26 ± 0.01[c]	1.84 ± 0.04[d]	3.40 ± 0.03[b]	5.11 ± 0.05[a]	–	Apple brandy
5.695	721	Ethyl vinyl carbinol	Alcohols	12.02 ± 0.05[b]	6.75 ± 0.02[c]	4.48 ± 0.02[d]	3.44 ± 0.03[d]	–	–	25.26 ± 1.52[a]	–	
11.017	946	(Z)-3-Nonen-1-ol	Alcohols	27.75 ± 0.74[a]	23.08 ± 0.54[b]	20.81 ± 0.54[c]	16.12 ± 0.06[d]	9.99 ± 0.21[e]	5.52 ± 0.11[f]	4.85 ± 0.11[f]	6.08 ± 0.09[f]	
13.775	960	1-Heptanol	Alcohols	–	8.59 ± 0.22[c]	10.48 ± 0.54[b]	9.37 ± 0.06[c]	9.00 ± 0.28[c]	2.16 ± 0.21[d]	15.36 ± 1.21[a]	–	Aromatic
15.295	968	3,5-Octadien-2-one	Alcohols	5.75 ± 0.11[a]	–	–	–	–	–	–	4.48 ± 0.06[b]	
16.06	969	1-Octen-3-ol	Alcohols	2.51 ± 0.07[b]	1.12 ± 0.04[c]	0.56 ± 0.01[d]	0.26 ± 0.01[d]	–	–	–	3.18 ± 0.02[a]	Fishy
20.652	1059	1-Octanol	Alcohols	10.18 ± 0.21[f]	35.52 ± 1.52[c]	40.32 ± 2.24[b]	46.86 ± 2.06[a]	41.06 ± 1.21[b]	23.32 ± 0.81[d]	14.81 ± 1.11[e]	–	Fruity
22.292	1060	1-Hexanol	Alcohols	2.68 ± 0.11[d]	3.38 ± 0.24[c]	3.97 ± 0.34[c]	6.91 ± 0.05[a]	5.92 ± 0.31[b]	5.72 ± 0.51[b]	5.42 ± 0.11[b]	–	Fruit wine
23.173	1136	Phenethyl alcohol	Alcohols	–	–	4.49 ± 0.24[c]	6.50 ± 0.06[c]	10.42 ± 1.27[b]	12.52 ± 1.28[b]	16.59 ± 1.88[a]	–	Rose
24.123	1159	1-Nonanol	Alcohols	–	1.07 ± 0.29c	1.31 ± 0.01c	1.25 ± 0.01c	1.67 ± 0.08c	2.07 ± 1.28b	3.00 ± 0.28a	–	
24.22	1175	(E,Z)-3,6-Nonadien-1-ol	Alcohols	–	8.38 ± 0.20[c]	9.30 ± 0.66[b]	12.19 ± 0.24[a]	2.23 ± 0.22[d]	8.00 ± 0.33[c]	7.99 ± 1.24[c]	–	
26.1	1258	1-Decanol	Alcohols	1.65 ± 0.08[c]	6.11 ± 0.29[b]	5.45 ± 0.05[b]	7.26 ± 0.25[a]	5.96 ± 0.34[b]	6.23 ± 0.29[b]	6.05 ± 0.85[b]	–	Sweet, flower, fruit
26.56	1357	2-Nonanol	Alcohols	1.17 ± 0.02[c]	1.60 ± 0.14[c]	3.06 ± 0.05[b]	3.16 ± 0.01[b]	3.77 ± 0.07[b]	4.26 ± 0.44[b]	8.88 ± 1.28[a]	–	Earthy
27.11	1670	Tridecan-1-ol	Alcohols	3.25 ± 0.27[a]	–	1.39 ± 0.01[c]	–	–	–	2.57 ± 0.22[b]	–	
28.21	1708	Cis-3-hexen-1-ol	Alcohols	7.91 ± 1.21[b]	11.80 ± 1.14[a]	10.55 ± 0.22[a]	11.91 ± 1.20[a]	–	5.90 ± 0.20[c]	0.92 ± 0.11[d]	–	Grass
29.151	1886	Cis-hept-4-enol	Alcohols	0.17 ± 0.01[d]	–	1.59 ± 0.01[b]	1.72 ± 0.01[b]	0.68 ± 0.18[c]	1.05 ± 0.01[c]	4.52 ± 0.03[a]	–	Cream
30.404	1965	Hept-3-(Z)-en-1-ol	Alcohols	–	1.12 ± 0.04[c]	1.2 ± 0.01[c]	1.72 ± 0.01[b]	2.33 ± 0.02[a]	1.84 ± 0.02[b]	–	–	
31.24	2033	2,6-Dimethyl-1,7-octadien-3-ol	Alcohols	–	–	9.10 ± 0.75[c]	13.41 ± 1.24[a]	8.09 ± 1.28[d]	8.00 ± 0.33[d]	10.63 ± 0.10[b]	–	
32.091	2065	Cedrol	Alcohols	–	–	–	–	1.05 ± 0.01[b]	1.58 ± 0.01[a]	1.79 ± 0.01[a]	–	Woody Fragrance
6.654	715	2-Pentenal	Aldehydes	11.96 ± 1.27[a]	5.16 ± 0.22[b]	–	–	–	–	–	1.81 ± 0.11[c]	
12.775	806	Hexanal	Aldehydes	1.93 ± 0.11[c]	1.62 ± 0.06[c]	0.72 ± 0.01[d]	1.03 ± 0.08[d]	0.15 ± 0.01[e]	0.92 ± 0.01[d]	3.07 ± 0.21[b]	16.30 ± 1.43[a]	Fishy
13.568	814	(E)-2-hexenal	Aldehydes	–	6.27 ± 0.52[b]	3.83 ± 0.12[c]	3.31 ± 0.22	9.63 ± 0.12[a]	3.47 ± 0.12[c]	3.43 ± 0.20[c]	6.10 ± 0.23[b]	Fresh fruit
15.295	878	2-Butenal	Aldehydes	0.46 ± 0.08[d]	3.31 ± 0.11[c]	3.78 ± 0.12[c]	1.99 ± 0.12	4.06 ± 0.22[c]	4.21 ± 0.26[c]	5.84 ± 0.62[b]	10.22 ± 1.20[a]	Smelly

