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Original article

Profiling the effects of starter cultures on biochemical compounds in fermented fish sauces and their relationships with sensory perceptions

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Summary In this study, the effect of *Lactiplantibacillus* (*L.*) *plantarum* XL23 (Lp) and *Saccharomyces* (*S.*) *cerevisiae* RC212 (Sc) on flavour formation in fermented fish sauce production was investigated. The levels of, and relationship between, non-volatile and volatile compounds responsible for taste and aroma were determined in fish sauce samples. Regarding non-volatiles, the results showed that free amino acids and organic acids were significantly higher in Lp and Lp + Sc compared to traditional fish sauce. In fatty acids, there was an irregular distribution between the groups. In terms of volatile compounds, *L. plantarum* supported the presence of acidic compounds (1413.31 ng mL^{-1}), while *S. cerevisiae* supported the presence of alcoholic compounds at high levels (3891.56 ng mL^{-1}). Significant correlations between components proved the accuracy of analytical and sensory analyses and demonstrated the reliability of multi-replicate statistical interactions. The results indicated that inoculation with starter cultures changed the taste and aroma in favour of the strains.

Keywords Allochthonous starter culture, descriptive sensory analysis, fish sauce, *Lactiplantibacillus plantarum* XL23, *Saccharomyces cerevisiae* RC212, volatile compounds.

Introduction

Fish sauce is a popular fermented condiment that traditionally flavours dishes in Asian countries and nowadays is used all over the world. Its traditional production involves fermenting fish with salt and various spices. Fermentation plays a crucial role in developing the nutritional and bioactive properties, as well as the distinctive sensory attributes, of the product (Daeschel *et al.*, 1987; Leroy & De Vuyst, 2004). Consequently, these characteristics, including aroma, taste, and texture, hold significant importance for consumers (Hu *et al.*, 2022). The flavour profile of fermented fish products is primarily influenced by volatile and non-volatile compounds (Giri *et al.*, 2010a), contributing to a salty and synergistically umami taste (Yimdee & Wang, 2016).

Flavour formation is associated with numerous biological and chemical reactions occurring during the fermentation process. The breakdown of macromolecules by microorganisms and the chemical metabolism of endogenous enzymes in fish contribute to the rich flavour profile of sauces (Akolkar *et al.*, 2010; Gao *et al.*, 2020; Wang *et al.*, 2020). Microbiota dynamics during fermentation play a critical role, especially in final flavour formation (Zang *et al.*, 2018; Chan *et al.*, 2023). Therefore, the relationship between genus abundance in microbiota and flavour has been intensively investigated in recent years (Wang *et al.*, 2020; Feng *et al.*, 2021; Gao *et al.*, 2023). In research, controlled production studies are carried out to obtain typical flavours through autochthonous production modifications or the application of allochthonous microorganisms (Sim *et al.*, 2015; Udomsil *et al.*, 2017; Hu *et al.*, 2020; Li *et al.*, 2022a, 2022b, 2023).

Lactiplantibacillus (*L.*) *plantarum* and *Saccharomyces* (*S.*) *cerevisiae* are renowned for their enzymatic prowess in food fermentation (Pérez-Díaz *et al.*, 2017). *Lactiplantibacillus plantarum* excels in protein and peptide hydrolysis, breaking down complex proteins and lipids into digestible forms, enhancing flavour and texture in

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fermented foods (Leroy *et al.*, 2013; Wang *et al.*, 2017; Liu *et al.*, 2022). *Saccharomyces cerevisiae* showcases expertise in amino acid decarboxylation, crucial for aroma development in wines and beers, and moderate protein hydrolysis, enhancing nutritional value and sensory appeal in fermented products (Chaves-López *et al.*, 2011; Gao *et al.*, 2017). Both strains contribute significantly to fermentation processes, enriching the flavour and quality of foods (Diez *et al.*, 2010; Yin *et al.*, 2020). Research on fish sauce production from starter cultures, including LAB and *Saccharomyces* spp., has focused on monitoring the fermentation process (Lee *et al.*, 2015; Duan *et al.*, 2016; Wang *et al.*, 2018), flavour (Kasankala *et al.*, 2012; Sun *et al.*, 2016; Zhao *et al.*, 2017), quality (Sun *et al.*, 2016; Ormancı *et al.*, 2018), and safety (Lee *et al.*, 2016; Zeng *et al.*, 2017; Sang *et al.*, 2021; Ma *et al.*, 2022).

Despite the existence of contemporary studies on sauce production, there are still limited results that can be practically applied. In our study, in addition to traditional fish sauce production, we used *L. plantarum* XL23, isolated from sourdough, and the commercially available *S. cerevisiae* RC212 strains for sauce production. *L. plantarum* XL23 was chosen for this study to enhance the reproducibility of the applied production and methodology, as it is known to be safe for use in fermented food production, increases bioactivity and functionality, and is easily obtainable for scientific and industrial applications related to food fermentation. The *L. plantarum* XL23 strain was selected for its ability to lower pH, making it suitable for producing modified fish sauce with a minimum of 3% salt in our test groups. This helps with protein hydrolysis and controls potentially harmful microbiota from the raw fish material, which is necessary due to the lower salt content compared to traditional production (20%). In addition to *L. plantarum* XL23, *S. cerevisiae* RC212 was selected due to its accessibility for reproducing similar scientific works, its industrial applications, and its safety.

Biochemically, our previous pilot studies revealed that *L. plantarum* (a different strain) could attain the sensory and physicochemical characteristics of traditionally produced fermented fish sauce. However, the flavour profile still required improvement. The sole use of *S. cerevisiae* at lower salt concentrations (3–7%) had no effect and could not adequately survive beyond 7% to produce fermented fish sauce. Reducing the salt in favour of *S. cerevisiae* limited the fermentation and maturation of the final product. Optimal production was observed when *S. cerevisiae* was employed as a co-culture with a new strain of *L. plantarum*, which has been tested and well-documented for its fermentation conditions used in the production of the tested groups in this study. The rich umami and roasted flavours characteristic of traditional fish sauce were only achieved with the combination of these strains, even when the salt was reduced to healthier levels compared to traditional fish sauce. However, consumer acceptance still needs to be assessed. For this reason, our objective in this study was to characterise the flavour and taste profile as influenced by volatile and non-volatile compounds in the presence of these strains in optimised fish sauce production, as well as to evaluate consumer perception and acceptance.

Materials and methods

Materials

Anchovies (*Engraulis encrasicolus*) from the Black Sea region were sourced from the wholesale fish market in Çanakkale, Türkiye. The fish were transported to the laboratory in ice-cooled boxes to maintain freshness. The main nutritional characteristics of the fish were as follows: protein content at 19.05%, moisture at 76.20%, fat at 3.35%, and ash at 2.10%. Additionally, other ingredients such as coarse sea salt, sugar, powdered black pepper, and red pepper were purchased from the local market. All reagents used in the analysis were of analytical grade.

Methods

Preparation of starter cultures

The *L. plantarum* XL23 strain used in this study was previously isolated from traditional sourdough (Gunduz *et al.*, 2022). *Saccharomyces cerevisiae* RC212 (Lalvin, Canada) was acquired as a commercial strain. To activate the strains, *L. plantarum* was cultured twice in De Man Rogosa and Sharpe (MRS) medium under anaerobic conditions at 30 °C for 2 days. Meanwhile, *S. cerevisiae* underwent two rounds of culturing in yeast extract peptone dextrose (YPD) medium under aerobic conditions at 30 °C for 24 h. Subsequently, cells in the logarithmic growth phase were harvested and suspended in physiological saline to achieve cell densities of 0.5 McFarland units (approximately 1.5×10^8 cfu mL^{-1}) using a densitometer (Bio-San, Türkiye). The suspended cultures were then inoculated into prepared sauce doughs to initiate the fermentation process.

Production of fish sauce

The fish were rinsed with ice water and minced. The minced fish was then mixed with 3% sea salt, 4% sugar, 1% paprika, and 0.5% black pepper (w/w), along with 2% (v/w) starter culture suspensions (1.5×10^8 cfu mL^{-1}). Samples inoculated with 2% *L. plantarum* (by volume) were designated as single-culture fermentation and labelled 'Lp'. For combined-culture fermentation, *L. plantarum* (1%) and

S. cerevisiae (1%) were used together and labelled 'Lp + Sc'. The control group, labelled 'Tfs', consisted of 20% sea salt, 4% sugar, 1% paprika, and 0.5% black pepper (all percentages by weight) prepared using traditional methods without the use of a starter culture.

The sauce doughs of all groups were thoroughly mixed with the ingredients and fermented at 37 °C for 90 days. The fermentation process was monitored with quality criteria (appearance, odour, and taste) and chemical properties (total nitrogen content, amino acid nitrogen content, and pH). The fermentation was completed in a transparent, sediment-free product with a distinct fish sauce aroma and taste, containing total nitrogen levels ≤10 g L^{-1} and amino nitrogen levels ≤40%, achieved within a pH range of 4.5–6.5 (Codex Alimentarius, 2018). At the end of the fermentation period, all groups underwent a two-step filtration process: first through double-layer cheesecloth and then through paper filters moistened with distilled water. The collected sauce samples were then placed in glass jars and tightly sealed. All produced sauce samples were stored at +4 °C until used for analysis.

Non-volatile compounds

Determination of free amino acids. For the detection of free amino acid (FAA) concentrations, the Jasem LC–MS/MS amino acid kit (Sem Laboratory Devices Marketing Industry, Istanbul, Türkiye) was utilised. Samples were prepared according to the kit's procedure by adding reagents and internal standards. The analysis was carried out using an Agilent Infinity 1260 HPLC system, comprising a dual pump, a degasser, and an autosampler coupled with 6460 triple quadrupole mass spectrometers (Agilent Technologies, Santa Clara, CA, USA).

