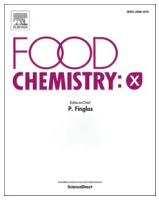
Food Chemistry: X 23 (2024) 101428

Contents lists available at ScienceDirect

Food Chemistry: X

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



Preservation strategies for processed grass carp products: Analyzing quality and microbial dynamics during chilled and ice temperature storage

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ARTICLE INFO

Keywords:
Processed fish paste products
Spoilage
High-throughput sequencing
Cold storage
Quality properties
Microflora

ABSTRACT

This study investigated the impact of ice temperature storage on quality and bacterial composition of processed fish paste products (PFP). Freezing curve revealed the ice temperature was −1 °C. Electric nose (e-nose) showed significant changes in volatile components within 8 days. Results of total volatile basic nitrogen (TVB-N) showed that PFP stored at 4 °C reached its limit after 2 days, whereas PFP stored at ice temperature remained stable for 6 days. Thiobarbituric acid reactive substances (TBARS) demonstrated delayed oxidation in PFP stored at ice temperature compared to 4 °C. TCA-soluble peptides indicated that the protein degradation was suppressed by ice temperature. Additionally, ice temperature inhibited microbial growth and altered bacterial composition. High-throughput sequencing revealed that *Pseudomonas*, *Brochothrix*, *Carnobacterium* were dominant at 4 °C, while *Acinetobacter*, *Pseudomonas*, *Janthinobacterium* and *Brochothrix* were dominant at ice temperature. In summary, ice temperature might be a potential method for maintaining the freshness of PFP.

1. Introduction

The grass carp (*Ctenopharyngodon idellus*) is a prominent Asian freshwater fish species, known for its delicious taste and substantial yields in aquaculture. Over five million tons of grass carp are produced in the world annually (Xie et al., 2018). In China, the surge in processed fish products, including those made from grass carp, aligns with national economic growth and shifts in consumer habits. Processed fish paste products (PFP) are produced by making fresh fish into a paste and incorporating additives. PFP can be used in various dishes such as soup and hotpot and needs to be cooked before serving. PFP does not undergo processes such as rinsing and filtration; thus, it retains the nutrients and flavor of the fish. However, the perishability of fish is a challenge. Factors like high water activity, a high nutrient content, and a neutral pH contribute to the rapid spoilage of aquatic products, exacerbated by endogenous enzymes, oxidation, and microbial growth, even under chilled storage. Moreover, cross-contamination during processing further affects their shelf life (Yu, Xu, Jiang, Yang, & Xia, 2017), with spoilage indicated by signs like mucus formation and an unpleasant odor.

The growth of the processed fish sector is closely linked with advancements in the Internet of Things (IoT) and cold-chain technology (Cao et al., 2023; Yin et al., 2023). Although freezing is commonly used for long-term storage, the resulting protein denaturation and ice crystal formation can affect the texture and nutritional value of fish (Zheng, Qu, Peng, Chen, & Hu, 2022). Chilling, in contrast to freezing, retains the sensory properties by delaying protein and lipid oxidation and hampering bacterial growth. Ice-temperature storage (i.e., maintaining the temperature between 0 °C and the freezing point), preserves the quality of meat products (Zhong et al., 2023). Ji et al. (2021) found that

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https://doi.org/10.1016/j.fochx.2024.101428
Received 8 January 2024; Received in revised form 12 April 2024; Accepted 28 April 2024
Available online 7 May 2024

lower ice temperatures slowed down gel deterioration and retained the physicochemical properties of chicken breast. Furthermore, these temperatures delayed the rise in total volatile basic nitrogen (TVB-N) levels in greater amberjack fillets, prolonging their shelf life by six days (Zhong et al., 2023).

Identifying and sourcing spoilage microorganisms is essential to estimate and extend the shelf-life of processed foods. Traditional microbial analysis methods are often inadequate for the real-time identification of diverse microflora (Zhong et al., 2023). High-throughput sequencing (HTS) of the 16S rDNA gene has emerged as a reliable method for analyzing microbial composition and dynamics, identifying specific spoilage organisms, and understanding the correlation between microbial metabolic pathways and spoilage. Research has linked spoilage processes and profiles in fish to their microbiota composition (Zhuang, Hong, Zhang, & Luo, 2021). In addition, Wang et al. (2017) identified *Acinetobacter*, *Pseudomonas*, *Aeromonas*, *Flavobacterium*, *Shewanella*, *Micrococcus*, *Carnobacterium* and *Moraxella* as the predominant bacteria in freshwater fish. In the initial 10 days of storage at 4 °C, *Acinetobacter hydrophila* and *Pseudomonas jessenii* populations surged, whereas *Shewanella putrefaciens* experienced delayed growth in sterile grass carp fillets. Zhuang et al. (2023) explored the connection between three spoilage bacteria (*Aeromonas rivipollensis*, *Shewanella putrefaciens*, and *Shewanella putrefaciens*) and fish quality deterioration. They found that variations in the quality of grass carp, such as bacterial levels, free amino acids, ammonia, putrescine, volatile organic compounds (VOCs), muscle glucose, and lactate, were primarily due to the breakdown of amino acids by bacteria and the consumption of carbon sources.

However, research on processed fish products, which can spoil from the inside and outside because of the high initial bacterial loads, is lacking. Understanding the storage characteristics of chilled grass carp products is necessary to optimize their transportation and processing. Therefore, this study aimed to investigate relationship of the changes in the quality and bacterial community of PFP during storage at 4 °C and ice temperature, combining methods of traditional microbial incubation and HTS based on 16S rDNA of bacteria, to develop effective preservation strategies for aquatic products.

2. Materials and methods

2.1. Sample preparation and storage

Cultured grass carp (weight: 1.5 ± 0.2 kg; length: 50.3 ± 5.2 cm) and pork fat were purchased from a market in Dalian, Liaoning, China, since November 2022 to March 2023. The raw material was transported to the National Engineering Research Center of Seafood lab within 30 min. The fish was immediately processed (decapitation, descaling, evisceration, and filleting). The fillets were ground in ice water using a meat grinder (QSJ-B02R, Bear, China) for 10 min. The ground fish was mixed with pork fat (50 g/kg), starch (80 g/kg), and water (50 g/kg). The samples were then weighed, vacuum-sealed, and stored at 4 °C and ice temperature (−1 °C) for testing.

2.2. Chemicals

Bromocresol green, K_2CO_3, trichloroacetic acid (TCA), malondialdehyde (MDA), and Folin-Ciocalteu's phenol reagent were sourced from Shanghai Macklin Biochemical Technology Co., Ltd. (China). Boric acid and ethylenediaminetetraacetic acid (EDTA) were purchased from Damao Chemical Reagent Factory (China); Arabic gum was procured from Shanghai Yuanye Bio-Technology Co., Ltd. (China); glycerol, from Sangon Biotech (Shanghai) Co., Ltd. (China); methyl red, from Shanghai Aladdin Biochemical Technology Co., Ltd. (China); and selective media from Qingdao Hope Bio-Technology Co., Ltd. (China).