Table 1 (*continued*)

Retention time	Retention index	Volatile aromatic compound	Classes	Estimated concentration ($\mu g \cdot g^{-1}$)								Aroma description
				R	RJ 1	RJ 2	RJ 3	RJ 4	RJ 5	J	CK	
15.586	906	Heptanal	Aldehydes	5.01 ± 0.03[b]	1.93 ± 0.04[c]	1.81 ± 0.02[c]	–	–	–	–	6.44 ± 0.20[a]	Rancid
16.202	912	Sorbic aldehyde	Aldehydes	6.38 ± 0.15[a]	5.44 ± 0.13[b]	2.92 ± 0.11[c]	3.11 ± 0.24	–	–	–	2.42 ± 0.10[c]	
16.221	913	(*E*)-2-heptenal	Aldehydes	4.51 ± 0.25[a]	1.69 ± 0.22[b]	1.17 ± 0.14[c]	–	–	–	–	2.05 ± 0.11[b]	Grass
20.432	964	Benzaldehyde	Aldehydes	3.20 ± 0.21[a]	1.76 ± 0.11[c]	–	–	–	–	–	2.30 ± 0.09[b]	Bitter almond
21.533	1005	Octanal	Aldehydes	12.02 ± 0.05[b]	5.73 ± 0.15[c]	3.39 ± 0.19[d]	2.45 ± 0.10[e]	1.79 ± 0.01[e]	2.17 ± 0.05[e]	2.26 ± 0.11[e]	16.37 ± 1.50[a]	Fishy aroma
22.143	1011	Oct-2(*E*)-enal	Aldehydes	32.49 ± 1.15[a]	10.52 ± 1.11[b]	6.19 ± 0.14[c]	4.54 ± 0.19[e]	2.78 ± 0.11[f]	4.32 ± 0.12[e]	5.61 ± 0.15[d]	7.17 ± 1.20[c]	Nutty and oily aroma
23.543	1013	(*E,E*)-2,4-heptadienal	Aldehydes	7.01 ± 0.18[b]	3.75 ± 0.12[c]	2.33 ± 0.11[d]	2.72 ± 0.11[d]	2.02 ± 0.13[d]	2.89 ± 0.15[d]	4.29 ± 0.01[c]	5.64 ± 1.10[a]	Fishy
25.675	1023	2-Butyl-2-octenal	Aldehydes	4.55 ± 0.11[a]	–	1.50 ± 0.21[c]	–	–	4.40 ± 0.11[a]	2.58 ± 0.01[b]	–	Fruit
26.227	1112	(*E*)-2-Nonenal	Aldehydes	13.96 ± 0.61[a]	5.89 ± 0.16[c]	4.27 ± 0.17[d]	3.45 ± 0.11[e]	–	–	3.50 ± 0.01[e]	7.76 ± 2.20[b]	Citrus
26.304	1120	(*E*)-Nona-2,6-dienal	Aldehydes	11.86 ± 1.21[a]	4.27 ± 0.15[b]	3.43 ± 0.13[c]	3.56 ± 0.12[c]	3.04 ± 0.11[c]	4.52 ± 0.13[b]	4.67 ± 0.14[b]	–	
