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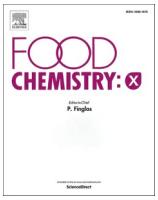

Application of *Ulva lactuca* polysaccharide in the preservation of refrigerated of *Lateolabrax maculatus* fillets

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ABSTRACT

This study aimed to explore the use of *Ulva lactuca* polysaccharide (ULP) as a preservative for perch (*Lateolabrax maculatus*) fillets stored under refrigeration at 4 °C. Fresh perch fillets were treated with ULP (7–10 kDa) and potassium sorbate, respectively, to evaluate their effectiveness in inhibiting bacterial growth and maintain freshness. A 0.5% ULP solution significantly decreased the pH value, total volatile basic nitrogen value, thiobarbituric acid value, and total bacterial count of perch fillets. ULP solution delayed the changes in whiteness and texture of fillets, as well as protein degradation. The acute toxicity experiment further evaluates the safety and reliability of ULP. Simultaneously, utilizing 16S rRNA techniques, the ULP solution inhibited microorganisms known for their strong spoilage capabilities, such as *Pseudomonas*, *Actinetobacter*, and *Shewanella*. Microorganisms with a weaker ability to cause corruption became the dominant bacteria, such as *Acetobacter*, *Lactobacillus,* and *Faecalibacterium,* thereby exerting a degree of inhibition against spoilage.

1. Introduction

The deterioration of aquatic products has consistently been a bottleneck in the sustainable development of the food industry. Discovering efficacious preservation strategies for aquatic products is a top priority (Jia, Roy, Pan, & Mraz, 2022). Perch, with its superior nutritional profile rich in protein, fatty acids, carbohydrates, trace elements, and vitamins, is also prone to spoiled (Hung Quang et al., 2023). The proliferation of spoilage microorganisms coupled with endogenous enzymes that cause the hydrolysis of muscle proteins and the oxidation of lipids, makes fresh perch fillets susceptible to spoilage (Jeon, Kamil, & Shahidi, 2002). Consequently, the short shelf life of perch presents a formidable obstacle to their widespread distribution and marketing, thereby reducing its commercial value (Howard et al., 2023). The escalating demand for chilled fish with an extended shelf life has spurred significant innovations, including the utilization of natural antimicrobial agents such as phenolic antioxidants, essential oils, natural polymers, and microorganisms (Baptista, Horita, & Sant'Ana, 2020). There is an ever-mounting emphasis on adopting natural, safe, and ecologically sound alternatives in the realm of antimicrobial intervention.

In recent decades, the emergence and proliferation of green tides have attracted significant attention due to their substantial ecological implications (Lv et al., 2022). Among the culprits, *Ulva lactuca* has emerged as a primary cause of these algal blooms, posing severe environmental challenges in China's aquatic environments (Xia et al., 2016). Recognizing the imperative need to mitigate the adverse impact of green tides on marine organisms and aquaculture, concerted efforts have been dedicated to explore the potential applications of seaweeds. Extensive scientific investigations have elucidated the presence of polysaccharide within the complex cell wall composition of *Ulva* algae, with water-

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soluble sulfated polysaccharide being a key component. The monosaccharide profile of *U. lactuca* polysaccharides (ULP) encompasses a diverse range, featuring glucose, rhamnose, arabinose, xylose, mannose, galactose, fucose, aminoglycoside, and glucuronic acid (Gomez et al., 2020). Furthermore, extracting ULP has revealed a multifaceted spectrum of bioactive compounds, offering a variety of physiological benefits, including antioxidative, immunomodulatory, hypoglycemic, hypolipidemic, antiaging, anticancer, antiradiation, anti-inflammatory, analgesic, and phytodefensive activities (Chen et al., 2022).

Currently, algae polysaccharides like chitosan, sodium alginate, and agar are known for their antimicrobial, antioxidant, and biocompatible functions, commonly used in food preservation and enhancing the physicochemical properties of foods (Tomadoni, Fabra, & Lopez-Rubio, 2022). The antibacterial properties of two fucoidan glycans from *Fucus vesiculosus* at different purification stages were observed, with a minimum inhibitory concentration of 4–6 mg/mL highlighting these glycans' effectiveness in reducing bacterial proliferation (Ayrapetyan et al., 2021). The extracted solution from waste macroalgae *Enteromorpha prolifera* polysaccharides (EPP) was used as a preservative for cherry tomatoes, significantly reducing the disease and rot compared to the untreated group. After 36 days, EPP-treated cherry tomatoes were at a commercially acceptable level three times higher than untreated group (Wu et al., 2023). To investigate the effects of polyphenols and polysaccharides derived from *Porphyra yezoensis*, Pacific white shrimp samples were subjected to treatment with 5 g/L polyphenols and 8 g/L polysaccharides. Subsequently, the samples were stored at a temperature of 4 ± 1 °C for eight days. The results indicated that the application of polyphenols, polysaccharides, and the combination of polyphenols and polysaccharides effectively inhibited lipid oxidation, protein hydrolysis, and microbial growth. Furthermore, these treatments efficiently maintained the sensory properties of the shrimp within acceptable limits compared to the control group (Li, Yang, & Li, 2017).

