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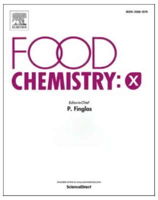

Quality improvement of the cured fish by replacing nitrite with chromogenic microorganisms: Insight into the effect on residual nitrosamine levels, color, and flavor characteristics

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ABSTRACT

To achieve enhanced red color and improved safety of cured fish, the three nitrite-alternative strains, namely, *Staphylococcus saprophyticus* CICC 24365 (SS), *Staphylococcus carnosus* ATCC 51365 (SP), and *Limosilactobacillus fermentum* CICC 25124 (LF), were employed in this study. Furthermore, the hemoglobin in grass carp muscle was largely retained by directly removing the head of fish to increase heme levels in fish muscle. The results demonstrated that the nitroso-heme contents in the SS and SP groups were 7.40 and 6.53 mg/kg, respectively, which exhibited a comparable level to that observed in the nitrite group (8.70 mg/kg). Compared to the nitrite group, the nitrite levels in the SS and SP groups decreased by 91.70 % and 89.97 %, respectively, while there was a notable decrease of 70.33 % and 74.64 % in the total amount of nitrosamines. In addition, inoculation fermentation can effectively enhance the quality of cured fish by augmenting the levels of free amino acids and aromatic compounds.

1. Introduction

Color is an important sensory property affecting consumer acceptability (Luo et al., 2020). Nitrite has emerged as the predominant additive in cured meat products due to its distinctive coloring, antioxidant properties, and antibacterial effects (Hospital et al., 2015). Nitrite generates nitric oxide in acidic conditions, which subsequently binds with heme iron to form nitrosomyoglobin, leading to the characteristic cherry-red color observed in cured meat products. However, under acidic conditions, a portion of nitrite will be converted into nitrous acid and subsequently react with amines to generate N-nitrosamine (Huang et al., 2024). Nitrosamines are a class of substances exhibiting significant carcinogenicity (Yang et al., 2022). Moreover, excessive intake of nitrite can induce the oxidation of hemoglobin in human blood to methemoglobin, resulting in impaired oxygen-carrying capacity and potentially leading to asphyxia in severe cases (Neth et al., 2021). Therefore, the search for nitrite alternatives has been a hot topic of meat products research (Luo et al., 2020).

Currently, there are two main types of nitrite substitutes: natural extracts and chromogenic microorganisms (Gou et al., 2023). In current research, the substitution of nitrite with microbial fermentation has emerged as the prevailing trend for obtaining wholesome, eco-friendly and delicious fermented meat products. Certain microorganisms found in nature exhibit exceptional color enhancement ability and antibacterial properties, and enabling the production of distinctive flavors in fermented meat products. In recent years, the utilization of microorganisms as substitutes for nitrite can be categorized into three groups based on the types of microorganisms involved: lactic acid bacteria, *Staphylococcus* and mixed strains (Gou et al., 2023). Zhang et al. (2007) investigated the impact of different concentrations of *Limosilactobacillus fermentum* inoculation on the coloration of Harbin red sausage and found that this strain effectively facilitated nitroso myoglobin production. Notably, sausages inoculated with 8 log CFU/g of *Limosilactobacillus fermentum* exhibited a pink hue comparable to that achieved by adding 60 mg/kg nitrite. Cruxen et al. (2017) found that *Staphylococcus xylosa* LQ3 exhibited lipolytic, proteolytic, nitrate reductase, and superoxide dismutase activities. Moreover, compared to the control group, sausages treated with *Staphylococcus xylosa* LQ3 displayed reduced levels of lipid oxidation and more vibrant red color.

Microbial fermentation as a substitute for nitrite is primarily investigated in animal meat due to its higher myoglobin content, facilitating the formation of abundant bright red nitroso myoglobin (Cruxen et al.,

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2017; Xu & Zhu, 2021). However, it is difficult for certain meats with low myoglobin content, such as fish, to achieve a distinct red color by forming abundant nitroso myoglobin. Therefore, to enhance the color of fish products, we proposed to increase the hemoglobin content by appropriately controlling the blood residue in fish muscle in previous research (Fang et al., 2024). Furthermore, we screened three microorganisms capable of converting hemoglobin into nitroso hemoglobin, intending to enhance the color rendering effect in cured grass carp. The results demonstrated that the inoculation of *Staphylococcus saprophyticus* CICC 24365 and *Staphylococcus carnosus* ATCC 51365 significantly enhanced the red color of cured grass carp, achieving a redness value comparable to that obtained by adding 0.15 g/kg nitrite (Fang et al., 2024).

To confirm the feasibility of these three microorganisms as nitrite substitutes, it is essential to investigate their effects on the edible and safety quality of cured fish. Therefore, this article explored the impact of inoculating chromogenic strains on the quality and safety of cured fish by assessing various indicators, including nitroso heme, flavor profile, free amino acids, biogenic amines, total volatile basic nitrogen, peroxidation value, thiobarbituric acid content, residual nitrite levels, N-nitrosamine, and other indicators. This study proposed the technological path of color improvement by regulating the residual amount of fish blood to make up for the insufficient content of myoglobin, and established a new color enhancement method for cured fish based on microbial fermentation, which provided theoretical basis and technical support for the non-nitrification transformation of the traditional cured fish production process, and held significant application value for promoting the green transformation of the traditional aquatic product processing industry.

2. Materials and methods

2.1. Materials

Mannitol salt agar, MRS broth and MRS agar were purchased from Qingdao Hi-Tech Industrial Park Haibo Biotechnology Co., Ltd. Plate count agar was purchased from China National Pharmaceutical Group Chemical Reagent Co., Ltd. Mannitol sodium chloride liquid culture medium was purchased from Shandong Topology Biotechnology Co., Ltd. Standard solution containing nine N-nitrosamine mixtures (the concentration of each N-nitrosamine is 2000 μg/mL) was purchased from Sigma Corporation in the United States. All other chemicals and solvents were of analytical grade.

2.2. Preparation of fermentation broth

Limosilactobacillus fermentum CICC 25124 (LF) and *Staphylococcus saprophyticus* CICC 24365 (SS) were provided by the China Center of Industrial Culture Collection (Beijing, China). *Staphylococcus carnosus* ATCC 51365 (SP) was obtained from Shanghai Bioresource Collection Center. All of these strains were preserved in the Laboratory of Food Processing Technology, School of Food Science and Technology, Jiangnan University. The SS and SP strains were incubated in mannitol sodium chloride liquid medium at 37 °C for 72 h, while the LF strain was incubated in MRS broth at 30 °C for 72 h to obtain bacterial suspensions. Cell precipitation was obtained by centrifugation of the bacterial suspension (4 °C, 10000 g, 10 min), followed by washing with sterile water, and then the cell concentration was adjusted to 7–8 log CFU/mL to obtain fermentation broth.

2.3. Preparation of cured fish

Raw material pretreatment: Fresh grass carp with an average weight of 2 ± 0.2 kg were obtained from a local supermarket (Wuxi, Jiangsu, China). Immediately following stunning, the heads were removed to retain blood within the fish muscle. They were gutted, gills removed, cleaned, and cut into standardized pieces measuring approximately 6 cm × 4 cm × 4 cm. Only the central section of the fish bodies was used for assessment.

Curing ingredients preparation: A curing mixture consisting of 6 % (*w*/w) salt and 1 % (w/w) glucose was prepared based on the weight of the fish pieces.

Curing treatment: The fish pieces were thoroughly mixed with curing ingredients, transferred to a sealed curing bucket, and cured at 4 °C for specified durations using an incubator (LRH-250, Yiheng Scientific Instrument Co., Ltd. Shanghai, China).

Curing groups: No nitrite was added to the curing ingredients in the control group (C1), while 0.15 g/kg of nitrite (based on the weight of fish chunks) was added to the curing ingredients in the nitrite group (N). Both groups C1 and N underwent a curing treatment for 6 days at 4 °C and were subsequently dried in a drying oven (DHG-9070 A, Yiheng Scientific Instrument Co., Ltd. Shanghai, China) at 43 °C for 16 h.

Fermentation groups: Inoculate activated SS, SP, and LF (7–8 log CFU/mL) into fish through immersion after the fish have undergone curing treatment for 3 days. Subsequently, ferment the fish in an incubator (BXS-150, Boxun Medical Biological Instrument Co., Ltd. Shanghai, China) at 20 °C for 3 days (relative humidity 70 %). Fermentation without inoculation served as the control group (C2). After fermentation, all groups (C2, SS, SP, and LF) were dried in a drying oven (DHG-9070 A, Yiheng Scientific Instrument Co., Ltd. Shanghai, China) at 43 °C for 16 h.

