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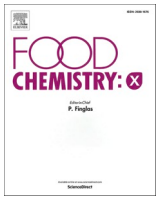

Regulating effects of low salt dry-curing pre-treatment on microbiota, biochemical changes and flavour precursors of grass carp (*Ctenopharyngodon idella*) fillets during storage at 4 °C

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ABSTRACT

Low salt dry-curing (LSD), as a healthier pre-treatment for the preservation of fishery products, is a potential technique substitute for excessively salty curing. The regulatory effects of 2 % and 3 % LSD on the quality evolution through an intrinsic correlation between microbiota succession and flavour precursors of refrigerated grass carp fillets were investigated in this study. The results showed that the LSD pre-treatment was effective in promoting proteolysis, free amino acid and fatty acid metabolism with the microbiota succession and quality evolution. Compared with unpre-treated samples, the 3 % LSD pre-treatment effectively extended the shelf life by 10 days within the acceptable quality attributes. Not only did the LSD pre-treatment lead to catalytic microbiota succession and inhibitive spoilage substance production but it also improved the flavour precursors, which are taste-active amino acids and polyunsaturated fatty acids (PUFAs). Moreover, considerable correlations between quality attributes, taste-active amino acids, PUFAs and microbiota were obtained.

1. Introduction

Grass carp (*Ctenopharyngodon idella*) is widely cultured because of its rapid growth rate and low cost of culture. In 2022, Chinese aquaculture production of grass carp was 5.9 million tonnes according to the China Fishery Statistical Yearbook of 2023 and ranked first in the world aquaculture fish species. Grass carp is widely popular with consumers from different countries because of its tenderness. Nowadays, pre-processed products from primary processing might become mainstream for fish consumption in the future with the popularity of 'fast food' culture. Thereinto, dry-curing pre-treatment fillets are a key process of cooking, prepared from fish flesh by grilling, air-frying or pan-frying. Salt dry-curing can suppress corruption and even provide a unique flavour to naturally fermented fish during storage, which is an important approach to maintaining freshness for the acceptability of consumers (Wang et al., 2020; Zhuang et al., 2021). The development of this flavour in fish is a dynamic and complex process that depends on the combined biochemical reactions of microbial succession and endogenous enzymes.

Salt-curing, as an ancient and effective method of food preservation, is designed to inhibit spoilage microbial metabolism and reduce water activity, which eventually maintains its freshness (Larsen & Elvevoll, 2008). Commonly, refrigeration decelerates the deterioration of aquatic products, but a single preservation technology is still relatively limited in prolonging the shelf life (Hao et al., 2021). Moreover, not only do traditional salt-curing aquatic products possessing excessive salt and low moisture conflict with healthy diet, but they also limit the development of the freshwater processing industry (Liu et al., 2013). Therefore, LSD pre-treatment is regarded as a safe and potential technique substitute for excessively salty curing.

The complex microbiota plays a vital role in the formation of flavour compounds during the refrigerated process of salt dry-curing pre-treatment fish fillets, and its metabolism affects the composition of flavour components and even determines consumer acceptance. The microbial metabolism, including protein hydrolysis and lipolysis, determines the type and content of flavour components in biochemical processes, thereby providing the sensory characteristics of the pre-fabricated fillets (Zhao et al., 2022; Zhuang et al., 2021). Previous studies have found that

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the composition of amino acids and oligopeptides during fish storage was impacted towards the endogenous proteolytic mechanism of muscle by autochthonous microbiota that hydrolysed the sarcoplasmic and myofibrillar proteins during fermentation (Zhuang et al., 2022). Besides, it was found that the degradation of amino acids is mainly driven by microbial metabolism, resulting in the corresponding volatile flavour of alcohols, acids and aldehydes, such as the degradation of leucine to 3-methylbutanal (Zhuang et al., 2022). Under the synergistic effect of microbial succession and enzymes, flavour compounds and essential nutrients are produced by amino acid metabolism, free fatty acid production and lipid oxidation (Chen et al., 2023; Wang et al., 2020). However, protein degradation and lipid oxidation caused by spoilage microorganisms are highly correlated with fish quality deterioration during refrigeration, and their quality indicators reflect whether they are acceptable. It is essential to clarify the relationship between the variations in flavour metabolites and quality indicators and functional microorganism succession of the LSD fillets.

LSD pre-treatment technology affects the functional microorganism succession of fish fillets. We studied the regulating effects of the technology on microbial succession, quality evolution and flavour compounds through high-throughput sequencing and chromatographic techniques. Although the metabolic development of the fillets is primarily impacted by microbial activities during refrigeration, identifying the core microbiota responsible for fermentation would help further analyse the flavour formation mechanism of the fillet microbiome. Despite the fact that the changes in flavour metabolism profile could elucidate the mechanism of flavour formation, the correlations between the core functional microbiota and flavour compounds in fish remain poorly characterised. This study aimed to investigate the effects of LSD pre-treatment regulation on the microbiota succession of grass carp fillets on flavour compounds metabolism and quality evolution and to promote technological advances in the aquatic processing industry. Clarifying the metabolic mechanism of flavour precursors will facilitate further targeted regulation of the quality of fish production.

2. Materials and methods

2.1. Materials

Grass carp were purchased from Nanchang Poyang Lake Agriculture and Fishery Industry Development Stock Co., Ltd. (Nanchang, China). Edible salt was purchased from a local store, Rainbow Department Store (Nanchang, China). An ammonia determination kit was obtained from Nanjing Jiancheng Bioengineering Institute. All organic reagents such as butanol, hexane and chloroform were chromatographic grade.

2.2. Sample preparation

According to our preliminary experiment, pre-treatment with 0 %–12.5 % (w/w) salt had an obvious influence on the salt-curing loss rate and total volatile basic nitrogen (TVB-N) of grass carp fillets, while the 2 % and 3 % salt-curing exhibited a reduction in the loss rate and efficient preservative effects (Figs. S2 and S3). Thus, the low salt ratio of 2 % and 3 % was selected to be used in this study.

Grass carp (weight of 1.5 ± 0.2 kg) were transported to the laboratory alive in a large container filled with lake water. After a temporary hold in domestic water for 2 h, grass carp were washed to remove sediment, scaled, beheaded, gutted after stunning and immediately washed to clean the surface and coelom. Then the fish were divided along the vertebrae into two pieces and sliced into similar-sized fillets (about 4 × 1.5 × 1.5 cm for each). All transportation and stunning methods used were consistent with the recommendation of the World Organisation for Animal Health. The fillets were randomly divided into three groups and subsequently cured with 0 % (control), 2 % and 3 % (w/w) salt on silicone oil papers for dry curing for 20 min. Finally, the fillets were placed into individually sealed polyethylene bags at 4 °C to prevent brine evaporation. The entire processing was carried out at an ambient temperature of less than 10 °C. Three samples of each group were selected randomly for analysis on days 0, 5, 10, 20 and 35 of refrigeration. Furthermore, lipid was extracted through chloroform/methanol/water (2:1:1, v/v/v) for lipid oxidation and fatty acid analysis.

2.3. Determination of quality attributes

The total viable count (TVC), pH and TVB-N of fillets were determined according to the China National Standards GB 4789.2-2016, GB 5009.237-2016 and GB 5009.228-2016 (Automatic Kjeldahl nitrogen method). While acid value (AV), peroxide value (POV) and fatty acid content of lipids were tested based on our study (Hu et al., 2023). The ammonia concentration (AC) was determined by a protein-free filtrate assay based on Zhuang et al. (2021). The AC was calculated in millimole of ammonia per 100 g of fish flesh (mmol/100 g).

2.4. Analysis of free amino acids

Free amino acids (FAAs) were determined according to the description of Larsen & Elvevoll. (2008). Briefly, 1 g of fish samples was homogenised with 10 mL of 15 % trichloroacetic acid (TCA) and centrifuged at 8,000 rpm. All the supernatant was combined and diluted to 25 mL after re-extractions and centrifugations. Subsequently, 5 mL of supernatant was adjusted to pH 2.0 and diluted to 10 mL. Finally, the supernatant was applied to an automatic amino acid analyser (S-433D, SYKAM, Germany) after filtration using a 0.22-μm membrane filter.

2.5. Analysis of biogenic amines

Biogenic amines (BAs) were monitored by pre-column derivatisation high-performance liquid chromatography (Zhuang et al., 2021). Briefly, 5 g of the samples adding an internal standard (1,7-diaminoheptane, Shanghai Macklin Biochemical Co., Ltd, Shanghai, China) was extracted with 5 % TCA and centrifuged after oscillating for 30 min. All the supernatants were combined after re-extractions. Then, the supernatant was adjusted to pH 12.0 after removing lipid with hexane, and butanol/chloroform (1:1, v/v) was added for further extraction. All the supernatant was combined after re-extractions and drying with nitrogen. Subsequently, 0.1-M HCl was added for reconstitution. After adding the derivative (Dansyl chloride, Sigma-Aldrich Trading Co., Ltd., Shanghai, China) and reaction at 60 °C for 30 min, ether was added for extraction, separated and dried again with nitrogen, and resuspended with acetonitrile. Finally, the target was determined by an Agilent HPLC (1260, Agilent, USA) equipped with a column (Agilent ZORBAXSB-C18). The BAs in fillets were identified and quantified according to a mixture solution including 5-component BAs (ANPEL Laboratory Technologies Inc., Shanghai, China).

2.6. DNA extraction and PCR amplification

The microbiota DNA of fish samples was extracted by using a FastDNA® spin kit (MP Biomedicals, Irvine, CA, USA) according to the manufacturer's protocol. The hypervariable region V3–V4 of the bacterial 16S rRNA gene was amplified with the primer pairs 338F 5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′ (Zhuang et al., 2020) using an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). PCR reactions were performed in triplicate. The PCR product was purified by an AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, CA, USA) and quantified by a Quantus™ fluorometer (Promega, USA) according to the manufacturer's instructions.

2.7. Illumina sequencing and data processing

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA) according to standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw sequencing reads were demultiplexed, quality filtered by fastp version 0.19.6, https://github.com/OpenGene/fastp) and merged by FLASH (version 1.2.11, https://ccb.jhu.edu/software/FLASH/index.shtml). Operational taxonomic units (OTU) were clustered with a 97 % similarity cutoff using UPARSE (version 11, http://www.drive5.com/uparse/), and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analysed by Ribosomal Database Project (RDP) Classifier (version 2.13, https://sourceforge.net/projects/rdp-classifier/) against the 16S rRNA database (Silva v138, https://www.arb-silva.de/) using a confidence threshold of 0.7. The alpha diversity estimators were calculated by MOTHUR (version 1.30.2, https://mothur.org/wiki/calculators/).