26.522	1170	1-Nonanal	Aldehydes	3.68 ± 0.61[c]	48.68 ± 3.22[a]	46.78 ± 2.22[a]	44.87 ± 3.61[a]	15.18 ± 8.11[b]	1.40 ± 0.01[d]	–	0.59 ± 1.20[d]	Animal fat
26.84	1175	(*E,Z*)-2,6-nonadien-1-al	Aldehydes	13.31 ± 0.61[a]	5.24 ± 0.09[b]	4.85 ± 0.31[b]	3.31 ± 0.22[c]	3.26 ± 0.04[c]	2.42 ± 0.09[d]	2.09 ± 0.04[d]	–	Cucumbers melons
27.97	1200	2,4-Decadienal	Aldehydes	5.41 ± 0.44[d]	3.57 ± 0.23[e]	17.69 ± 1.21[b]	12.55 ± 0.98[c]	2.23 ± 0.01[e]	0.63 ± 0.01[f]	–	33.89 ± 2.11[a]	Sweet
28.13	1205	Decanal	Aldehydes	1.7 ± 0.02[b]	1.26 ± 0.01[b]	0.80 ± 0.01[c]	–	–	–	–	12.7 ± 2.10[a]	Marine products
29.44	1212	2-Decenal	Aldehydes	4.27 ± 0.09[b]	4.46 ± 0.42[b]	6.72 ± 0.41[a]	3.43 ± 0.09[c]	–	4.91 ± 1.01[b]	–	2.66 ± 0.10[d]	
31.45	1345	(*E,E*)-2,4-heptadienal	Aldehydes	5.23 ± 0.06[a]	5.26 ± 0.41[a]	3.56 ± 0.09[b]	3.20 ± 0.21[b]	–	–	2.88 ± 0.05[b]	–	Chicken
35.85	1401	(*E,E*)-2,4-nonadienal	Aldehydes	6.16 ± 0.24[b]	–	–	–	–	–	–	1.50 ± 0.08[a]	Chicken soup
42.54	1410	(*E*)-2-dodecenal	Aldehydes	2.15 ± 0.11[c]	9.38 ± 0.23[a]	6.72 ± 0.51[b]	–	2.33 ± 0.31[c]	1.90 ± 0.22[c]	–	–	Grass
2.768	576	Acetic acid	Acids	25.59 ± 2.36[a]	16.76 ± 2.33[b]	13.84 ± 1.38[c]	11.65 ± 1.31[c]	6.05 ± 0.33[d]	5.50 ± 0.03[d]	–	4.64 ± 0.09	
3.288	615	β-methylvaleric acid	Acids	3.80 ± 0.12[b]	4.67 ± 0.11[a]	2.64 ± 0.12[c]	3.43 ± 0.13[b]	3.39 ± 0.11[b]	2.16 ± 0.18[c]	–	–	Herbs
9.154	865	2-Methyl-propanoic acid	Acids	3.64 ± 0.32[a]	–	–	–	–	–	–	–	Sour
14.123	974	Hexanoic acid	Acids	3.34 ± 0.55[a]	3.25 ± 0.33[a]	1.36 ± 0.02[b]	0.60 ± 0.02[b]	–	–	–	–	Coconut oil
26.961	1073	Heptanoic acid	Acids	–	–	–	–	–	–	–	2.08 ± 0.12[a]	Slightly decayed fat
13.688	644	1-Penten-3-one	Ketones	–	–	–	–	–	–	–	3.82 ± 0.32[a]	Garlic, pungent