2.4. Determination of a* value

The a* value of cured fish samples was determined using a colorimeter (UltraScan Pro1166, Hunterlab, USA. Aperture opening size: 25 mm; Optical geometry: d/8°; Observer angle: 10°; Illuminant type: pulse xenon lamp).

2.5. Determination of nitroso heme

The nitroso heme content was determined following the protocol described by Hyun-Wook et al. (2015) with slight modifications. The pigment in the cured fish was extracted by an acetone-distilled water solvent system. The extract was filtered with filter paper and the absorbance was measured at 540 nm. Nitroso heme content is quantified in units of μg/kg.

2.6. Determination of pH

Two grams of cured fish sample were mixed with 18 mL of deionized water and the mixture was homogenized. The pH was determined using a pH meter (FiveEasy Plus 28, Mettler Toledo, Shanghai, China. Type of probe: InLab ® Routine Pro-ISM, Electrode model: composite electrode).

2.7. Determination of moisture content

Two grams of cured fish sample were dried at 105 °C until a constant weight was achieved. The difference in weight before and after drying was utilized to determine the total moisture content of the sample.

2.8. Determination of thiobarbituric acid (TBARS)

Thiobarbituric acid was determined using the method described by Xu et al. (2021) with slight modifications. The crushed fish samples (5 g) were combined with 10 %TCA solution (20 mL), followed by homogenization at 10000 r/min for 1 min and subsequent centrifugation at 10000 *g* for 10 min at 4 °C. The supernatant (5 mL) was mixed with 0.02 M TBA solution (5 mL), followed by heating in boiling water for 20 min. After cooling, the absorbance at 532 nm was measured. A standard curve was prepared using 1,1,3,3-tetramethyloxypropane (MAD) at concentrations ranging from 0 to 10 ppm. TBARS were quantified as MAD equivalent mg/kg of samples.

2.9. Determination of total volatile basic nitrogen (TVB-N)

The total volatile basic nitrogen content was determined using the method described by Xu and Zhu (2021) with slight modifications. The cured fish (4 g) was mixed with distilled water (20 mL), followed by homogenization at 10000 r/min for 1 min and subsequent centrifugation at 10000 *g* for 10 min at 4 °C. The supernatant (10 mL) was combined with magnesium oxide solution (10 g/L, 10 mL) and subjected to distillation for 5 min using a semimicro Kjeldahl bottle. Subsequently, the resulting distillate was mixed with boric acid solution (10 g/L, 10 mL), and then titrated with hydrochloric acid (0.01 mol/L). Finally, the total volatile basic nitrogen content was determined based on the amount of hydrochloric acid consumed.

2.10. Determination of TCA-soluble peptides

The TCA-soluble peptides were determined using the method described by Tian et al. (2021) with slight modifications. The fish samples (2 g) were mixed with 5 % TCA solution (18 mL), homogenized at 10000 r/min for 2 min and subsequently incubated at 4 °C for 1 h. The homogenate was centrifuged at 8000 *g* for 5 min at 4 °C, followed by determination of the TCA-soluble peptide content in the supernatant using the Lowry method. The results were expressed as μmol Tyr/g.

2.11. Determination of free amino acids

The free amino acids were determined using the method described by Hua et al. (2020) with slight modifications. The fish samples (5 g) were mixed with 5 % TCA solution (25 mL). After homogenizing at 10000 r/min for 2 min, 2 mL of 1 M KOH was added. The mixture was then centrifuged at 8000 *g* for 10 min at 4 °C. The supernatant was filtered (0.45 μm, pore size; Acrodisc syringe filter, Pall, USA), and the resulting filtrate was transferred into a small bottle for subsequent use. Free amino acids were determined by cation exchange chromatography using the Biochron 20 automatic amino acid analyzer (Amersham Pharmacia LKB, Biotech Biocom, Uppsala, Sweden).

2.12. Determination of volatile compounds

Volatile compounds were extracted by solid phase microextraction (SPME) using the method of Tian et al. (2021). Volatile compounds were analyzed by GC–MS (TSQ 8000, Thermo Fisher Scientific, Waltham, MA, USA) using a 30 mm × 0.25 mm × 0.25 μm DB-WAX column. The gas chromatography and mass spectrometer conditions were established in accordance with the method described by Gao et al. (2016). The volatile flavor compounds were identified using the NIST2005 and Willey 7 standard libraries, while semi-quantification was performed with the use of a 2,4,6-trimethylpyridine internal standard (Sigma Chemical, St Louis, MO, USA). The results were expressed as μg/kg of the sample.

2.13. Electronic nose

Electronic nose was determined according to the method described by Tian et al. (2021). The chopped fish samples (2 g) were placed in a 20 mL headspace vial, and the overall odor differences between the samples were measured and analyzed using a rapid electronic nose equipped with two chromatographic columns (DB-5 and DB-1701, 10 m × 0.25 mm × 0.25 μm, Agilent) and a flame ionization detector. The detection parameters referred to the method described by Hua et al. (2020).

2.14. Microbial analysis

The microbial analysis was determined using the method described by Tian et al. (2021). The total viable bacteria were cultured on aerobic plate counting agar (PCA) at 30 °C for 48 h. *Staphylococcus* was cultured on Manitol Salt Agar (MSA) at 37 °C for 48 h. Lactic acid bacteria were cultured on de Man Rogosa and Sharpe (MRS) agar at 30 °C for 72 h. Coliform was cultured on Violet Red Bile agar (VRBA) at 37 °C for 24 h. *Pseudomonas* was cultured on Pseudomonas CFC Selective agar at 37 °C for 48 h. The results were expressed as log CFU/g of the sample.

2.15. Determination of biogenic amine

Biogenic amine was determined according to the method described by Tian et al. (2021). The fish samples (2 g) were mixed with 0.6 M trichloroacetic acid solution (10 mL), homogenized at 10000 r/min for 2 min and then centrifuged at 10000 *g* for 10 min at 4 °C to obtain the supernatant. The remaining substrate was re-extracted with 10 mL of 0.6 M perchloric acid solution following the same homogenization and centrifugation procedure. The two parts of supernatant were combined and proceeded with the filtration step. The filtrate was diluted to 25 mL with 0.6 M perchloric acid solution and stored at 0–4 °C for biogenic amine analysis. The Agilent ZORBAX SB-C18 chromatography column (4.6 × 150 mm, 5 μm; Agilent Technologies, Inc., CA, USA) was used for analysis at a wavelength of 254 nm. The results were expressed as mg/kg of the sample.

2.16. Determination of nitrite content

Nitrite content was determined according to the method described by Xu et al. (2021) with slight modifications. The minced fish (1 g) was mixed with distilled water (30 mL) and heated in a boiling water bath for 20 min. Then, it was filtered with filter paper (No. 4, Whatman PLC., Kent, UK), followed by dilution with distilled water to a final volume of 50 mL. The filtrate (20 mL) was mixed with 1 mL of sulfonic acid (30 mmol/L) and 1 mL of N-(1-naphthyl) ethylenediamine dihydrochloride (5 mmol/L). The solution was thoroughly mixed and subsequently diluted to 25 mL with deionized water. The reaction mixture was allowed to stand for 20 min at room temperature (25 ± 1 °C). The absorbance of the sample solution was measured at 540 nm and the nitrite content in the sample could be calculated using a standard curve prepared with sodium nitrite. The result was expressed as mg/kg.

2.17. Determination of nitrosamine

The nitrosamine was extracted using the method described by Liao et al. (2019) with slight modifications. The fish sample (10 g) was mixed with 15 mL of acetonitrile and homogenized for 3 min. The mixture was stored at −20 °C for 30 min. Then 4 g of $MgSO_4$ and 1 g of NaCl were added, and the mixture was vigorously shaken for 30 s. The solution was then centrifuged at 3800 *g* for 10 min at 4 °C. The supernatant (6 mL) was mixed with 50 mg of N-propylethylenediamine (PSA), 150 mg of C18E, and 900 mg of anhydrous sodium sulfate. The mixture was vortexed for 1 min and subsequently centrifuged again at 3800 *g* for 10 min at 4 °C. Then 5 mL of the supernatant was transferred to a nitrogen vaporizer (WD-12, Allsheng Instruments co. LTD., Hangzhou, China) and concentrated to a final volume of 1 mL at room temperature (25 °C). The concentrated samples were subsequently passed through a 0.22 μm membrane filter (Sartorius NY, Goettingen, Germany) for GC–MS/MS analysis. Detailed GC–MS/MS analysis conditions can be found in the method described by Qiu et al. (2017).

2.18. Statistical analysis

Each independent experiment was repeated three times. Statistical analysis of the data was performed using SPSS 26.0. Variance and Duncan's multi-range test were used for the average difference. ($p <$ 0.05 indicates significance).