In this study, different concentrations of ULP (7–10 kDa) were used to preserve perch fillets. The structure of ULP was initially characterized by Fourier transform infrared spectroscopy, X-ray diffraction, and thermal stability analyses. During cold storage with ULP treatment, parameters such as the pH, whiteness, total volatile basic nitrogen (TVB-N) value, thiobarbituric acid (TBA) value, total bacterial count, texture, and protein degradation of perch fillets were measured to assess the preservation effects. Furthermore, an acute toxicity experiment assessed ULP's safety and reliability, while 16S rRNA sequencing explored the bacterial flora composition of ULP as a bacteriostatic agent during cold storage. This study demonstrated the effectiveness of seaweed polysaccharide as a preservative, providing a novel solution for utilizing *U. lactuca* and preserving *Lateolabrax maculatus* fillets.

2. Materials and methods

2.1. Preparation of ULP

U. lactuca powder was purchased from Fuzhou Fudovang Biotechnology Co., Ltd. The powder was dissolved in distilled water at a mass ratio of 1:40, followed by ultrasound extraction at 60 °C, 45 kHz, and 200 w/h for 1 h. The supernatant was then filtered and concentrated, with the product being precipitated in ethanol overnight. The precipitate was centrifuged, lyophilized, crushed and weighed, and then the enzyme was digested with neutral protease (Solarbio, China) for two hours, and the enzyme was inactivated at 100 °C. The supernatant was again centrifuged, dialyzed for 2 days, and lyophilized to obtain *Ulva lactuca* polysaccharide, which was then prepared into a 1% polysaccharide solution. After concentration and ethanol precipitation, the supernatant was concentrated. Preliminary experiments using a dialysis bag determined the molecular weight segments of ULP to be between 7 and 10 kDa in this experiment.

2.2. Fourier transform infrared (FT-IR) analysis of ULP

A 5 mg ULP sample was accurately weighed, and 100 mg of potassium bromide dried after high-temperature treatment was added. The mixture was then stirred well and pressed after thorough grinding. The FT-IR spectra were recorded with Thermo Nicolet Nexus 670, scanned from 4000 cm^{-1} to 500 cm^{-1}.

2.3. X-ray diffraction (XRD) analysis of ULP

XRD measurements were conducted with PANalytical's X'pert3 Powder X-ray diffraction system. A voltage of 40 kV and current of 40 mA were applied while scanning within 10°- 90° in the 2θ range to observe the crystalline structure of ULP particles.

2.4. Analysis of the thermal properties of ULP

A 5 mg ULP sample was placed in the sample cell of the thermogravimetric analyzer, and the measurement temperature range was set between 30 and 1000 °C with the heating rate set to 10 °C/min to obtain the thermogravimetric (TG), differential thermogravimetric analysis (DTA), and derivative thermogravimetric (DTG) curves of ULP.

2.5. Pretreatment of perch

Live perch (*Lateolabrax maculatus*), weighing approximately 650 ± 50 g, were acquired from a local seafood market for the experiment. The head, tail, skin and internal organs were removed from the fish to prepare for subsequent analysis. Following the preparation process, two fillets were extracted from the back of each specimen. These fillets were promptly placed on ice and transported to the laboratory within a maximum of 30 min to maintain freshness. In the normal control (NC) group, the perch fillets were immersed in sterile water, while in the treatment group, they were submerged in a 1% potassium sorbate (PS) solution. In the treated group, the perch fillets were immersed in solutions containing 0.5%, 1%, and 2% ULP. After precisely 30 min of immersion, the fillets were removed and allowed to air dry. For the purpose of preservation, the treated perch fillets were individually packed into sterile bags, properly labeled, and stored in a refrigerator set at a constant temperature of 4 ± 1 °C. Because the acceptance of perch is generally <8 days of 4 °C, the experimental period is set to 8 days (Kaur & Singh, 2021). To monitor the effects of the different treatments over time, samples are taken from the 4 °C refrigerator every 2 days, starting from Day 0 until Day 8.

2.6. Determination of pH of perch fillets

Two grams of chopped perch fish fillet were placed in a 50 mL centrifuge tube, mixed with 18 mL of distilled water, and left for 30 min. The pH value of perch fish meat was determined 3 times every 2 days.