Table 1 (*continued*)

Retention time	Retention index	Volatile aromatic compound	Classes	Estimated concentration (µg·g^{-1})								Aroma description
				R	RJ 1	RJ 2	RJ 3	RJ 4	RJ 5	J	CK	
15.84	943	1-Octen-3-one	Ketones	–	–	–	–	0.88 ± 0.11^c	4.13 ± 0.52^b	5.41 ± 0.21^a	–	Metal
16.276	983	5-Methylheptan-3-one	Ketones	5.75 ± 0.11^a	3.81 ± 0.17^c	4.28 ± 0.15^b	4.21 ± 0.42^b	1.16 ± 0.12^e	1.04 ± 0.13^e	2.74 ± 0.17^d	–	Herbs
15.978	1041	3-Ethenyl-cyclohexanone	Ketones	3.05 ± 0.62^a	1.53 ± 0.12^c	–	–	–	–	2.10 ± 0.12^b	–	
17.007	1052	5-Methylheptan-3-one	Ketones	–	20.54 ± 5.33^a	11.29 ± 1.33^b	11.20 ± 2.13^b	–	–	10.44 ± 1.33^b	–	
17.187	1054	2-Nonanone	Ketones	–	–	–	–	–	0.65 ± 1.03^b	0.66 ± 1.33b^a	5.22 ± 0.02^a	Animal fat
21.73	1089	(*E,E*)-3,5-octadien-2-one	Ketones	–	–	–	–	–	–	–	5.28 ± 0.52^a	Fat
27.303	1159	Heptylidene acetone	Ketones	22.18 ± 2.33^a	16.09 ± 1.33^b	12.40 ± 2.11^c	9.48 ± 1.08^d	20.61 ± 2.03^a	14.68 ± 2.73^b	6.42 ± 0.33^e	0.73 ± 0.10^f	Citrus, dairy products
28.676	1261	2-Undecanone	Ketones	29.23 ± 3.43^b	35.25 ± 2.43^a	38.78 ± 2.90^a	25.61 ± 2.88^b	5.97 ± 0.12^c	0.61 ± 0.11^d	–	–	Fruit, cheese
29.942	1266	2-Tridecanone	Ketones	2.55 ± 0.08^a	2.03 ± 0.02^b	1.64 ± 0.10^c	2.43 ± 0.12^a	1.17 ± 0.04^d	–	–	–	Coconut, nutty, herbal
30.653	1268	Nonyl methyl ketone	Ketones	–	–	–	–	–	–	–	1.76 ± 0.11^a	Rutaceae
31.865	1284	Dihydro-5-pentyl-furanone	Ketones	–	–	3.84 ± 0.01^d	4.67 ± 0.12^b	4.26 ± 0.02^c	4.92 ± 0.08^b	6.77 ± 0.19^a	–	Peach, apricot
36.875	1447	Hydroxyacetone	Ketones	–	–	–	–	3.00 ± 0.19^a	3.20 ± 0.09^a	–	–	Fragrant and sweet
2.987	158	Ethyl pyruvate	Esters	–	0.60 ± 0.03^d	1.47 ± 0.02^d	31.28 ± 2.39^c	142.45 ± 8.33^a	152.54 ± 9.44^a	103.91 ± 7.65^b	–	Apples, mandarin oranges
5.048	592	Ethyl acetate	Esters	–	–	–	–	12.55 ± 0.43^c	17.23 ± 0.46^b	21.48 ± 1.35^a	–	Grapes, cherries, pineapples
5.156	675	Hexyl formate	Esters	12.77 ± 0.53^c	22.23 ± 3.33^a	22.61 ± 2.73^a	18.40 ± 2.63^b	–	–	6.82 ± 0.12^d	–	Grass
10.683	757	Ethyl propanoate	Esters	–	–	–	2.97 ± 0.30^d	11.34 ± 0.12^c	15.34 ± 0.13^b	17.55 ± 0.29^a	–	Pineapples
11.234	876	Isopentyl acetate	Esters	–	–	–	–	23.51 ± 1.39^a	24.10 ± 1.32^a	0.60 ± 0.01^b	–	Bananas, pears
12.456	964	Vinyl acetate	Esters	–	0.27 ± 0.02^c	0.52 ± 0.01^c	0.67 ± 0.00^c	0.81 ± 0.12^c	1.67 ± 0.12^b	2.90 ± 0.02^a	–	Fruit
13.677	1013	Ethyl caprylate	Esters	–	–	–	–	16.87 ± 1.30^a	5.38 ± 0.07^c	10.20 ± 0.92^b	–	Fruits
15.585	1259	Ethyl phenylacetate	Esters	–	–	–	11.11 ± 1.32^c	15.12 ± 1.39^b	15.20 ± 1.42^b	16.71 ± 1.45^a	–	Honey
16.464	1284	Caprylyl acetate	Esters	–	–	–	–	1.53 ± 0.00^c	2.16 ± 0.01^b	2.81 ± 0.21^a	–	Irritant
18.574	1322	2-Cyclohexaneethyl acetate	Esters	–	–	–	–	3.63 ± 0.12^b	3.87 ± 0.13^b	5.36 ± 0.33^a	–	Rose
20.543	1407	1-Norbornanemethanol acetate	Esters	–	1.89 ± 0.02^d	2.45 ± 0.12^c	2.02 ± 0.00^d	5.56 ± 0.10^b	6.68 ± 0.12^a	–	–	
21.642	1473	1,3-Nonanediol, 1-acetate	Esters	6.86 ± 0.11^a	5.38 ± 0.21^b	5.00 ± 0.52^b	5.31 ± 0.21^b	–	–	–	2.84 ± 0.12^c	Herbal aroma
30.653	1675	Ethyl laurate	Esters	–	–	–	–	1.08 ± 0.00^b	–	2.03 ± 0.01^a	–	Fruit
32.645	1779	Ethyl myristate	Esters	–	–	–	2.07 ± 0.12^b	3.90 ± 0.08^a	3.67 ± 0.03^a	0.52 ± 0.00^c	1.83 ± 0.15^b	Orris oil
35.564	1978	Ethyl palmitate	Esters	–	–	–	–	0.67 ± 0.00^b	1.18 ± 0.04^a	1.25 ± 0.01^a	–	Cream

Table 1 (*continued*)