The analytical conditions of the HPLC were as follows: the injection volume was 3 μL; the column used was the Jasem analytical column specified for amino acid analysis; the column temperature was set to 30 °C; the mobile phases consisted of Jasem's mobile phase A and B with gradient elution. The gradient program involved an initial LC gradient of 22% A for 1 min, followed by a ramp to 78% A over 3 min and held for 0.5 min; thereafter, the column was equilibrated at 22% A for 3 min. The flow rate was maintained at 0.7 mL min^{-1}, and the total running time was 7.5 min.

Mass spectrometric detection was performed using the Agilent 6460 triple quadrupole MS equipped with an ESI source in the positive ion mode. The MS detector parameters were as follows: a drying gas temperature of 150 °C, a drying gas flow of 10 L min^{-1}, a nebuliser pressure of 40 psi, and a capillary voltage of 2000 V (+). The FAA content was expressed in mg 100 g^{-1} of total protein.

Determination of free fatty acids. Lipids were extracted from the samples using ether, and free fatty acids (FFA) were separated from total lipids according to the standards set by the International Olive Council (IOC, 2017). The supernatant was transferred into a sample injection vial, and analysis was conducted using an Agilent 7820A GC-FID (Agilent Technologies, Santa Clara, CA, USA). For the analysis, 1 μL of FAME (Fatty Acid Methyl Ester) was injected in split mode (1:50).

The analytical conditions of the GC-FID were as follows: an HP88 Agilent column (100 m × 0.25 mm ID, 0.20 μm film thickness) was used; the column temperature was initially set to 120 °C for 1 min and then increased to 175 °C at a rate of 10 °C min^{-1}, followed by an increase to 210 °C at a rate of 2 °C min^{-1}, with a final hold at 230 °C for 5 min. Both the injector and detector temperatures were set to 280 °C. Hydrogen (purity >99.99%) served as the carrier gas, with a flow rate of 0.2 mL min^{-1}. The identification of FFA was conducted by comparing retention times with FAME standards, and results were expressed as percentages.

Determination of organic acids. The water extract of the fermented fish sauces was analysed by injecting it into an HPLC-UV system (Shimadzu, Japan). The device and detection parameters were as follows: an injection volume of 10 μL; a chromatographic column composed of C18, measuring 25 cm × 4 mm × 5 μm; the column temperature set to 35 °C; the detector wavelength at UV-214 nm; and the mobile phase consisting of a 0.2 M KH_2PO_4 solution (pH 2.4, with orthophosphoric acid), with a flow rate of 0.8 mL min^{-1}. Results were expressed as g L^{-1}.

Extraction, identification, and quantification of volatile compounds

The volatile compounds of the fermented fish sauce were isolated using Solid Phase Microextraction (SPME) and subsequently analysed via gas chromatography–mass spectrometry (GC–MS; Agilent Technologies, Wilmington, DE, USA). The sample was added to amber vials along with NaCl and an internal cyclohexanol standard (purity ≥99.9%, Sigma-Aldrich Co., Ltd, USA), and the vials were sealed with a PTFE/silicone septum. Subsequently, the vials were gradually heated in a water bath to 50 °C to stabilise the volatiles in the headspace, allowing them to be collected on a SPME needle (2 cm to 50/30 μm DVB/Carboxen/PDMS, Supelco, Bellafonte). After 1 h, the needle was removed from the vial and injected into a GC–MS system consisting of an HP 6890 GC coupled with a 7895C mass selective detector (Hosoglu *et al.*, 2020).

The GC–MS conditions were set up as follows: an HP5 MS column (30 m × 0.25 mm ID, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was

used. The oven programme started with an initial temperature of 40 °C for 1 min, followed by a ramp from 40 to 250 °C at a rate of 4 °C min^{-1}, with a final hold at 250 °C for 10 min. Helium was used as the carrier gas with a flow rate of 1 mL min^{-1}. The capillary direct interface temperature was set to 280 °C, the ionisation energy was 70 eV, the mass range was 35–350 m/z, and the scan rate was 4.45 scans s^{-1}.

For the identification of volatile compounds, the National Institute of Standards and Technology (NIST) and Wiley Mass Spectral Data Records were utilised. The concentration of compounds identified in the fermented fish sauce samples was determined based on the area ratio of each compound to an Internal Standard (IS), as described by Avsar *et al.* (2004). Volatile compounds were expressed in ng mL^{-1}.

Sensory analysis

Sensory analysis was conducted following the method outlined by Ritthiruangdej & Suwonsichon (2006) and Ormancı *et al.* (2018), with slight modifications. The fermented fish sauce samples were assessed by nine experienced panellists, comprising three females and six males aged between 25 and 55 years. Before commencing the sensory analysis, the panellists underwent brief training, which included familiarisation with various fermented foods, sauces, and salt-cured fish samples, as well as identification of the sensory attributes of these products (Data S1). Guidance was also provided on the assessment of our sauce samples, and instructions were given on how to complete the sensory analysis form (Data S2).

The sensory evaluation was conducted using a nine-point hedonic scale where aroma, basic taste, aftertaste, and related attributes were rated from 1 (not perceived) to 9 (strongly perceived). To minimise bias and deviations, unique codes representing each sauce group, along with their corresponding parallel samples, were randomly assigned and then provided to each panellist in different orders. Panellists were provided with unsalted crackers and water to neutralise their palates between tastings of the various fermented fish sauce groups.

Statistical analysis

All data in this study were obtained in triplicate. One-way analysis of variance (ANOVA) was conducted using SPSS 27 statistical software (SPSS Inc., Chicago, IL, USA) to ascertain the differences between fish sauce groups, with deviations expressed as mean ± SD. The Tukey test was utilised for multiple comparisons between groups ($P < 0.05$). The Kruskal–Wallis test was employed to compare means of non-parametric data on volatile compounds and sensory analyses. When significant effects were observed, Dunn's test was used for comparison, with significance set at $P < 0.01$ and $P < 0.05$. Pearson correlation coefficient was utilised to determine the relationship between the quantities of volatile and non-volatile compounds and the sensory criteria of the sauce groups. Principal component analysis (PCA) was conducted using SPSS to assess the interaction of multivariate data. Hierarchical cluster analysis (HCA) of all volatile compounds in comparison with sensory criteria was carried out using Clustvis. Two-way clustering was applied to volatile compounds and sensory analyses.

Results and discussion

Free amino acids

Free amino acids play a crucial role in imparting umami, sweet, bitter, salty, and sour flavours and serve as precursors to numerous volatile flavour compounds. Fish sauce typically contains around 10% amino acids, with approximately 70% of them present in free form (Curtis, 2009). The composition of resulting FAAs in fermented foods may vary depending on the food matrix and the fermentation capacity of the strains (Li *et al.*, 2021).

In this study, the FAA compositions of the fish sauce groups are summarised in Table 1. The concentrations of total free amino acids (TFAAs) were determined to be 9822.50 mg 100 g^{-1} in Tfs, 12187.08 mg 100 g^{-1} in Lp, and 12889.48 mg 100 g^{-1} in Lp + Sc ($P < 0.05$). These results suggest that lactic acid fermentation increases the levels of FAAs and their related derivatives through proteolysis and metabolic synthesis (Pei *et al.*, 2022). Similar findings were reported in a study where the amount of TFAA in traditional fish sauce was 12065.0 mg 100 mL^{-1}, while starter culture inoculations (*Tetragenococcus halophilus* and *Virgibacillus* spp.) increased the levels to the range of 12433.0–13420.0 mg 100 mL^{-1} (Udomsil *et al.*, 2017).

The predominant FAAs in all samples were found to be glutamic acid (Glu), lysine (Lys), alanine (Ala), leucine (Leu), and aspartic acid (Asp), consistent with findings from previous studies (Kong *et al.*, 2012; Xiao *et al.*, 2020; Zhu *et al.*, 2021; Cheng *et al.*, 2022). Glu, associated with the umami flavour and formed as a result of glutamine deamination (DeMasi *et al.*, 1990), was the most detected FAAs in the starter culture inoculated sauces. Glu levels were determined as 1899.44, 1472.89, and 1103.36 mg 100 g^{-1} in Lp + Sc, Lp, and Tfs, respectively ($P < 0.05$; Table 1). Another notable observation of starter inoculation was the significantly elevated presence of ornithine (Orn) and cystine (Cys) compared to the Tfs group, where they were either absent or present at very low levels ($P < 0.05$) (Table 1). This suggests that the starter microorganisms may play a role in the metabolism of these amino acids.