2.7. Thiobarbituric acid reactant (TBA)

The TBA value was measured according to the method of Nowzari, Shabanpour, and Ojagh (2013), with a slight modification. Two grams of chopped perch fillet was accurately weighed and placed in 5 mL of ultrapure water. An equal amount of 20% trichloroacetic acid (TCA) solution was added, and the mixture was homogenized. The sample was left to stand at room temperature for 30 min and filtered through filter paper. The sample was heated in a thermostatic water bath at 80 °C for 40 min and then cooled in cold water, and the absorbance was measured at 532 nm. For the preparation of the standard curve, 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, China) was used as the standard. The standard was dissolved in a 20% TCA solution and subsequently diluted to a concentration of 3.15 μg/mL, equivalent to 1 μg of malondialdehyde (MDA) per. To quantify the TBA value, the amount of MDA formed as a

result of lipid oxidation, the concentration was expressed as milligrams of MDA per kilogram of sample.

2.8. Total volatile basic nitrogen (TVB-N)

The TVB-N of fish was measured according to the method of Khulal, Zhao, Hu, and Chen (2016), with a slight modification. Two grams of chopped perch fish meat were weighed, and 20 mL of distilled water was added to the digestion tube. The sample solution was shaken to ensure that it was dispersed uniformly and impregnated for 30 min. After the end of the impregnation, 0.25 g of magnesium oxide was added and immediately connected to a distiller. An automatic Kjeldahl nitrogen instrument (Kjeltec 800, Denmark) was used for determination 3 times every 2 days.

2.9. Determination of total bacterial count of perch fillets

We weighed 2.5 g of clipped perch fillet samples and placed them in a conical flask containing 22.5 mL of sterile saline. The samples were shaken for 2 min, and homogenate was subjected to serial decimal dilution. Subsequently, 1 mL diluted sample was pipetted for the determination of total viable counts using the plate pouring method. This method facilitates the enumeration of viable microorganisms by providing suitable conditions for their growth and colony formation on agar plates.

2.10. Texture analysis

Textural analysis was conducted used for measurements by a texture analyzer (TA-XT. Plus, SMS, Surrey, UK). The fish flesh in the center of the perch fillets was cut into 1 cm^3 cubes along the transverse stripe of the back muscles. The samples were tested three times by using a texture analyzer and a flat-bottomed cylindrical probe to simulate the human teeth during food chewing (the test conditions were as follows: the speed before the test was 5 mm/s, the test speed was 1 mm/s, the speed after the test was 5 mm/s, the compression ratio was 50%, and the residence time interval was 5 s. Type of load-bearing probe: Auto 5 g, data acquisition speed: 200 Hz, ambient temperature: 12–16 °C). A sample was measured 3 times. The measured textural data included hardness, springiness, resilience, and chewiness.

2.11. Determination of whiteness of perch fish

The whiteness of fish was measured according to the method of Otero, Perez-Mateos, and Lopez-Caballero (2017), and the back muscle of perch was taken.The L* (lightness), a* (redness), and b* (yellowness) values were determined by a colorimeter, and the whiteness value was calculated. Whiteness was calculated based on L*, a*, and b* values, according to Eq. (1).

$$\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^*} \quad (1)$$

2.12. Sensory analysis

Sensory analysis of bass fillets was carried out using the quantitative emotional consumer test as described by Mörlein (2019). During storage, sensory evaluation was conducted by a team of 10 trained laboratory members (5 males and 5 females). The team members evaluated the color, smell and texture of the bass samples, and then rated them on a 10-point happiness scale (1 = dislike them very much; 10 = like it very much). The average score of all members represents sensory preference.

2.13. Extraction myofibrillar fibrillar protein

Following the method outlined by Chin, Go, and Xiong (2009), with slight modifications. The 3.0 g bass flesh was taken and mixed with 15 mL of prepared 10 mmol/L Tris-HCl (pH 7.2), homogenized. The homogenate was then centrifuged at 4 °C, 10000 r/min for 15 min. After centrifugation, the supernatant was removed, and 15 mL of prepared 10 mmol/L Tris-HCl buffer solution (containing 0.6 mol/L NaCl, pH 7.2) was added to the precipitate. The mixture was homogenized again and centrifuged (4 °C, 10000 r/min) for 10 min. The supernatant obtained was the solution of myofibrillar protein, which was stored at −80 °C.