Retention time	Retention index	Volatile aromatic compound	Classes	Estimated concentration ($\mu g \cdot g^{-1}$)								Aroma description
				R	RJ 1	RJ 2	RJ 3	RJ 4	RJ 5	J	CK	
36.674	1986	Ethyl 9-hexadecenoate	Esters	–	–	–	–	–	0.98 ± 0.02[a]	0.54 ± 0.01[b]	–	
39.645	2037	Dibutyl phthalate	Esters	–	0.39 ± 0.05[b]	0.49 ± 0.00[b]	–	–	0.66 ± 0.00[a]	–	–	
12.065	933	2-Nonyne	Alkanes	3.75 ± 0.06[a]	–	–	–	–	–	–	2.63 ± 0.00[b]	
17.642	980	1-Methylethylidene-cyclohexane	Alkanes	3.80 ± 0.12[b]	2.16 ± 0.01[c]	1.11 ± 0.02[d]	–	–	–	2.65 ± 0.07[c]	7.76 ± 0.11[a]	
21.674	1102	1,3-Dimethyl-2-(1-methylethyl)-cyclopentene	Alkanes	–	7.91 ± 0.81[a]	6.61 ± 0.32[b]	4.52 ± 0.01[c]	–	0.66 ± 0.00[e]	2.48 ± 0.06[d]	–	
21.765	1171	4-Ethyl-phenol	Alkanes	2.75 ± 0.04[a]	2.11 ± 0.15[b]	1.56 ± 0.03[c]	1.24 ± 0.05[d]	–	–	1.64 ± 0.01[c]	2.32 ± 0.02[b]	
26.981	1204	Di-t-butylacetylene	Alkanes	4.16 ± 0.34[a]	1.25 ± 0.00[c]	–	–	2.02 ± 0.13[b]	2.42 ± 0.22[b]	2.88 ± 0.11[b]	0.67 ± 0.04[c]	
27.112	1332	1-Tridecene	Alkanes	–	–	–	–	–	–	1.83 ± 0.00[b]	13.47 ± 0.76[a]	
27.267	1387	Cyclododecyne	Alkanes	4.17 ± 0.01[a]	1.79 ± 0.00[c]	0.2 ± 0.00[d]	3.12 ± 0.07[b]	1.57 ± 0.01[c]	1.64 ± 0.02[c]	0.29 ± 0.00[d]	1.58 ± 0.01[c]	
28.527	1400	Tetradecane	Alkanes	8.30 ± 0.69[a]	4.60 ± 0.05[c]	3.17 ± 0.06[d]	–	–	–	–	5.68 ± 0.22[b]	
28.75	1419	2,6,10-Trimethyltridecane	Alkanes	5.55 ± 0.25[a]	3.52 ± 0.01[b]	1.83 ± 0.01[d]	–	–	2.95 ± 0.03[c]	2.96 ± 0.05[c]	2.11 ± 0.12[d]	
26.981	1423	5-Ethenyl-2-methoxy-phenol	Alkanes	–	2.12 ± 0.06[b]	1.39 ± 0.01[d]	1.70 ± 0.04[c]	1.12 ± 0.02[d]	1.83 ± 0.03[c]	2.79 ± 0.02[a]	1.30 ± 0.01[d]	
27.112	1504	1-Propyloctyl-benzene	Alkanes	–	–	0.91 ± 0.00[a]	–	–	0.59 ± 0.00[b]	–	0.54 ± 0.02[b]	
27.267	1653	2,6,10,14-Tetramethyl-pentadecane	Alkanes	40.30 ± 2.34[b]	37.64 ± 2.93[c]	37.04 ± 5.01[c]	28.59 ± 4.33[d]	8.45 ± 0.33[e]	6.53 ± 0.23[f]	–	55.62 ± 5.05[a]	
28.527	1758	Butylidene cyclohexane	Alkanes	3.20 ± 0.03[c]	0.97 ± 0.01[e]	1.92 ± 0.21[d]	4.40 ± 0.08[b]	3.32 ± 0.01[c]	5.31 ± 0.23[a]	–	1.30 ± 0.01[e]	
···28.75	1882	6-(Z)-1-butenyl-1,4-cycloheptadiene	Alkanes	–	–	–	–	–	–	–	5.31 ± 0.21[a]	
30.613	1954	2-Methyltetracosane	Alkanes	6.06 ± 0.13[a]	2.23 ± 0.01[c]	0.56 ± 0.01[d]	3.34 ± 0.01[b]	–	–	–	3.02 ± 0.05[b]	
33.01	2090	Heptadecane	Alkanes	–	3.00 ± 0.01[b]	3.03 ± 0.01[b]	3.35 ± 0.00[b]	–	–	–	92.30 ± 7.01[a]	
35.156	2254	Nonadecane	Alkanes	3.15 ± 0.06[a]	2.23 ± 0.03[c]	1.62 ± 0.00[d]	2.79 ± 0.01[b]	0.42 ± 0.00[e]	0.66 ± 0.00[e]	2.73 ± 0.18[b]	–	
37.64	2473	(N)-docosane	Alkanes	0.40 ± 0.06[b]	0.60 ± 0.03[b]	0.35 ± 0.00[b]	0.51 ± 0.00[b]	0.36 ± 0.01[b]	0.74 ± 0.02[b]	0.70 ± 0.01[b]	20.20 ± 1.99[a]	
39.918	2753	Pentacosane	Alkanes	0.70 ± 0.02[b]	0.63 ± 0.00[b]	0.72 ± 0.00[b]	0.45 ± 0.00[b]	–	–	–	15.17 ± 2.32[a]	
39.401	2975	1,8,11,14-Heptadecatetraene	Alkanes	3.15 ± 0.02[a]	1.63 ± 0.04[c]	2.14 ± 0.01[b]	1.12 ± 0.05[d]	2.06 ± 0.00[b]	1.90 ± 0.01[c]	2.33 ± 0.07[b]	1.29 ± 0.03[d]	
41.42	3064	3-Propyl-cyclohexene	Alkanes	2.75 ± 0.03[b]	1.96 ± 0.02[c]	1.31 ± 0.01[d]	–	–	–	–	4.60 ± 0.05[a]	
42.606	3326	(N)-pentadecane	Alkanes	1.05 ± 0.01[b]	0.56 ± 0.00[c]	0.42 ± 0.03[c]	–	–	–	–	2.18 ± 0.33[a]	

Table 1 (*continued*)