Table 1 The free amino acid content of fish sauce samples fermented by starter culture inoculations or traditional method (mg 100 g^{-1})

	Tfs	Lp	Lp + Sc
Umami			
Glutamic acid	1103.36 ± 0.62^{c}	1472.89 ± 1.37^{b}	1899.44 ± 0.27^{a}
Aspartic acid	763.51 ± 0.33^{c}	1358.87 ± 5.87^{a}	1177.64 ± 0.15^{b}
Sweet			
Alanine	1061.97 ± 0.31^{c}	1438.14 ± 0.80^{a}	1339.38 ± 0.12^{b}
Threonine	118.22 ± 0.03^{c}	132.64 ± 0.11^{a}	125.83 ± 0.05^{b}
Serine	927.26 ± 0.71^{a}	532.29 ± 0.21^{c}	568.93 ± 0.21^{b}
Glycine	373.44 ± 0.20^{c}	690.08 ± 2.85^{b}	734.33 ± 0.21^{a}
Proline	479.61 ± 0.15^{c}	644.89 ± 0.08^{b}	645.42 ± 0.25^{a}
Bitter			
Arginine	596.54 ± 0.51^{a}	94.93 ± 0.07^{b}	68.4 ± 0.02^{c}
Valine	775.67 ± 0.01^{c}	1054.62 ± 0.24^{a}	996.05 ± 0.44^{b}
Methionine	240.91 ± 0.11^{c}	334.71 ± 0.11^{b}	389.52 ± 0.12^{a}
Isoleucine	524.17 ± 0.50^{c}	748.51 ± 0.10^{b}	783.56 ± 0.71^{a}
Leucine	635.06 ± 0.44^{c}	1133.01 ± 0.01^{a}	1126.14 ± 0.32^{b}
Histidine	281.13 ± 0.27^{b}	338.34 ± 5.07^{a}	346.6 ± 5.40^{a}
Tyrosine	114.24 ± 0.41^{a}	19.07 ± 0.02^{c}	27.56 ± 0.06^{b}
Phenylalanine	497.03 ± 0.16^{a}	451.71 ± 0.56^{c}	492.41 ± 0.01^{b}
Lysine	1323.81 ± 0.60^{b}	1138.54 ± 0.44^{c}	1551.75 ± 0.63^{a}
Others			
Ornithine	6.57 ± 0.01^{c}	568.6 ± 0.02^{b}	577.44 ± 0.12^{a}
Cystine	n.d.^{c}	35.24 ± 0.01^{b}	39.08 ± 0.04^{a}
Taurine	n.d.	n.d.	n.d.
Total free amino acids	9822.50 + 0.15^{c}	12187.08 + 4.68^{b}	12889.48 + 2.91^{a}

n.d., not detected; Lp + Sc, co-culture fermentation with *L. plantarum* and *S. cerevisiae*; Lp, starter culture fermentation with *L. plantarum*; Tfs, traditional fish sauce sample. Data represent mean of triplicated analyses ± SD (n = 3). Different superscript letters (a, b, c) in the same row represent statistical differences among fish sauce groups ($P < 0.05$).

Additionally, Lys, serine (Ser), arginine (Arg), and tyrosine (Tyr) levels were decreased in *L. plantarum* fermentation and co-fermentation with *S. cerevisiae* (Table 1), likely due to the catabolism of these amino acids by the starter microorganisms (Jung *et al.*, 2016).

Free fatty acids

Free fatty acids serve as precursor compounds to produce volatile compounds through oxidation and lipolysis (Olivares *et al.*, 2011; Xu *et al.*, 2014; Flores, 2018). In this study, the details of the FFAs composition are summarised in Table 2. The FFAs profile of the samples was predominantly composed of saturated fatty acids (SFAs). The concentrations of total saturated free fatty acids (TSFA) among FFAs were 76.29%, 60.15%, and 57.56% in Lp + Sc, Tfs, and Lp, respectively. The most dominant SFA was palmitic acid (C16), and its concentration was in the range of 29.80–35.15%, followed by stearic acid (C18) (14.67–19.27%) in all sauce groups. The distribution of FFAs was consistent with the results previously reported in similar fish sauce studies (Dincer *et al.*, 2010; Gao *et al.*, 2016; Li & Xu, 2021).

Monounsaturated fatty acids (MUFAs) ranged between 10.72% and 31.02%, while polyunsaturated fatty acids (PUFAs) ranged between 8.93% and 26.68% in all sauce groups (Table 2). Changes in unsaturated fat content and dominant fatty acids between the groups in our study are consistent with findings of other researchers (Majumdar *et al.*, 2016; Chen *et al.*, 2017; Li & Xu, 2021). In this study, the total free unsaturated fatty acids were found to be considerably lower than SFAs ($P < 0.05$). This result may be explained by the susceptibility of unsaturated fatty acids to lipolytic activity or oxidation. The level of lipolysis and oxidative reactions can vary depending on the factors such as raw materials, fermentation conditions, dominant microbiota, and interactions with endogenous enzymes (Chen *et al.*, 2017; Li & Xu, 2021). Furthermore, in this study, starter cultures with different lipolytic activities (Stepaniak, 2004; Kuncharoen *et al.*, 2020) were used for sauce production, leading to significant differences in FFAs composition compared to Tfs ($P < 0.05$).

Organic acids

Organic acids (OAs) are crucial flavour compounds that contribute significantly to sour taste through their concentration of H+ ions. These compounds likely play a role in the intensity of bitterness, umami, saltiness, and sweetness (Guo *et al.*, 2020; Shi *et al.*, 2022). In this study, the OAs composition of all sauce groups is presented in Table 3.

A total of four OAs, including lactic, citric, acetic, and formic acids, were detected in the sauce groups. The highest OA content was determined in the Lp group, while the lowest OA content was found in the Tfs group ($P < 0.05$). In the Lp and Lp + Sc groups, lactic acid was the predominant OA, with concentrations of 19.83 and 17.07 g L^{-1}, respectively, while in the Tfs group, citric acid had the highest concentration (6.68 g L^{-1}) (Table 3). Among the groups, lactic acid is associated with the metabolic activity of *L. plantarum*, whereas citric acid is associated with salt-tolerant bacteria in the Tfs (Jung *et al.*, 2016; Wang *et al.*, 2019). Our findings are consistent with those of other researchers who studied various bacteria (*L. fermentum* PCC, *L. plantarum* 299v, and *Lactococcus lactis* subsp. *cremoris* D) and yeasts (*Torulaspora delbrueckii*, *S. cerevisiae*, *Pichia kluyveri*, *Kluyveromyces marxianus*) (Leroy & De Vuyst, 2004; Gao *et al.*, 2019, 2020).

Table 2 Free fatty acid profiles of fish sauce samples fermented by starter culture inoculations or traditional method (percentage of total fatty acid)

		Tfs	Lp	Lp + Sc
C14:0	Myristic acid	2.94 ± 0.01^{a}	2.56 ± 0.05^{b}	1.61 ± 0.11^{c}
C15:0	Pentadecanoic acid	0.51 ± 0.09^{b}	0.38 ± 0.01^{b}	7.18 ± 0.4^{a}
C16:0	Palmitic acid	31.80 ± 0.33^{b}	29.80 ± 0.11^{b}	35.15 ± 1.44^{a}
C17:0	Margaric acid	0.98 ± 0.1^{a}	0.93 ± 0.01^{a}	n.d.^{b}
C18:0	Stearic acid	14.67 ± 0.05^{b}	19.27 ± 0.02^{a}	18.90 ± 0.65^{a}
C20:0	Arachidic acid	2.48 ± 0.19^{c}	0.41 ± 0.03^{b}	3.77 ± 0.61^{a}
C22:0	Behenic acid	2.26 ± 0.08^{a}	1.92 ± 0.01^{ab}	1.74 ± 0.25^{b}
C24:0	Lignoceric acid	4.50 ± 0.07^{b}	2.28 ± 0.07^{c}	7.93 ± 1.15^{a}
∑*SFA*		60.15 ± 0.54^{b}	57.56 ± 0.09^{c}	76.29 ± 0.86^{a}
C14:1	Myristoleic acid	0.78 ± 0.02^{b}	0.76 ± 0.03^{b}	1.73 ± 0.24^{a}
C15:1	cis-10-pentadecanoic acid	1.46 ± 0.06^{a}	0.24 ± 0.03^{c}	1.07 ± 0.14^{b}
C16:1	Palmitoleic acid	2.96 ± 0.01^{a}	2.37 ± 0.07^{b}	n.d.^{c}
C17:1	cis-10-heptadecanoic acid	0.62 ± 0.1^{a}	0.39 ± 0.01^{b}	n.d.^{c}
C18:1n9c	Oleic acid	6.34 ± 0.19^{b}	25.60 ± 2.43^{a}	2.97 ± 0.23^{b}
C18:1n9t	Elaidic-trans acid	n.d.^{b}	1.37 ± 0.04^{a}	n.d.^{b}
C20:1	cis-11-eicosenoic acid	1.02 ± 0.09^{b}	0.33 ± 0.09^{c}	4.95 ± 0^{a}
∑*MUFA*		13.17 ± 0.28^{b}	31.02 ± 2.49^{a}	10.72 ± 0.12^{b}
C18:2n6c	Linoleic acid	2.02 ± 0.06^{b}	3.71 ± 0.02^{a}	1.88 ± 0.07^{c}
C22:2	cis-13,16-docosadienoic	5.16 ± 0.11^{a}	n.d.^{b}	n.d.^{b}
C18:3n3	Linolenic acid	6.45 ± 0.16^{a}	4.33 ± 0^{b}	5.12 ± 0.69^{b}
C22:6n3	Docosahexaenoic acid (DHA)	13.05 ± 0.03^{a}	0.89 ± 0.08^{c}	6.00 ± 0.12^{b}
∑*PUFA*		26.68 ± 0.25^{a}	8.93 ± 0.1^{c}	13.00 ± 0.74^{b}

n.d., not detected; Lp + Sc, co-culture fermentation with *L. plantarum* and *S. cerevisiae*; Lp, starter culture fermentation with *L. plantarum*; Tfs, traditional fish sauce sample. Data represent mean of triplicated analyses ± SD (n = 3). Different superscript letters (a, b, c) in the same row represent statistical differences among fish sauce groups ($P < 0.05$).

Table 3 Organic acid content of fish sauce samples fermented by starter culture inoculations or traditional method (g L^{-1})

	Tfs	Lp	Lp + Sc
Lactic acid	3.73 ± 0.07^{c}	19.83 ± 0.28^{a}	17.07 ± 0.22^{b}
Citric acid	6.68 ± 0.09^{c}	11.99 ± 0.15^{a}	11.29 ± 0.21^{b}
Acetic acid	0.88 ± 0^{c}	10.03 ± 0.04^{b}	10.31 ± 0.15^{a}
Formic acid	3.47 ± 0.4^{b}	3.68 ± 0.01^{a}	3.54 ± 0.03^{b}
Total organic acids	14.76 + 0.12^{c}	45.53 + 0.46^{a}	42.21 + 0.25^{b}

Lp + Sc, co-culture fermentation with *L. plantarum* and *S. cerevisiae*; Lp, starter culture fermentation with *L. plantarum*; Tfs, traditional fish sauce sample. Data represent mean of triplicated analyses ± SD (n = 3). Different superscript letters (a, b, c) in the same row represent statistical differences among fish sauce groups ($P < 0.05$).