2.3. Freezing curve

The freezing curve was monitored using a thermometer (SE309, Center, China). Spherical plastic molds with a diameter of 30 mm were filled with PFP (about 25 g). Samples were initially chilled at 4 °C for 2 h, then frozen at −40 °C without demolding. Thermometer probes were inserted into the sample centers, recording temperatures every 2 s.

2.4. Quality changes

2.4.1. Whiteness

The color (i.e., the L*, a*, and b* values, representing brightness, red and blue, and yellow and green, respectively) of PFP was measured using a colorimeter (UltraScan PRO, HunterLab, USA) based on Cao et al. (2022). Whiteness was calculated using the following equation:

$$\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}$$

2.4.2. Volatile compounds

The volatile compounds of the PFP were identified using an electronic nose (e-nose) (PEN3.0, AIRSENSE, Germany), following the method of Huang et al. (2023). Briefly, 3.0 g of PFP was equilibrated at 25 °C for 15 min in a glass injection bottle before testing. The sensors incorporated in the equipment were R1 (aromatic components), R2 (nitrogen oxides), R3 (ammonia and aromatic components), R4 (hydrides), R5 (short-chain alkanes), R6 (methyl groups), R7 (inorganic sulfides), R8 (alcohols, aldehydes, ketones), R9 (organic sulfides and aromatic compounds), and R10 (long-chain alkanes).

2.4.3. Total volatile base nitrogen (TVB-N)

TVB-N was quantified using the micro-diffusion method from GB/T 5009.228–2016, with modifications. Samples (10.0 g) were mixed with 100 mL of deionized water, stirred for 30 min, and filtered using an 80-mesh filter cloth. The diffusion dishes were prepared with a water-soluble adhesive (10.0 g of Arabic gum, 10 mL of deionized water, 5 mL of glycerol, and 5.0 g of K_2CO_3), a boric acid solution (1 mL), and a mixed indicator (1 drop). After adding 1 mL of filtrate and saturating with potassium carbonate, dishes were incubated at 37 ± 1 °C for 2 h. After incubation, samples were titrated with 0.01 mol/L hydrochloric acid. A mixture of methyl red and bromocresol green was used as an indicator. TVB-N was calculated using the following equation:

$$\text{TVB} - \text{N} = \frac{(V_1 - V_2) \times c \times 14}{m \times \left(\frac{V}{V_0}\right)} \times 100$$

where V_0 is the volume of the sample solution (100 mL); V_1 is the volume of the hydrochloric acid standard titration solution consumed by the sample (mL); V_2 is the volume of the hydrochloric acid standard titration solution consumed by the blank sample (mL); V is the volume of filtrate added into the dish (1 mL); and c is the concentration of the hydrochloric acid standard solution (mol/mL).

2.5. Spoilage bacteria count

The spoilage bacteria count was determined using the method specified in the Chinese National Food Safety Standard, Food Microbiology Test-Determination of Aerobic Plate Count, GB/T 4789.2–2016. A 25 g sample was aseptically weighed, mixed, and homogenized in 225 mL of sterile saline for 3 min using a sterile homogenizer (Scientz-11, Scientz Biotechnology Co., Ltd). Subsequently, the suspension was repeatedly diluted (1:10) with sterile saline until an appropriate concentration for counting was achieved. Approximately 100 μL of the diluent was spread onto several prepared solid media. Various cultivated microorganisms were inoculated onto the corresponding selective media. To determine total viable counts (TVC), samples were incubated

in plate count agar (PCA) at 30 °C for 3 days; for psychrotrophic counts, samples were incubated in PCA at 4 °C for 7 days; for *Enterobacteriaceae* counts, samples were incubated in violet red bile glucose agar (VRBGA) at 37 °C for 2 days; for *Pseudomonas* counts, samples were incubated in *Pseudomonas* CFC selective agar at 20 °C for 2 days; for *Aeromonas* counts, samples were incubated in ampicillin MacConkey agar (AMA) at 28 °C for 2 days; for *Vibrio* counts, samples were incubated in thiosulfate citrate bile salts sucrose agar (TCBS) at 30 °C for 2 days; for *Lactobacillus* counts, samples were incubated in MRS agar at 37 °C for 2 days; and for *Brochothrix* counts, samples were incubated in STAA agar at 25 °C for 2 days.

2.6. Thiobarbituric acid reactive substances (TBARS)

The thiobarbituric acid reactive substances (TBARS) value was determined following the method of Zou et al. (2022). Briefly, 5.0 g of the sample was thoroughly combined with 50 mL of a TCA mixed solution (containing 37.5 g of TCA and 0.5 g of EDTA, diluted to 500 mL). This mixture was agitated in a constant temperature oscillator (THZ-82, Zhiborui Instrument Co., Ltd) at 50 °C for 30 min, and filtered using double-layer quantitative slow-speed filter paper. Next, 5 mL of the filtrate was combined with 5 mL of a TBA solution (0.02 mol/L), and the resulting mixture was heated in a water bathed at 90 °C for 30 min. After cooling to room temperature, the absorbance of the mixture was measured at 532 nm. The TBARS value, expressed as mg MDA/kg of PFP, was calculated using the following equation:

$$\mathrm{MDA} = \frac{c \times V \times 1000}{m \times 1000}$$

where c is the concentration of malondialdehyde in the sample solution, V is the constant volume of the sample solution, and m is the sample's mass in the final sample solution.

2.7. TCA-soluble peptide

The TCA-soluble peptide content was assessed following the method of Yang, Liu, Sang, and Sun (2023), with slight modifications. Briefly, 4.0 g of the sample was homogenized in 36 mL of a 10% TCA solution (*w/v*) at 9510 ×g for 2 min, followed by centrifugation at 4230 ×*g* for 10 min. Subsequently, the protein content in the supernatant was quantified using the Lowry's method and expressed as μmol tyrosine/g.

2.8. High-throughput sequencing

Total microbial DNA extraction and amplification of the target fragment followed the methodology of Jiang et al. (2022). The quality of the DNA extraction was assessed through 1.2% agarose gel electrophoresis. VAHTSTM DNA Clean Beads from Vazyme, China were utilised to purify and recover the amplified DNA fragment. Subsequently, the resulting DNA was quantitated using a Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher, USA) and a microplate reader (FLx800, BioTek, USA).

The sequencing library was prepared using the TruSeq Nano DNA LT Library Prep Kit from Illumina (USA). The library underwent final fragment selection and purification through 2% agarose gel electrophoresis. Finally, the DNA products were combined and subjected to paired-end sequenced on the Illumina MiSeq platform.

2.9. Statistical analysis

The experimental data were collected in triplicate, except for the five sets of samples used to determine the bacterial composition. SPSS 22.0 was used for data analysis, and data were expressed as mean ± standard deviation. One-way ANOVA-Duncan test was used for multiple comparisons, and an unpaired test was used for detecting inter-group differences. A significance level of 95% ($p < 0.05$) was used. Principal component analysis (PCA) was performed using the built in PCA application of Origin 2021. The alpha diversity index was analyzed using QIME2 (2019.4). The species accumulation curves and principal coordinate analysis (PCoA) plots based on the Bray-curtis distance were obtained using R.