Retention time	Retention index	Volatile aromatic compound	Classes	Estimated concentration (µg·g^{-1})								Aroma description
				R	RJ 1	RJ 2	RJ 3	RJ 4	RJ 5	J	CK	
43.167	3473	Octadecane	Alkanes	0.85 ± 0.01^d	1.93 ± 0.04^c	4.81 ± 0.13^b	4.66 ± 0.21^b	0.18 ± 0.00^e	0.25 ± 0.00^e	1.13 ± 0.00^d	17.53 ± 2.39^a	
43.248	3643	Tetradecyl-oxirane	Alkanes	1.55 ± 0.00^b	1.06 ± 0.06^c	0.32 ± 0.00^d	–	–	–	–	4.60 ± 0.00^a	
43.357	3765	1-Nonadecene	Alkanes	1.55 ± 0.05^a	1.33 ± 0.02^c	1.17 ± 0.14^d		0.98 ± 0.00^e	1.44 ± 0.14^b	1.48 ± 0.14^b	0.94 ± 0.00^e	
43.62	3790	Heneicosane	Alkanes	0.53 ± 0.01^d	0.65 ± 0.02^d	0.72 ± 0.05^d	0.37 ± 0.03^d	2.05 ± 0.05^b	1.70 ± 0.21^c	2.42 ± 0.05^b	5.35 ± 0.24^a	Coconut
2.559	463	Trimethylamine	Others	–	–	–	–	–	–	–	3.97 ± 0.06^a	Putrid
4.827	742	2-Ethylfuran	Others	15.93 ± 1.85^a	7.69 ± 0.06^b	5.33 ± 0.52^c	2.23 ± 0.32^d	1.22 ± 0.12^e	1.09 ± 0.21^e	1.11 ± 0.01^e	8.19 ± 0.12^b	Meaty
6.406	972	Dimethyl trisulfide	Others	–	–	–	–	–	–	–	7.50 ± 0.32^a	Fishy, sulfur
10.698	991	2-Pentylfuran	Others	12.91 ± 0.32^a	5.45 ± 0.09^b	1.91 ± 0.06^e	3.49 ± 0.35^c	0.98 ± 0.31^f	1.96 ± 0.06^e	2.66 ± 0.09^d	12.33 ± 2.34^a	Ham
15.431	1048	2-Pent-2-enylfuran	Others	29.85 ± 3.35^a	18.95 ± 1.30^c	19.65 ± 2.01^c	15.74 ± 2.11^d	9.14 ± 1.27^e	10.29 ± 1.33^e	16.62 ± 2.31^d	23.15 ± 2.39^b	

Note1 Aroma descriptions and estimated concentrations (µg•g^{-1}) are included. The data shown represent the means of three biological replicates. Retention indices were referred from the studies of previous research (Giri et al., 2010; Liang et al., 2023; Moreira et al., 2013; Wu et al., 2014; Zhu et al., 2021).

Note2: Different letters in the same row represent significant differences ($P < 0.05$). "–" indicates that the substance was not detected.
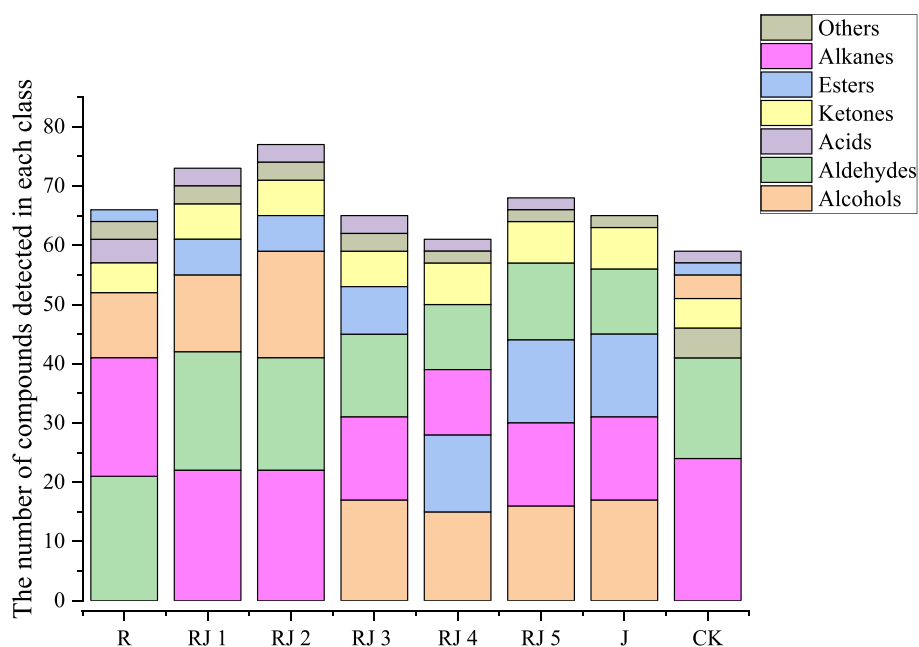

Fig. 2. Stacked bar chart of the numbers of compounds from each class detected in the treatment groups and the control group. Note: CK: Tuna cooking liquid; group R: RP26; group J: JGM9-1; group RJ1: Multiple strains of RP26 and JGM9-1 at a ratio of 100:1; group RJ2: Multiple strains of RP26 and JGM9-1 at a ratio of 20:1; group RJ3: Multiple strains of RP26 and JGM9-1 at a ratio of 10:1; group RJ4: Multiple strains of RP26 and JGM9-1 at a ratio of 5:1; group RJ5: Multiple strains of RP26 and JGM9-1 at a ratio of 1:1.