Volatile compounds

Volatile compounds may be synthesised directly during fermentation or may arise from collaborative reactions between metabolic compounds (Macedo *et al.*, 2017). Volatile compounds in Tfs and starter culture-inoculated groups (Lp and Lp + Sc) are shown in Table 4. A total of seventy-eight volatile compounds, including twelve esters, twelve alcohols, eleven aldehydes/ketones, ten acids, eight hydrocarbons, four alkanes/alkenes, and twenty-one other compounds, were identified from all sauce groups. The diversity of volatile compounds was highest in Tfs (41), followed by Lp + Sc (31) and Lp (30). Despite the low diversity in starter culture-inoculated groups, the total volatile amounts were determined to be higher than in the Tfs. In the Lp + Sc and Lp groups, the total amount of volatile compounds was determined as 7972.82 and 5235.13 ng mL^{-1}, respectively, while it was determined as 1982.51 ng mL^{-1} in Tfs. In previous studies, researchers have observed a similar relationship between volatile compound variety and amount in sauces produced with autochthonous flora and allochthonous starter culture, as seen in our results (Li *et al.*, 2022a, 2022b, 2023; Han *et al.*, 2023).

Table 4 Volatile compounds of fish sauce samples fermented by starter culture inoculations or traditional method (ng mL^{-1})

	Tfs	Lp	Lp + Sc	Odour description
Esters				
Ethyl hydrogen oxalate	n.d.^{b}	n.d.^{b}	66.97 + 9.66^{a}	
Ethyl formate	n.d.^{a}	36.15 + 10.69^{a}	48.42 + 48.42^{a}	Sharp, rum-like, fruity, pleasant
Isopropenyl acetate	37.38 + 1.82^{a}	n.d.^{b}	n.d.^{b}	Fruity, ethereal, pears
Ethyl acetate	n.d.^{a}	n.d.^{a}	10.48 + 10.48^{a}	Characteristic, ether-like, pineapple
Hexamethylcyclotrisiloxane	n.d.^{b}	86.50 + 13.46^{b}	779.28 + 58.34^{a}	
Methyl butyrate	60.76 + 52.36^{a}	n.d.^{a}	n.d.^{a}	Apple
Bis(trimethylsilyl) diethyl silicate	n.d.^{b}	169.38 + 19.05^{a}	n.d.^{b}	
Ethyl lactate	n.d.^{b}	n.d.^{b}	416.51 + 9.86^{a}	Buttery, fruity butterscotch, milk cream
Trimethylsilyl trimethylsiloxy salicylate	n.d.^{b}	n.d.^{b}	70.30 + 6.37^{a}	
Isopropyl formate	4.36 + 0.08^{a}	n.d.^{b}	n.d.^{b}	Pleasant, fruity, ether-like
Ethyl DL-Leucate	n.d.^{a}	n.d.^{a}	38.71 + 32.79^{a}	
Methyl 3-hydroxybutyrate	5.35 + 1.01^{a}	n.d.^{b}	n.d.^{b}	
Subtotal	107.85 + 53.19^{c}	292.03 + 43.20^{b}	1430.67 + 5.82^{a}	
Alcohols				
4-amino-1-pentanol	n.d.^{a}	n.d.^{a}	25.41 + 25.41^{a}	
2-heptanol	n.d.^{a}	n.d.^{a}	584.93 + 584.93^{a}	Mild alcohol
Ethanol	n.d.^{a}	n.d.^{a}	273.65 + 273.65^{a}	Pleasant, fragrant, weak, ethereal, vinous
1,3-Butanediol	n.d.^{b}	564.68 + 95.39^{a}	n.d.^{b}	Odourless, sweet flavour, bitter aftertaste
4-Methoxy-1-butanol	n.d.^{a}	n.d.^{a}	233.51 + 233.51^{a}	
1-Penten-3-ol	257.71 + 215.14^{a}	n.d.^{a}	n.d.^{a}	Fried onion
3-methyl-2-butanol	n.d.^{b}	n.d.^{b}	163.07 + 35.35^{a}	
2,3-butanediol	n.d.^{b}	244.58 + 244.58^{ab}	513.65 + 46.84^{a}	Sweet, odourless, fruity creamy buttery
2-furanmethanol	305.05 + 35.62^{b}	465.07 + 103.35^{ab}	1352.15 + 832.66^{a}	Faint burning
Benzenemethanol	16.79 + 0.07^{a}	9.78 + 1.13^{a}	702.83 + 668.34^{a}	Slightly pungent-aromatic, fruity
Phenylethyl alcohol	n.d.^{b}	52.81 + 1.05^{a}	42.36 + 42.36^{ab}	Mild, warm, rose, honey-like
Octaethylene glycol	n.d.^{b}	25.07 + 16.41^{a}	n.d.^{b}	
Subtotal	579.55 + 250.69^{b}	1361.99 + 462.20^{b}	3891.56 + 1437.66^{a}	
Aldehydes/Ketones				
3-Methylbutanal	198.46 + 5.68^{a}	n.d.^{b}	n.d.^{b}	Weak suffocating, apple
2-Methyl-2-butenal	4.72 + 0.53^{a}	n.d.^{b}	n.d.^{b}	Powerful green ethereal aroma
3-Methyl-2-butenal	10.57 + 0.37^{a}	n.d.^{b}	n.d.^{b}	Pungent, almond, mild-buttery aroma
Hydroxyacetone	20.11 + 0.53^{a}	n.d.^{b}	n.d.^{b}	Pungent, sweet-caramellic, etheral
Methional	197.73 + 12.55^{b}	287.75 + 18.33^{ab}	320.32 + 93.58^{a}	Powerful, onion, meat-like
Benzaldehyde	24.24 + 17.75^{b}	43.83 + 15.17^{ab}	122.79 + 73.24^{a}	Sweet, strong almond
Benzeneacetaldehyde	207.85 + 1.39^{ab}	170.99 + 46.49^{b}	266.14 + 43.52^{a}	Sweet, hyacinth, clover, honey, cocoa, lilac
4-Pentenal	11.81 + 0.06^{a}	n.d.^{b}	n.d.^{b}	High strength, roasted
3-Ethylbenzaldehyde	n.d.^{a}	n.d.^{a}	54.85 + 54.85^{a}	Floral, green
Benzeneacetaldehyde, alpha-ethylidene-	4.73 + 0.39^{a}	n.d.^{b}	n.d.^{b}	Cocoa, honey, woody, caramel, smoky
5-Methyl-2-phenyl-2-hexenal	4.43 + 0.39^{a}	n.d.^{b}	n.d.^{b}	Sweet, roasted, cocoa
Subtotal	684.64 + 0.62^{ab}	502.57 + 49.65^{b}	764.10 + 155.48^{a}	
Hydrocarbones				
N-ethyl-1,3-dithioisoindoline	n.d.^{b}	456.54 + 99.63^{a}	170.75 + 110.26^{b}	
Toluene	13.65 + 0.10^{a}	n.d.^{b}	n.d.^{b}	Sweet, pungent, benzene-like
Nonane	12.76 + 0.09^{a}	n.d.^{b}	n.d.^{b}	Gasoline-like
Decane	18.50a	n.d.^{b}	n.d.^{b}	Gasoline-like
1,2,4-trimethoxybutane	n.d.^{b}	244.88 + 76.99^{a}	n.d.^{b}	
1,3,5,7,9-pentaethylcyclopentasiloxane	n.d.^{b}	128.81 + 11.40^{a}	12.18 + 12.18^{b}	
Pentadecane	8.24 + 1.77^{a}	n.d.^{b}	n.d.^{b}	
4-methyldecane	4.65 + 0.00^{a}	n.d.^{b}	n.d.^{b}	Pungent, acrid
Subtotal	57.8 + 2.44^{b}	830.23 + 34.04^{a}	182.93 + 122.45^{b}	
Alkanes/Alkenes				
2-aminopyridine	17.16 + 0.36^{a}	n.d.^{b}	n.d.^{b}	Characteristic
Cyclopentene	67.84 + 16.91^{a}	n.d.^{b}	n.d.^{b}	Gasoline, Slightly sweetish

Table 4 (*Continued*)