3. Results and discussion

3.1. Freezing curve

The freezing point is the temperature at which water in food freezes, this temperature depends on the composition of the food. If the storage temperature is controlled in the range from the freezing point to 0 °C, ice crystal formation can be prevented. The freezing curve of PFP (Fig. 1), reveals that the temperature of the center of PFP decreased to −1.2 °C. Subsequently, from 432 s to 1550 s, the freezing curve stabilized. Afterward, a faster and continued decline in the center's temperature was observed. These results suggest that a temperature range between 0 °C and − 1.2 °C is suitable for ice storage, ensuring that the PFP remains fresh without freezing. After a comprehensive evaluation of freezing point considerations and refrigerator settings, storage temperatures of 4 °C and − 1 °C (ice temperature) were selected.

3.2. Bacterial counts

Monitoring the presence of spoilage and pathogenic microorganisms is essential for ensuring the quality of aquatic products. To quantify the bacteria, the colonies were counted using the traditional method. The microbiological changes are depicted in Fig. 2, revealing a significant increase ($p < 0.05$) increase in all bacterial species. The temperature and environmental conditions during fish growth influenced the initial TVC. Song, Liu, Shen, You, and Luo (2011) reported that the TVC of freshwater fish species ranged between 2.00 and 6.00 lg CFU/g.

Fig. 2A illustrates the TVC for PFP during storage. For samples stored at 4 °C, an upward trend in TVC was observed. Nevertheless, this trend was interrupted on days 2–4, and this interruption became more pronounced at ice temperature. This might be attributed to the lower temperature and reduced oxygen levels, which suppressed the growth of aerobic and cold-sensitive bacteria. According to DjordjeviĆ et al. (2019), the presence of *Enterobacteriaceae* decreased in an oxygen-deprived environment. Here, this led to a steady increase in TVC, reaching 8.27 lg CFU/g at 4 °C and 7.39 lg CFU/g at ice temperature, indicating severe spoilage.

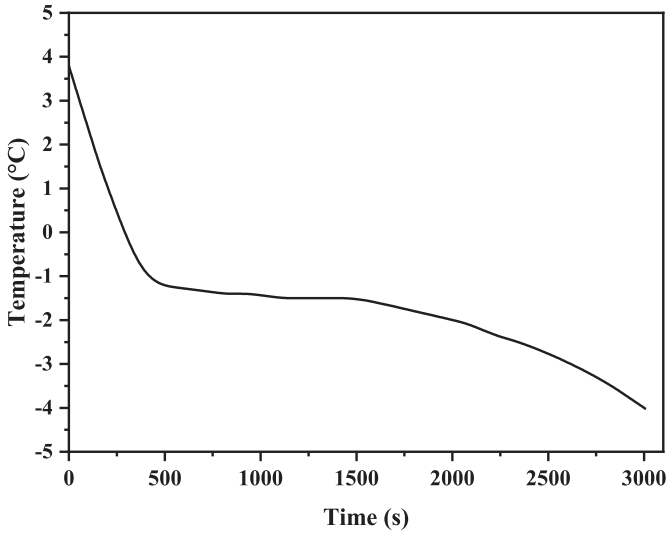

Fig. 1. Freezing curve of PFP.

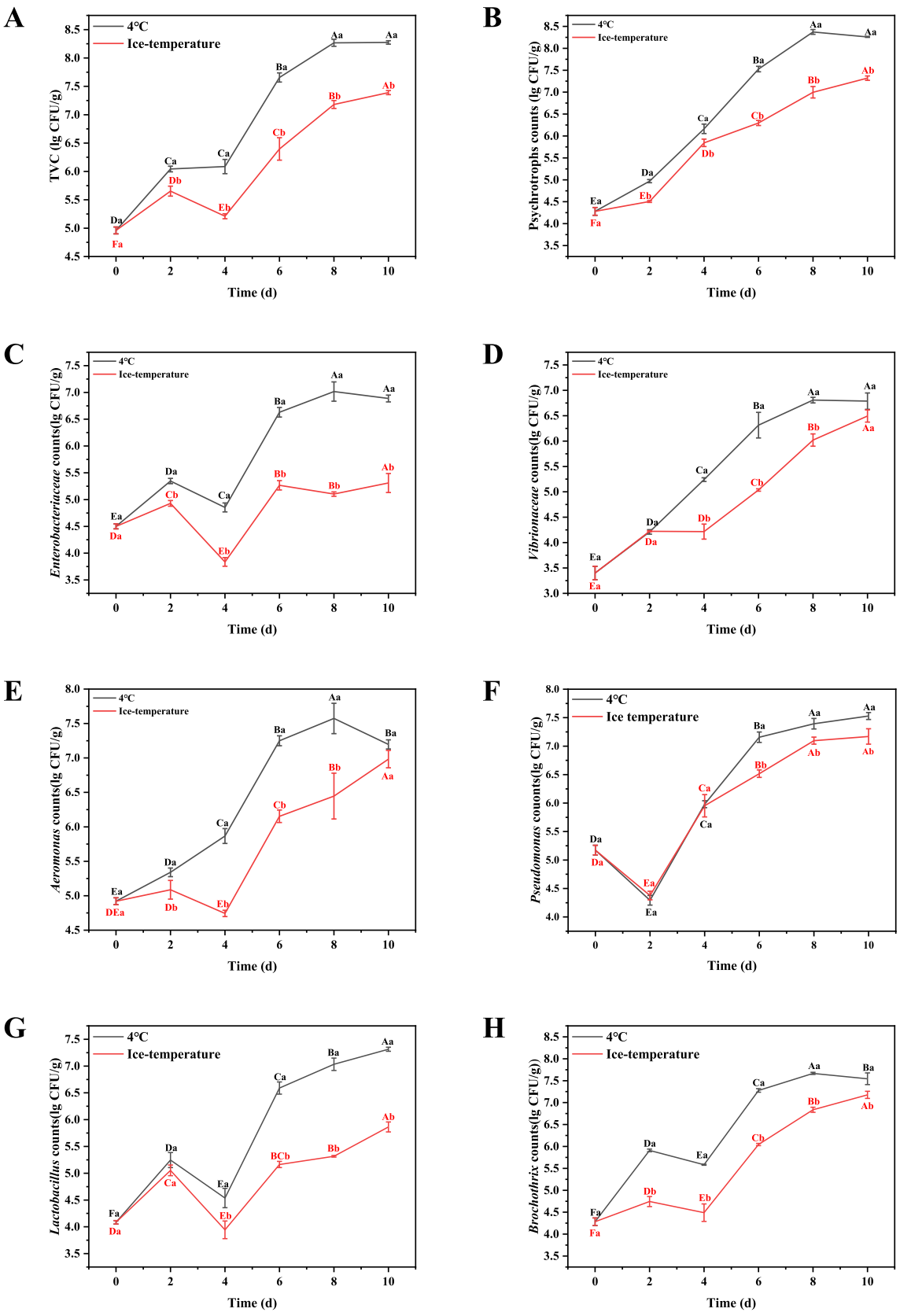

Fig. 2. Microbial counting of PFP during storage.
Note. Different upper case letters represent the significant differences within the storage time ($p<0.05$). Different lower case letters represent the significant differences within the storage temperature ($p<0.05$).