produced by RP26 through the fermentation process were (*E*)-2-dode-cenal (grassy aroma), 2-butyl-2-octenal (fruity aroma), and 2-undeca-none (fruity aroma). The fermentation of lactic acid bacteria can degrade saturated aldehydes into ketones and enals via enzymatic re-actions, which was an important mechanism for the formation of aromatic compounds in the fermented product (Smit et al., 2004). For example, *Lactobacillus* spp. can convert enones into enals, such as 2-butyl-2-octenal and (*E*)-2-dodecenal, by enone reductase (Cheng, Huynh-Ba, Blank & Robert, 2008; Zhu et al., 2021). Ketones are formed by the enzymatic oxidation of free fatty acids into β-ketoacids and by
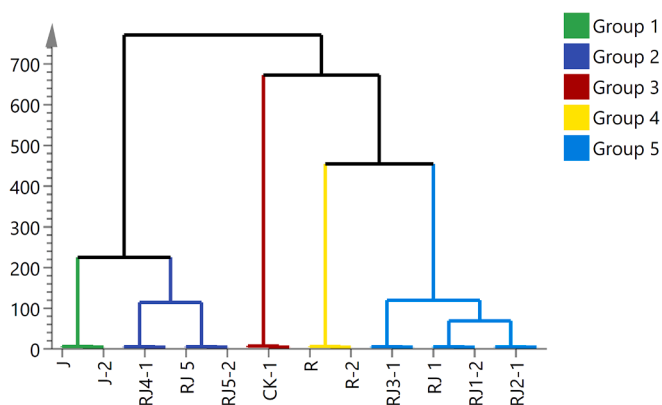
Fig. 3. HCA of volatile aromatic compounds in tuna cooking liquid during fermentation with bacteria and yeast. Note: CK: Tuna cooking liquid; group R: RP26; group J: JGM9-1; group RJ1: Multiple strains of RP26 and JGM9-1 at a ratio of 100:1; group RJ2: Multiple strains of RP26 and JGM9-1 at a ratio of 20:1; group RJ3: Multiple strains of RP26 and JGM9-1 at a ratio of 10:1; group RJ4: Multiple strains of RP26 and JGM9-1 at a ratio of 5:1; group RJ5: Multiple strains of RP26 and JGM9-1 at a ratio of 1:1. Data represent the means of two biological replicates,

losing a single carbon atom and their subsequent decarboxylation to ketones, such as 2-undecanone (Ziino et al., 2005).

Fifteen volatile aromatic compounds were strongly correlated with *Cyberlindnera fabianii* JGM9-1. The characteristic aromatic compounds consumed by JGM9-1 through fermentation were decanal (fishy aroma), hexanal (fishy aroma), 1-octen-3-ol (fishy aroma), trimethylamine (stinky aroma), and dimethyltrisulfide (stinky aroma). Furthermore, the characteristic aromatic compounds produced by JGM9-1 through fermentation were phenethyl alcohol (floral aroma), ethyl myristate (floral aroma), ethyl phenylacetate (floral aroma), ethyl pyruvate (fruity aroma), ethyl propanoate (fruity aroma), ethyl laurate (fruity aroma), ethyl caprylate (fruity aroma), 1-octanol (fruity aroma), ethyl acetate (fruity aroma), and 3-methyl-1-butanol (fruity aroma). After fermentation with *Cyberlindnera fabianii* JGM9-1, the most abundant volatile aromatic compounds were alcohols and esters. According to their
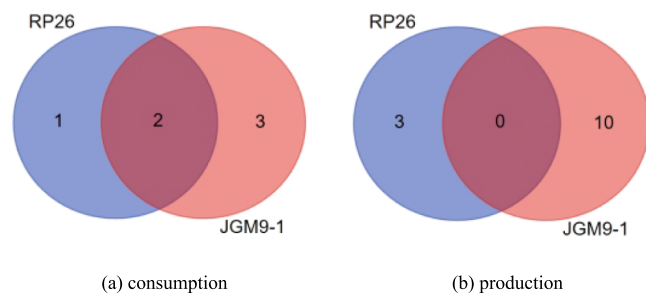
characteristics, these volatile aromatic compounds impart floral and fruity aromas. Higher alcohols are produced by yeast through either sugar or amino acid metabolic pathways using the Ehrlich mechanism (Delač Salopek et al., 2022). Ketones and aldehydes are reduced in amino acid metabolic pathways, resulting in the production of higher alcohols. Amino acids play an important role in volatile aromatic compound production by converting isoleucine and leucine into corresponding 3-methyl-1-butanol and phenethyl alcohol. 3-Methyl-1-butanol provides an apple brandy aroma, and phenethyl alcohol provides a rose aroma (Stewart, 2017). Yeast can also esterify acids with a small molecular weight with alcohols to produce esters. Short-chain esters are volatile at room temperature and have a low threshold value (Zhou et al., 2016). Moreover, some esters, such as ethyl pyruvate (fruity aroma), ethyl caprylate (fruity aroma), and phenethyl alcohol (floral aroma), have strong volatility (Stefanovic et al., 2018), and usually have an important influence on the overall aroma of the fermented tuna cooking liquid.