3. Results and discussion

3.1. pH, a* value, and moisture content

As shown in Table 1, the a* values of SS and SP groups were 5.64 ± 0.52 and 5.42 ± 0.55, respectively, which were comparable to those of the N group with an addition of 150 ppm nitrite. Nevertheless, the a* value of LF group did not exhibit a significant enhancement compared to C2 group, aligning with our prior research findings (Fang et al., 2024). The pH values of each group ranged from 5.98 to 6.16, with no significant differences observed. The pH variation during fermentation is primarily associated with the proliferation of microorganisms. For instance, lactic acid bacteria can produce a substantial quantity of lactic acid via glycolysis as they grow, leading to a reduction in the pH level of fermented food (Tian et al., 2021). The pH value of the LF group did not exhibit a lower level compared to that of other groups. This could be attributed to the potential inhibition of *Limosilactobacillus fermentum* growth in a high-salt environment, as well as the possible reaction between non-protein nitrogen generated from protein hydrolysis in fish and lactic acid during late-stage fermentation, leading to an elevation in pH (Chen et al., 2016). The moisture content of the fermentation groups was significantly lower than that of the curing groups, potentially attribute to water evaporation and water consumption by microbial growth during the fermentation process (Tian et al., 2021).

3.2. Nitroso heme and nitrite residues

To evaluate the impact of different treatments on color enhancement and nitrite residue in cured fish, we quantified the levels of nitroso heme content and nitrite residue in each group (Fig. 1**A&B**). Nitroso heme, the predominant pigment found in cured meat products, exhibits a stable red color (Huang et al., 2024). The group N showed a significantly higher nitroso heme content (8.70 mg/kg) compared to that in group C1 ($p < 0.05$), suggesting that nitrite primarily enhances color by facilitating the formation of nitroso heme. The nitroso heme content of the SS and SP groups was 7.40 and 6.53 mg/kg, respectively, which closely approximated that of the group N. Our previous research demonstrated that SS, SP, and LF all exhibit nitric oxide synthase (NOS) activity (Fang et al., 2024). NOS can facilitate the conversion of free L-arginine into NO and L-citrulline, subsequently leading to the formation of nitroso heme through the reaction between heme with NO (Huang et al., 2024). The high nitroso heme content observed in the SS and SP groups may be attributed to the role of nitric oxide synthase in facilitating nitroso heme formation. However, only a small amount of nitroso heme was detected in the LF group. This may be attributed to the high salt content in cured fish, which likely reduces the color development ability of LF. (Fang et al., 2024).

Maintaining low levels of nitrite residues in cured meat products is crucial due to the significant safety risk posed by high amounts of nitrite residues in food (Gou et al., 2023). The Codex General Standard for Food Additives specifies that the nitrite content in heat-treated meat products should not exceed 80 mg/kg. The maximum allowable limit of nitrite in fermented meat products was set at 30 mg/kg according to GB 2760–2024 (National Health Commission of China, 2024). It is noticed that the nitrite content in group N (53.96 mg/kg) was at a relatively high concentration. Trace amounts of nitrite residue were also detected in the remaining five groups even without the addition of nitrite, potentially originating from the salt used during the curing process (Liao et al., 2019). The nitrite content in the SS and SP groups was significantly lower, with reductions of 91.70 % and 89.97 %, respectively, compared to the N group. This phenomenon can be attributed to the relatively lower initial nitrite content in the SS and SP groups, as well as the nitrite reductase activity of SS and SP, which plays a role in degrading nitrite (Fang et al., 2024; Liao et al., 2018). Therefore, the SS and SP groups maintained a relatively low level of nitrite content while forming nitroso heme content comparable to that of the N group, which can greatly improve the safety of cured fish (Huang et al., 2024).

Table 1
The pH, a* value and moisture content in different cured fish samples.

	C1	N	C2	SS	SP	LF
a*	2.45 ± 0.29b	6.46 ± 0.59a	2.83 ± 0.95b	5.64 ± 0.52a	5.42 ± 0.55a	2.33 ± 0.06b
pH	6.16 ± 0.01a	6.14 ± 0.01ab	6.12 ± 0.01bc	6.06 ± 0.01d	5.98 ± 0.02e	6.09 ± 0.03 cd
Moisture content (%)	51.12 ± 1.33a	49.89 ± 1.17ab	42.29 ± 2.73c	44.62 ± 1.68bc	43.10 ± 3.98c	43.56 ± 1.31c

Note: Values are means ± SD ($n = 3$). C1:Curing control group; N: Nitrite curing group; C2: Fermentation control group; SS: *Staphylococcus saprophyticus* inoculated fermentation group; SP: *Staphylococcus carnosus* inoculated fermentation group; LF: *Limosilactobacillus fermentum* inoculated fermentation group. Different lowercase letters represent significant differences between groups in the same line. ($p < 0.05$).

3.3. Nitrosamine

Under acidic conditions, nitrite can be converted into unstable nitrous acid, which subsequently decomposes into nitrous anhydride (N_2O_3). Nitrous anhydride can directly react with amine compounds, leading to the formation of carcinogenic N-nitrosamine (Zhang et al., 2007). As shown in Table 2, a total of six nitrosamines were detected in the six groups of cured fish samples, namely NDMA, NMEA, NDEA, NPYR, NMOR, and NDpheA. Notably, NDMA and NDEA exhibit significant carcinogenic potential. The NDMA content in the N group was measured at 0.24 μg/kg, whereas the SS group exhibited a significantly lower NDMA content (0.15 μg/kg) compared to the N group ($p < 0.05$). Additionally, no detectable levels of NDMA were observed in the SP group. The NDEA levels in the SS and SP groups were 0.12 and 0.15 μg/kg, respectively, representing a reduction of 72.73 % and 65.91 % compared to those in the N group. This phenomenon may be attributed to the relatively low nitrite content in the SS and SP groups, thereby reducing the formation of nitrosamines. Alternatively, it could also be due to microbial fermentation exerting a certain degree of degradation on nitrosamines (Li et al., 2019). Furthermore, a substantial amount of NPYR (0.89 μg/kg) was detected in group N. The total amount of nitrosamines in group N reached 2.09 μg/kg, which was significantly higher compared to the other five groups ($p < 0.05$). Therefore, the SS and SP groups maintained a relatively low level of nitrosamine content while achieving a similar red color to that of the N group. This enhancement in safety levels for cured fish makes it highly valuable for practical applications.

3.4. Microbial count

The counts of all detected microorganisms were shown in Table 3. The total bacterial count of groups C1 and N was found to be lower compared to the other four fermentation groups, potentially attributed to the inhibitory effect of low temperature on microbial growth. No significant differences were observed in the counts of *Staphylococcus* (7.61, 7.82, 8.06 log CFU/g) and lactic acid bacteria (5.66, 6.18, 5.59 log CFU/g) among the SS, SP and LF groups. This phenomenon can potentially be attributed to the inhibitory effect of high salt and abundant oxygen on the growth of lactic acid bacteria, while *Staphylococcus* thrived and proliferated rapidly, ultimately establishing dominance within this particular environment (Hongchen et al., 2023; Zhang et al., 2023). Therefore, despite the inoculation of *Limosilactobacillus fermentum* in the LF group, a substantial increase in lactic acid bacteria was not observed. The presence of *Pseudomonas* was not detected in any of the groups. No Coliforms were detected in groups C1 and N, whereas a limited number of Coliforms were observed in each fermentation group (2.49 to 2.78 log CFU/g). This phenomenon may be attributed to the