Psychrotrophs were the most prominent microflora during storage (Fig. 2B). For samples stored at 4 °C, psychrotrophs increased from 4.28 lg CFU/g (Day 0) to 8.37 lg CFU/g (Day 6) before ceasing to grow. Meanwhile, at ice temperature, psychrotrophs counts increased until Day 10 (7.32 lg CFU/g), without reaching a plateau, indicating inhibition by the ice-cold environment ($p < 0.05$). Most microbial activities were inhibited during refrigeration. However, psychrotrophs such as *pseudomonas* and *Shewanella* remained active, and contributed to the spoilage of refrigerated fish.

The contribution of *Enterobacteriaceae* to fish microflora should be considered because they can potentially contaminate the product during processing. Fig. 2C displays the *Enterobacteriaceae* count, which increased slowly during storage. Then, they decreased on Days 2–4, possibly because of their slower growth compared to other spoilage bacteria (Papadopoulos, Chouliara, Badeka, Savvaidis, & Kontominas, 2003). Subsequently, the *Enterobacteriaceae* counts rapidly increased again. For samples stored at 4 °C, *Enterobacteriaceae* counts reached the peak on Day 8 (7.02 lg CFU/g), followed by a slower growth rate. At ice temperature, the *Enterobacteriaceae* counts showed a slower increase after Day 6, indicating a 1.71 lg CFU/g lower count than that of the group stored at 4 °C. *Vibrionaceae,* including *vibrio parahaemolyticus*, *vibrio alginolyticus*, and *vibrio harveyi*, can contaminate aquatic products and cause vibriosis, potentially leading to severe cases of the disease (Santhana Lakshmi, Ranjani, & Hemalatha, 2023). As depicted in Fig. 2D, the growth of *Vibrionaceae* was little affected by low temperatures; it continued to increase during storage, reaching 6.79 lg CFU/g (4 °C) and 6.49 lg CFU/g (ice temperature), from an initial count of 3.4 lg CFU/g, the lowest among all bacterial species. The growth of *Aeromonas,* a foodborne pathogen with pathogenic potential for both fish and humans (Lee et al., 2023), is shown in Fig. 2E. At 4 °C, the counts of *Aeromonas* increased from 4.92 lg CFU/g to a maximum of 7.20 lg CFU/g on Day 8. Storage at ice temperature inhibited *Aeromonas,* with counts reaching 6.98 lg CFU/g on Day 10. The growth of *Pseudomonas*, a major contributor to spoilage bacterium in grass carp and a conditional pathogen (Zhang et al., 2020), is illustrated in Fig. 2F. The growth of *Pseudomonas* was slightly inhibited by the ice temperature (4.8%), despite having the highest initial count among all species (5.13 lg CFU/g). *Lactobacillus* and *Brochothrix,* both gram-positive bacteria, grew notably during storage. Fig. 2G displays the counts of *Lactobacillus*, which were sensitive to storage temperature. On the 10th day, the counts of samples stored at ice temperature were observed to be 19.8% lower than those of samples stored at 4 °C. Fig. 2H illustrates the counts of *Brochothrix*, a predominant spoilage microorganism in aquatic products (Illikoud et al., 2019). The ice temperature also suppressed the growth of *Brochothrix*. For samples stored at 4 °C, *Brochothrix* reached a peak of 7.67 lg CFU/g. By contrast, for samples stored at ice temperature, the final count of *Brochothrix* was 7.18 lg CFU/g, lower than the count of *Brochothrix* on the 6th day at 4 °C (7.28 lg CFU/g).

In conclusion, storage at ice temperature effectively inhibited the growth of spoilage bacteria, as demonstrated by the bacterial counts. Zhong et al. (2023) reported that microbial growth in amberjack fillets stored near the freezing point was delayed, extending the shelf life of the fish. Our results suggest that the 8th day marks a critical point at which the samples enter the late stage of spoilage.