2.14. Determination of the total carbonyl content of myofibrillar proteins

The total carbonyl content was measured using the method described by Vuorela et al. (2005). One milliliter of the extracted myofibrillar fibrillar protein solution from perch fillet was transferred into a 50 mL centrifuge tube, 1 mL of 10 mmoL/L (2,4-dinitrophenyl)-hydrazine solution was added, and the tube was reacted at room temperature for 1 h. Every 15 min, the mixture was vortexed, followed by the addition of 1 mL of 20% TCA solution to halt the reaction. Following centrifugation to collect the precipitate, it was washed with 1 mL of an ethyl acetate: alcohol (1:1) solution. After washing, the precipitate was centrifuged, and the procedure was repeated three times. Then, 3 mL of 6 mol/L guanidine hydrochloride solution was added to the precipitate and incubated at 37 °C for 20 min. At the end of the reaction, the supernatant was centrifuged, and absorbance was measured at 370 nm, allowing the carbonyl content to be calculated as nmol/mg protein, which was calculated by Eq. (2).

$$\text{Carbonyl content} = \varepsilon^*a^*b \quad (2)$$

where ε is the molecular absorbance coefficient, 22,000 L/moL·cm^{-1}; a is the width of the cuvette; and b is the concentration of myofibrillar protein solution.

2.15. Determination of total sulfhydryl content of myofibrillar proteins

The sulfhydryl content determination was based on Ellman's method (Ellman, 1959), with a slight modification. One milliliter of the extracted myofibrillar protein of perch fish flesh was stirred with 9 mL of 0.2 mmol/L Tris-HCl solution, and 4 mL of the above mixture was mixed with 0.4 mL of 5,5′-dithiobis-(2-nitrobenzoic acid) and put into the reaction at 40 °C for 25 min. Then, the absorbance value was measured at 412 nm, which was calculated by Eq. (3)

$$\text{Sulfhydryl content (nmoL/mg protein)} = (A^*n)/(\varepsilon^*\rho) \times 10^6 \quad (3)$$

where A is the absorbance at 412 nm; N is the number of dilutions; ε is the molar absorbance coefficient, 13,600/L/(moL·cm^{-1}); and ρ is the concentration of myofibrillar protein (mg/mL).

2.16. Determination of ca $^{2+}$-ATPase activity

One gram of minced perch was weighed, and 9 mL of saline was added. Homogenization was carried out for 30 s to obtain the protein solution, which was used for determining Ca^{2+}-ATPase activity using the Ca^{2+}-ATPase Assay Kit (Jiancheng Bioengineering, Nanjing, China), while the bicinchoninic acid (BCA) Assay Kit (Lablead, China) was used for quantifying the protein concentration.

2.17. Acute toxicity experimental study in mice

The maximum tolerated dose (MTD) (gavage amount of 5000 mg/kg) used the method described by Liu, Zhao, Wen, and Zhao (2022). ICR mice (SPF grade) were provided by the Zhejiang Laboratory Animal Center, weighing 18–22 g and 4 weeks of age. Twenty mice in the ULP group (5000 mg/mL) were in the ICR clean-grade, with 10 males and 10 females. Twenty mice in the NC group were in the ICR clean-grade, with 10 males and 10 females. The experiments were conducted for 14 days,

with mice's body weights recorded bi-daily, and the organ weights and blood biochemistry were analyzed at the end of the fourteenth days.

2.18. Extraction of bacterial DNA and sequencing of amplified 16S rRNA region of samples

Perch fillets from the NC and ULP groups on Day 0, Day 4, and Day 8 of refrigeration were selected, and the samples were renamed as follows: NC1 (Day 0), NC2 (Day 4), NC3 (Day 8), ULP1 (Day 0), ULP2 (Day 4), and ULP3 (Day 8). The samples were shaken at 100 r/min in a constant temperature shaker at 37 °C for 15 min. The supernatant was discarded, and the precipitate was placed into a sterile centrifuge tube to obtain the bacterial bodies of perch fillets at different refrigeration times. After pulverization with liquid nitrogen, the sample's genomic DNA was extracted via the cetyl trimethylammonium bromide method. Subsequently, the purity and concentration of the DNA were assessed via agarose gel electrophoresis. An appropriate amount of the DNA sample was then transferred to a 1.5 mL centrifuge tube. To obtain a concentration of 1 ng/μL, the sample DNA was diluted with sterile water. For sequencing purposes, the genomic DNA served as the template. Specific primers containing barcodes were utilized based on the desired sequencing region. PCR amplification was performed using the diluted genomic DNA as the template and the designated primers with barcodes corresponding to the chosen sequencing region. For sequencing library construction, we used the TruSeq® DNA PCR-Free Sample Preparation Kit. Following library construction, the constructed library was quantified using Qubit and quantitative PCR (Q-PCR) methods.