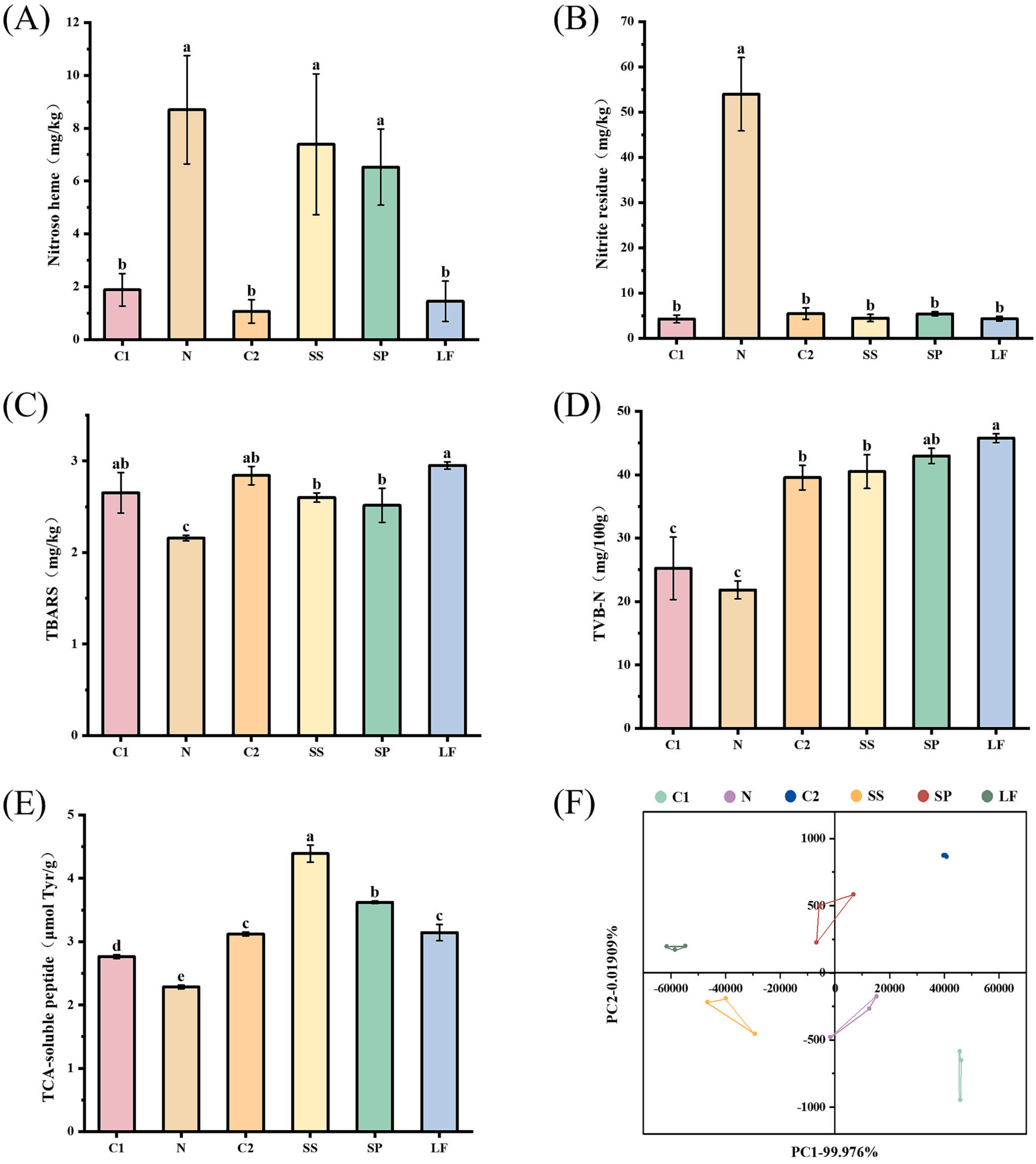

Fig. 1. Quality indicators of different cured fish samples. **N**itroso heme content (A); Nitrite residue (B); TBARS value (C); TVB-N content (D); TCA-soluble peptide content (E); Electronic nose (F). C1:Curing control group; N: Nitrite curing group; C2: Fermentation control group; SS: *Staphylococcus saprophyticus* inoculated fermentation group; SP: *Staphylococcus carnosus* inoculated fermentation group; LF: *Limosilactobacillus fermentum* inoculated fermentation group. Different lowercase letters represent significant differences between the groups ($p < 0.05$).

inhibitory effect of low-temperature curing on the proliferation of Coliform (Tian et al., 2021).

3.5. TBARS

The levels of TBARS value can serve as indicators for assessing the extent of lipid oxidation in meat products (Xu & Zhu, 2021). As shown in the Fig. 1C, the TBARS value of group N exhibited a slight decrease compared to the other groups, potentially attributed to the capacity of nitrite to form nitrite-fat complexes with lipids, thereby inhibiting lipid oxidation (Zang et al., 2023). The TBARS values of SS and SP groups were slightly lower than those of the C2 group, which may be attributed to *Staphylococcus saprophyticus* CICC 24365 and *Staphylococcus carnosus* ATCC 51365 converting a portion of heme in fish into nitroso heme during fermentation, thereby maintaining iron ions in a relatively stable divalent state and consequently reducing oxidation (Zhang et al., 2007).

Table 2
The content of N-nitrosamine in different cured fish samples.

Fermentation group	N-nitrosamine content(μg/kg)					
	C1	N	C2	SS	SP	LF
NDMA	0.16 ± 0.04ab	0.24 ± 0.05a	0.18 ± 0.01ab	0.15 ± 0.01b	NE	0.15 ± 0.02b
NMEA	0.33 ± 0.16a	0.45 ± 0.05a	0.37 ± 0.03a	0.31 ± 0.05a	0.26 ± 0.08a	0.26 ± 0.03a
NDEA	0.18 ± 0.01bc	0.44 ± 0.05a	0.23 ± 0.03b	0.12 ± 0.02c	0.15 ± 0.06bc	0.15 ± 0.04bc
NPYR	0.30 ± 0.05b	0.89 ± 0.12a	0.25 ± 0.05bc	0.15 ± 0.04c	0.22 ± 0.04bc	0.16 ± 0.03c
NMOR	0.08 ± 0.02a	0.07 ± 0.01a	0.04 ± 0.01a	0.06 ± 0.03a	0.06 ± 0.05a	NE
NEpheA	0.06 ± 0.02^{a}	NE	NE	NE	NE	NE
Total content	0.84 ± 0.37b	2.09 ± 0.21a	0.80 ± 0.46b	0.62 ± 0.12b	0.53 ± 0.18b	0.52 ± 0.24b

Note: Values are means ± SD (n = 3). C1:Curing control group; N: Nitrite curing group; C2: Fermentation control group; SS: *Staphylococcus saprophyticus* inoculated fermentation group; SP: *Staphylococcus carnosus* inoculated fermentation group; LF: *Limosilactobacillus fermentum* inoculated fermentation group. NE: Non-existence. Different lowercase letters represent significant differences between groups in the same line. ($p < 0.05$).

Table 3
The number of microorganisms in different cured fish samples.

Microbial count (log CFU/g)	C1	N	C2	SS	SP	LF
Staphylococcus	4.64 ± 0.04c	4.99 ± 0.57c	6.25 ± 0.61b	7.61 ± 0.04a	7.82 ± 0.08a	8.06 ± 0.08a
Lactic acid bacteria	3.76 ± 0.01c	3.77 ± 0.03c	5.05 ± 0.56b	5.66 ± 0.01ab	6.18 ± 0.58a	5.59 ± 0.10ab
Pseudomonas	<1	<1	<1	<1	<1	<1
Coliform	<1	<1	2.78 ± 0.16a	2.56 ± 0.07a	2.62 ± 0.15a	2.49 ± 0.20a
Total bacteria	5.65 ± 0.10d	5.68 ± 0.01d	7.65 ± 0.04c	8.58 ± 0.08b	8.89 ± 0.05a	8.67 ± 0.03b

Note: Values are means ± SD (n = 3). C1:Curing control group; N: Nitrite curing group; C2: Fermentation control group; SS: *Staphylococcus saprophyticus* inoculated fermentation group; SP: *Staphylococcus carnosus* inoculated fermentation group; LF: *Limosilactobacillus fermentum* inoculated fermentation group. Different lowercase letters represent significant differences between groups in the same line. ($p < 0.05$).

Xu and Zhu (2021) indicated that *Limosilactobacillus fermentum* can exhibit strong antioxidant capacity in fermented meat products. However, the TBARS value of the LF group was comparable to those of C2 group. This phenomenon may be attributed to the fact that *Limosilactobacillus fermentum* did not become the dominant bacteria in the LF group, leading to the proliferation of miscellaneous bacteria and ultimately intensifying the oxidation of the LF group. On the other hand, it may also be attributed to the fact that there is insufficient capacity of a small quantity of *Limosilactobacillus fermentum* to convert residual hemoglobin in the fish into nitroso hemoglobin, resulting in the formation of Fe^{3+} when Fe^{2+} comes into contact with air (Nguyen & Phan, 2018). The Fe^{3+} serve as catalysts for lipid oxidation, facilitating the formation of superoxide, hydrogen peroxide, and hydroxyl radicals, thereby augmenting oxidative processes (Roth et al., 2009).

3.6. TVB-n

The effect of different treatments on the TVB-N content in cured fish was shown in Fig. 1D. TVB-N refers to alkaline nitrogen-containing substances, such as ammonia and amines, which are generated through protein degradation (Wang et al., 2019). It is commonly utilized for evaluating the degree of product spoilage. The TVB-N content in group N was 21.83 mg/100 g, which was lower compared to the cured control group C1 (25.23 mg/100 g), potentially attributed to the antimicrobial properties of nitrite that inhibit microbial protein degradation (Zhang et al., 2016). The TVB-N content in the fermentation groups exhibited a significant increase compared to that in the two curing groups ($p < 0.05$). This phenomenon can be attributed to the optimal higher fermentation temperature (25 °C), which enhances protease activity and facilitates the growth and metabolism of microorganisms. During the fermentation process, these microorganisms decompose proteins to produce ammonia and amines (Wang et al., 2019).