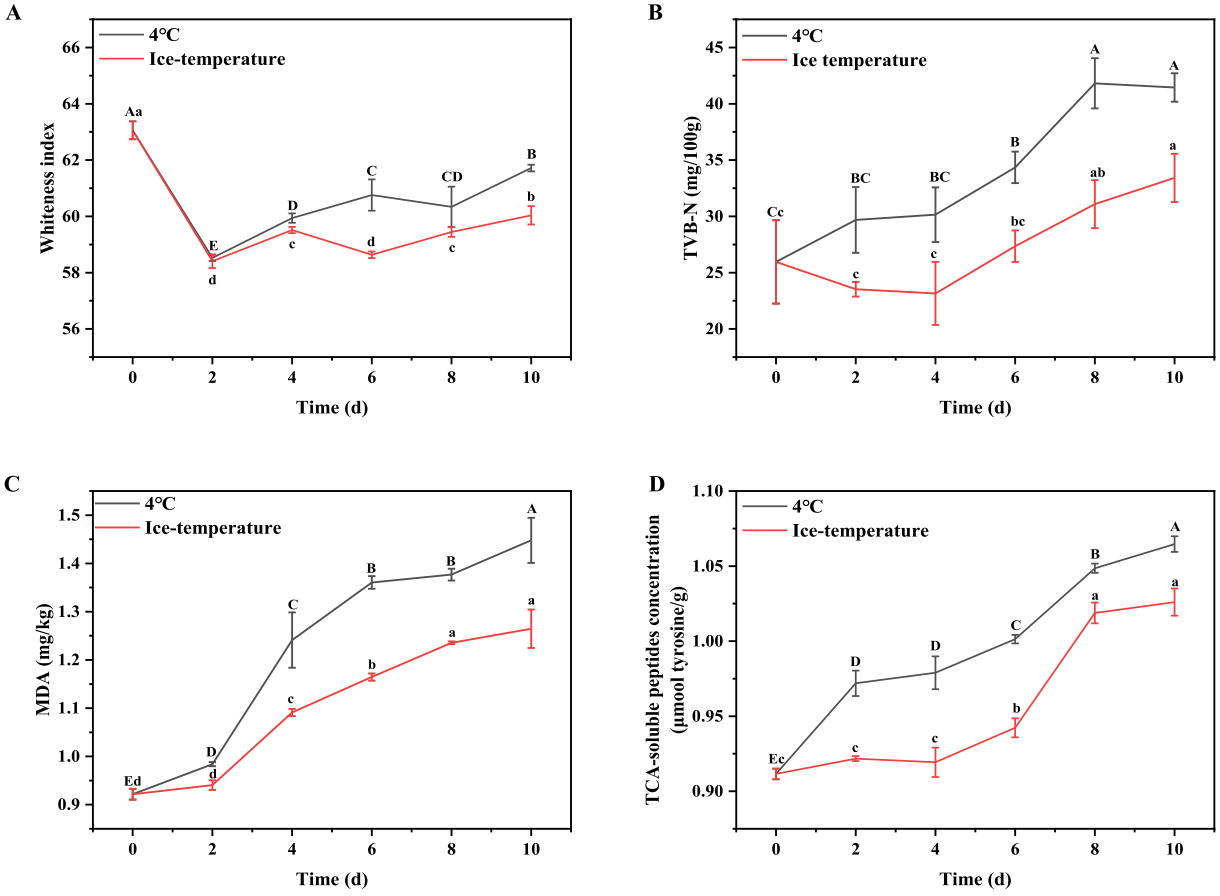

Fig. 3. Changes of the whiteness (A), TVB-N (B), TBARS (C), and TCA-soluble peptides in PFP storaged at different temperature.
Note. Different upper case letters represent the significant differences within the storage time ($p<0.05$). Different lower case letters represent the significant differences within the storage temperature ($p<0.05$).

3.3. Quality changes

3.3.1. Whiteness

Whiteness affects both the sensory qualities and commercial value of PFP. Consumers generally prefer products with higher whiteness, which is closely linked to the structure of muscle, protein composition, and water bonding (Liu et al., 2020). The whiteness of PFP experienced fluctuations when stored at different temperatures compared to fresh fish products ($p < 0.05$), followed by an increase over time (Fig. 3A). Samples stored at 4 °C and ice temperature exhibited similar trends. The primary reasons for the decrease in whiteness during early storage were endogenous enzymes and lipid oxidation (Liu et al., 2020). According to Cao et al. (2022), the decline in whiteness of the myofibrillar protein (MP) gel during storage is associated with a lower free water content caused by MP oxidative denaturation. With continued storage, the whiteness of PFP began to recover, potentially due to myoglobin decomposition. Furthermore, myofibrillar protein denaturation resulted in water and oil exudation, increasing the moisture content on the surface of samples and enhancing its whiteness (Liu et al., 2020).

3.3.2. E-nose analysis

An e-nose equipped with 10 metal-oxide gas sensors was used to detect the aromas of PFP. These sensors detected specific volatile classes. As shown in Figs. 4A and B, the PFP samples stored at 4 °C and ice temperature exhibited higher levels of nitrogen oxides, aroma components, inorganic sulfides and alcohols, indicating intensified spoilage. Moreover, samples stored at ice temperature for 10 days showed a notable presence of short-chain alkanes.

Principal component analysis (PCA) is a widely used analytical method in food science. Here, PCA was used to reduce the data complexity of by decreasing the dimensionality of multi-bit spatial matrices and retaining only a few principal components that represent the characteristics of the original variables as the feature values of the samples. Figs. 4C and D present three-dimensional PCA plots for PFP based on e-nose data. Under both storage temperature conditions, the combined contribution of all the principal components exceeded 99%, indicating that PCA captured information from multiple indicators (Ni, Wang, Zhan, Tian, & Li, 2021). Here, samples in the later stages of storage were distinguishable from those stored for shorter periods. As shown in Figs. 4C and D, separating the PFP samples stored for different periods was difficult, except for samples after 8 days. The PCA plot of the PFP stored at 4 °C overlapped for the first 6 days, differentiating these samples from spoiled samples, based on the odor detected by the e-nose. Samples stored for 8 and 10 days were separated from those of other storage durations along PC1 (64.0%). Furthermore, samples on the 8th day and 10th day were always along PC3 (3.0%), which could be attributed to the formation of similar volatile compounds and suggests that the e-nose can detect the freshness of PFP. For samples stored at ice temperature, the PFP on the 8th day overlapped with the 4th day along PC1 (68.2%). Only the samples on the 10th day were separated from the others, indicating spoilage and the accumulation of volatile compounds. Spoilage, primarily driven by bacterial activity, was the main cause of odor changes. Consistent with the total bacterial counts, an increase in bacteria led to the accumulation of objectionable odors, resulting in flavor deterioration.

Various alcohols, aldehydes, and ketones produced by microorganisms such as *Pseudomonas* spp., *Shewanella* spp., Enterobacteriaceae, *B. thermosphacta*, LAB, and *Photobacterium phosphoreum* accumulate during fish and meat spoilage (Parlapani, Mallouchos, Haroutounian, & Boziaris, 2014). Therefore, measures to control bacteria in aquatic