2.19. 16S rRNA sequence analysis

Based on the barcode sequence and PCR amplification primer sequences, each sample's data were separated from the downstream data. The barcode and primer sequences were truncated to obtain spliced raw tags. To ensure data quality, tags with consecutive high-quality bases accounting for <75% of the tags' length were filtered out. The purpose of this filtering process was to extract valid data. To gain insight into the species richness and evenness within the samples, as well as the common and unique operational taxonomic units (OTUs) among different samples or subgroups, various analyses were performed. These included alpha diversity calculation, beta diversity analysis, and the generation of Venn diagrams. Additionally, OTUs were compared using multiple sequences, and their distribution was visually represented through PCoA, PCA dimensionality reduction analysis, and a sample clustering tree. To further investigate differences in community structure among grouped samples. *t*-test statistical analysis was employed, this analysis method evaluates the significance of differences in species composition and community structure within the grouped samples.

2.20. Statistical analysis

All experiments were performed three times. The average and standard deviation values were recorded. All data were statistically analyzed by t-test, in which $p < 0.01$ was considered significantly different and $p < 0.05$ was considered different.

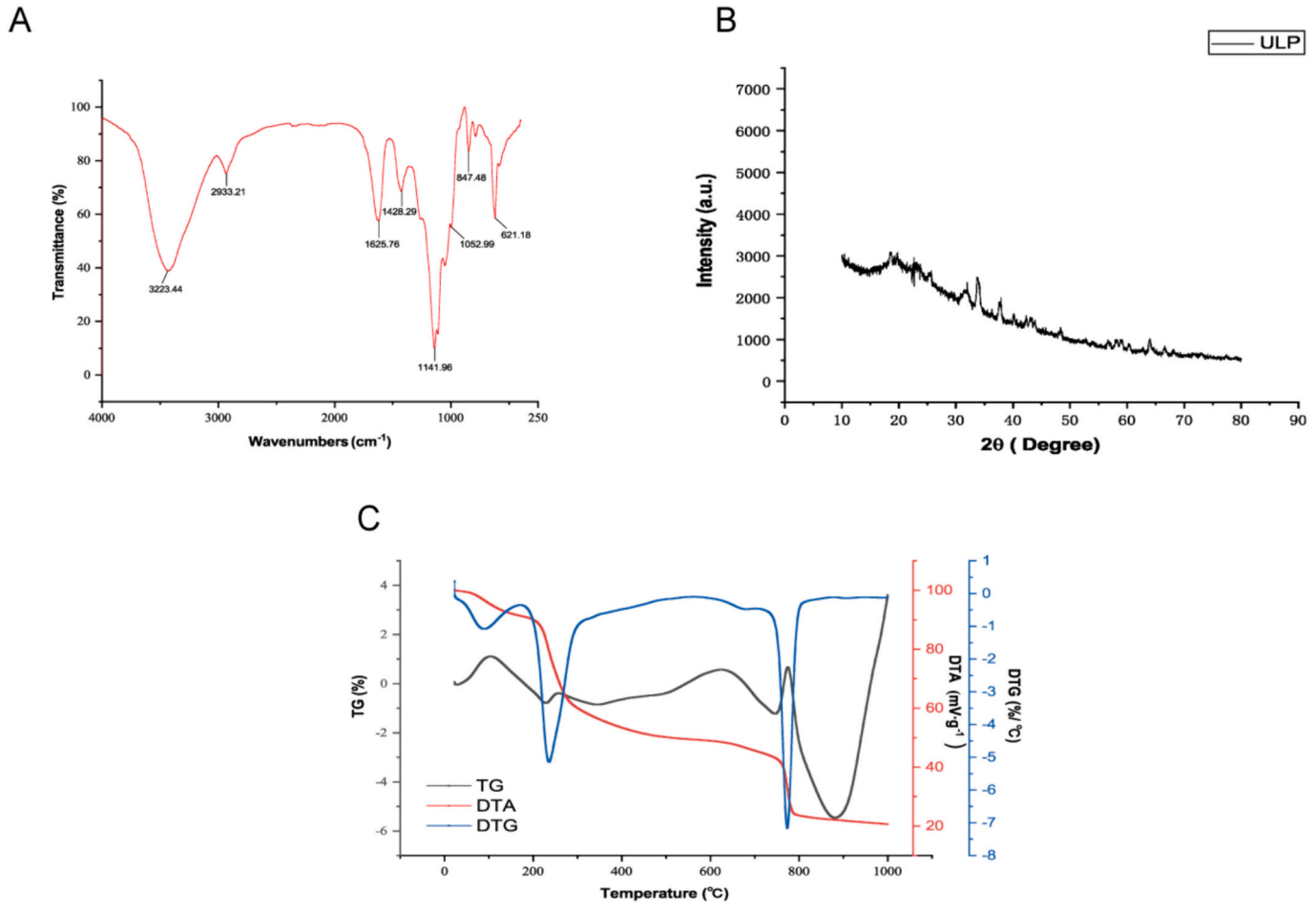

Fig. 1. Structural identification of ULP. (A) Fourier transform infrared (FT-IR) of ULP, (B) X-ray diffraction (XRD) of ULP, (C) Thermal properties of ULP.