	Tfs	Lp	Lp + Sc	Odour description
1-heptadecene	23.65 + 0.21[a]	n.d.[b]	n.d.[b]	Flowery, anise
2,6,10,14-tetramethylhexadecane	1.98 + 0.18[a]	n.d.[b]	n.d.[b]	Fishy
Subtotal	110.63 + 17.67[a]	n.d.[b]	n.d.[b]	
Acids				
Acetic acid	100.05 + 16.35[b]	1172.85 + 325.99[a]	1259.51 + 21.38[a]	Vinegar like
Propanoic acid	2.64 + 0.02[a]	19.84 + 19.84[a]	0a	Pungent, disagreeable, rancid
Butanoic acid	12.14 + 0.05[b]	125.91 + 24.50[a]	35.75 + 8.61[b]	Unpleasant, rancid, obnoxious, acrid
Isovaleric acid	131.88 + 1.28[a]	n.d.[b]	n.d.[b]	Rancid cheese
3-Methylpentanoic acid	n.d.[a]	n.d.[a]	27.60 + 27.60[a]	Sour, herbaceous, slightly green
3-Furoic acid	20.68 + 3.53[a]	n.d.[b]	n.d.[b]	
Hexanoic acid	n.d.[b]	42.67 + 9.45[a]	33.21 + 33.21[ab]	Fatty, cheesy, like goats, barnyard animals
Heptanoic acid	3.57 + 0.01[a]	n.d.[b]	n.d.[b]	Disagreeable, rancid, tallow-like
Propylmalonic acid	n.d.[b]	52.04 + 8.36[a]	n.d.[b]	
Tridecanoic acid	11.37 + 0.40[a]	n.d.[b]	n.d.[b]	Soapy
Subtotal	282.33 + 20.80[b]	1413.31 + 339.14[a]	1356.07 + 54.34[a]	
Other compounds				
2-Butanamine, (S)	n.d.[b]	27.57 + 8.68[a]	n.d.[b]	Ammonia
Methyl Mercaptan	26.5 + 1.10[a]	n.d.[b]	n.d.[b]	Garlic, rotten cabbage, smelly socks
5-Methyl-2-phenylindole	n.d.[a]	7.16 + 7.16[a]	n.d.[a]	
Trans-4-(2-(5-nitro-2-furyl)vinyl)-2-quinolinamine	n.d.[b]	132.50 + 3.03[a]	n.d.[b]	
Octamethylcyclotetrasiloxane	n.d.[b]	77.56 + 49.88[a]	n.d.[b]	
2-Methyltetrahydro-3-furanone	12.48 + 1.21[a]	n.d.[b]	n.d.[b]	Buttery
4,6-Dimethylpyrimidine	n.d.[a]	n.d.[a]	4.35 + 4.35[a]	
2,6-Dimethylpyrazine	17.01 + 2.98[a]	n.d.[b]	n.d.[b]	Coffee-like
2,5-bis(trimethylsilyloxy)benzaldehyde	n.d.[b]	208.21 + 1.74[a]	n.d.[b]	
4-ethenyl-1,2-dimethylbenzene	4.5 + 0.62[a]	n.d.[b]	n.d.[b]	
2-Methyl-1-phenylpropene	n.d.[a]	241.67 + 223.63[a]	n.d.[a]	
3-Methylfuran	n.d.[a]	n.d.[a]	29.42 + 29.42[a]	
Trans-Caryophyllene	9.98 + 0.50[a]	n.d.[b]	n.d.[b]	Cloves, turpentine
2-Methoxyphenol	7.37 + 1.94[a]	n.d.[b]	n.d.[b]	
2-Acetylpyrrole	55.54 + 0.31[a]	n.d.[b]	63.31 + 6.68[a]	Nutty, popcorn, bready, walnut, licorice
3-Methylpyrrole	n.d.[a]	7.91 + 7.91[a]	n.d.[a]	Roasted
1,4,7,10,13-Pentaoxacyclopentadecane	n.d.[c]	30.29 + 4.28[b]	59.57 + 11.79[a]	
1,4,7,10,13,16-hexaoxacyclooctadecane	n.d.[c]	81.76 + 7.93[b]	118.15 + 6.58[a]	
2-Ethyl-4,5-dimethylphenol	6.1 + 0.17[a]	n.d.[b]	n.d.[b]	
Octaethylene glycol monododecyl ether	n.d.[a]	20.37 + 20.37[a]	n.d.[a]	
2,4-bis(1,1-dimethylethyl)phenol	20.23 + 0.38[b]	n.d.[c]	72.69 + 10.49[a]	
Subtotal	159.71 + 8.21[b]	835 + 172.72[a]	347.49 + 10.47[b]	
Total	1982.51 + 353.62[c]	5235.13 + 656.21[b]	7972.82 + 1432.64[a]	

n.d., not detected; Lp + Sc, co-culture fermentation with *L. plantarum* and *S. cerevisiae*; Lp, starter culture fermentation with *L. plantarum*; Tfs, Traditional fish sauce sample. Data represent mean of triplicated analyses ± SD (n = 3). Different superscript letters (a, b, c) in the same row represent statistical differences among fish sauce groups ($P < 0.05$).

In this study, volatile alcohols were the first group to attract attention among volatile compounds. Volatile alcohol formation typically results from lipid oxidation (Pham *et al.*, 2008). The total amount of volatile alcohols peaked in the Lp + Sc group and was determined to be 3891.56 ng mL^{-1} ($P < 0.05$). These compounds were lower in Lp and Tfs, with concentrations of 1361.99 and 579.55 ng mL^{-1}, respectively (Table 4). This difference may be attributed to the alcoholic fermentation ability of *S. cerevisiae*. Similar results were also reported in some fermented fish produced using *S. cerevisiae* (Gao *et al.*, 2022). The main volatile alcohols in the alcoholic compounds group were 2-furanmethanol (1352.15 ng mL^{-1}) and benzenemethanol (702.83 ng mL^{-1}), which had the highest total concentrations in the Lp + Sc group ($P < 0.05$). In this study, 2-furanmethanol showed a significant negative correlation with myristic, margaric, and palmitoleic acids (r: −0.990 to −0.999; $P < 0.05$), while benzenemethanol showed significant positive

correlations with pentadecanoic, myristoleic, and cis-11-eicosenoic acids (r: 0.990 to 1.000; $P < 0.05$; Fig. 1). In previous studies, 2-furanmethanol formation was observed in fish sauce and fermented fish produced using koji (Giri *et al.*, 2010b; Han *et al.*, 2023). Both mentioned alcohols are responsible for the formation of the burning taste and aroma, which are related to caramelisation (Prado *et al.*, 2021). Furthermore, in our study, *S. cerevisiae* alone was effective in the formation of 4-amino-pentanol, 2-heptanol, and ethanol volatiles ($P < 0.05$). These compounds, mainly characterised by mild alcoholic, ethereal, and pleasant aromas, showed a significant negative correlation only with margaric acid (r: −0.999; $P < 0.05$; Fig. 1). Specific to the Lp group, two compounds, 1,3-butanediol and octaethylene glycol, were identified. This is in agreement with the findings of Coulibaly *et al.* (2017) who reported that *L. plantarum* was responsible for the formation of 1,3-butanediol. Moreover, 1,3-butanediol, as the most abundant alcoholic compound specific to only *L. plantarum*, showed a strong negative correlation with phenylalanine (Phe) (r: −0.996; $P < 0.05$). On the other hand, in contrast to the starter culture inoculated groups, the only alcoholic compound specific to the Tfs group was 1-Penten-3-ol, which was responsible for the fried onion odour tones (Han *et al.*, 2023).

Volatile acids are the most common compounds found in our sauce groups after alcohols. They are formed through amino acid metabolism, fat hydrolysis, and oxidation (Raksakulthai, 1993; Li *et al.*, 2013; Zhu *et al.*, 2019). The concentration of these compounds was 282.33 ng mL^{-1} in the Tfs group, 1356.07 and 1431.31 ng mL^{-1} in the Lp + Sc and Lp groups, respectively ($P < 0.05$; Table 4). The most predominant compound in the starter culture-inoculated groups was acetic acid, which was ten times higher than that found in Tfs. This is related to the fact that acetic acid is the main metabolite of different fermentation pathways of yeast and lactic acid bacteria (Freer, 2002; Andrea *et al.*, 2019). Similarly, a study on suan-yu production revealed that volatile acetic acid is also a common compound in samples produced with different starter culture strains (Gao *et al.*, 2016). In our study, non-volatile acetic acid formation was positively correlated with volatile acetic acid formation (r: 0.999; $P < 0.05$), while it was significantly negatively correlated with other volatile acids (r: −0.993 to −1.000; $P < 0.01$) (Fig. 1). On the other hand, volatile acetic acid formation in the sauce groups showed significant positive correlations with glycine (Gly), proline (Pro), isoleucine (Iso), leucine (Leu), histidine (His), ornithine (Orn), cysteine (Cys), stearic acid (r: 0.990 to 1.000; $P < 0.01$) while showed significant negative correlations with serine (Ser), arginine (Arg), tyrosine (Tyr), and cis-13,16-docosadienoic (r: −0.989 to −1.000; $P < 0.01$; Fig. 1). This suggests that our starter culture strains utilise these FAAs and FFAs for volatile acid production. Similar results regarding volatile acids were also emphasised by Li *et al.* (2013) and Zhu *et al.* (2019). In the Tfs group, the diversity of volatile acid compounds was high, dominated by isovaleric acid (131.88 ng mL^{-1}) and acetic acid (100.05 ng mL^{-1}), while the amount of other compounds was significantly less than these two compounds (Table 4).

Esters are typically formed from carboxylic acids and alcohols through esterification catalysed by transferase enzymes (Petričević *et al.*, 2018). Generally, in our sauces, esters were found to follow alcohols and acids and were present in low quantities (Table 4). The highest amount of esters was found in Lp + Sc with 1430.67 ng mL^{-1}, followed by Lp with 292.03 ng mL^{-1} and Tfs with 107.85 ng mL^{-1} (Table 4). The high quantity in Lp + Sc is thought to be related to *S. cerevisiae*, which promotes ester synthesis, particularly the synthesis of medium-chain fatty acid ethyl esters (Saerens *et al.*, 2010; Lv *et al.*, 2023). The most dominant ester compounds in Lp + Sc were hexamethylcyclotrisiloxane (779.28 ng mL^{-1}) and ethyl lactate (416.51 ng mL^{-1}) (Table 4). The presence of hexamethylcyclotrisiloxane (Han *et al.*, 2023) and ethyl lactate (Kong *et al.*, 2014) in the fermentation medium was also reported in previous studies with *S. cerevisiae*. In this study, hexamethylcyclotrisiloxane and ethyl lactate showed significant negative correlations (r: −0.998 and − 0.996; $P < 0.05$) only with margaric and palmitoleic acids (Fig. 1). However, no other significant correlations were found between these two volatile compounds and FAAs and OAs. No significant presence of esters was observed in Tfs and Lp. Bis(trimethylsilyl) diethyl silicate in Lp and methyl butyrate in Traditional fish sauce sample were more abundant than other ester compounds (Table 4).