2.8. Statistical analysis

All the experiments were carried out in triplicate and results were expressed as means ± standard deviations. Except for the Illumina sequencing data, the presentation of other experimental results was processed using Prism 8.0.2 (GraphPad Software, Boston, MA, USA). Significance tests were performed by SPSS 26.0 software (SPSS Co., Ltd., Chicago, IL, USA) using one-way analysis of variance followed by the Duncan test. Pearson's correlation heatmap shows the linear correlation level between two variables constructed by the R language matrix (Lucent Technologies, Inc., New Jersey, USA). In detail, the linear correlation between changes in flavour precursors, biochemical indicators, and microbial abundance was visualised.

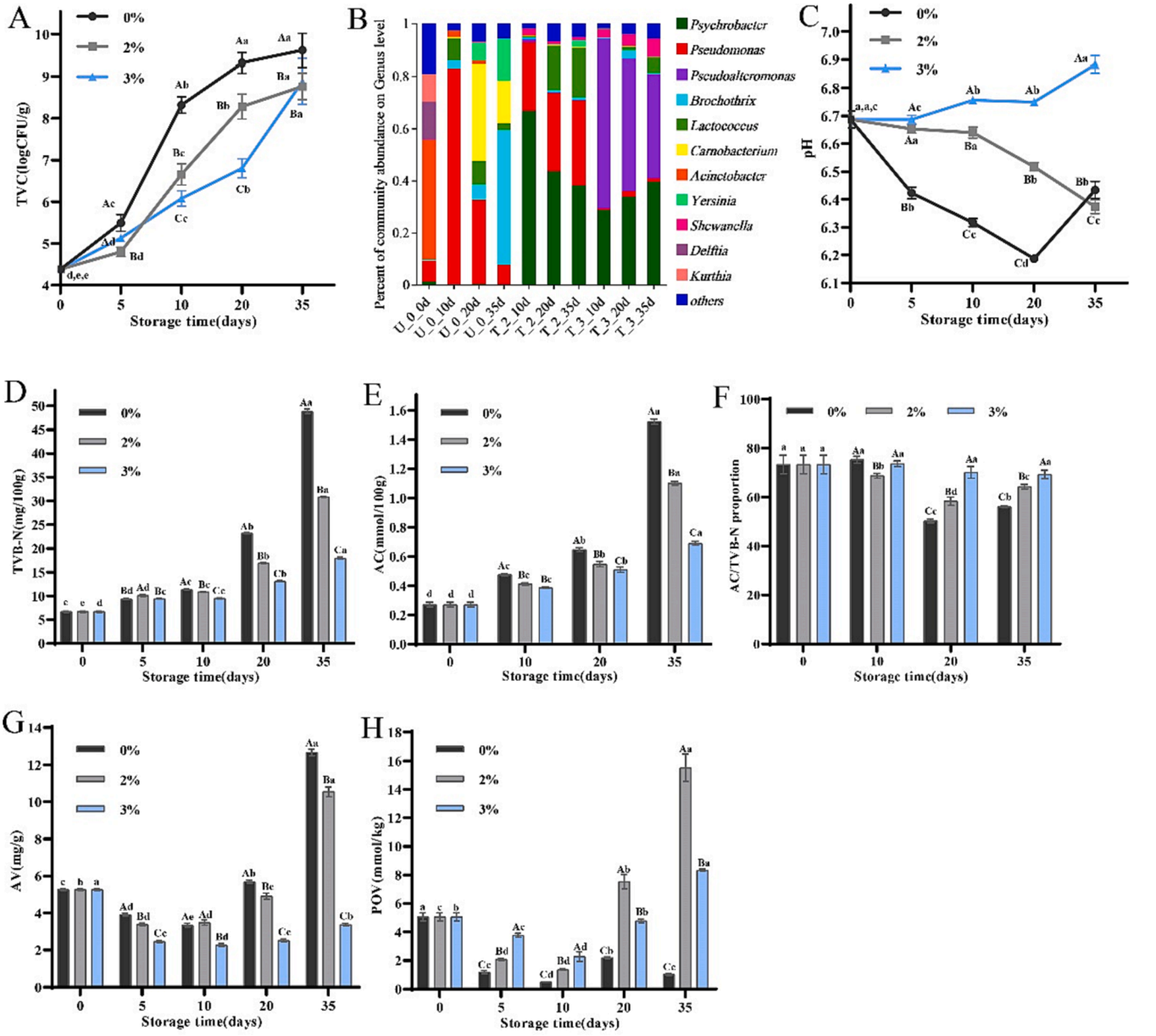

Fig. 1. Changes in TVC (A), composition and relative abundance of microbiota at the genus level (B), pH value (C), TVB-N content (D), AC (E), proportion values of ammonia to TVB-N (F), AV value of lipid (G) and POV value of lipid (H) of fresh and LSD pre-treated fillets during refrigeration. Lines and bars represent the standard deviation from three determinations (mean ± SD). Different capital letters in the same storage time indicate significant differences ($P < 0.05$) between different curing salinity, and different lowercase letters in the same curing salinity indicate significant differences ($P < 0.05$) between different storage time.

3. Results and discussion

3.1. Microbiome analysis

3.1.1. Microbial enumeration

The maximum edible limit of fish TVC is 7.0 log CFU/g (Zhuang et al., 2021). As shown in Fig. 1A, the initial TVC value of the control group was 4.38 log CFU/g, and the TVC values were 5.49–9.62 log CFU/g, 4.80–8.76 log CFU/g and 5.13–8.88 log CFU/g during 35 days for the control, 2 % and 3 % groups, respectively. The LSD pre-treatment inhibited microbial activity by reducing free water content in fillets, thereby reducing its degradation and damage to structural proteins (Yang, Yan & Xie, 2023). Clearly, the 2 % and 3 % groups showed extremely significant decreases in TVC from days 10 to 20. Previous studies showed that high-salt curing as a preservation method effectively inhibited microbiota growth (Olatunde & Benjakul, 2018; Yang et al., 2020). However, our results indicated that LSD pre-treatment could also be prospective in inhibiting bacterial metabolic activities of fillets.

3.1.2. Analysis of microbial succession

We conducted metagenomic sequencing to study the diversity and composition changes of microbiota during the refrigeration of fillets. To maximise the quality and reliability of the analysis, a table of OTU averages generated by a minimum sample reading was used for data mining. Based on the RDP naïve Bayesian classifier, the representative OTU sequences with 97 % similar levels were analysed, and the corresponding species classification information of each OTU was obtained (Table 1). The average values of the sequencing number, base number and mean length of all samples were similar to the detected values, which were 52,554, 22,529,207 and 428.70, respectively, indicating that the difference caused by the sequencing depth across samples was minimised. The coverage values of all samples exceeded 0.998, suggesting that the sequencing reads had thoroughly detected the type of bacterial phylotypes. A decrease in Sobs indices meant that the community richness of fillets was less than that at the fresh as storage time proceeded. A similar decreasing trend of richness was also uncovered in previous research (Liu et al., 2018; Zhuang et al., 2022). The evenness and diversity of the microbiota in the 2 % and 3 % groups were higher than those of the control group, according to Shannoneven and Shannon, respectively. This phenomenon may be a result of the edible salt promoting the growth of functional microbiota (Halophiles) related to the formation of fillet flavour.

Fig. 1B shows the microbiota composition and the relative abundance at the genus level in fish samples during refrigeration. *Acinetobacter* (46.09 %), *Delftia* (14.33 %), *Kurthia* (10.63 %) and *Pseudomonas* (7.86 %) were the top four bacterial genera in grass carp fish. Such *Acinetobacter* and *Pseudomonas* are commonly present in fresh fish (Zhao, et al., 2022). However, the abundance of *Pseudomonas*, *Carnobacterium* and *Brochothrix* relative to the total microbiota in the control group increased dramatically to 82.82 % (day 10), 36.79 % (day 20) and 51.64 % (day 35), respectively. Microbiota composition changed dramatically, where only a section of the bacteria, namely spoilage bacteria, participated in the decay process (Huang et al., 2017). Significantly, the control group exceeded the maximum edible limit (TVC: 8.32 log CFU/g) until day 10. The growth and abundance of spoilage bacteria (e.g. *Pseudomonas, Carnobacterium* and *Brochothrix*) preferred to increase and result in spoilage of fillets (Dallagnol et al., 2021; Fall et al., 2010). On day 10, the 2 % group approached decay (TVC: 6.66 log CFU/g), and the abundance of *Psychrobacter* and *Pseudomonas* reached 66.69 % and 26.05 %, respectively. The significant decrease in *Pseudomonas* abundance is extremely related to the salt-curing loss rate of pre-treated fillets (Yang, Yan & Xie, 2023), which started decaying on day 20, and *Lactococcus* also became one of the main microbiota. Except for *Psychrobacter*, the abundance of microbiota increased significantly during refrigeration, which was consistent with the results of the TVC and previous study (Zhuang et al., 2022). *Pseudomonas* had high spoilage activities for inducing fish spoilage as compared with other spoilage bacteria (Gram et al., 2002), whose abundance changes in the control and 2 % groups indicated a slower spoilage process after LSD pre-treatment.

As for the 3 % group, when the fillet approached decay on day 20 (TVC: 6.8 log CFU/g), there was a significant difference in the microbiota composition from the control and 2 % groups. Meanwhile, the abundance of *Pseudomonas*, *Pseudoalteromonas* and *Lactococcus* accounted for 2.09 %, 50.56 % and 1.42 %, respectively, while *Psychrobacter* (33.84 %) significantly increased. This result may be due to the 3 % LSD pre-treatment has effectively inhibited the growth of *Pseudomonas* and *Lactococcus* during refrigeration, resulting in differential microbiota among different groups. Besides, LSD pre-treatment could remove the part of free water in the cell space of fillets and inhibit microbial activity to regulate bacterial composition and reduce the abundance of spoilage bacteria, which may further inhibit the migration of immobilised water trapped in structural protein network to free water and effectively delay the degradation of myofibrillar protein (Zhao et al., 2021). It is worth noting that the 3 % group also presented an increase in *Shewanella* and *Lactococcus* from days 20 to 35. This phenomenon further confirmed that TVC increased significantly at latter periods and indicated the gradual decrease of antibacterial efficiency in the later state of 3 % LSD salinity. Compared with the control group, the 2 % group showed a higher inhibitory effect on *Pseudomonas*, reducing the abundance of *Pseudomonas* from 40.29 % to 37.66 % on day 10, whereas the 3 % group had a more significant effect on it. These results indicated that the 3 % LSD pre-treatment could maintain grass carp fillets edible for the first 20 days of the chill-stored period.