3.7. TCA-soluble peptide

The TCA-soluble peptide can serve as reliable indicators for assessing the degradation of fish proteins (Tian et al., 2021). As shown in the Fig. 1E, the TCA-soluble peptide content in group N was significantly lower than that in group C1, suggesting that the addition of nitrite could reduce the extent of fish protein hydrolysis. This may be attributed to the antibacterial properties of nitrite, which could lead to a reduction in microbial proteolytic enzyme secretion. The fermentation groups exhibited a higher level of TCA-soluble peptide content compared to the curing groups, with the SS and SP groups demonstrating 1.4- and 1.2-times higher TCA-soluble peptide content than the C2 group, while no significant difference was observed between the LF group and the C2 group ($p > 0.05$). The extent of protein degradation may be influenced by the type of microorganisms inoculated. Hua et al. (2020) also found that the degree of protein hydrolysis in fish-chili paste varied when different fermentation agents were used. Moreover, soluble peptides can undergo metabolic processes to yield amino acids, which can directly serve as taste compounds or flavor precursors (Geng & Wang, 2019).

3.8. Free amino acid

As shown in Table 4, the results revealed the presence of a total of 17 free amino acids across six different samples. The total concentration of free amino acids in the four fermentation groups exceeded that in the two curing groups, potentially attributed to the enhanced protein hydrolysis activity exhibited by microorganisms during the fermentation process. Aspartate and glutamic acid are crucial umami amino acids in fermented products, exhibiting thresholds of 3 and 5 mg/100 g, respectively. The aspartic acid content in the SS and SP groups was 1.62 and 1.66 times higher than that in the fermentation control group C2. Similarly, the glutamic acid content in the SS and SP groups was 1.61 and 1.51 times higher than that in the fermentation control group C2, respectively. These findings suggest that inoculation with SS and SP fermentation can significantly enhance the umami of cured fish. Yu et al. (2022) also found that inoculation with *Staphylococcus* could increase the content of umami amino acids in shrimp sauce, which may be related to the higher protease activity in *Staphylococcus*. Serine, glycine, threonine, alanine, and proline are amino acids known for their sweet taste (Hua et al., 2020). Among these five sweet amino acids, except for glycine, the fermentation groups exhibited higher levels of the remaining four sweet amino acids compared to the curing group. Arginine is a typical bitter amino acid (Qian et al., 2022). Compared with C2 group, arginine content in SS and SP groups was decreased by 29.34 % and 41.98 %, respectively. This phenomenon may be attributed to the decomposition of arginine by nitric oxide synthase in SS and SP (Huang et al., 2024). In conclusion, inoculating SS and SP in cured fish not only achieves a redness comparable to that achieved by adding nitrite but

Table 4

The content of free amino acid in different cured fish samples.

Free amino acid (mg/100 g)	C1	N	C2	SS	SP	LF
Aspartate	11.84 ± 1.42 cd	10.21 ± 1.80d	14.42 ± 1.89bc	23.36 ± 0.99a	24.00 ± 1.41a	16.96 ± 0.62b
Glutamic acid	23.51 ± 2.91b	24.06 ± 9.41b	28.21 ± 0.30b	45.42 ± 4.89a	42.53 ± 2.00a	29.85 ± 2.09b
Serine	6.14 ± 0.55a	5.98 ± 0.24a	6.30 ± 0.54a	6.87 ± 2.21a	7.67 ± 1.33a	6.46 ± 0.42a
Glycine	60.28 ± 4.07a	54.44 ± 11.99ab	62.47 ± 6.88a	34.33 ± 1.76c	49.51 ± 7.77ab	42.87 ± 0.76bc
Alanine	21.16 ± 3.32d	14.17 ± 2.70e	24.43 ± 1.67 cd	46.06 ± 4.97a	31.98 ± 2.65b	28.92 ± 1.48bc
Threonine	23.28 ± 1.54a	21.42 ± 9.43a	27.77 ± 2.73a	24.10 ± 4.71a	28.53 ± 0.67a	29.12 ± 0.35a
Proline	10.75 ± 0.20b	10.10 ± 1.10b	12.43 ± 0.07ab	11.94 ± 0.91ab	11.52 ± 0.83ab	13.93 ± 2.93a
Histidine	202.92 ± 2.01b	195.35 ± 6.13b	338.43 ± 45.98a	217.01 ± 22.28b	234.33 ± 1.22b	221.70 ± 7.58b
Lysine	17.52 ± 3.07ab	11.50 ± 1.24ab	14.31 ± 4.07ab	18.78 ± 7.60a	9.47 ± 1.00b	16.85 ± 1.45ab
Tyrosine	20.37 ± 1.60bc	20.95 ± 6.38abc	31.74 ± 5.74a	28.39 ± 3.84abc	17.79 ± 0.51c	29.71 ± 7.27ab
Cysteine	8.86 ± 3.29a	3.47 ± 0.19a	6.42 ± 3.98a	5.10 ± 1.40a	4.78 ± 0.70a	5.50 ± 4.32a
Valine	17.16 ± 4.52 cd	14.55 ± 2.06d	22.04 ± 0.06bc	40.94 ± 4.27a	21.01 ± 1.53bcd	26.80 ± 4.25b
Methionine	13.43 ± 3.89a	8.58 ± 0.58a	11.37 ± 1.51a	13.29 ± 10.96a	12.59 ± 0.31a	16.79 ± 6.24a
Phenylalanine	15.21 ± 1.31b	12.77 ± 0.67b	14.74 ± 2.27b	32.59 ± 9.30a	20.52 ± 2.36b	20.67 ± 1.29b
Isoleucine	12.83 ± 0.57b	10.46 ± 0.52b	11.73 ± 1.66b	22.34 ± 5.91a	13.39 ± 0.82b	14.64 ± 0.61b
Leucine	21.55 ± 0.69b	17.79 ± 1.09b	20.99 ± 1.48b	49.09 ± 13.15a	25.57 ± 1.08b	26.57 ± 0.46b
Arginine	28.04 ± 2.51a	22.88 ± 3.00bc	25.80 ± 3.67ab	18.23 ± 0.31 cd	14.97 ± 2.43d	18.33 ± 0.99 cd
Total free amino acids	514.85 ± 12.45bc	458.68 ± 35.39c	673.61 ± 43.29a	637.85 ± 26.45a	570.16 ± 16.84b	565.66 ± 16.67b

Note: Values are means ± SD (n = 3). C1:Curing control group; N: Nitrite curing group; C2: Fermentation control group; SS: *Staphylococcus saprophyticus* inoculated fermentation group; SP: *Staphylococcus carnosus* inoculated fermentation group; LF: *Limosilactobacillus fermentum* inoculated fermentation group. Different lowercase letters represent significant differences between groups in the same line. ($p < 0.05$).

also enhances the levels of umami and sweet amino acids in cured fish, thereby positively impacting its taste profile.

3.9. Biogenic amine

A total of eight biogenic amines were identified in the six groups of cured fish samples (Table 5), with histamine, tyramine, and phenylethylamine being the predominant causative agents of foodborne illnesses (Xu & Zhu, 2021). The established maximum limits for tyramine and phenylethylamine in food are reported to be 800 mg/kg and 30 mg/kg, respectively (Omer et al., 2021). The tyramine content in the cured fish samples of all groups was within the limit range, while the phenylethylamine content in the naturally fermented C2 group was 37.28 mg/kg, surpassing the maximum limit and posing certain safety risks. It is reported that the histamine content in food should not exceed 100 mg/kg, as exceeding this limit may lead to potential toxicity (Ran & Chen, 2017). Among the six groups of cured fish samples, only the SS group exhibited histamine content exceeding 100 mg/kg, with levels reaching 139.01 mg/kg. Wu et al. (2019) conducted a screening of histamine-producing microorganisms in cured fish samples and identified *Staphylococcus saprophyticus* as the strain exhibiting the highest histamine accumulation ability among the isolated strains, which aligns with the findings of our current experiment. The total content of biogenic amines in group N was found to be lower compared to that in group C1, suggesting that nitrite exhibits inhibitory effects on the formation of biogenic amines in cured fish. The SS group was the only one that exhibited a total biogenic amine content exceeding 200 mg/kg, thereby presenting a significant food safety concern.