The other group of compounds detected in sauces is aldehydes and ketones. Aldehydes and ketones are formed through amino acid catabolism and lipid oxidation (Feng *et al.*, 2015; Shen *et al.*, 2021). Aldehydes are known to be strong odorants in foods due to their low odour threshold (Varlet *et al.*, 2007). For example, straight and branched-chain aldehydes often provide 'herbaceous', 'grassy', and 'pungent' flavours, while unsaturated aldehydes are associated with 'herbal' and 'fishy' notes (Giri *et al.*, 2010b). In this study, the total aldehyde/ketone concentrations in all fish sauce groups were determined to be close to each other and in the range of 502.57–764.10 ng mL^{-1}. Almost all the compounds in the aldehydes and ketones were detected in the Tfs group ($P < 0.05$). Only three compounds, methional, benzaldehyde, and benzeneacetaldehyde, were determined to be common in all groups (Table 4). Benzeneacetaldehyde is the most abundant aldehyde in the Tfs group, while methional is the most abundant aldehyde in both the Lp and Lp + Sc groups. These

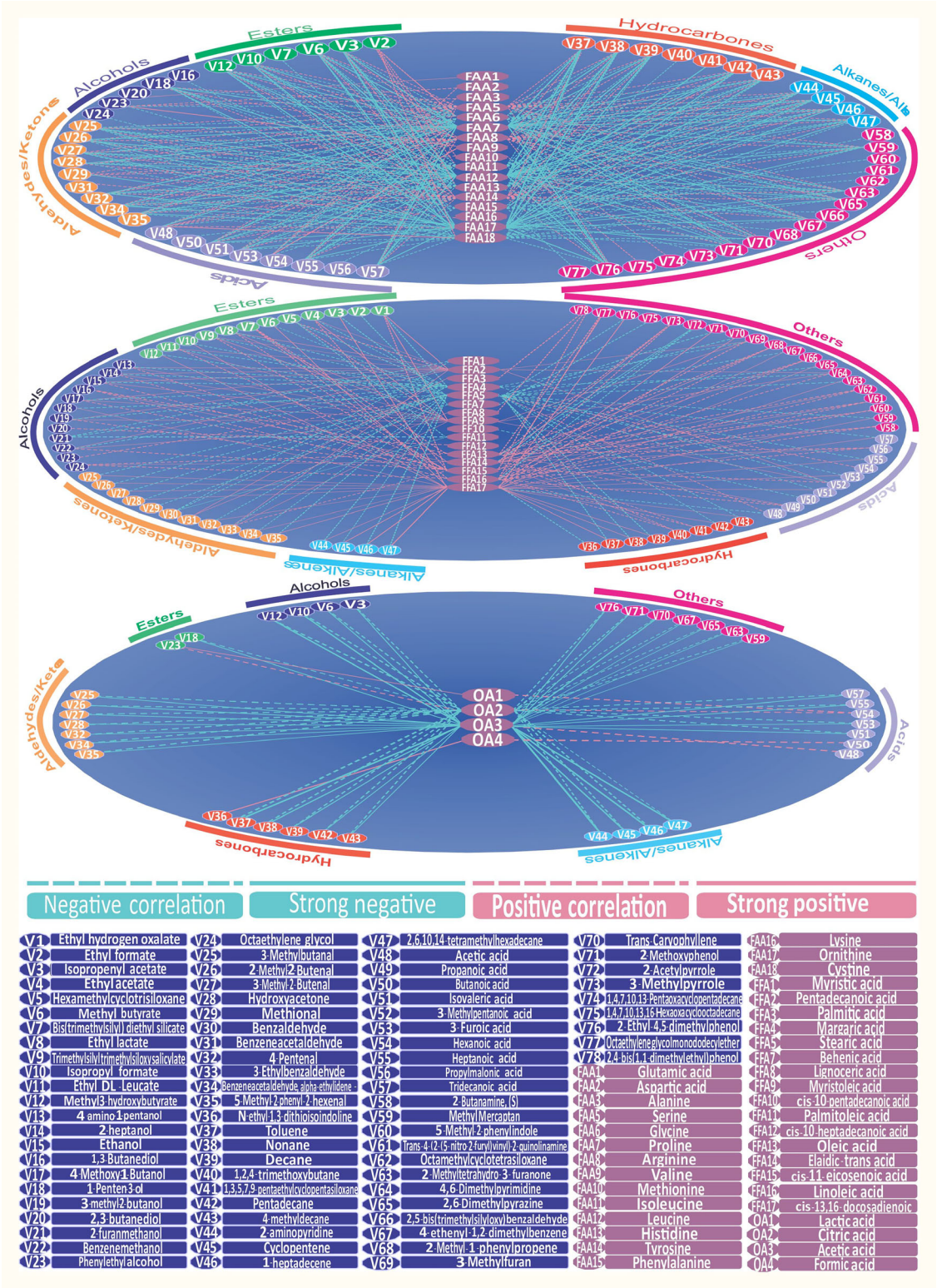

Figure 1 Correlation networks constructed by Pearson's correlation coefficient illustrating significant ($P < 0.05$, $P < 0.01$) relationships between volatile compounds and free amino acids (FAA) (a), free fatty acids (FFA) (b) and organic acids (OA) (c).

volatiles are characterised by a sweet, floral, and persistent odour (Prado *et al.*, 2021). Most of the volatiles in aldehydes and ketones group showed strong negative correlations with FAAs such as Gly, Pro, Iso, Leu, His, Orn, and Cys (r: −0.993 to −1000; $P < 0.05$, $P < 0.01$; Fig. 1). They also showed a strong negative correlation with OAs including citric and acetic acids and with stearic acid as the only FFA (r: −0.990 to −1.000; $P < 0.05$, $P < 0.01$; Fig. 1). Similar findings to those obtained for aldehydes and ketones were also observed for alkanes and alkenes in Tfs. Alkanes and alkenes are compounds formed by lipid oxidation (Drumm & Spanier, 1991), and they have low aroma impact due to their high odour thresholds (Wang *et al.*, 2021). This group of compounds was only detected in the Tfs group and is considered to have a slight effect on Tfs ($P < 0.05$; Table 4).

Hydrocarbons are compounds formed as a result of lipid oxidation (García *et al.*, 1991; Narváez-Rivas *et al.*, 2012). In previous studies on similar fermented products, it was reported that the contributions of various hydrocarbons to flavour may be limited due to their low content and high threshold values (Li *et al.*, 2022a, 2022b). In this study, total hydrocarbon concentrations were 57.80, 182.93, and 830.23 ng mL^{-1} in Tfs, Lp + Sc, and Lp, respectively (Table 4). The most diversity was found in Tfs, although the highest amount of compounds was found in Lp. Toluene, nonane, decane, pentadecane, and 4-methyldecane were found only in Tfs. These compounds correlated with many FAAs and OAs but showed a strong negative correlation only with stearic acid (r: −0.997; $P < 0.05$; Fig. 1). Nonane, decane, and pentadecane have been reported to be present in fish sauces and fermented meat products in previous studies (Sakpetch *et al.*, 2022; Han *et al.*, 2023). High concentrations of *N*-ethyl-1,3-dithioisoindoline, 1,2,4-trimethoxy-butane, and 1,3,5,7,9-pentaethylcyclopentasiloxane found in starter culture-inoculated groups were observed to be directly related to *L. plantarum* ($P < 0.05$). Among these, *N*-ethyl-1,3-dithioisoindoline showed a significant negative correlation with cis-10-pentadecanoic acid (r: −0.998; $P < 0.05$) and a positive correlation with formic acid (r: 0.999; $P < 0.05$; Fig. 1). 1,2,4-trimethoxy-butane and 1,3,5,7,9-pentaethylcyclopentasiloxane showed significant positive correlations with elaidic-trans acid and linoleic acid (r: 0.988 to 1.000; $P < 0.05$, $P < 0.01$) while showed significant negative correlations with Phe (r: −0.996 and − 1.000; $P < 0.05$, $P < 0.01$). In addition to these correlations, 1,2,4-trimethoxy-butane correlated only with oleic acid (r: 0.990; $P < 0.05$; Fig. 1).

Other compounds detected in the sauce groups, aside from those listed above, mainly comprised amines, furans, benzenes, ethers, carbonyls, pyrazines, alkaloids, terpenes, and phenolic compounds. These compounds were found at a concentration of 159.71 ng mL^{-1} in the Tfs group, 347.49 ng mL^{-1}, and 835 ng mL^{-1} in Lp + Sc and Lp, respectively ($P < 0.05$; Table 4). In all cases, it was thought that these compounds were involved in the formation of sensory characteristics in all groups.

Sensory profiling

Microorganisms, key enzymes, and chemical reactions in the fermentation environment play crucial roles in shaping the sensory characteristics of fish sauce (Sun *et al.*, 2016; Zang *et al.*, 2020; Nguyen *et al.*, 2021; Zhu *et al.*, 2021; Gao *et al.*, 2022). Flavour serves as a key sensory indicator of fish sauce and significantly influences consumer preferences. In this study, sensory profile analysis was conducted, classifying sensory attributes into basic tastes, aroma, and aftertastes. The results of descriptive sensory analyses of fish sauces are presented in Fig. 2a, while significant interactions, as determined by principal component analysis considering principal component 1 (PC1) and principal component 2 (PC2), are depicted in Fig. 2b.