The Venn diagram in Fig. 5(a) indicates that six volatile aromatic compounds were consumed by RP26 and JGM9-1 strains in the fermentation process. Among them, two volatile aromatic compounds were consumed by both microbial species. Thirteen volatile aromatic compounds produced by the combination of the RP26 and JGM9-1 strains through the fermentation process are presented in Fig. 5(b). RP26 consumed three compounds with greasy and stinky aromas and simultaneously produced three compounds with grassy and fruity aromas. JGM9-1 consumed five compounds with fishy and stinky aromas and simultaneously produced 10 compounds with floral and fruity aromas. The results showed that multiple-strain fermentation may be a more efficient method for improving the aroma profile of the fermented products than single-strain fermentation. Compared with single-strain fermentation, the combination of yeast and lactic acid bacterial strains removed more unpleasant aromatic compounds including greasy, stinky, and fishy aromas, and added more pleasant aromatic compounds that contributed to fruity, grassy, and floral aromas.

In addition, *Lactobacillus* and yeast can produce multicopper oxidase to degrade trimethylamine, dimethyltrisulfide, and oxidized amines into aldehydes, ammonia, and H_2O_2 (Stefanovic et al., 2018). According to Callejon, multicopper oxidase that degrades amines can be isolated and purified from *L. plantarum* Jl6 strains, and the product encoded by the
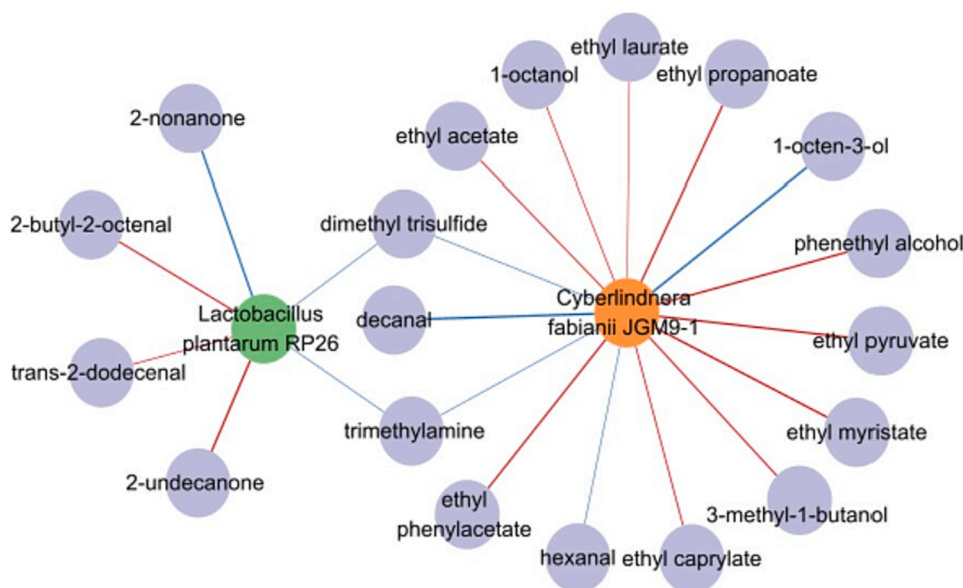
Fig. 4. Correlation network visualization based on significant correlations between characteristic volatile aromatic compounds and microbes. Note: Orange nodes represent *Cyberlindnera fabianii* JGM9-1, green nodes represent *Lactobacillus plantarum* RP26, and purple nodes represent characteristic volatile aromatic compounds. The red and blue solid lines indicate positive and negative correlations, respectively. Line width is proportional to the strength of correlation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(a) consumption

(b) production

Fig. 5. Venn diagram of characteristic volatile aromatic compounds produced and consumed by both microbes. Note: Blue shading corresponds to the characteristic volatile aromatic compounds of RP26, red shading corresponds to the characteristic compounds of JGM9-1, and purple shading corresponds to the characteristic compounds of both strains. (a) Characteristic volatile aromatic compounds consumed by microbial species during the deodorization process. (b) Compounds produced by microbial species during the deodorization process. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

L. plantarum Sufl gene is important in this reaction (Callejón et al., 2014). This may be the reason for the decline of trimethylamine and dimethyltrisulfide in tuna cooking liquid during fermentation.

4. Conclusions

In this study, the strains RP26 and JGM9-1 were used in the fermentation and deodorization of tuna cooking liquid. We investigated the dynamic changes in characteristic volatile aromatic substances during fermentation, using HCA analysis, VIP value analysis, and OAV analysis, allowing us to, for the fist time, determine the characteristic volatile aromatic compounds consumed and produced by these two different strains. These findings help in the clarification of the metabolic mechanisms of microbial fermentation and deodorization in tuna cooking liquid. In future experiments, we will study the metabolic pathways of the aromatic compounds of the JGM9-1 and RP26 strains.

CRediT authorship contribution statement

Wenjing Ma: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Zhangcheng Liang:** Data curation, Validation, Methodology, Software, Writing – original draft. **Bing He:** Data curation, Methodology, Software. **Yuxi Wu:** Data curation, Visualization. **Yan Chen:** Investigation. **Zhigang He:** Funding acquisition, Supervision, Writing – review & editing, Resources. **Bingyan Chen:** Data curation. **Xiaozi Lin:** Funding acquisition, Project administration, Writing – review & editing, Resources. **Lianyu Luo:** Data curation.

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.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100900.

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