3.10. Volatile compounds

The types and contents of volatile compounds in cured fish treated with different methods are shown in Table 6. A total of 96 volatile compounds were detected in six groups, including 23 alcohols, 11 aldehydes, 5 esters, 13 ketones, 6 acids, 20 alkanes, and 18 other compounds. Significant variations were observed in the composition of alcohols, aldehydes, ketones, and acids among cured fish samples treated with different methods. Moreover, fermented groups exhibited higher levels of alcohols, ketones, and acids compared to cured groups, suggesting that fermentation treatment could facilitate the generation of flavor compounds.

Esters are crucial compounds that affect the flavor of cured fish. Generally speaking, they are formed by microorganisms with high glycolytic or lipolytic activity through acid and alcohol esterification, thereby imparting a fruity aromatic (Shen et al., 2021). The noteworthy observation was that the ester content in group N was significantly lower, with only trace amounts of octyl formate detected. This phenomenon can potentially be attributed to the antibacterial properties of nitrite, which effectively hinder the proliferation of microorganisms and subsequently impede ester formation (Zhang et al., 2019). In addition, acids were detected in only three inoculated fermentation groups, of which acetic acids were the main acid compounds in the fermentation groups and were produced by fatty acid oxidation and alanine metabolism (Gao et al., 2016). A substantial quantity of isovaleric acid was detected in the SS group, which was not found in the other groups, thus signifying its distinctive flavor compound. The isovaleric acid can be generated through the catabolism of leucine by *Staphylococcus* (Beck

Table 5

The content of biogenic amine in different cured fish samples.

Biogenic amine(mg/kg)	C1	N	C2	SS	SP	LF
Tryptamine	1.41 ± 0.35ab	1.09 ± 0.13b	1.34 ± 0.15b	2.35 ± 0.67a	1.88 ± 0.15ab	1.77 ± 0.75ab
Phenylethylamine	4.01 ± 0.69b	2.72 ± 0.35b	37.28 ± 12.02a	3.39 ± 2.70b	9.40 ± 6.98b	2.40 ± 0.70b
Putrescine	1.02 ± 0.07bc	0.29 ± 0.13d	2.14 ± 0.47a	1.52 ± 0.14b	1.18 ± 0.06bc	0.90 ± 0.31c
Cadaverine	1.10 ± 0.48c	0.59 ± 0.18c	0.38 ± 0.09c	27.96 ± 4.11a	12.37 ± 3.12b	14.08 ± 3.66b
Histamine	3.09 ± 0.86c	1.17 ± 0.80c	4.01 ± 0.45c	139.01 ± 27.59a	40.84 ± 7.92b	36.82 ± 4.09b
Tyramine	36.20 ± 8.53ab	23.18 ± 11.89ab	37.92 ± 9.73ab	40.02 ± 2.85a	26.35 ± 5.47ab	21.28 ± 4.63b
Spermidine	11.04 ± 2.88ab	8.92 ± 3.55ab	14.76 ± 4.43a	8.74 ± 1.07b	5.68 ± 0.69b	5.60 ± 0.80b
Spermine	9.60 ± 1.43ab	6.77 ± 2.62b	16.25 ± 4.30a	9.85 ± 1.24ab	7.38 ± 0.83b	11.99 ± 9.15ab
Total content	67.47 ± 11.83 cd	44.72 ± 4.65d	114.07 ± 16.68b	232.85 ± 30.97a	105.08 ± 15.16bc	94.83 ± 13.34bc

Note: Values are means ± SD (n = 3). C1:Curing control group; N: Nitrite curing group; C2: Fermentation control group; SS: *Staphylococcus saprophyticus* inoculated fermentation group; SP: *Staphylococcus carnosus* inoculated fermentation group; LF: *Limosilactobacillus fermentum* inoculated fermentation group. Different lowercase letters represent significant differences between groups in the same line. ($p < 0.05$).

Table 6
The content of flavor compounds in different cured fish samples.

μg/kg	Sample groups					
	C1	N	C2	SS	SP	LF
Alcohol(23)						
Tertiary butano	31.12 ± 4.10b	31.65 ± 8.14b	24.14 ± 9.25b	68.71 ± 22.92a	44.50 ± 5.00b	27.56 ± 2.61b
Ethyl alcohol	10.16 ± 2.30d	7.60 ± 0.53d	13.31 ± 1.07d	81.09 ± 14.94c	200.60 ± 6.50a	166.59 ± 29.32b
1-Pentene-3-ol	11.11 ± 4.42a	7.27 ± 1.67a	8.93 ± 0.06a	NE	NE	6.92 ± 0.14a
Isoamylol	10.67 ± 1.98d	9.09 ± 2.13d	79.62 ± 9.42d	745.91 ± 126.73b	1208.92 ± 79.87a	518.53 ± 141.81c
Pentanol	49.15 ± 11.40a	30.89 ± 2.25b	55.25 ± 8.43a	34.34 ± 2.51b	9.40 ± 0.13c	42.97 ± 6.95ab
Hexyl alcohol	614.37 ± 190.97a	248.92 ± 38.67 cd	427.58 ± 54.05abc	291.12 ± 74.68bcd	89.61 ± 5.71d	495.57 ± 143.04ab
1-Octen-3-ol	326.95 ± 97.95b	106.78 ± 8.96c	479.43 ± 54.34a	308.01 ± 71.44b	152.11 ± 30.36c	355.38 ± 128.26ab
N-heptanol	33.96 ± 10.78a	10.57 ± 0.76b	35.03 ± 8.86a	23.13 ± 4.25ab	10.51 ± 0.73b	38.29 ± 1.55a
Trans-2-octene-1-ol	31.79 ± 9.55bc	12.52 ± 1.21d	51.97 ± 8.20a	41.39 ± 11.71ab	20.95 ± 7.45 cd	36.03 ± 11.54abc
N-nonyl alcohol	12.24 ± 4.44c	NE	16.26 ± 4.55bc	22.60 ± 2.64ab	12.09 ± 0.78c	28.06 ± 3.60a
2,5-Dimethyl-2,5-hexanediol	3.57 ± 0.73a	NE	NE	4.70 ± 0.42a	3.60 ± 0.15a	NE
Benzyl alcohol	12.27 ± 3.12a	5.67 ± 1.03b	4.44 ± 1.17b	12.87 ± 1.24a	4.62 ± 0.70b	6.62 ± 1.12b
Phenylethanol	7.80 ± 1.33d	7.60 ± 0.87d	13.20 ± 1.55d	146.98 ± 6.83b	316.41 ± 29.56a	72.57 ± 6.45c
2-Ethylhexanol	2.19 ± 0.16a	NE	3.06 ± 0.50a	18.67 ± 14.46a	9.56 ± 0.85a	15.68 ± 9.35a
1-Nonen-4-ol	12.37 ± 2.18bc	6.68 ± 0.16c	9.82 ± 2.36c	20.48 ± 1.76ab	NE	25.56 ± 6.56a
Heptamethylene glycol	1.72 ± 1.54a	2.79 ± 0.94a	0.55 ± 0.64a	0.69 ± 0.03a	NE	NE
2,4-dimethylcyclohexanol	NE	NE	4.73 ± 2.47a	NE	NE	NE
N-octanol	NE	NE	41.52 ± 1.85a	32.42 ± 1.55b	NE	33.60 ± 1.56b
Butanol	NE	NE	NE	18.20 ± 1.52b	22.44 ± 0.86a	14.83 ± 1.81b
3-Methylthiopropanol	NE	NE	NE	22.91 ± 1.55b	36.32 ± 3.16a	13.16 ± 1.65c
Isopropanol	NE	NE	NE	6.59 ± 0.00a	NE	4.75 ± 0.21b
N-butanol	NE	NE	NE	NE	NE	5.22 ± 2.87a
Octanol	NE	NE	NE	NE	NE	3.92 ± 1.72a
Total alcohols	1162.31 ± 337.53b	482.28 ± 55.71c	1236.89 ± 171.56b	1830.68 ± 345.34a	2125.94 ± 143.24a	1856.07 ± 481.05a
Aldehyde(11)						
Acetaldehyde	4.73 ± 0.59a	NE	3.11 ± 0.79a	NE	NE	4.60 ± 0.15a
Isovaleraldehyde	2.39 ± 0.15d	1.86 ± 0.02d	4.09 ± 0.44d	14.61 ± 0.48b	25.95 ± 3.25a	9.12 ± 1.82c
Glutaraldehyde	35.61 ± 10.11a	14.38 ± 1.10b	NE	NE	NE	NE
N-hexanal	613.50 ± 119.30a	294.58 ± 31.17b	209.93 ± 15.78b	41.63 ± 7.23c	13.83 ± 1.34c	36.15 ± 1.70c
Heptanal	30.15 ± 7.60a	NE	14.64 ± 0.20b	NE	NE	NE
N-octanal	20.64 ± 6.88a	5.47 ± 0.19c	14.57 ± 2.47ab	8.63 ± 0.18bc	NE	4.70 ± 0.06c
Nonanal	104.93 ± 30.02a	30.45 ± 5.94c	69.15 ± 2.69b	32.87 ± 12.10c	13.04 ± 4.04c	27.13 ± 7.33c
Trans-2-octenal	13.20 ± 3.57ab	2.04 ± 0.06c	17.62 ± 2.78a	13.28 ± 0.40ab	NE	11.21 ± 0.41b
Trans-2,4-decadienal	6.82 ± 0.63a		7.60 ± 2.91a			
2-Methylbutanal	NE	NE	NE	NE	8.80 ± 0.49a	5.91 ± 1.01b
Phenylacetaldehyde	NE	NE	NE	NE	35.29 ± 6.93a	NE
Total aldehydes	827.32 ± 179.62a	347.48 ± 31.06b	333.44 ± 32.15b	84.97 ± 28.42c	92.30 ± 12.28c	74.92 ± 36.61c
Esters(5)						
Octyl formate	20.03 ± 6.69ab	3.96 ± 0.06c	29.04 ± 0.28a	28.57 ± 6.80a	15.14 ± 0.23bc	31.85 ± 6.48a
Heptyl formate	NE	NE	36.14 ± 0.65a	NE	8.56 ± 1.00b	NE
Ethyl acetate	NE	NE	NE	2.88 ± 0.32b	6.13 ± 1.30a	2.93 ± 0.42b
Isoamyl acetate	NE	NE	NE	10.90 ± 2.80a	15.82 ± 10.55a	3.05 ± 0.35a
Propyl nonyl lactone	NE	NE	NE	6.85 ± 0.49a	NE	4.42 ± 0.57b
Total esters	20.03 ± 6.69ab	3.96 ± 0.06b	43.46 ± 18.99a	32.79 ± 20.63ab	37.74 ± 6.59ab	29.14 ± 20.82ab
Ketone(13)						
Acetone	72.54 ± 14.86b	88.92 ± 26.53b	59.77 ± 25.69b	197.44 ± 82.84a	119.98 ± 24.26b	76.57 ± 15.68b
2,3-Butanedione	6.31 ± 0.26d	6.45 ± 1.13d	33.59 ± 10.29c	92.87 ± 1.86a	67.75 ± 23.33b	50.08 ± 2.34bc
2,3-Pentanedione	8.35 ± 3.36a	7.69 ± 0.03a	7.06 ± 0.50a	NE	NE	6.61 ± 0.15a
2-Heptanone	9.58 ± 2.94ab	4.11 ± 0.53b	12.80 ± 1.81ab	11.53 ± 0.47ab	6.09 ± 1.60b	19.32 ± 11.58
3-Hydroxy-2-butanone	17.49 ± 3.40d	19.85 ± 2.31d	82.53 ± 1.87 cd	392.07 ± 58.71a	324.64 ± 168.69ab	1179.05 ± 38.40bc
2,5-Octadione	163.69 ± 39.25a	71.54 ± 6.57b	154.29 ± 14.76a	63.13 ± 1.73bc	26.50 ± 1.31c	98.23 ± 12.93b
6-Methyl-2-heptanone	1.19 ± 0.16b	NE	2.22 ± 0.73a	NE	NE	NE
1-Octene-3-one	8.20 ± 0.27b	NE	11.02 ± 2.25a	NE	NE	NE
Dihydro-2-methyl-3 (2H) - thiophene ketone	NE	NE	4.73 ± 0.12c	252.95 ± 3.35a	NE	50.04 ± 15.39b
3-Octanone	NE	NE	26.58 ± 13.53b	NE	NE	59.70 ± 2.53a
Methylacetone	NE	NE	NE	9.22 ± 0.38ab	12.39 ± 2.17a	7.71 ± 1.31b
2,3-Heptanedione	NE	NE	NE	2.23 ± 0.27a	2.39 ± 0.35a	NE
2-Hydroxy-3-pentanone	NE	NE	NE	4.07 ± 0.39a	NE	NE
Total ketones	285.25 ± 43.69 cd	196.01 ± 31.89d	384.15 ± 31.25bcd	845.32 ± 167.65a	558.95 ± 169.50b	462.36 ± 192.19bc
Acids(6)						
Isobutyric acid	NE	NE	NE	195.41 ± 33.67a	220.99 ± 20.66a	114.71 ± 32.03b
Glacial acetic acid	NE	NE	NE	289.82 ± 57.73a	230.92 ± 60.55ab	129.29 ± 34.15b
Isovaleric acid	NE	NE	NE	456.78 ± 185.62a	NE	NE
S-methyl-N, N-diethyldithiocarbamate	NE	NE	NE	6.17 ± 1.16a	5.93 ± 0.55a	4.88 ± 0.40a
4-Methyl-3-pentenoic acid	NE	NE	NE	16.35 ± 0.55a	NE	12.97 ± 0.83b
2-Methylhexanoic acid	NE	NE	NE	NE	344.29 ± 87.67a	187.27 ± 3.80b