The basic tastes are salty, sour, sweet, bitter, and umami. When analysing the sauce groups in terms of basic taste, it was observed that the salty taste was dominant in Tfs (7.78), while the sour taste was dominant in the starter culture-inoculated groups (6.33 in Lp; 6.39 in Lp + Sc). The salty taste in Tfs is attributed to the high salt concentration (20%) used in production. Furthermore, the negative correlations observed in sweet FAAs (r: −0.988 to −0.999; $P < 0.05$) and positive correlations in bitter FAAs (r: 0.996 to 0.998; $P < 0.05$) statistically support the perception of salty taste (Fig. 3). Additionally, the sour taste was distinct in the starter culture-inoculated groups and was mainly due to the presence of OAs and related compounds (Tables 3 and 4). These compounds led to a decrease in the pH value (to about 4.5) and consequently contributed to the sensory perception of sour taste. In this study, particularly acetic acid (r: 1.000; $P < 0.01$), stearic acid (r: 0.996; $P < 0.05$), and citric acid (r: 0.990; $P < 0.05$) showed strong positive correlations with sour taste. In addition to these non-volatile acids, significant correlations were also found with five FAAs and FFAs (Fig. 1). Regarding basic taste, the other tastes that followed the dominant taste in the groups were umami (4.72) and sour (3.61) in Tfs, and salty (5.72 in Lp; 5.17 in Lp + Sc) and umami (4.06 in Lp; 4.17 in Lp + Sc) in the starter culture-inoculated groups. Umami is a taste often associated with seafood and is influenced by other basic tastes. With the addition of the starter culture, the perception of umami taste was perceived as slightly lower than in Tfs. This was thought to be due to an increase in sour taste, which may slightly overshadow the perception of umami, and was supported by the strong negative correlations found for lactic and citric acids (r: −0.996 to −1.000; $P < 0.05$, $P < 0.01$). On the other hand, umami taste also showed strong correlations with seven other non-volatile

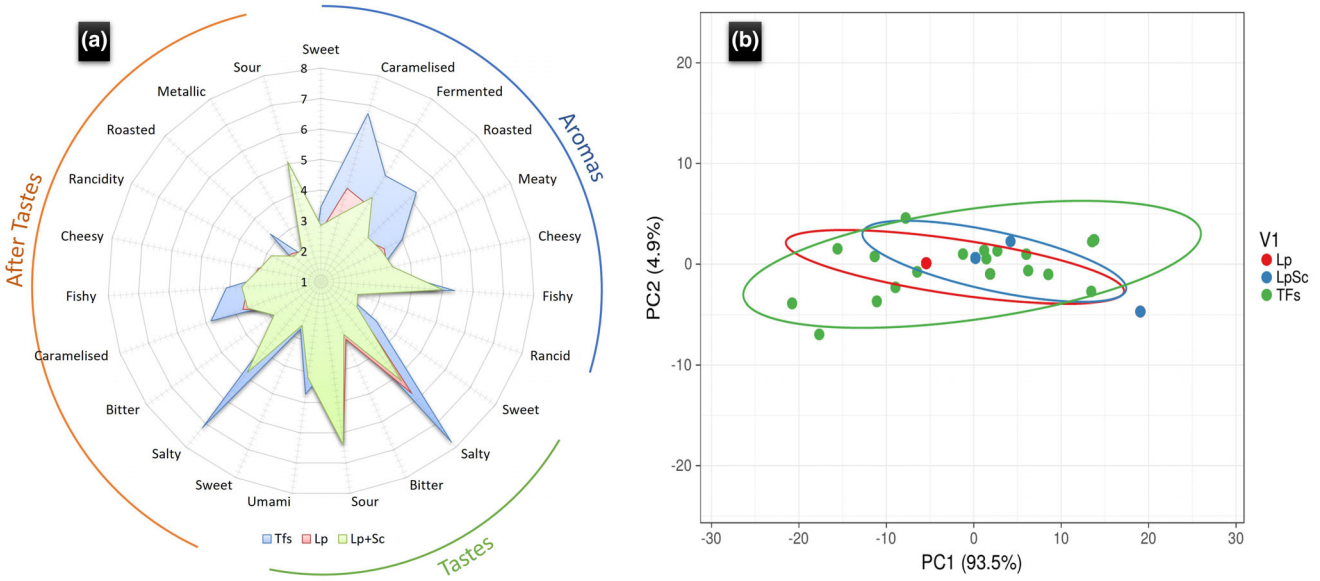

Figure 2 Sensory characteristics of fish sauces as determined by trained panellists (a), and principal component analysis illustrating variations between groups according to production method (b).

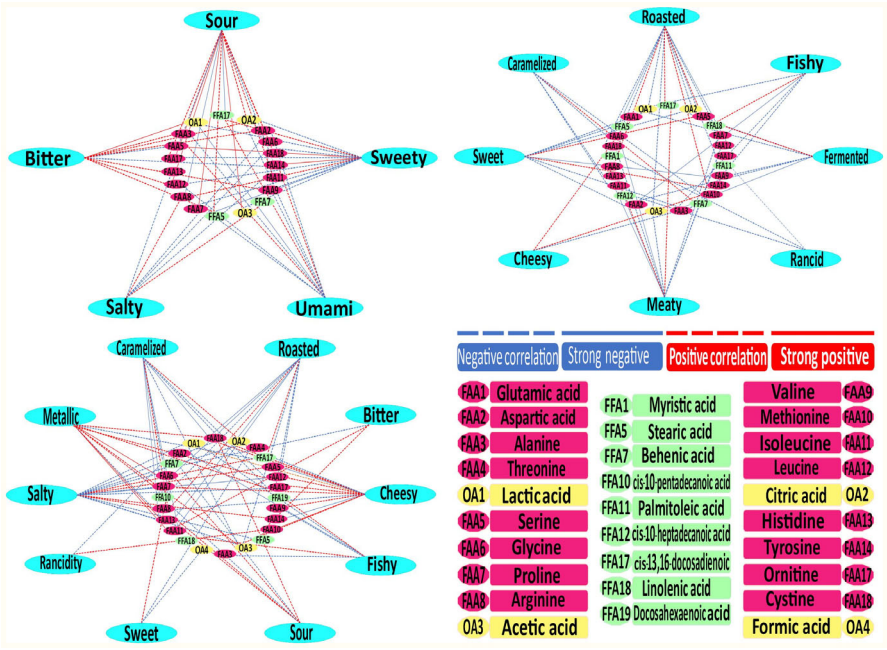

Figure 3 Correlation networks constructed by Pearson's correlation coefficient illustrating relationships between basic taste (a), aroma (b), and aftertaste (c) profiles with the most significant ($P < 0.05$, $P < 0.01$) amino acids (pink), fatty acids (green), and organic acids (yellow) across all fish sauce groups.

compounds (Figs 3 and 4). Besides, in the sensory profile results, it was observed that sweet and bitter tastes were similar in all groups ($P > 0.05$).

The aromas in the sauce groups were evaluated in terms of eight categories: sweet, caramelised, fermented, roasted, meaty, cheesy, fishy, and sour. The aromas

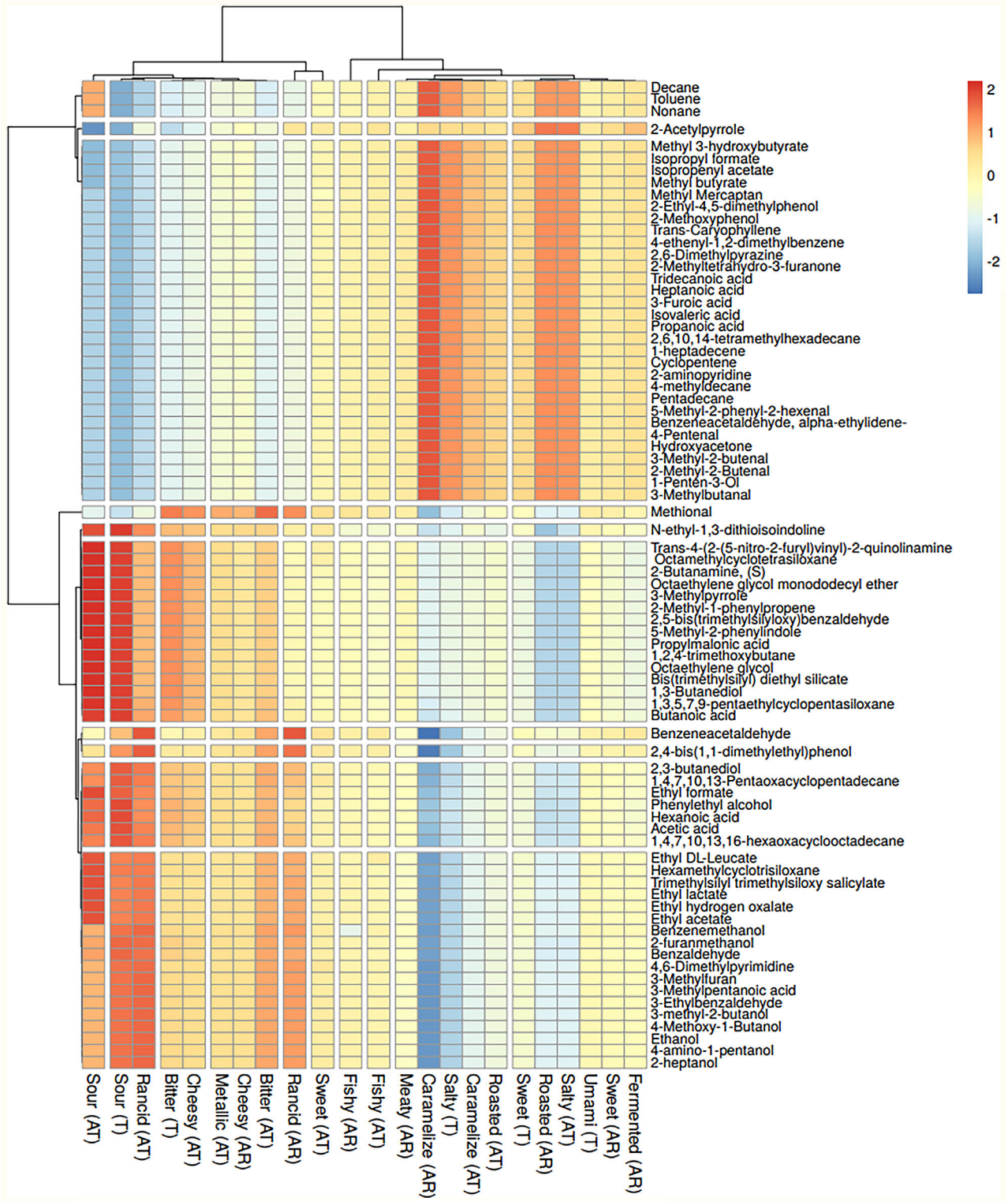

Figure 4 Hierarchical cluster analysis of sensory parameters (AR, aroma; AT, aftertaste; T, basic taste) and volatile compounds of fish sauces. Rows are centred; unit variance scaling was applied to rows. Both rows and columns were clustered using correlation distance and average linkage.