3. Results and discussion

3.1. Content and structure of ULP

The absorption spectrum of ULP revealed several distinctive peaks at specific wavenumbers, each corresponding to various molecular vibrations. The FT-IR spectra ranging from 4000 cm^{-1} to 500 cm^{-1} showed the characteristic photoenergetic groups of ULP (Fig. 1A). The broad stretching peak at 3423 cm^{-1} is attributed to the O—H stretching vibration, a typical characteristic of polysaccharides. The peak at 2933 cm^{-1}, indicating weaker C—H stretching and bending vibration absorptions, is characteristic of such molecular interactions. The absorption peak at 1625.76 cm^{-1} represented the expansion and contraction vibration of C═O in carboxylic acids, indicating the presence of glyoxylate. The absorption peak at 1428.29 cm^{-1} was caused by the C—O expansion and contraction vibration. The two absorption peaks at wavelengths of 1141 cm^{-1} and 1052 cm^{-1} were caused by the expansion and contraction vibrations of the C-O-C glycosidic bond on the pyran ring, and the peaks at 847 cm^{-1} were attributed to the C-O-C glycosidic bond on the pyran ring. Moreover, the absorption peak at 847.48 cm^{-1} can be attributed to α-type glycosidic linkages within the polysaccharides (Ellman, 1959). Bands across the 1300–500 cm^{-1} region arose from the vibrational modes associated with the sulfate groups' C-O-S and S-O-S stretching vibrations. These vibrations manifested as absorption peaks at wavenumbers 1249 cm^{-1} and 609 cm^{-1}, respectively. Thus, it could be concluded that ULP comprises various types of bonds, including O—H, C—H, C═O, C—O, C-O-C, C—H, C—H, C-O-S, and S-O-S.

XRD is a widely acknowledged technique for assessing the crystalline and amorphous properties of biopolymers (Liu et al., 2022). The majority of polysaccharides exhibited either amorphous or semicrystalline structures. Notably, the intermolecular bonds within the amorphous region were weaker than those within the crystalline region (Wei et al., 2023). As a result, amorphous polysaccharides possess enhanced solubility and water absorption properties. The XRD results recorded between 10° and 90° indicated that the polymer tended to be amorphous in nature, and the characteristic sharp peaks suggested that the monosaccharides may have crystallized in combination with impurities such as peptides and ions, which displayed sharp characteristic peaks. No peaks were observed in other ranges, indicating that the ULP (7–10 kDa) structure was amorphous and crystalline (Fig. 1B). Valerio Filho et al. (2023) reported that ULPs were also amorphous with crystals present in the structure.

TG-DTG-DTA thermal analysis is a widely utilized technique to assess the thermal or oxidative stability, measure weight variations, and determine crucial parameters such as the melting temperature and glass transition temperature of biopolymers. These analytical methods offer valuable insights into the thermal properties and behavior of biopolymers (Zhang et al., 2016). TG curves revealed that the initial thermal decomposition stage of ULP occurred within the 0–200 °C range, involving the breakdown of free water, bound water, and heat-sensitive compounds within the polysaccharides. As the temperature rose, the second stage was concentrated at 200–800 °C, in which the long chain of ULP broke down and decomposed. In the third stage ranging from 800 to 1000 °C, the ULP decomposition was essentially completed, but some residues still remained. The DTA curve indicated that ULP undergoing a process characterized by four peaks of heat absorption and decomposition, with the peaks near 100 °C likely due to water evaporation. The DTG curve illustrated the rate of weight loss, pinpointing the temperature at which this loss peaked. The maximum weight loss of ULP was at 220 °C and 780 °C, which was mainly due to thermal degradation (Fig. 1C).

3.2. Effect of ULP on the quality change of perch fillets during refrigeration

3.2.1. pH, TBA, TVB-N, total bacterial count

Changes in the pH value of perch fillets during refrigeration at 4 °C are shown in Fig. 2A. All treatment groups exhibited a trend of initial decrease followed by an increase throughout the refrigeration period. This may be attributed to the degradation of glycogen into lactic acid and the simultaneous catabolism of ATP and phosphocreatine into phosphate ions, leading to a decrease in the pH value (Cai et al., 2022). As fish storage time extends, the decomposition of proteins into trimethylamine and other substances causes a gradual increase in pH (Li et al., 2012). From Day 2 to Day 8, the pH of ULP treatment group decreased significantly ($p < 0.05$) compared to the NC group, this phenomenon can be ascribed to the structural features of ULP that contain a large amount of acidic functional groups, reduced the surface pH of perch fillets (Jannat-Alipour, Rezaei, Shabanpour, Tabarsa, & Rafipour, 2019).