(*continued on next page*)

Table 6 (*continued*)

μg/kg	Sample groups					
	C1	N	C2	SS	SP	LF
Total acids	NE	NE	NE	959.08 ± 254.31a	802.14 ± 164.96ab	380.75 ± 171.73b
Alkanes(20)						
N-octane	6.17 ± 2.42c	NE	9.98 ± 1.67abc	11.34 ± 2.83ab	7.66 ± 1.70bc	13.19 ± 2.72a
2,4-Dimethylheptane	1.37 ± 0.21b	1.42 ± 0.27b	1.27 ± 0.36b	2.75 ± 0.22b	1.73 ± 0.07b	5.18 ± 1.89a
N-dodecane	13.40 ± 2.01ab	7.03 ± 2.06b	6.32 ± 0.79b	22.85 ± 13.44ab	29.14 ± 4.95ab	43.22 ± 28.48a
N-decadecane	1.78 ± 0.53b	NE	1.57 ± 0.14b	NE	NE	3.22 ± 0.06a
Tridecane	2.77 ± 0.69b	NE	2.48 ± 0.16b	5.71 ± 2.95b	5.69 ± 1.32b	18.36 ± 5.22a
4,7-DimethyluNEecane	9.27 ± 0.27ab	2.98 ± 1.99c	NE	12.31 ± 0.49a	NE	6.85 ± 0.60b
UNEecane	NE	2.87 ± 1.30b	3.83 ± 0.14ab	5.37 ± 0.71ab	3.17 ± 0.02b	6.45 ± 2.07a
2,6,11-Trimethyldodecane	NE	2.09 ± 0.23b	NE	70.04 ± 27.09a	4.85 ± 0.24b	51.27 ± 1.52a
Pentyl cyclopropane	NE	4.84 ± 0.35b	NE	NE	12.44 ± 0.36a	
Isobutylene oxide	NE	NE	1.76 ± 0.17b	6.66 ± 0.66b	3.56 ± 0.06b	41.02 ± 3.73a
Trichloromethane	NE	NE	2.95 ± 0.37a	NE	NE	NE
N-heptane	NE	NE	1.86 ± 0.29a	NE	NE	2.05 ± 0.13a
N-pentadecane	NE	NE	NE	5.34 ± 0.01b	4.88 ± 0.28b	6.33 ± 0.47a
4-Methyloctane	NE	NE	NE	44.15 ± 1.69a	NE	26.69 ± 1.88b
2,3,3,4-Tetramethylpentane	NE	NE	NE	53.08 ± 3.02a	1.61 ± 0.10b	NE
2,6-Dimethyl-Nonane	NE	NE	NE	42.83 ± 3.45a	NE	33.34 ± 2.44b
2,3,6,7-Tetramethyloctane	NE	NE	NE	17.01 ± 2.51a	NE	13.02 ± 0.94a
5-Methyldecane	NE	NE	NE	14.98 ± 0.81a	NE	9.47 ± 0.03b
4-MethyluNEecane	NE	NE	NE	2.79 ± 0.18a	1.89 ± 0.17b	28.14 ± 3.20a
Tetradecane	NE	NE	NE	9.41 ± 0.74a	6.05 ± 0.66b	8.27 ± 0.45a
Total alkanes	27.20 ± 8.24c	15.32 ± 2.45c	27.76 ± 5.46c	223.43 ± 151.93ab	69.28 ± 5.81bc	235.64 ± 123.69a
Other compounds(18)						
2,4-Dimethyl-1-heptene	4.08 ± 1.44b	4.77 ± 4.87b	4.39 ± 2.63b	4.23 ± 1.45b	1.69 ± 0.24b	22.03 ± 3.92a
2-Methyltetrahydrofuran	13.14 ± 1.46a	7.00 ± 3.93b	NE	NE	NE	NE
Ethylbenzene	2.12 ± 0.17a	2.43 ± 0.17a	2.57 ± 0.77a	3.89 ± 2.03a	NE	2.50 ± 0.28a
P-xylene	9.30 ± 1.25c	6.10 ± 0.12d	2.89 ± 1.52e	2599 ± 1.27a	24.55 ± 1.60a	13.48 ± 0.81b
2-n-Amylfuran	9.32 ± 2.93b	3.47 ± 0.68c	11.68 ± 1.11b	10.38 ± 0.23b	2.03 ± 0.30c	17.72 ± 1.66a
2-Ethylfuran	3.19 ± 0.05a	NE	3.87 ± 0.38a	NE	NE	2.39 ± 1.23a
Toluene	2.83 ± 00.37b	NE	NE	19.62 ± 0.67a	NE	5.61 ± 3.34b
Ortho xylene	3.80 ± 0.47a	4.87 ± 0.39a	NE	3.56 ± 0.62a	NE	NE
Naphthalene	NE	2.03 ± 0.41b	NE	2.56 ± 0.77b	1.62 ± 0.28b	46.96 ± 4.34a
Phenol	NE	NE	2.55 ± 0.25d	7.60 ± 0.74b	12.67 ± 0.72a	6.18 ± 0.08c
4-Methyl-5- (beta hydroxyethyl) thiazole	NE	NE	7.63 ± 2.04a	NE	12.06 ± 2.50a	11.87 ± 5.27a
2,3,5-Trimethylpyrazine	NE	NE	NE	12.99 ± 0.83a	7.91 ± 0.05b	6.43 ± 0.16c
N. N-Dibutylfamide	NE	NE	NE	3.69 ± 0.24a	NE	1.35 ± 0.32b
Benzothiazole	NE	NE	NE	2.05 ± 0.26a	1.65 ± 0.84a	1.61 ± 0.18a
Methyl *tert* butyl ether	NE	NE	NE	22.75 ± 4.57a	NE	18.08 ± 2.56a
2-Chloro-*N*-methylaniline	NE	NE	NE	5.60 ± 0.01a	NE	5.01 ± 0.08a
Orthochloroaniline	NE	NE	NE	5.98 ± 0.08a	NE	5.56 ± 0.10a
4-Tert butylphenol	NE	NE	NE	11.89 ± 0.24a	NE	4.83 ± 0.33b
Total other compounds	42.44 ± 8.87c	24.37 ± 7.52c	30.72 ± 2.98c	97.73 ± 31.33ab	61.00 ± 4.96bc	116.47 ± 52.06a