Table 1
Alpha diversity estimation of the microbiota of fresh and LSD pre-treated fillets during refrigeration.

Samples	Sequencing no.	Base no.	Mean length	Sobs	Shannoneven	Shannon	Coverage
U_0_0d	56,777	24,261,818	427.32	299.67	0.39	2.17	0.9980
U_0_10d	49,862	21,390,173	428.98	36.00	0.21	0.76	0.9997
U_0_20d	53,128	22,790,056	428.97	63.67	0.43	1.75	0.9993
U_0_35d	55,580	23,842,854	428.99	41.33	0.40	1.48	0.9997
T_2_10d	46,461	19,926,760	428.89	56.33	0.46	1.86	0.9995
T_2_20d	54,618	23,413,075	428.67	71.00	0.49	2.08	0.9994
T_2_35d	51,287	21,985,518	428.68	62.00	0.47	1.96	0.9997
T_3_10d	46,834	20,087,428	428.91	118.33	0.34	1.62	0.9986
T_3_20d	55,016	23,593,664	428.85	67.00	0.52	2.19	0.9997
T_3_35d	55,981	24,000,727	428.73	75.00	0.56	2.40	0.9998
Average	52,554	22,529,207	428.70	89.03	0.43	1.83	0.9993

U_0_0d: fresh fillets; U_0_10d: uncured fillets on day 10; U_0_20d: uncured fillets on day 20; U_0_35d: uncured fillets on day 35; T_2_10d: 2% salt dry-cured fillets on day 10; T_2_20d: 2% salt dry-cured fillets on day 20; T_2_35d: 2% salt dry-cured fillets on day 35; T_3_10d: 3% salt dry-cured fillets on day 10; T_3_20d: 3% salt dry-cured fillets on day 20; T_3_35d: 3% salt dry-cured fillets on day 35.

3.2. pH, TVB-N and AC analysis of different salinities pre-treatment fillets

Changes in quality attributes, namely pH, TVB-N and AC of grass carp fillets after LSD pre-treatment during refrigeration were analysed. Fillets undergo several biochemical reactions during storage, resulting in their quality deterioration (Zhuang et al., 2023). The initial pH of grass carp fish was similar to that reported by Yu et al. (2018) (Fig. 1C). After 20 days of refrigeration, the pH of the control group decreased significantly ($P < 0.05$), and the subsequent increase might be caused by the accumulation of basic amino acids and their metabolites, such as Lys, ammonia and BAs. The pH in the 2 % group remained stable for the first 10 days due to the production of the basic amino acids that neutralised lactic acid, and the subsequent decrease was attributed to the activation of phosphokinase and the production of numerous phosphates (Zhuang et al., 2021). However, the increased pH was explained by FAAs metabolism in the 3 % group from the fifth day of refrigeration. Chen et al. (2020) found that the pH was related to the inhibition of *Pseudomonas* growth in 6 % salting combined with vacuum-packing the Russian sturgeon. Thus, the 2 % and 3 % groups effectively reduced the pH change rate compared with the control group, which verified that LSD pre-treatment delayed the deterioration of fillets.

Furthermore, the changes in TVB-N reflected the total volatile basic compounds produced by spoilage microbiota and its metabolic enzymes involved in protein hydrolysis and FAAs metabolism (Ge, Xu & Xia, 2015; Larsen & Elvevoll, 2008). The change range of TVB-N was 6.67–48.86 mg/100 g (Fig. 1D), and there was a significant increase ($P < 0.05$) of the TVB-N in all samples (except day 10 of the 3 % group) with refrigeration time. However, significant inhibition was shown in the 2 % and 3 % groups compared with the control group on corresponding days and decreased by 27.0 % and 43.5 % on day 20, respectively. According to changes in TVC, 20 mg/100 g was the maximum acceptable limit for TVB-N in fresh fish (Zhu et al., 2024), and the shelf life of 0 %, 2 % and 3 % salt dry-curing fillets at chilled storage would be at most 5, 10 and 20 days, respectively. Further, LSD pre-treatment reduced TVB-N significantly by inhibiting the metabolism of spoilage bacteria on flavour precursors such as FAAs, which may explain why the trend of the AC was consistent with the TVB-N as well (Zhuang et al., 2021). Similarly, several studies have confirmed that spoilage bacteria were a key generator of TVB-N in chill-stored fillets (Jia et al., 2019; Zhuang et al., 2020). Thus, a lower TVB-N content could be caused by the bacteriostatic effects of salt-curing on spoilage bacteria.

Generally, ammonia and amines in TVB-N are putrefactive substances of aquatic products, mainly converting FAAs into free ammonia and ammonia ions by deamination of spoilage microorganisms (Zhuang et al., 2020). As shown in Fig. 1E, the initial AC of the control group was 0.27 mmol/100 g and then increased significantly with the refrigeration period, which was 0.96 mmol/100 g for the final concentration. The AC of the 2 % and 3 % groups were significantly reduced ($P < 0.05$). Obviously, there is no significant difference in the AC/TVB-N proportion of the 3 % group (Fig. 1F). The AC/TVB-N of control and 2 % groups first significantly decreased and then slightly rebounded, and the decrease was attributed to the quicker increase of TVB-N than of AC, indicating that the composition of TVB-N compounds, such as BAs, might become more diverse. Furthermore, the changes of AC/TVB-N proportions in the control and 2 % groups were subjected to changes in the abundance of spoilage bacteria. In the latter stage of refrigeration, the *Carnobacterium* and *Bromothrix* of the control group had a higher abundance, and the *Pseudomonas* abundance of the 2 % group was still above than that of the control group. Thus, *Carnobacterium* and *Bromothrix* may have a higher activity for FAAs deamination than *Pseudomonas* at the later stage of spoilage.

3.3. FAAs and BAs analysis of different salinities pre-treatment fillets

Fermentation promoted protein oxidation, but the effect was lowered when functional microbiota such as *Lactobacillus fermentum* was replaced as a key microbial group, causing the inhibition of protein oxidation and changing the meat composition in terms of flavour (Gao et al., 2022; Wen et al., 2021). Besides, the degree of protein oxidation of chill-stored fish was lower than that of frozen during a longer storage period, and the bacterial growth and protease that caused its degradation were more active (Shen et al., 2014). This study utilised LSD pre-treatment technology to regulate the bacteriota composition in fillets, thereby improving protein degradation and fish flavour. The protein degradation might be caused by bacterial activity and endogenous enzymes exerting proteolytic effects on the myofibril, leading to myofibrillar protein fragmentation, fibre rupture and detachment (Feng, Bansal & Yang, 2016). FAAs are a main contributor to flavour and are degraded into putrefactive substances by complex biochemical reactions involving spoilage bacteria, such as proteolysis, deamination and decarboxylation (Zhao, Hu & Chen, 2022). The relationship between the volatilome profile and metabolite based on the dynamic evolution of FAAs was explored during storage. As shown in Table 2, there were no umami FAAs in the control group, but the umami FAAs of all samples significantly increased with refrigeration time. Further, the sweet and bitter FAAs in the control group increased significantly at first and then decreased while their content was higher than the 2 % and 3 % groups during the first 20 days, which could be caused by the regulation of microbial succession and proteolysis in fillets by LSD pre-treatment. The sweet/bitter FAAs of the control group were dramatically reduced and lower than those in the 2 % and 3 % groups on day 35, which was possibly due to spoilage bacteria degrading the FAAs by deamination and decarboxylation, while their metabolic activities might have been inhibited by NaCl. Moreover, the contents of Pro, Gly, Ala, Val, Met, Ile, Leu, Phe, His and Cys in the control group were higher than those in the 2 % and 3 % groups during the first 20 days, indicating that proteolysis in the control group was more active. Several studies have reported that *Pseudomonas* and *Carnobacterium* have a strong potential for protein hydrolysis (Dallagnol et al., 2021; Zhuang et al., 2023). Furthermore, the FAAs' preferable increase observed in this study may be related to *Pseudomonas* and *Carnobacterium*. Besides, *Pseudomonas* also has a strong FAAs metabolism potential (Zhuang et al., 2022). The contents of Thr, Lsy, Gly, Ala, Try and His in the control group decreased significantly ($P < 0.05$) and were lower than those in the 2 % and 3 % groups on day 35, which could be ascribed to the abnormal activity of deamination and decarboxylation through spoilage bacteria in the latter period of refrigeration. However, no Ser was detected in the control group except for fresh fillets, and Arg was only found in the 3 % group during refrigeration, confirming further that changes in FAAs were influenced by edible salt regulating microbiota succession and its metabolic activity. Contents of Asp and Glu significantly increased with the refrigeration period (except for Asp of the 0 % group), which could be mainly attributed to spoilage microbiota preferring to utilise FAAs with a sweet- or bitter-tasting rather than umami.

BAs, as putrefactive substances, are often used as indicators of fish spoilage because they are closely related to the decarboxylation of spoilage bacteria caused by FAAs' degradation. As shown in Table 2, no BAs were detected in fresh fillets. Contents of phenethylamine (PHE), putrescine (PUT), cadaverine (CAD) and tyramine (TYR) in the control group significantly increased during refrigeration ($P < 0.05$), with their contents on day 20 at 9.41 mg/kg, 52.35 mg/kg, 61.85 mg/kg and 17.92 mg/kg, respectively, indicating abnormal activity of FAAs decarboxylation of spoilage bacteria. At present, the contents of the main FAAs were the highest in the control group because of the higher abundance of *Carnobacterium* causing extensive proteolysis (Dallagnol et al., 2021). BAs of the 2 % and 3 % groups were significantly inhibited compared with the control group, indicating that LSD pre-treatment helped to maintain the taste of FAAs in fillets. Furthermore, CAD is primarily formed by Lys decarboxylase acting on L-lysine (Xue et al., 2020), and PUT originated from the degradation of Arg and Glu in the corresponding arginine deiminase pathway and arginine decarboxylase pathway of spoilage bacteria such as *Pseudomonas* (Zhuang et al., 2022).