The TBA value measures the extent of oxidative decomposition in animal lipid. A higher TBA value indicates a greater degree of lipid oxidation (Wu, Bak, Goran, & Tatiyaborworntham, 2022). Specifically, when the TBA value in meat samples exceeds 0.5 mg/kg, it leads to the production of oxidative odors (Yu, Greish, McGill, Ray, & Ghandehari, 2012). Relative to the NC group, the ULP groups were effective in suppressing lipid oxidation in the perch fillets, with the 0.5% ULP treatment group being more efficient. On Day 8, the TBA values of the ULP groups were below 0.5 mg/kg (Fig. 2B). This result indicated that ULP has excellent antioxidant capacity to prevent lipid peroxidation and reduce malondialdehyde production in meat samples.

The TVB-N measurement acts as an indicator of meat freshness. Notably, the level of freshness in meat samples is inversely correlated with the TVB-N value. Meat is considered completely spoiled when its TVB-N exceeds 30 mg N/100 g (Kostaki, Giatrakou, Savvaidis, & Kontominas, 2009). The NC group approached the limit value of 30 mg N/100 g on Day 5, while the PS-treated and 0.5% ULP-treated groups reached the limit value around Day 8 (Fig. 2C). This could be because the 0.5% ULP solution-treated group retarded the rate of protein degradation (Li et al., 2021).

A colony count of 6 Log CFU/g indicates complete spoilage (Zhang et al., 2021). In this study, the initial total bacterial count was 3.1 Log CFU/g, indicating the fillets were of good quality. On Day 8, the total number of bacteria reached 7.61 Log CFU/g in the NC group,which indicated that the samples were at the end of their microbiological shelf-life, while those in the PS-treatment, 0.5% ULP-treatment groups were 5.52 Log CFU/g, and 5.80 Log CFU/g, respectively. The results indicate that the 0.5% ULP solution could effectively retard the growth of bacteria (Fig. 2D).

3.2.2. Texture analysis and characterization of protein

Texture characteristics can better reflect the histological characteristics of perch fillets (Cardinal et al., 2011). With increased storage time, the resilience of fish fillets during storage showed a decreasing trend (Fig. 3A). Additionally, their chewiness also exhibited a downward trend (Fig. 3B). The 0.5% ULP group was more effective in delaying the decrease of chewiness, and this trend is consistent with the resilience index discussed previously. The hardness exhibited a decreasing trend throughout the cold storage process. However, the addition of 0.5% ULP was able to slow the decrease in hardness compared to the NC group (Fig. 3C). This could be attributed to the 0.5% ULP slowing the degradation of oxidized proteins in fish lipid. The springiness of fillets treated with different methods exhibited an initially increasing and then decreasing trend during the storage period. Specifically, from Days 0 to 2, the samples may still show stiffness due to the treatment (Fig. 3D). However, from Days 2 to 8, the effect of bacteria and enzymes during storage leads to the degradation of proteins, which breaks the covalent cross-linking structure between the proteins, reduces the intermuscular force and increases the intermuscular space, leading to a decrease in

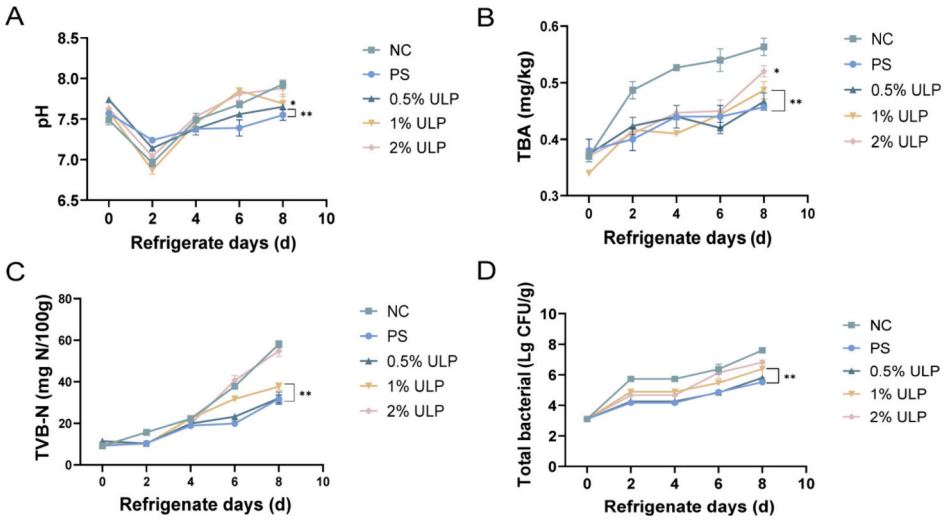

Fig. 2. Effects of ULP treatment on physicochemical property of perch fillets. (A) pH value, (B) TBA value, (C) TVB-N value, (D) Total bacteria. *$p < 0.05$, **$p < 0.01$, compared to the NC group.