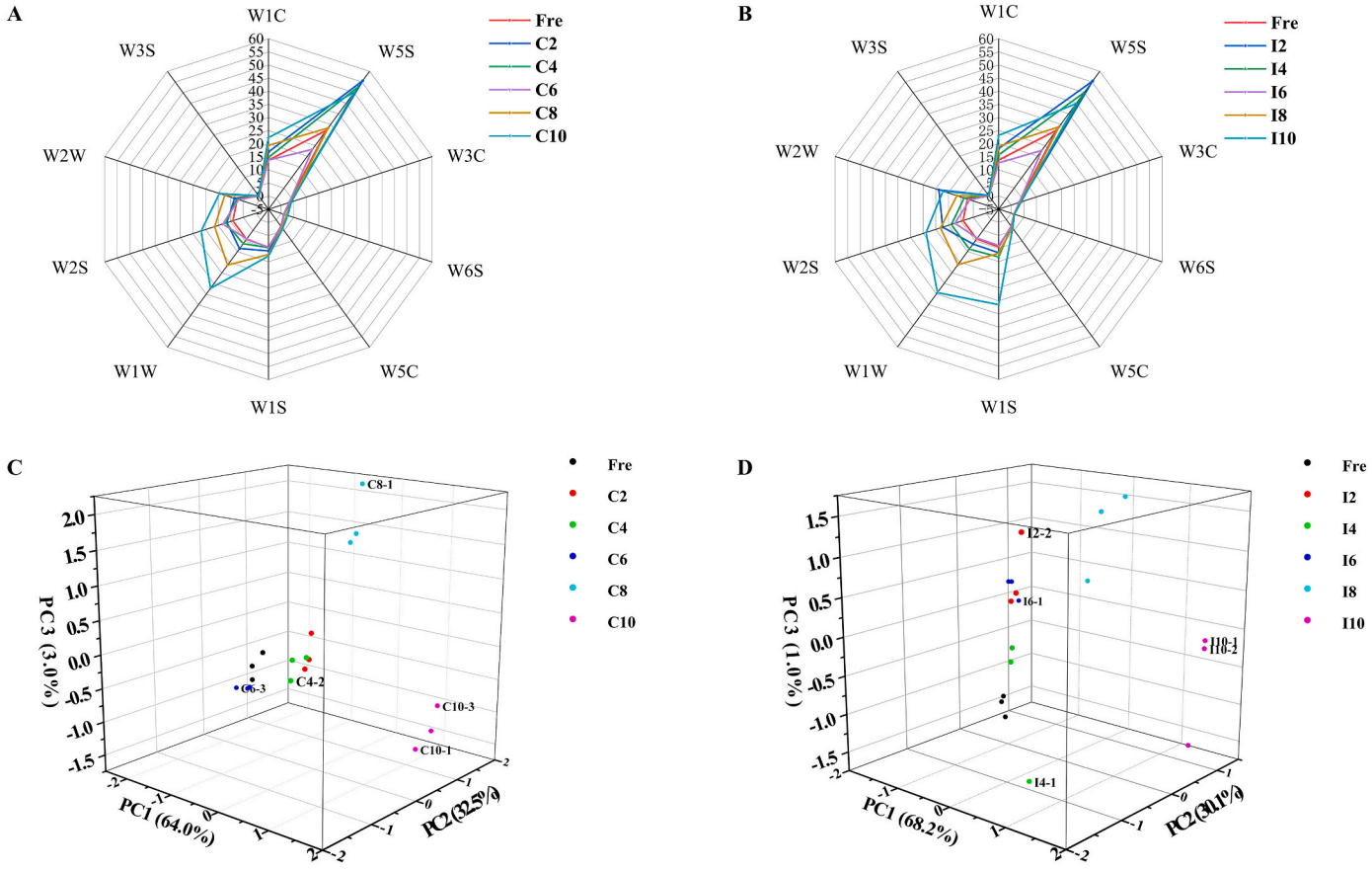

Fig. 4. Radar plots of PFP in different periods storaged at 4 °C (A) and ice temperature (B). Three-dimensional PCA plots performed on PFP with the results of e-nose at 4 °C (C) and ice temperature (D).

products should be implement to prevent spoilage.

3.3.3. TVB-N

Endogenous enzymes and spoilage bacteria degrade proteins and amino acids, resulting in the accumulation of TVB-N. This accumulation subsequently affects the sensory attributes of seafood. TVB-N serves as a direct indicator for assessing the quality and shelf life of prepared aquatic products. Fig. 3B illustrates the changes in the TVB-N content in PFP during storage, reflecting the freshness of PFP. The initial TVB-N content in PFP was 25.95 ± 3.70 mg/100 g which rose as the storage duration increased. On the 2nd day of storgae at 4 °C, the TVB-N had already exceeded the safety limit specified by the Chinese National Food Safety Standard (30 mg/100 g, GB/T 10136–2015). By contrast, samples stored at ice temperatures remained within the safety limits for up to 6 days. TVB-N in samples stored at 4 °C reached its peak on the 8th day (41.82 ± 3.23 mg/100 g), with a significantly higher content than that of the ice storage group (31.08 ± 2.14 mg/100 g). Likewise, Zhong et al. (2023) observed that storing greater amberjack at ice temperature lowered the TVC and TVB-N contents compared to storage at room temperature and refrigeration (4 °C). Thus, we can conclude that ice-temperature storage effectively delays the protein degradation caused by the growth and metabolism of spoilage bacteria. Hence, the quality assurance period for PFP stored at 4 °C is 2 days, whereas ice temperature extends the storage period to 6 days.

3.4. TBARS

Grass carp is a valuable source of polyunsaturated fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, these fatty acids are susceptible to oxidation, which may lower the quality of PFP (Cao et al., 2019). The content of malonic dialdehyde (MDA), a secondary metabolite resulting from the oxidation of fatty acids in meat, was assessed and expressed as TBARS. As depicted in Fig. 3C, the initial TBARS of the samples was 0.92 ± 0.01 mg MDA/kg, which increased over time. After 8 days, the TBARs of samples stored at 4 °C reached 1.37 ± 0.01 mg MDA/kg, a value significantly higher than those of the samples stored at ice temperature, indicating a more pronounced oxidation in PFP stored at 4 °C. The recommended TBARS for high-quality fish products is typically below 5–8 mg MDA/kg (Ovissipour et al., 2017). As reported by Cha et al. (2023), TBARS is related to protein, amino acids, and aldehydes. The content of MDA (an aldehyde) in PFP is consistent with the results of the e-nose analysis. Furthermore, the W2S response increased gradually with a longer storage time. Despite a notable increase in oxidation during storage, the samples still met the requirements for high-quality fish products.

3.5. TCA-soluble peptides

The TCA-soluble peptide content is an indicator of the small-molecule peptide content in PFP, offering insights into the extent of protein hydrolysis. These small molecular peptides result from the combined action of microorganisms and endogenous proteases (Liu et al., 2023). The TCA-soluble peptide content increased with a longer storage time (Fig. 3D), concurrent with the proliferation of spoilage bacteria. By the end of the storage period, TCA-soluble peptides indicated 1.06 μmol tyrosine/g (at 4 °C) and 1.03 μmol tyrosine/g (at ice temperature). A comparative analysis revealed that storing samples at ice temperature delayed the increase in TCA-soluble peptides, suggesting suppression of endogenous proteases and spoilage bacteria. In the initial storage stages, endogenous proteases played a dominant role in protein degradation. Subsequently, proteases and peptidases produced by microorganisms became the primary contributors to protein degradation in fish during storage (Liu et al., 2023). The microbiological counts agreed with the trend of TCA-soluble peptides, indicating that bacteria substantially affected protein degradation. Previous studies have consistently identified *Pseudomonas* as a predominant species in spoiled freshwater fish, confirming earlier assumptions about the predominant spoilage bacteria (Zhuang et al., 2021).

3.6. Microflora changes identified by high-throughput sequencing

3.6.1. Bacterial richness and diversity

Paired-end sequencing of DNA fragments of the bacterial community was conducted using the Illumina MiSeq platform. Quality control, denoising, splicing, and chimerism removal were performed using the DATA2 method. Sequences with a 97% similarity were clustered into operational taxonomic units (OTUs). After processing, a total of 5,576,246 sequences were obtained, with an average length of 428.64 bp. Taxonomic data were annotated using the Greengene database. Figs. 5A-H present the alpha diversity index, showing sequencing coverage rates exceeding 99% for all samples, indicating appropriate sequencing depth and reliable results. Based on this assessment, the microbiological diversity of the samples was evaluated following the approach outlined by Zhong et al. (2023).

We employed species accumulation curves to measure and predict the extent of bacterial richness in the community for various sample capacities. These curves are widely used to determine the optimal sample size for assessing bacterial richness, as suggested by Chao and Shen (2004). Figs. 6A and B present the species accumulation curves for PFP stored at 4 °C and ice temperature, both of which showed a plateau, indicating a sufficient sample capacity.