Table 2
Changes in FAAs and BAs content of fresh and LSD pre-treated fillets during refrigeration.

Free amino acids (mg/10 g)	Raw	0 %			2 %			3 %		
		Day 10	Day 20	Day 35	Day 10	Day 20	Day 35	Day 10	Day 20	Day 35
Asp	ND	0.03 ± 0.00Bc	0.10 ± 0.01Ca	0.06 ± 0.00Cb	0.42 ± 0.02Ac	0.65 ± 0.02Ab	2.83 ± 0.08Aa	0.37 ± 0.02Ab	0.49 ± 0.02Ba	0.19 ± 0.01Bc
Glu	ND	0.55 ± 0.01Cc	3.11 ± 0.10Ab	4.56 ± 0.12Ba	1.62 ± 0.03Ac	2.90 ± 0.07Ab	9.07 ± 0.23Aa	1.10 ± 0.02Bc	1.68 ± 0.05Ba	1.51 ± 0.06Cb
Umami FAAs	ND	0.58 ± 0.01Cc	3.21 ± 0.10Bb	4.62 ± 0.12Ba	2.05 ± 0.05Ac	3.55 ± 0.09Ab	11.90 ± 0.31Aa	1.47 ± 0.02Bc	2.17 ± 0.06Ca	1.70 ± 0.07Cb
Thr	1.03 ± 0.02c,c,c	1.54 ± 0.03Aa	1.22 ± 0.04Bb	0.14 ± 0.00Cd	1.02 ± 0.02Cc	1.31 ± 0.04Bb	2.95 ± 0.08Aa	1.29 ± 0.02Bb	1.41 ± 0.05Aa	0.82 ± 0.02Bd
Lys	3.65 ± 0.09a,a,a	2.33 ± 0.04Ac	3.21 ± 0.11Bb	0.56 ± 0.01Cd	1.80 ± 0.04Bc	2.11 ± 0.20Cb	3.61 ± 0.10Aa	2.00 ± 0.04Bc	3.53 ± 0.10Aa	2.74 ± 0.07Bb
Ser	0.64 ± 0.02a,c,a	ND	ND	ND	1.22 ± 0.02Ab	1.27 ± 0.03Ab	2.04 ± 0.05a	0.56 ± 0.01Bb	0.49 ± 0.02Bc	ND
Pro	1.00 ± 0.08d,c,d	5.44 ± 0.12Ab	7.82 ± 0.20Aa	3.72 ± 0.06Bc	3.94 ± 0.08Bb	3.76 ± 0.06Bb	5.14 ± 0.12Aa	3.45 ± 0.12Cb	3.96 ± 0.21Ba	2.34 ± 0.20Cc
Gly	3.02 ± 0.09d,d,d	15.55 ± 0.31Aa	14.38 ± 0.45Ab	5.10 ± 0.13Cc	8.80 ± 0.20Bb	7.12 ± 0.26Cc	9.50 ± 0.24Ba	5.88 ± 0.11Cc	9.71 ± 0.34Bb	11.67 ± 0.27Aa
Ala	2.07 ± 0.03d,d,c	5.03 ± 0.10Ab	7.44 ± 0.23Aa	4.04 ± 0.10Bc	3.04 ± 0.07Bc	3.77 ± 0.15Bb	7.64 ± 0.19Aa	1.47 ± 0.04Cd	3.01 ± 0.09Cb	3.86 ± 0.12Ba
Tyr	0.43 ± 0.01a,c,b	0.19 ± 0.00Bb	0.06 ± 0.00Cc	ND	0.28 ± 0.01Ad	0.82 ± 0.04Ab	2.85 ± 0.07Aa	0.26 ± 0.00Ac	0.46 ± 0.02Bb	0.62 ± 0.04Ba
Sweet FAAs	11.84 ± 0.36d,c,c	30.08 ± 0.60Ab	34.15 ± 1.00Aa	13.55 ± 0.30Cc	20.12 ± 0.45Bb	20.16 ± 0.79Cb	33.72 ± 0.86Aa	14.92 ± 0.28Cb	22.58 ± 0.75Ba	22.06 ± 0.59Ba
Val	0.56 ± 0.01c,c,b	0.55 ± 0.01Ac	1.28 ± 0.04Ab	1.52 ± 0.04Ba	0.47 ± 0.01ABc	0.94 ± 0.04Bb	3.01 ± 0.08Aa	0.40 ± 0.01Bc	0.67 ± 0.02Ca	0.67 ± 0.01Ca
Met	0.12 ± 0.00c,c,c	0.22 ± 0.00Ab	0.67 ± 0.02Aa	0.66 ± 0.02Ba	0.14 ± 0.01Bc	0.38 ± 0.02Bb	1.79 ± 0.10Aa	0.12 ± 0.00Bc	0.22 ± 0.01Cb	0.35 ± 0.01Ca
Ile	0.48 ± 0.01c,c,a	0.46 ± 0.01Ac	1.11 ± 0.03Ab	1.26 ± 0.03Ba	0.39 ± 0.01Ac	0.78 ± 0.03Bb	2.61 ± 0.06Aa	0.31 ± 0.01Bc	0.50 ± 0.02Ca	0.40 ± 0.01Cb
Leu	0.75 ± 0.02c,c,a	0.96 ± 0.02Ab	1.96 ± 0.06Ac	1.98 ± 0.05Bc	0.71 ± 0.01Bc	1.46 ± 0.07Bb	5.02 ± 0.13Aa	0.51 ± 0.01Cc	0.79 ± 0.02Ca	0.62 ± 0.01Cb
Phe	0.32 ± 0.00c,c,b	0.29 ± 0.00Ac	1.05 ± 0.03Ab	1.24 ± 0.03Ba	0.21 ± 0.00Bc	0.85 ± 0.04Bb	3.36 ± 0.10Aa	0.22 ± 0.01Bc	0.34 ± 0.01Cb	0.44 ± 0.02Ca
His	9.43 ± 0.24c,c,c	22.3 ± 0.48Aa	22.55 ± 0.71Aa	11.8 ± 0.28Cb	18.93 ± 0.36Ba	18.09 ± 0.18Bb	19.37 ± 0.50Ba	17.71 ± 0.26Cb	21.53 ± 0.62Aa	21.96 ± 0.51Aa
Arg	1.63 ± 0.07a	ND	ND	ND	ND	ND	ND	1.23 ± 0.02b	1.59 ± 0.04a	ND
Cys	ND	0.17 ± 0.00b	0.27 ± 0.03a	0.12 ± 0.00c	0.12 ± 0.00b	0.13 ± 0.03b	0.36 ± 0.02a	ND	ND	ND
Bitter FAAs	13.30 ± 0.38d,d,c	25.03 ± 0.53Ab	28.88 ± 0.90Aa	18.63 ± 0.45Cc	20.96 ± 0.40Bc	22.65 ± 0.40Cb	35.54 ± 0.89Aa	20.50 ± 0.30Bb	25.64 ± 0.73Ba	24.46 ± 0.57Ba
Biogenic amines (mg/kg)										
Phe	ND	0.61 ± 0.04c	9.41 ± 0.21b	60.25 ± 0.63a	ND	ND	ND	ND	ND	ND
Put	ND	2.02 ± 0.26c	52.35 ± 0.51Ab	179.36 ± 4.92Aa	ND	2.14 ± 0.16Cb	52.88 ± 2.20Ba	ND	5.56 ± 0.49Bb	31.59 ± 1.89Ca
Cad	ND	ND	0.54 ± 0.11c	61.85 ± 0.93Ab	ND	23.86 ± 0.84Bb	222.07 ± 5.34Ba	ND	ND	7.75 ± 0.41C
His	ND	ND	ND	80.14 ± 2.52	ND	ND	ND	ND	ND	ND
Tyr	ND	2.02 ± 0.16c	17.92 ± 0.56b	91.38 ± 2.69Aa	ND	ND	1.70 ± 0.19B	ND	ND	ND

Note: Data are expressed as mean ± SE. ND: not detected. Same capital letters within the same storage time in the same row indicate no significant differences ($P > 0.05$) between different curing salinity, and same lowercase letters within the same salting salinity in the same row indicate no significant differences ($P > 0.05$) between different storage times.

Moreover, HIS, as the most toxic BAs in aquatic products, only appeared in the later refrigeration period. His was metabolised under the catalysis of its decarboxylase contributed by *Pseudomonas* (Zhuang et al., 2022), indicating that His metabolism of spoilage microbiota was affected by NaCl and was selective. Buňková et al. (2009) found that TYR was derived from the catabolism of tyrosine decarboxylase contributed by *Lactococcus*, and supersession of Phe was related to a low abundance of *Lactobacillus* (Landete, Pardo & Ferrer, 2007). The deamination and decarboxylation reactions of the LSD pre-treated fillets during refrigeration were associated with *Carnobacterium, Pseudomonas* and *Lactococcus*. Thus, it is necessary to inoculate the major bacterial genera mentioned above into fish flesh individually and comprehensively explore the metabolite profiles related to the dynamic changes of flavour precursors by simulating the inactivation kinetics.

3.4. Lipid oxidation

The change in lipid oxidation was shown by AV and POV, among which was the dynamic accumulation of free fatty acids (FAs) and hydroperoxides. The combination of AV and POV can carefully reflect the degree of lipid oxidation in refrigerated fillets. As summarised in Fig. 1G and 1H, the initial AV and POV of fillets were 5.26 mg/g and 5.06 mmol/kg, respectively. Both AV and POV (except for the control group on day 35) decreased significantly and then increased with time. On day 35 of refrigeration, compared with the control group, the AV of the 2 % and 3 % groups decreased by 2.13 mg/g and 9.30 mg/g, respectively, while the POV increased by 14.49 mmol/kg and 7.31 mmol/kg, respectively, indicating that LSD pre-treatment could inhibit decomposition of lipid into rancidity products but accelerate its oxidation. Because fillets had

an uneven distribution of salt ions and adequate endogenous lipoxygenase during refrigeration, enzymatic oxidation and metal ions promoted free radical chain reaction. As salt ions diffused evenly, the integrity of the fillet cell membrane was destroyed by NaCl, which promoted the entry of oxidants into liposomes and accelerated its oxidation process, resulting in decreased AV and increased POV (Zeng et al., 2023). According to the acceptability limit of POV (7.5 mmol/kg) and AV (2.50 mg/g) value for fish oil (Hu et al., 2022), the shelf life of fillets was less than 10 days in the control and 2 % groups and 20 days in the 3 % group.