Note: Values are means ± SD (n = 3). C1:Curing control group; N: Nitrite curing group; C2: Fermentation control group; SS: *Staphylococcus saprophyticus* inoculated fermentation group; SP: *Staphylococcus carnosus* inoculated fermentation group; LF: *Limosilactobacillus fermentum* inoculated fermentation group. NE: Non-existence. Different lowercase letters represent significant differences between groups in the same line. ($p < 0.05$).

et al., 2004). Alcohols are mainly produced by the oxidative decomposition of lipids (Tian et al., 2021). The analysis of Table 6 revealed that isoamyl alcohol, n-hexyl alcohol, and 1-octene-3-alcohol emerged as the predominant alcohol compounds within each group. Among them, 1-octene-3-ol was identified as a fishy substance, and the content of 1-octene-3-ol in group N was significantly lower compared to that in group C1, which may be attributed to the fact that the presence of nitrite can impede the formation of 1-octene-3-ol through its inhibitory effect on lipid oxidation (Olivares et al., 2009). The levels of 1-octene-3-ol in the SS and SP groups were significantly lower compared to those in the C2 group, potentially attributed to enzymatic modification of its molecular structure by microorganisms, resulting in its conversion into an odorless constituent (Fukami et al., 2004). The isoamyl alcohol and hexanol were senior alcohols with distinctive aromatic odors. The content of isoamyl alcohol in the inoculated fermentation groups was significantly higher compared to that in groups C1 and C2, suggesting that inoculation fermentation can confer a distinctive flavor to cured fish. Furthermore, the total content of alcohol compounds in group N was the lowest (482.28 μg/kg), potentially due to the inhibitory effect of nitrite on oxidation. The formation of aldehydes primarily arises from the oxidation of unsaturated fatty acids and the degradation of amino acids (Qian et al., 2022). Caproaldehyde and nonylaldehyde were the main aldehydes in each group, which had an unpleasant odor such as the smell of fat (Tian et al., 2021). The levels of caproaldehyde and nonylaldehyde were higher in groups C1 and N, whereas they were lower in groups SS, SP, and LF. These findings suggest that fermentation can effectively mitigate the unpleasant odor of cured fish. A similar result was observed in fermented bream (Tian et al., 2021). Ketones are primarily generated through the oxidation of unsaturated fatty acids, microbial metabolic processes, or degradation of amino acids. The contents of ketones in SS, SP and LF groups were 2.20 times, 1.46 times and 1.20 times higher than those in the C2 group, respectively. The predominant ketones observed in each group were 3-hydroxy-2-butanedione and 2,5-octanedione. Specifically, 3-hydroxy-2-butanone exhibited sensory attributes of fruit, wood, and milk flavors, whereas 2,5-octanedione was associated with a fish flavor. The presence of 3-hydroxy-2-butanone was detected in each group, with significantly higher levels observed in the SS and SP groups, which were 4.75 times and 3.93 times higher than those in the fermentation control C2 group, respectively. These findings indicate that *Staphylococcus saprophyticus* and *Staphylococcus carnosus* have a substantial capacity for metabolizing 3-hydroxy-2-butanone during fermentation, thereby facilitating the development of distinctive flavors in cured fish. This phenomenon may be attributed to the heightened lipase and protease activity exhibited by *Staphylococcus*, thereby facilitating the generation of ketone flavor compounds in cured fish (Frederic et al., 2010). The concentration of 2,5-octanedione in group N was significantly lower than that in group C1, while the concentrations in groups SS, SP and LF were also significantly lower than that in group C2. These results suggest that inoculated fermentation can achieve a similar effect to nitrite in reducing fishy odor. In conclusion, the levels of alcohols, ketones, and acids in cured fish exhibited varying degrees of increase following inoculated fermentation. Moreover, inoculated fermentation effectively reduced the presence of unpleasant odor compounds such as straw and fat taste while simultaneously enhancing the content of pleasant compounds to improve the flavor quality of cured fish.

3.11. Electronic nose

The electronic nose is a technology capable of rapidly and non-invasively detecting the odors of volatile substances, which can effectively distinguish odor differences among various cured fish samples (Madhubhashini et al., 2024). As shown in the Fig. 1F, the total variance contribution rate of the first principal component (PC1) and the second principal component (PC2) is 99.99 %, indicating that these two components collectively capture the odor information of cured fish samples with high comprehensiveness. The results demonstrated that the odor of cured fish samples prepared by different treatment methods exhibited significant differences. The electronic nose principal component analysis diagram was divided into four quadrants, and the C1 and N groups were located in the fourth quadrant with a relatively close proximity, indicating that the odor of these two groups were similar. The C2 group was located in the first quadrant, distinctly separated from the SS, SP and LF groups, suggesting that inoculation and fermentation significantly influenced the odor characteristics of the cured fish samples.

4. Conclusion

This study explored the effects of the replacement of nitrite with chromogenic strains on the color enhancement and quality of cured fish. The results revealed that cured fish inoculated with *Staphylococcus saprophyticus* CICC 24365 and *Staphylococcus carnosus* ATCC 51365 exerted a red color similar to that adding 150 ppm nitrite and effectively reduced the nitrosamine levels and residual risk of nitrite. Conversely, *Limosilactobacillus fermentum* CICC 25124 did not exhibit a significant impact on color development in cured fish. In addition, the inoculation of *Staphylococcus saprophyticus* CICC 24365 and *Staphylococcus carnosus* ATCC 51365 enhanced the levels of free amino acids, elevated the content of isoamyl alcohol and 3-hydroxy-2-butanone as well as other aromatic compounds in the cured fish, while suppressing the generation of undesirable odors such as fishy and fatty odors. However, *Staphylococcus saprophyticus* CICC 24365 caused a significant accumulation of histamine with content of 139.01 mg/kg. Future research need be conducted to ensure the production of cured fish with superior quality that is both safe and healthy based on enhanced color rendering.

CRediT authorship contribution statement

Di Fang: Writing – original draft, Validation, Investigation, Formal analysis, Conceptualization. **Yanshun Xu:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, Conceptualization. **Xiaojing Zhang:** Writing – review & editing, Formal analysis. **Hong Sabrina Tian:** Writing – review & editing, Formal analysis.

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.

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Data availability

Data will be made available on request.

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