We assessed bacterial richness using the Chao 1 and observed species indices, while the Shannon and Simpson indices reflected the microbial diversity, following the method of Li et al. (2023). The Good's coverage index was used to determine the sample coverage degree. For PFP products stored at 4 °C, bacterial richness increased during the initial 4 days and then decreased in the subsequent 4 days. The Shannon and Simpson indices indicated a significant decrease in bacterial diversity during the first 6 days, followed by a slight increase in the last 2 days. This trend aligned with findings by Huang et al. (2020) and Li et al. (2023), who reported that the bacterial diversity in catfish fillets and hake fillets decreased during storage. By contrast, samples stored at ice temperature exhibited stable bacterial richness, which was lower than that observed in samples stored at 4 °C. During the last 4 days of storage at ice temperature, the samples had a lower Simpson index, suggesting higher bacterial diversity. This could be attributed to the lower storage temperature, which inhibited the growth of spoilage organisms and delayed potential quorum sensing in PFP. Similar results were reported by Zhong et al. (2023), indicating that lower storage temperatures lead to higher microbial flora diversity.

3.6.2. Bacterial community composition

Figs. 6C–F depict changes in bacterial communities of the PFP samples in terms of relative abundance. The analysis was focused on the top 20 phyla and genera. *Proteobacteria* were initially predominant at the phylum level (Figs. 6C and D), accounting for 69.81% of the bacteria and remaining the primary genus throughout storage. *Bacteroidetes* and *Firmicutes* initially represented 18.27% and 4.90% of the bacteria, respectively. Over time, *Firmicutes* increased to 52.88% at 4 °C and 21.00% at ice temperature, while *Bacteroidetes* decreased to 0.09% at 4 °C and 0.29% at ice temperature. Therefore, *Proteobacteria* and *Firmicutes* were identified as the predominant spoilage bacteria at the phylum level.

At the genus level (Figs. 6E and F), the initial bacterial community in the samples exhibited the following proportions: *Flavobacterium* (16.07%), *Psychrobacter* (12.57%), *Janthinobacterium* (9.83%), *Acinetobacter* (9.81%), *Stenotrophomonas* (9.73%), and *Pseudomonas* (6.54%). During storage at 4 °C, the levels of *Pseudomonas* and *Janthinobacterium* reached their peak on the 4th day, increasing to 26.89% and 15.84%, respectively, and then declined. At the end of storage, *Brochothrix* (26.93%), *Carnobacterium* (13.56%), and *Pseudomonas* (9.73%) were the dominant genera. When the PFP was stored at ice temperature,

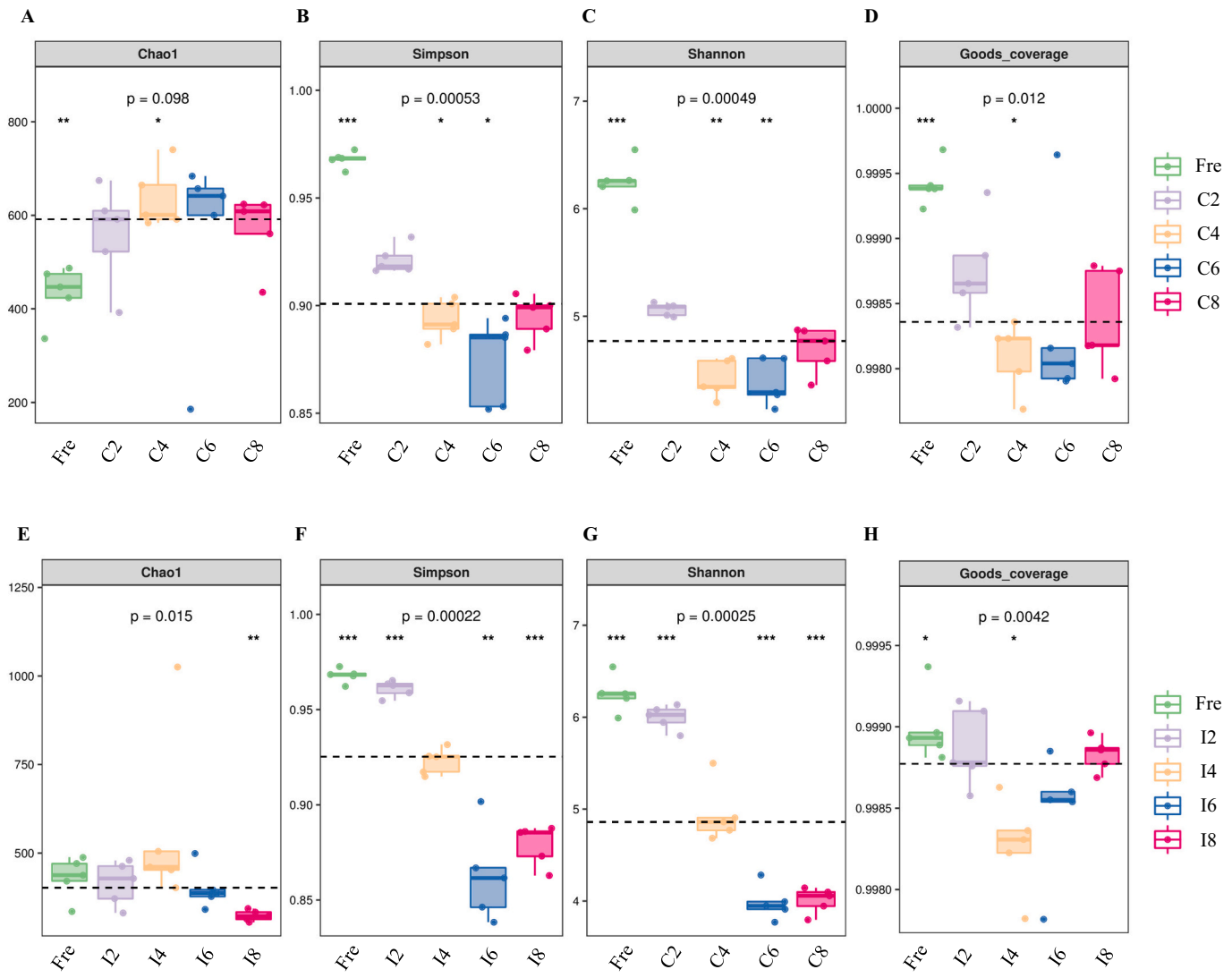

Fig. 5. Alpha diversity index of PFP during storaged at 4 °C (A-D) and ice temperature (*E*-H).

Acinetobacter, *Pseudomonas*, *Janthinobacterium* and *Brochothrix* substantially increased over time. No significant differences were observed in the predominant bacteria of the two temperature groups. These findings were consistent with previous research, which identified *Pseudomonas* as the primary microbial community in various food items, including chilled pork and Russian sturgeon fillets (Li et al., 2019; Shen et al., 2020). Moreover, Liu, Li, Li, and Luo (2018) reported that *Janthinobacterium* predominated in spoiled bighead carp fillets. Furthermore, *Carnobacterium* was identified as the predominant spoilage bacteria in grouper fillets (Yang, Yan, & Xie, 2023) and chilled monkfish (Wang, Kong, Yu, Jiang, & Han, 2023). The spoilage activity of *Brochothrix* was confirmed in salmon slices (Li et al., 2023). In summary, *Pseudomonas*, *Brochothrix*, *Carnobacterium* were the primary spoilage bacteria in PFP stored at 4 °C, whereas *Acinetobacter*, *Pseudomonas*, *Janthinobacterium* and *Brochothrix* predominated in PFP stored at ice temperature.