3.5. FAs evolution of different salinities pre-treatment fillets stored at 4 °C

Table 3 shows the FAs' content evolution of grass carp fillets at LSD for refrigeration. There were significant differences in the evolution of FAs between the control and LSD groups ($P < 0.05$). The content of saturated FAs (SFAs), monounsaturated FAs (MUFAs) and PUFAs (except for the 3 % group) decreased and rebounded regularly with time,

Table 3
Changes in FAs content of fresh and LSD pre-treated fillets during refrigeration.

Fatty acids (mg/g)	Raw	0 %			2 %			3 %		
		Day 10	Day 20	Day 35	Day 10	Day 20	Day 35	Day 10	Day 20	Day 35
C12:0	0.53 ± 0.03a,b,b	0.47 ± 0.05Aab	0.41 ± 0.03Cb	0.18 ± 0.02Ac	0.45 ± 0.01Ac	0.84 ± 0.02Ba	0.10 ± 0.01Bd	0.45 ± 0.01Ac	2.18 ± 0.05Aa	0.11 ± 0.00Bd
C14:0	6.45 ± 0.10a,a,a	5.53 ± 0.11Ab	5.75 ± 0.21Ab	3.95 ± 0.06Ac	4.87 ± 0.10Cb	5.03 ± 0.08Bb	2.91 ± 0.05Bc	5.17 ± 0.12Bc	6.00 ± 0.14Ab	2.75 ± 0.08Cd
C15:0	0.93 ± 0.02a,a,a	0.80 ± 0.05Ab	0.81 ± 0.04ABb	0.56 ± 0.02ABc	0.79 ± 0.00Ab	0.76 ± 0.03Bb	0.54 ± 0.01Bc	0.82 ± 0.02Ab	0.87 ± 0.04Aab	0.57 ± 0.01Ac
C16:0	100.33 ± 0.27a,a,a	81.79 ± 0.82Bc	94.66 ± 0.19Ab	59.66 ± 0.80Ad	85.26 ± 0.86Ac	91.49 ± 0.26Cb	55.66 ± 0.48Bd	86.84 ± 0.55Ac	93.52 ± 0.31Bb	55.03 ± 0.30Bd
C17:0	0.92 ± 0.05c,b,b	1.07 ± 0.04Bb	1.22 ± 0.10ABa	0.87 ± 0.02Ac	1.00 ± 0.03Bb	1.17 ± 0.06Ba	0.79 ± 0.01Bc	1.29 ± 0.02Aa	1.35 ± 0.02Aa	0.78 ± 0.00Bc
C18:0	22.23 ± 0.16b,a,a	20.31 ± 0.83Ac	23.71 ± 0.25Aa	12.98 ± 0.19Ad	16.27 ± 0.20Bc	19.13 ± 0.71Bb	9.83 ± 0.07Bd	13.40 ± 0.19Cc	18.66 ± 0.37Bb	8.63 ± 0.06Cd
C21:0	1.62 ± 0.03a,a	0.95 ± 0.03Ab	0.69 ± 0.03c	ND	0.57 ± 0.01Bb	ND	ND	ND	ND	ND
C22:0	6.95 ± 0.04a,a,c	6.01 ± 0.15Cb	6.67 ± 0.33Ba	4.20 ± 0.10Bc	6.43 ± 0.19Bb	6.62 ± 0.21Bab	4.09 ± 0.09Bc	7.42 ± 0.13Ab	8.13 ± 0.13Aa	4.80 ± 0.08Ad
C23:0	2.39 ± 0.03a,a,a	1.47 ± 0.08Bb	0.97 ± 0.06Bc	0.89 ± 0.02Bc	1.91 ± 0.04Ab	1.14 ± 0.06Bc	0.93 ± 0.03Bd	1.88 ± 0.06Ab	1.58 ± 0.14Ac	1.15 ± 0.02Ad
C24:0	1.20 ± 0.04c,ab,c	1.17 ± 0.05Bab	1.09 ± 0.06Cb	0.65 ± 0.02Bc	1.13 ± 0.02Bb	1.25 ± 0.04Ba	0.69 ± 0.01Bc	1.36 ± 0.02Ab	1.68 ± 0.07Aa	1.02 ± 0.02Ad
SFAs	143.59 ± 0.72a,a,a	119.60 ± 2.12Ac	136.01 ± 0.91Ab	83.97 ± 1.18Ad	118.72 ± 0.52Ac	127.47 ± 1.41Bb	75.59 ± 0.72Ba	118.65 ± 0.80Ac	134.01 ± 0.98Ab	74.87 ± 0.49Bd
C16:1	28.62 ± 0.64a,a,a	24.59 ± 0.77Ac	27.09 ± 0.65Ab	18.80 ± 0.44Ad	24.31 ± 0.33Ab	23.10 ± 0.59Bb	14.87 ± 0.41Bc	25.36 ± 0.13Ab	23.79 ± 0.52Bc	14.20 ± 0.09Bd
C18:1	241.31 ± 0.42a,a,a	193.16 ± 2.68Cc	221.68 ± 2.04Bb	147.38 ± 0.16Ad	200.45 ± 3.79Bc	209.67 ± 1.33Cb	132.59 ± 0.69Bd	238.43 ± 2.09Ab	238.62 ± 1.62Ab	145.98 ± 0.94Ab
C20:1	1.28 ± 0.03a,a,b	0.89 ± 0.00Bb	1.23 ± 0.11Ba	0.72 ± 0.01Cc	0.92 ± 0.03Bc	0.98 ± 0.02Cb	0.83 ± 0.02Bd	1.35 ± 0.00Ab	1.50 ± 0.05Aa	0.90 ± 0.02Ac
C24:1	1.58 ± 0.04a,a,c	1.46 ± 0.03Cb	1.37 ± 0.07Bb	0.86 ± 0.00Bc	1.56 ± 0.03Ba	1.47 ± 0.05Bb	0.87 ± 0.01Bc	1.69 ± 0.03Ab	1.93 ± 0.06Aa	1.12 ± 0.01Ad
MUFAs	272.80 ± 1.00a,a,a	220.11 ± 1.88Cc	251.39 ± 2.87Bb	167.76 ± 0.49Ad	227.24 ± 3.89Bc	235.22 ± 1.86Cb	149.17 ± 1.10Cd	266.84 ± 2.25Ab	265.86 ± 2.11Ab	162.22 ± 1.05Bc
C18:2n-6	127.25 ± 0.62a,a,a	100.52 ± 0.76Cc	119.29 ± 1.04Bb	79.41 ± 0.72Ad	109.97 ± 1.28Bc	117.10 ± 1.54Bb	79.59 ± 0.70Ad	116.83 ± 0.66Ac	123.68 ± 0.53Ab	78.09 ± 0.66Ad
C18:3n-6	13.47 ± 0.39a,a,b	10.85 ± 0.17Cc	12.43 ± 0.01Bb	8.35 ± 0.37ABd	12.81 ± 0.11Bab	12.57 ± 0.40Bb	8.00 ± 0.27Bc	14.66 ± 0.17Aa	15.18 ± 0.24Aa	8.84 ± 0.21Ac
C18:3n-3	7.64 ± 0.16a,b,c	6.37 ± 0.11Cc	6.93 ± 0.34Cb	4.88 ± 0.19Bd	8.38 ± 0.15Ba	8.27 ± 0.15Ba	4.89 ± 0.14Bc	10.78 ± 0.12Ab	11.38 ± 0.38Aa	5.94 ± 0.08Ad
C20:2n-6	1.55 ± 0.06a,a,a	1.27 ± 0.01Cb	1.42 ± 0.09Aa	0.94 ± 0.04Ac	1.34 ± 0.03Bb	1.29 ± 0.03Ab	0.90 ± 0.03Ac	1.45 ± 0.02Ab	1.32 ± 0.04Ac	0.82 ± 0.01Bd
C20:3n-6	7.21 ± 0.32a,a,b	6.27 ± 0.07Bb	6.39 ± 0.17Bb	4.09 ± 0.04Bc	6.54 ± 0.20Bb	6.72 ± 0.28Bab	4.78 ± 0.21Ac	7.15 ± 0.12Ab	7.86 ± 0.19Aa	4.76 ± 0.08Ac
C20:4n-6	13.88 ± 0.23a,a,c	12.31 ± 0.15Cb	11.42 ± 0.31Cc	6.92 ± 0.11Cd	13.94 ± 0.48Ba	13.95 ± 0.32Ba	8.28 ± 0.24Bb	16.74 ± 0.88Ab	19.36 ± 0.32Aa	11.63 ± 0.15Ad
C22:2n-6	1.34 ± 0.04a,b,c	1.16 ± 0.04Cb	1.35 ± 0.05Ca	0.88 ± 0.03Cc	2.04 ± 0.15Ba	2.19 ± 0.14Ba	1.14 ± 0.04Bb	3.50 ± 0.07Aa	3.38 ± 0.16Aa	1.72 ± 0.04Ab
C20:5n-3	6.71 ± 0.16a,b,c	6.47 ± 0.07Ca	5.63 ± 0.12Cb	3.27 ± 0.16Cc	7.40 ± 0.17Ba	7.50 ± 0.08Ba	4.68 ± 0.15Bc	8.97 ± 0.13Ab	10.73 ± 0.12Aa	6.57 ± 0.15Ac
C22:6n-3	6.61 ± 0.15a,a,c	5.87 ± 0.14Cb	5.20 ± 0.34Cc	3.23 ± 0.13Cd	6.35 ± 0.15Ba	5.92 ± 0.26Bb	3.57 ± 0.09Bc	7.24 ± 0.07Ab	7.81 ± 0.22Aa	4.61 ± 0.06Ad
PUFAs	185.70 ± 0.12a,a,b	151.13 ± 1.52Cc	170.10 ± 2.11Cb	111.99 ± 1.46Cd	168.99 ± 1.56Bc	175.54 ± 2.74Bb	115.86 ± 1.10Bd	187.33 ± 1.68Ab	200.72 ± 1.38Aa	123.01 ± 0.86Ac
n-3LCPUFAs	20.98 ± 0.47a,b,c	18.72 ± 0.32Cb	17.78 ± 0.54Cb	11.39 ± 0.45Cc	22.15 ± 0.41Ba	21.71 ± 0.21Bab	13.15 ± 0.35Bc	26.99 ± 0.12Ab	29.92 ± 0.06Aa	17.13 ± 0.28Ad
n-6LCPUFAs	163.16 ± 0.42a,a,b	131.13 ± 1.19Cc	150.90 ± 1.49Bb	99.66 ± 0.99Cd	145.93 ± 0.40Bc	152.53 ± 0.51Bb	101.80 ± 0.83Bd	158.89 ± 1.66Ac	169.47 ± 1.37Aa	105.06 ± 0.71Ad
UFAs	458.50 ± 0.89a,a,b	371.24 ± 0.37Cc	421.49 ± 4.87Bb	279.76 ± 1.88Bd	396.23 ± 3.04Bc	410.76 ± 4.40Cb	265.04 ± 2.15Cd	454.17 ± 3.80Ab	466.58 ± 3.46Aa	285.23 ± 1.64Ac