perceived by the panellists were supported by strong correlations between volatile and non-volatile compounds (Figs 1 and 2). The aroma characteristics of the Tfs group, where caramelised (6.72), fishy (5.39), and roasted (5.28) aromas were predominant, were noted by the panellists as more pronounced than in the starter culture-inoculated groups. These aromas are commonly detected in many fish sauces as a natural result of fermentation (Ritthiruangdej & Suwonsichon, 2006; Yimdee & Wang, 2016). In our study, the variety of volatile compounds formed in Tfs, especially aldehydes and ketones, is thought to play a crucial role in the formation of specific aromas in fish sauce (Table 4). Previous reports indicate that the variety of aldehydes and ketones enhance aromas of fish sauce (Wang *et al.*, 2020; Lestari *et al.*, 2023). In this study, the caramelised aroma characterising Tfs was strongly associated with three volatile compounds (especially methional with r: −1.000; $P < 0.01$) and five non-volatile compounds (r: −0.990 to 0.995; $P < 0.05$; Figs 3 and 4).

In the starter culture-inoculated groups, the abundance of volatile compounds shaped aroma in favour of the starter culture strains. With the presence of *L. plantarum* and *S. cerevisiae*, the aroma was significantly influenced, and the fishy aroma became prominent. The fish aroma was determined as 4.83 in Lp and 5.00 in Lp + Sc ($P > 0.05$). Following the dominant fish aroma were caramelised (4.17) and fermented (3.94) aromas in Lp, and fermented (4.22) and cheesy (3.39) aromas in Lp + Sc. The fish aroma can originate from a wide variety of metabolites. In a study on fresh fish aromas, certain volatile compounds belonging to alcohol, acid (especially acetic acid), hydrocarbon, and aldehyde/ketone groups were reported to be responsible for the natural fish aroma (Liu *et al.*, 2021). The fishy aroma in the starter culture-inoculated groups in our study can be attributed to the high content of alcohols and acids (especially acetic acid) (Table 4 and Fig. 4). Additionally, the perceived fishy aroma related to the presence of these volatiles was thought to be associated with the metabolism of some FAAs such as Asp, Ala, and Val (r: −0.995 to −1.000; $P < 0.05$, $P < 0.01$), FFAs such as linolenic acid, and docosahexaenoic acid (DHA) (r: 0.991 to 0.997; $P < 0.05$), and lactic acid (r: −0.990; $P < 0.05$).

The lowest aroma observed was bitterness in both Tfs and starter culture-inoculated groups ($P > 0.05$; Fig. 3). As is known, the formation of rancid aroma and taste generally occurs under the influence of oxidation of unsaturated and some saturated fatty acids (Li & Xu, 2021). Interactions of instrumental analysis results with sensory analysis scores showed that palmitoleic acid (r: −0.998; $P < 0.05$) and cis-10-heptadecanoic acid (r: −0.992; $P < 0.05$) were negatively correlated with the rancid aroma (Fig. 3). In this study, all these chain reactions, which provide strong sensory discriminations between sauces, also lead to the clustering of many volatile compounds according to the sensory characteristics perceived in sauces, as shown in Fig. 4.

Aftertaste was analysed under the headings of sweet, salty, bitter, caramelised, fishy, cheesy, sour roasted, metallic, and rancid. Salinity was emphasised as the predominant taste in Tfs (7.17) and Lp (4.72), while sourness prevailed in Lp + Sc (5.08) ($P > 0.05$; Fig. 2). Similar to the perception of salty taste, aftertaste saltiness was associated with FAAs such as Gly (r: −0.988; $P < 0.05$), Arg (r: −0.996; $P < 0.05$), His (r: −0.988; $P < 0.05$), and Cys (r: −0.992; $P < 0.05$; Fig. 3). Salty aftertaste also showed strong correlations with FAAs such as Ser (r: 0.999; $P < 0.05$), Pro (r: −0.999; $P < 0.05$), Leu (r: −1.000; $P < 0.01$), Tyr (r: 0.999; $P < 0.05$), and Orn (r: −0.999; $P < 0.05$), as well as FFAs such as stearic acid (r: −0.999; $P < 0.05$) and cis-13,16-docosadienoic acid (r: 0.999; $P < 0.05$), and OAs such as lactic acid (r: −0.993; $P < 0.05$), citric acid (r: −0.997; $P < 0.05$), and acetic acid (r: −0.998; $P < 0.05$). Sour perception in aftertaste showed significant positive correlations with FAAs such as Gly, Iso, His, Cys, (r: 0.994 to 0.997; $P < 0.05$), as well as showed significant negative correlations with FAA such as Arg (r: −0.988; $P < 0.05$) and FFA such as behenic acid (r: −0.989; $P < 0.05$). The dominant aftertaste impressions were characterised by panellists as caramelised (4.83) and fishy (4.11) for Tfs, sour (4.39) and caramelised (3.72) for Lp, and salty (4.83) and caramelised (3.61) for Lp + Sc groups (Fig. 2). Metallic, bitter, and roasted aftertastes were perceived less prominently in all samples ($P > 0.05$). Furthermore, the significant correlations detected between these data components demonstrate the accuracy of both analytical and sensory analyses and the reliability of multi-replicate statistical interactions. In other words, the analytically determined amounts of amino acids, fatty acids, organic acids, and volatile compounds were verified by the panellists based on the statistically determined correlation results. This demonstrates the overall accuracy and reliability of the tests performed.

Conclusion

Fish sauce is a fermented product characterised by its unique microbiota and enzymes present in the fermentation environment. Understanding how fish fermentation can be shaped and optimised using easily accessible fermentative microorganisms to meet consumer demand, whilst utilising raw materials that are overproduced or not intended for direct human consumption, not only contributes to the development of the fishing and related food industries by improving the evaluation of natural resources, but also holds

significant economic potential. This potential includes strengthening domestic markets and expanding exports to adapt to evolving market and production dynamics influenced by various factors, as well. Therefore, it is thought that the findings of this research have significant implications for the fishing and food industries, particularly in countries such as Türkiye, the Mediterranean, Asia, and other countries worldwide where the seafood industry is experiencing rapid growth and high production rates.

In light of this information, the use of starter cultures in production can shape flavour and ensure standardisation. In this study, the effect of *L. plantarum* and *S. cerevisiae* on the taste and aroma of fish sauce was investigated through descriptive sensory analysis, supported by correlations with data obtained from precise instrument analysis. As a result, it was found that the use of *L. plantarum* and *S. cerevisiae* as starter cultures in fish sauce production alters aroma and taste in favour of these microorganisms through breakdown products and metabolites. Additionally, it is believed that the data presented here could be useful in designing the sensory profile of new fermented foods.

Further research into how microbial communities involved in the fermentation process are shaped by the presence of starter cultures and their effects on product quality will deepen the understanding of fermentation dynamics. Moreover, exploring alternative fermentation substrates or improving existing ones can provide insights into optimising fermentation efficiency and product consistency. Furthermore, evaluating the nutritional profile of fermented fish products and their health benefits at low salt levels can open up avenues for marketing and product development. Future research efforts addressing these areas can contribute to continuous improvement and innovation in the field of novel fermented seafood.

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Author contributions

Selin Ozge Dinc: Conceptualization; software; validation; formal analysis; writing – original draft; writing – review and editing; visualization. **Fatma Colakoglu:** Project administration; conceptualization; methodology; validation; writing – review and editing; supervision. **Ibrahim Ender Kunili:** Conceptualization; methodology; validation; writing – review and editing; supervision; visualization; formal analysis. **Hasan Basri Ormanci:** Writing – review and editing; formal analysis; visualization.

Conflict of interest

The authors declare that there is no conflict of interest.

Ethical guidelines

Ethics approval was not required for this research.

Consent to participate

Written informed consent was obtained from the sensory panellists.

Peer review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ijfs.17392.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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The study provides data showing that *L. plantarum* exhibits proteolytic activity in low-salt meat fermentation. In our research, it was cited to demonstrate that our end products are fermented and to examine the effects of L. plantarum on proteins in the environment.

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The study explains the formation mechanisms and product properties of volatile compounds based on sensory, molecular, and metagenomic analyses. This publication is included in our research because it provides a solid basis for the association between volatile compounds and sensory perceptions.

Wang, M., Wang, C., Yang, C. *et al*. (2021). Effects of *Lactobacillus plantarum* C7 and *Staphylococcus warneri* S6 on flavour quality and bacterial diversity of fermented meat rice, a traditional Chinese food. *Food Research International*, **150**, 110745.

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Our study proves the existence of significant correlations between non-volatile compounds and sensory characteristics. This research contains important data to demonstrate the relationship between the natural flora in traditional fish sauce and the proteolytic activity of our cultures in starter applications and flavour.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

File S1 Attributes and definitions in descriptive sensory analysis.

File S2 Fish sauce sensory analysis form.