3.6.3. Beta diversity analysis

Figs. 6G and H present the results of PCoA for bacteria, using classical multidimensional scaling to arrange the distance matrix of the samples, as described by Ramette (2007). For storage at 4 °C, spoiled samples were primarily segregated from the others along Axis 2, which accounted for 23.7% of the variance. No significant differences were observed in the bacterial communities between the 6th and 8th days of storage. During storage, the samples were predominantly separated along Axis 1, representing a dissimilar in bacterial composition with a variance of 55.7%. Likewise, samples stored at ice temperature exhibited a comparable distinction to the 4 °C samples. All the samples were primarily differentiated along Axis 1, contributing to 74.8% of the variance, except for the 6th and 8th day samples, which exhibited similar bacterial communities. This finding agreed with Zhong et al. (2023), which reported that bacterial community variations decreased after storage at various temperatures.

4. Conclusion

This study investigated the influence of storage temperature on the quality deterioration and microbial composition of PFP. Regardless of the storage temperature, *Proteobacteria* and *Firmicutes* remained the predominant microbial phyla. An evaluation of the quality characteristics revealed that lower storage temperatures helped preserve the color and odor of PFP. Moreover, lower temperatures—particularly ice temperature—also helped delay the oxidation and degradation of nutritional components. Overall, this study provides a comprehensive understanding of the patterns in the bacterial community of PFP, offering valuable insights into the safety of processed aquatic products. The bacterial analysis at the genus level revealed that *Pseudomonas* and *Brochothrix* were the primary contributors to PFP spoilage at both storage temperatures. Furthermore, *Carnobacterium* spoiled the samples

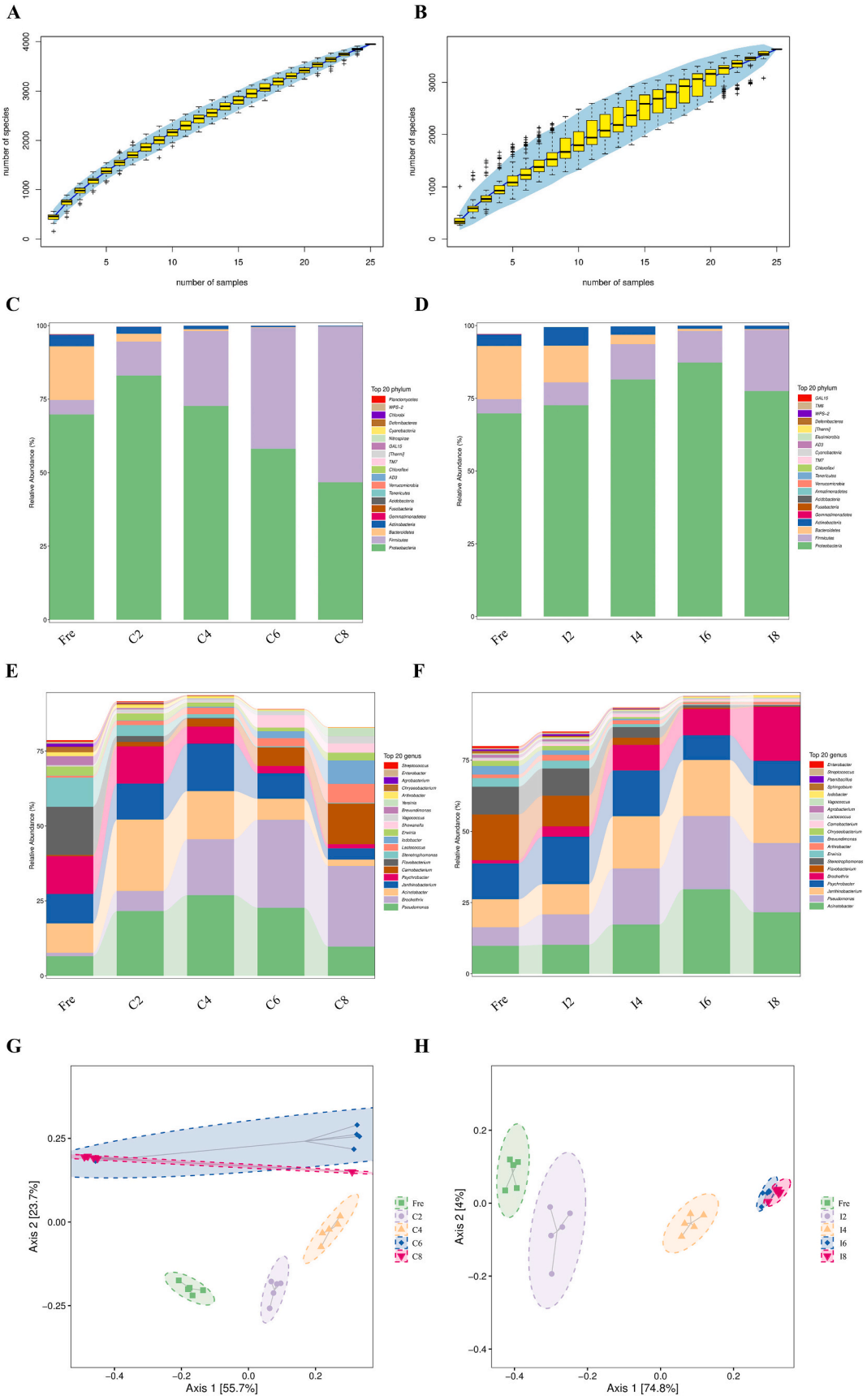

Fig. 6. Species accumulative curves of the prefabricated fish paste products storaged at 4 °C (A) and ice temperature (B); Bacterial communities in the prefabricated fish paste products storage at 4 °C (phylum: C, genus: E) and ice temperature (phylum: D, genus: F); PCoA of the prefabricated fish paste products storage at 4 °C (G) and ice temperature (H).

stored at 4 °C, whereas *Acinetobacter* and *Janthinobacterium* deteriorated the samples stored at ice temperature. However, this study did not delve into the quorum sensing among spoilage bacteria or propose specific solutions for extending the shelf life. Therefore, future studies may find it worthwhile to explore effective and safe bacteriostatic measures to maintain the freshness of PFP.

CRediT authorship contribution statement

Lin Zhang: Conceptualization, Data curation, Writing – original draft. **Lin Han:** Software, Formal analysis. **Jinye Yang:** Validation. **Qinxiu Sun:** Writing – review & editing, Conceptualization. **Ke Li:** Funding acquisition, Writing – review & editing. **Sangeeta Prakash:** Writing – review & editing. **Xiuping Dong:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

This work was funded by the National Key R&D Program of China (2023YFD2100200) and the Xingliao Talent Program of Liaoning Province (XLYC2202047), and supported by Key Laboratory of Cold Chain Food Processing and Safety Control (Zhengzhou University of Light Industry), Ministry of Education (2023).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101428.

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