Note: Data are expressed as mean ± SE. ND: not detected. Same capital letters within the same storage time in the same row indicate no significant differences ($P > 0.05$) between different curing salinity, and same lowercase letters within the same salting salinity in the same row indicate no significant differences ($P > 0.05$) between different storage times.

which reached its minimum value at day 35. Furthermore, the content of MUFAs decreased significantly (decreased by 6.94 mg/g in 3 % group < 21.41 mg/g in control group < 37.58 mg/g in 2 % group at day 20), among which the abundant vaccenic acid (C18:1) (decreased by 1.11 % in 3 % group < 8.13 % in control group < 13.11 % in 2 % group at day 20) and palmitoleic acid (C16:1) (decreased by 5.34 % in control group < 16.88 % in 3 % group < 19.29 % in 2 % group at day 20) followed in order of the most abundant MUFAs. By contrast, the PUFAs in the 3 % group had a contrary evolution trend compared with the control group at day 20, of which n-3LCPUFAs and n-6LCPUFAs contents increased by 8.94 mg/g and 6.31 mg/g, respectively. Moderate intake of food containing PUFA n-6 and n-3 is beneficial for residents' health. Furthermore, C20:4n-6, C20:5n-3, C18:3n-3 and C22:2n-6 in the LSD groups increased significantly ($P < 0.05$) compared with the control group on day 20. A plausible explanation for their contents evolution rate could be attributed to the specific enzymes involved in the metabolic activity of dominant bacterial genera to synthesise PUFAs, such as lipase, and the random distribution of substrate FAs on the glyceryl position. Commonly, NaCl, as a pro-oxidant, accelerates excessive oxidation of lipids in fish products, producing off-flavours and being refused by consumers (Zeng et al., 2023). Interestingly, moderate lipid oxidation is desirable during shelf life, such as a significantly higher PUFAs content in fish sausage containing salt (1.25 %, w/w) and tocopherol (Feng et al., 2020). Indeed, Corral, Salvador & Flores. (2015) confirmed that the key aroma compounds in dry-cured sausages mostly derived from the degradation of FAs and amino acids. Therefore, LSD pre-treatment could not only extend shelf-life but also be beneficial for the quality of fish products. Moreover, substituting for high-salt curing in aquatic products is also one of the major challenges for public health concerns related to a high-salt diet.

3.6. Correlation analysis

The metabolic profile change of LSD pre-treatment combined with chill-stored fillets during storage played a vital role in studying their microbial succession, flavour compounds and food quality. The results of microbiota succession and biochemical monitoring indicated that the LSD groups had significant protective effects against quality changes as compared with the control group, which inhibited the growth of spoilage bacteria and the formation of spoilage substances. In addition, there were strong connections between metabolite profiles and microbiota, but correlations between them and flavour precursors are rarely investigated. Therefore, this study developed more complete connections between FAAs, UFAs, biochemical indicators and microbial abundance changes of grass carp fillets by using the Pearson correlation test and then further explored the relationship between flavour components and metabolites by flavour precursors evolution.

Pearson correlation network analysis revealed the effects of LSD pre-treatment on the correlations between microbiota, biochemical indicators and their connections during refrigerated fillets. As shown in Fig. 2A, the dominant bacterial genera were *Psychrobacter*, *Pseudoalteromonas* and *Pseudomonas*. Among them, *Psychrobacter* was negatively correlated with *Brochothrix* and *Carnobacterium*, and *Pseudoalteromonas* was also negatively correlated with *Pseudomonas* and *Lactococcus*, but *Pseudomonas* and *Lactococcus* were positively correlated. Furthermore, in Fig. 2B, the connectivity within the dominant bacterial genera and biochemical indicators in fillets are visually presented, especially the strong positive correlation between the dominant bacterial genera and more PUFAs, and FAAs. Thus, the LSD pre-treatment improved the contents of flavour precursors in fillets by regulating microbial succession.

AV, TVC, CAD, TVB-N, PUT and AC were significantly ($P < 0.01$) correlated with Val, Met, Ile, Leu, Phe, Glu and Ala, and their correlation coefficients were greater than 0.61 (Fig. 3A). Among them, strong positive correlations between multiple FAAs and TVC might be due to the proteolysis of spoilage microbiota such as *Carnobacterium*, while the strong positive correlation with CAD, TVB-N, PUT and AC was attributed to the strong degradation potential of FAAs (Arg) by *Brochothrix* and *Pseudomonas* (Huang et al., 2017; Zhuang et al., 2021). Moreover, these biochemical indicators were negatively correlated with main UFAs and Arg, which might be related to proteolysis (lipase) and FAAs degradation by deamination and decarboxylation of spoilage microbiota. The Arg was degraded into putrefactive substances through the ADI and ADC metabolic pathways (Zhuang et al., 2022), which suggested that the metabolic potential of microbiota might be a hazard to fish products. Conclusively, LSD pre-treatment reduced the production of nitrogen-containing small molecule metabolites and excessive lipid oxidation, which was attributed to the regulation of microbial succession by salt

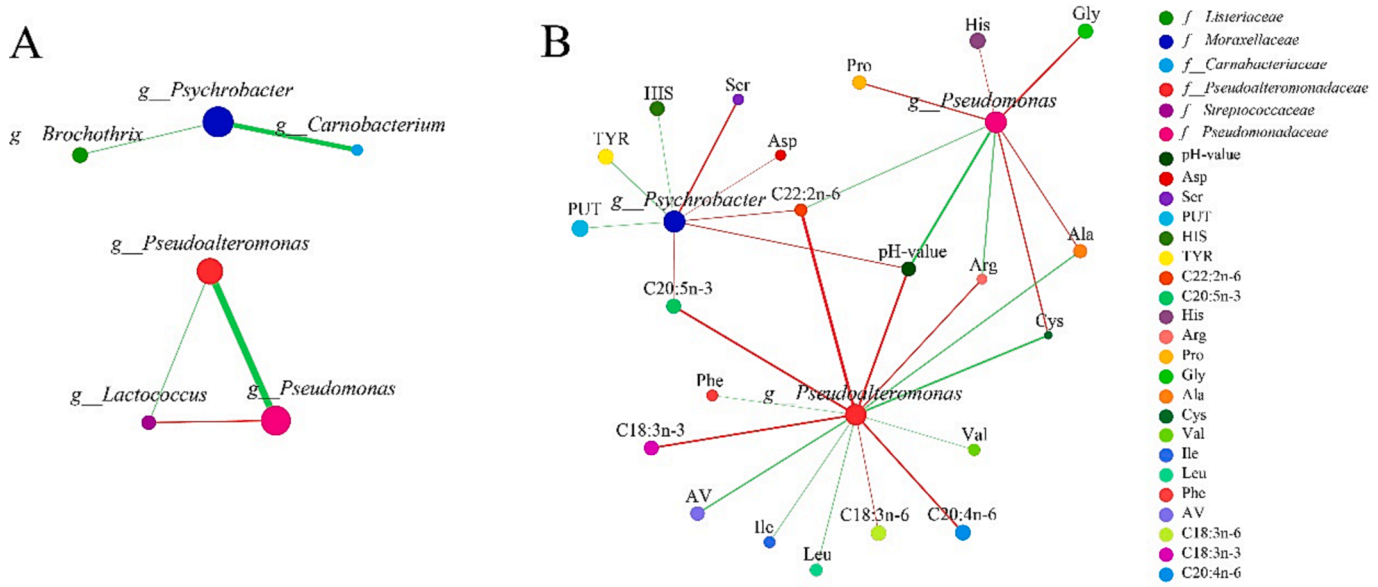

Fig. 2. Correlation Network analysis applied to microbiota composition (A), and between biochemical indicators, flavour precursors and dominant microbiota composition (B) of fresh and LSD pre-treated fillets during refrigeration. Size and colour of the nodes represent relative abundance of the fresh and LSD pre-treated fillets microbiota. Connecting in red and green denote positive and negative correlations, respectively. The width reflects the strength of the correlation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

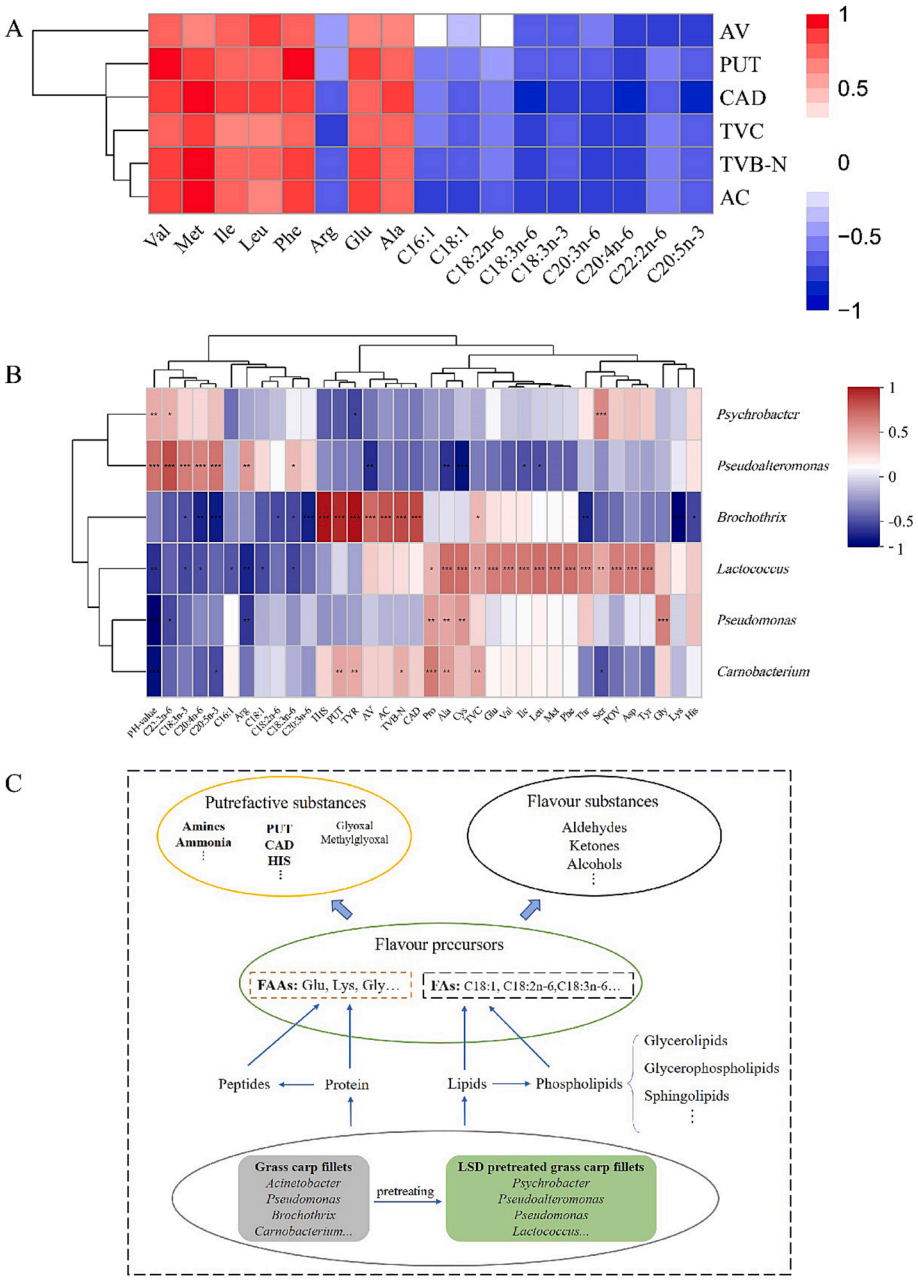

Fig. 3. Pearson correlation analysis between biochemical indicators and flavour precursors (A), and between the biochemical indicators, flavour precursors and microbiota composition (B) of fresh and LSD pre-treated fillets during refrigeration. Predicted metabolic network for flavour precursors in fresh and LSD pre-treated fillets during refrigeration (C). (***: correlation P value $\leq$ 0.001; **: 0.001 $<$ P value $\leq$ 0.01; *: 0.01 $<$ P value $\leq$ 0.05.).

ions.

The relative abundance of *Brochothrix* was significantly ($P < 0.01$) correlated with HIS, PUT, TYR, AV, AC, TVB-N and CAD, and their correlation coefficients were more than 0.72 (Fig. 3B), and negatively correlated with flavour precursors such as C20:5n-3, C20:3n-6, Lys and His. Protein is hydrolysed into nitrogen-containing metabolites such as amino acids or peptides, which can be further metabolised into putrefactive substances (ammonia and amines) (Nowak & Czyzowska, 2011). Previous suggested that flavour compounds are mainly produced by proteolysis, free FAs production and lipid oxidation under the synergistic action of the bacteria and enzymes (Zhao, Hu & Chen, 2022). However, the strong correlation between *Brochothrix* and biochemical indicators was presumed to be active deamination, decarboxylation and excessive lipid oxidation in the latter stage of refrigeration. The abundance of *Lactococcus* was also positively correlated with POV, FAAs (except for Arg), which might be beneficial to the development of flavoured fillets as it improved the content of umami- and sweet-tasting FAAs, moderates lipid oxidation and inhibited the metabolic activity of *Brochothrix* (Fall et al., 2010). *Pseudomonas* had a strong negative correlation with Arg and pH, and a strong positive correlation with Pro, Ala, Cys and Gly, which were consistent with its strong degradation of FAAs and protein hydrolysis potential (Zhuang et al., 2022). In addition, the Arg metabolism and pH changes of fillets were regulated by *Pseudomonas* and *Lactococcus*. As the predominant spoilage bacteria in the latter stage of the control group (Fig. 1B), *Carnobacterium* had a strong positive correlation with PUT, TYR, TVC, TVB-N, Pro and Ala, and a negative correlation with pH, C20:5n-3 and Ser, indicating that protein degradation, FAAs deamination, decarboxylation and lipolysis of the samples pre-treated by LDS pre-treatment were largely regulated by *Carnobacterium*. The abundance of *Pseudoalteromonas* and *Psychrobacter*

in the 3 % group was dominant during refrigeration (Fig. 1B). Although *Psychrobacter* was not involved in the putrefactive substances from FAAs, *Pseudomonas* and *Brochothrix* were involved in protein-rich fish deterioration (Yang, 2014), which indicates that the contribution of *Psychrobacter* to fillet deterioration is limited. *Pseudoalteromonas,* requiring Na^+ ions for growth, was positively correlated with Arg, pH and numerous UFAs, and negatively correlated with AV and a few FAAs. *Pseudoalteromonas* mainly participates in protein hydrolysis and lipolysis, which increases the taste-active amino acids and volatile flavour compounds of fillets. This result also serves as a reminder that the application of the LSD pre-treatment technique should not be regarded as only prolonging shelf life in aquatic product processing and storage. However, further studies of the spoilage mechanism of LSD pre-treatment fish fillets during refrigeration through targeted metabolomics and bacterial adaptive response are necessary.

The evolution of flavour precursors in LSD pre-treated fillets is presented in the network pathway shown in Fig. 3C. The formation and degradation of flavour precursors is a complicated biochemical reaction that is mainly affected by proteolysis and lipolysis of specific microbiota. According to the microbiota succession and compounds in fillets, the main flavour precursors can be divided into three categories as follows: FAs, FAAs and flavour peptides. Aldehydes, alcohols and ketones are known vital flavour compounds, which are mainly produced by the oxidative degradation of UFAs and the Strecker degradation of FAAs on the involvement of the dominant microbial metabolism and enzymes and are essential to improve the content of flavour precursors in the processing and preservation of fillets. Furthermore, this reminds us that LSD pre-treatment promotes the improvement of flavour precursors in fillets while also ensuring that their quality attributes are qualified.

4. Conclusion

This study comprehensively evaluated the effects of LSD pre-treatment on quality, FAAs, FAs and bacterial genus of chill-stored fillets. LSD pre-treatment effectively regulated the community abundance and succession of bacteria, and slowed the accumulation of putrefactive substances, namely TVC, TVB-N, AC, BAs and AV, thus promoting the benign development of quality attributes. The correlations between bacterial genera, quality evolution and flavour precursors were also obtained. It was possible to infer which bacterial genera were involved in quality degradation and flavour precursor liberation. In particular, *Lactococcus* and *Pseudoalteromonas* showed positive effects on the metabolism of various flavour precursors such as active-amino acids and PUFAs, while the growth and metabolic activities of spoilage bacteria *Pseudomonas*, *Carnobacterium* and *Brocothrix* were inhibited by 3 % LSD pre-treatment to prolong the shelf life of fillets. Therefore, they can be used as a core functional microorganism. The current study provides novel insights for utilising microbial resources to develop flavoured fish and their preservation that might fuel freshwater fish processing development.

CRediT authorship contribution statement

Qingxi Liang: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xiangfei Hu:** Writing – review & editing, Methodology, Formal analysis. **Bizhen Zhong:** Writing – review & editing, Methodology, Conceptualization. **Xiaoliang Huang:** Investigation. **Hui Wang:** Supervision, Resources. **Chengwei Yu:** Writing – review & editing. **Jinlin Li:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Zongcai Tu:** Resources, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101188.

References

Buňková, L., Buňka, F., Hlobilová, M., Vaňátková, Z., Nováková, D., & Dráb, V. (2009). Tyramine production of technological important strains of Lactobacillus, Lactococcus and Streptococcus. *European Food Research and Technology, 229*(3), 533–538. https://doi.org/10.1007/s00217-009-1075-3

Chen, J., Zhang, Y., Huang, X., Dong, M., Dong, X., Zhou, D., & Qin, L. (2023). Integrated volatolomics and metabolomics analysis reveals the characteristic flavor formation in Chouguiyu, a traditional fermented mandarin fish of China. *Food Chemistry, 418*, Article 135874. https://doi.org/10.1016/j.foodchem.2023.135874

Chen, Y., Cai, W., Shi, Y., Dong, X., Bai, F., Shen, S., & Zhu, X. (2020). Effects of different salt concentrations and vacuum packaging on the shelf-stability of Russian sturgeon (Acipenser gueldenstaedti) stored at 4 °C. *Food Control, 109*, Article 106865. https://doi.org/10.1016/j.foodcont.2019.106865

Corral, S., Salvador, A., & Flores, M. (2015). Elucidation of key aroma compounds in traditional dry fermented sausages using different extraction techniques. *J Sci Food Agric, 95*(6), 1350–1361. https://doi.org/10.1002/jsfa.6830

Dallagnol, A. M., Pescuma, M., Espínola, N. G., Vera, M., & Vignolo, G. M. (2021). Hydrolysis of raw fish proteins extracts by Carnobacterium maltaromaticum strains isolated from Argentinean freshwater fish. *Biotechnology Reports, 29*, e00589.

Fall, P. A., Leroi, F., Cardinal, M., Chevalier, F., & Pilet, M. F. (2010). Inhibition of Brochothrix thermosphactaand sensory improvement of tropical peeled cooked shrimp byLactococcus pisciumCNCM I-4031. *Letters in Applied Microbiology, 50*(4), 357–361. https://doi.org/10.1111/j.1472-765X.2010.02801.x

Feng, X., Bansal, N., & Yang, H. (2016). Fish gelatin combined with chitosan coating inhibits myofibril degradation of golden pomfret (Trachinotus blochii) fillet during cold storage. *Food Chemistry, 200*, 283–292. https://doi.org/10.1016/j.foodchem.2016.01.030

Feng, X., Tjia, J. Y. Y., Zhou, Y., Liu, Q., Fu, C., & Yang, H. (2020). Effects of tocopherol nanoemulsion addition on fish sausage properties and fatty acid oxidation. *Lwt, 118*, Article 108737. https://doi.org/10.1016/j.lwt.2019.108737

Gao, S., Liu, Y., Zhang, L., Tan, Y., Li, B., Hong, H., & Luo, Y. (2022). Sodium chloride-induced oxidation of bighead carp (Aristichthys nobilis) fillets: The role of mitochondria and underlying mechanisms. *Food Research International, 152*, Article 110915. https://doi.org/10.1016/j.foodres.2021.110915

Ge, L., Xu, Y., & Xia, W. (2015). The function of endogenous cathepsin in quality deterioration of grass carp (Ctenopharyngodon idella) fillets stored in chilling conditions. *International Journal of Food Science & Technology, 50*(3), 797–803. https://doi.org/10.1111/ijfs.12713

Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B., & Givskov, M. (2002). Food spoilage–interactions between food spoilage bacteria. *International Journal of Food Microbiology, 78*(1–2), 79–97. https://doi.org/10.1016/s0168-1605(02)00233-7

Hao, R., Roy, K., Pan, J., Shah, B. R., & Mraz, J. (2021). Critical review on the use of essential oils against spoilage in chilled stored fish: A quantitative meta-analyses. *Trends in Food Science & Technology, 111*, 175–190. https://doi.org/10.1016/j.tifs.2021.02.054

Hu, X., Jiang, Q., Wang, H., Li, J., & Tu, Z. (2023). Insight into the effect of traditional frying techniques on glycosylated hazardous products, quality attributes and flavor characteristics of grass carp fillets. *Food Chemistry, 421*, Article 136111. https://doi.org/10.1016/j.foodchem.2023.136111

Hu, X., Peng, B., Wang, S., Tu, Z., Li, J., Wang, H., & Zhong, B. (2022). Oxidative stabilities of grass carp oil: Possible mechanisms of volatile species formation in hydroperoxylated metabolites at high temperature. *European Food Research and Technology, 248*(8), 2079–2095. https://doi.org/10.1007/s00217-022-04032-9

Huang, Z., Liu, X., Jia, S., & Luo, Y. (2017). Antimicrobial effects of cinnamon bark oil on microbial composition and quality of grass carp (Ctenopharyngodon idellus) fillets during chilled storage. *Food Control, 82*, 316–324. https://doi.org/10.1016/j.foodcont.2017.07.017

Jia, S., Li, Y., Zhuang, S., Sun, X., Zhang, L., Shi, J., & Luo, Y. (2019). Biochemical changes induced by dominant bacteria in chill-stored silver carp (Hypophthalmichthys molitrix) and GC-IMS identification of volatile organic

compounds. *Food Microbiol, 84*, Article 103248. https://doi.org/10.1016/j.fm.2019.103248

Landete, J. M., Pardo, I., & Ferrer, S. (2007). Tyramine and phenylethylamine production among lactic acid bacteria isolated from wine. *International Journal of Food Microbiology, 115*(3), 364–368. https://doi.org/10.1016/j.ijfoodmicro.2006.10.051

Larsen, R., & Elvevoll, E. O. (2008). Water uptake, drip losses and retention of free amino acids and minerals in cod (Gadus morhua) fillet immersed in NaCl or KCl. *Food Chemistry, 107*(1), 369–376. https://doi.org/10.1016/j.foodchem.2007.08.031

Liu, D., Liang, L., Xia, W., Regenstein, J. M., & Zhou, P. (2013). Biochemical and physical changes of grass carp (Ctenopharyngodon idella) fillets stored at -3 and 0°C. *Food Chemistry, 140*(1–2), 105–114. https://doi.org/10.1016/j.foodchem.2013.02.034

Liu, X., Li, D., Li, K., & Luo, Y. (2018). Monitoring bacterial communities in ε-Polylysine-treated bighead carp (Aristichthys nobilis) fillets using culture-dependent and culture-independent techniques. *Food Microbiol, 76*, 257–266. https://doi.org/10.1016/j.fm.2018.06.001

Nowak, A., & Czyzowska, A. (2011). In vitro synthesis of biogenic amines by Brochothrix thermosphacta isolates from meat and meat products and the influence of other microorganisms. *Meat science, 88*(3), 571–574. https://doi.org/10.1016/j.meatsci.2011.02.015

Olatunde, O. O., & Benjakul, S. (2018). Natural Preservatives for Extending the Shelf-Life of Seafood: A Revisit. *Comprehensive Reviews in Food Science and Food Safety, 17*(6), 1595–1612. https://doi.org/10.1111/1541-4337.12390

Shen, S., Jiang, Y., Liu, X., Luo, Y., & Gao, L. (2014). Quality assessment of rainbow trout (Oncorhynchus mykiss) fillets during super chilling and chilled storage. *Journal of Food Science and Technology, 52*(8), 5204–5211. https://doi.org/10.1007/s13197-014-1539-8

Wang, Y., Li, C., Zhao, Y., Li, L., Yang, X., Wu, Y., & Yang, D. (2020). Novel insight into the formation mechanism of volatile flavor in Chinese fish sauce (Yu-lu) based on molecular sensory and metagenomics analyses. *Food Chemistry, 323*, Article 126839. https://doi.org/10.1016/j.foodchem.2020.126839

Wen, R., Sun, F., Wang, Y., Chen, Q., & Kong, B. (2021). Evaluation the potential of lactic acid bacteria isolates from traditional beef jerky as starter cultures and their effects on flavor formation during fermentation. *Lwt, 142*, Article 110982. https://doi.org/10.1016/j.lwt.2021.110982

Xue, C., Hsu, K.-M., Ting, W.-W., Huang, S.-F., Lin, H.-Y., Li, S.-F., & Ng, I. S. (2020). Efficient biotransformation of l-lysine into cadaverine by strengthening pyridoxal 5'-phosphate-dependent proteins in Escherichia coli with cold shock treatment. *Biochemical Engineering Journal, 161*, Article 107659. https://doi.org/10.1016/j.bej.2020.107659

Yang, W., Shi, W., Qu, Y., Wang, Z., Shen, S., Tu, L., & Wu, H. (2020). Research on the quality changes of grass carp during brine salting. *Food Science & Nutrition, 8*(6), 2968–2983. https://doi.org/10.1002/fsn3.1599

Yang, X. (2014). Moraxellaceae. 826-833. 10.1016/b978-0-12-384730-0.00441-9.

Yang, Z., Yan, J., & Xie, J. (2023). Effect of vacuum and modified atmosphere packaging on moisture state, quality, and microbial communities of grouper (Epinephelus coioides) fillets during cold storage. *Food Research International, 173*, Article 113340. https://doi.org/10.1016/j.foodres.2023.113340

Yu, D., Regenstein, J. M., Xia, W., Yang, F., Jiang, Q., & Wang, B. (2018). The effects of edible chitosan-based coatings on flavor quality of raw grass carp (Ctenopharyngodon idellus) fillets during refrigerated storage. *Food Chemistry, 242*, 412–420. https://doi.org/10.1016/j.foodchem.2017.09.037

Zeng, J., Song, Y., Fan, X., Luo, J., Song, J., Xu, J., & Xue, C. (2023). Effect of lipid oxidation on quality attributes and control technologies in dried aquatic animal products: A critical review. *Critical Reviews in Food Science and Nutrition, 1–22*. https://doi.org/10.1080/10408398.2023.2224451

Zhao, D., Chong, Y., Hu, J., Zhou, X., Xiao, C., & Chen, W. (2022). Proteomics and metagenomics reveal the relationship between microbial metabolism and protein hydrolysis in dried fermented grass carp using a lactic acid bacteria starter culture. *Current Research in Food Science, 5*, 2316–2328. https://doi.org/10.1016/j.crfs.2022.11.016

Zhao, D., Hu, J., & Chen, W. (2022). Analysis of the relationship between microorganisms and flavour development in dry-cured grass carp by high-throughput sequencing, volatile flavour analysis and metabolomics. *Food Chemistry, 368*, Article 130889. https://doi.org/10.1016/j.foodchem.2021.130889

Zhao, X., Chen, L., Wongmaneepratip, W., He, Y., Zhao, L., & Yang, H. (2021). Effect of vacuum impregnated fish gelatin and grape seed extract on moisture state, microbiota composition, and quality of chilled seabass fillets. *Food Chemistry, 354*, Article 129581. https://doi.org/10.1016/j.foodchem.2021.129581

Zhu, C., Zeng, X., Chen, L., Liu, M., Zheng, M., Liu, J., & Liu, H. (2024). Changes in quality characteristics based on protein oxidation and microbial action of ultra-high pressure-treated grass carp (Ctenopharyngodon idella) fillets during magnetic field storage. *Food Chemistry, 434*, Article 137464. https://doi.org/10.1016/j.foodchem.2023.137464

Zhuang, S., Li, Y., Hong, H., Liu, Y., Shu, R., & Luo, Y. (2020). Effects of ethyl lauroyl arginate hydrochloride on microbiota, quality and biochemical changes of container-cultured largemouth bass (Micropterus salmonides) fillets during storage at 4 °C. *Food Chemistry, 324*, Article 126886. https://doi.org/10.1016/j.foodchem.2020.126886

Zhuang, S., Liu, X., Li, Y., Zhang, L., Hong, H., Liu, J., & Luo, Y. (2021). Biochemical changes and amino acid deamination & decarboxylation activities of spoilage microbiota in chill-stored grass carp (Ctenopharyngodon idella) fillets. *Food Chemistry, 336*, Article 127683. https://doi.org/10.1016/j.foodchem.2020.127683

Zhuang, S., Liu, Y., Gao, S., Tan, Y., Hong, H., & Luo, Y. (2023). Mechanisms of fish protein degradation caused by grass carp spoilage bacteria: A bottom-up exploration from the molecular level, muscle microstructure level, to related quality changes. *Food Chemistry, 403*, Article 134309. https://doi.org/10.1016/j.foodchem.2022.134309

Zhuang, S., Tan, Y., Hong, H., Li, D., Zhang, L., & Luo, Y. (2022). Exploration of the roles of spoilage bacteria in degrading grass carp proteins during chilled storage: A combined metagenomic and metabolomic approach. *Food Research International, 152*, Article 110926. https://doi.org/10.1016/j.foodres.2021.110926