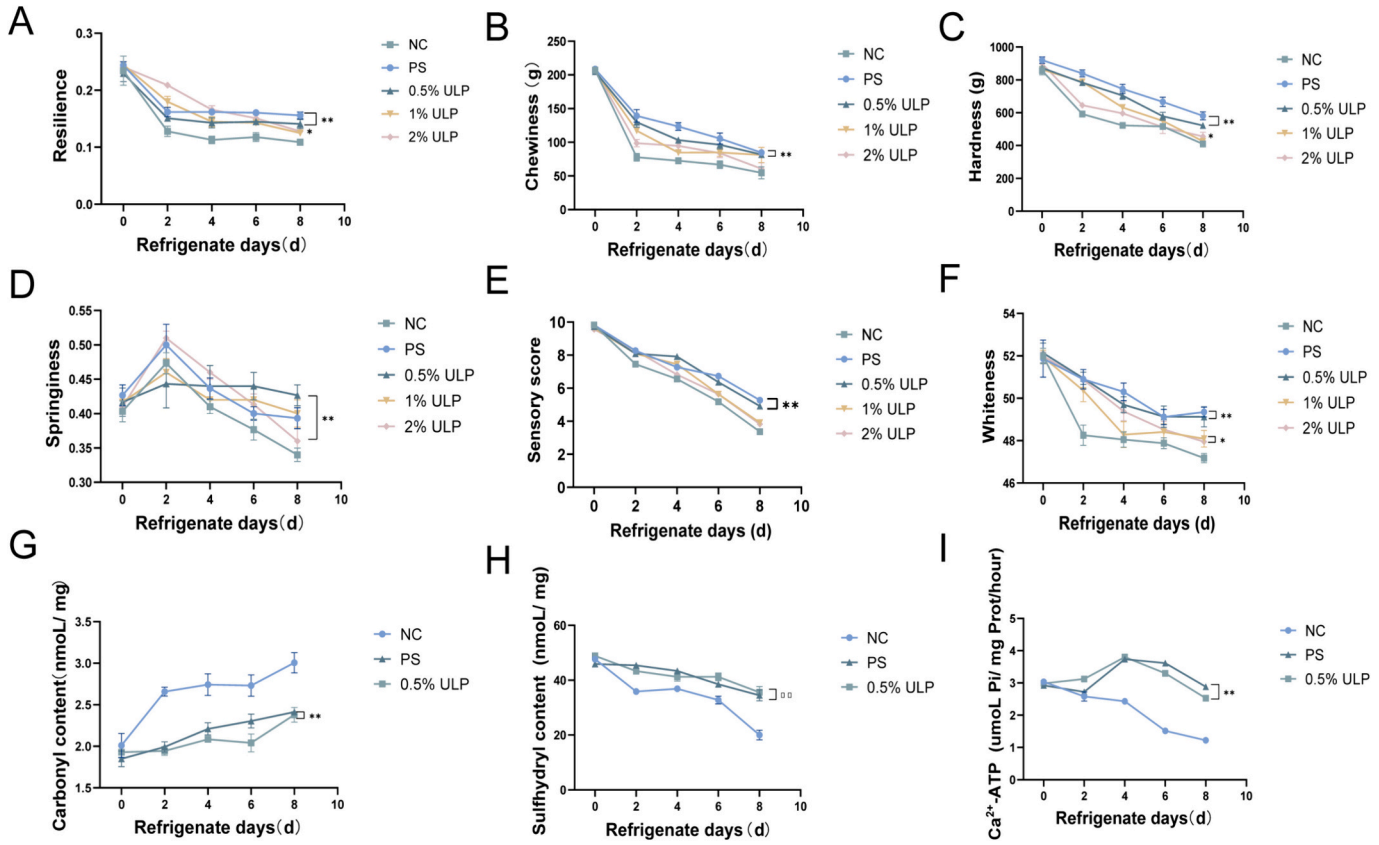

Fig. 3. Changes in texture and characterization of protein during cold storage. (A) Resilience, (B) Chewiness, (C) Hardness, (D) Springiness, (E) Sensory score, (F) Whiteness, (G) Carbonyl content, (H) Sulfhydryl content, (I) Ca^{2+}-ATPase activity. *$p < 0.05$, **$p < 0.01$, compared to the NC group.

springiness (Wei et al., 2021).

Sensory evaluation is an intuitive method for assessing changes in the quality of stored fish (Jia et al., 2022). As the storage time increases, the sensory scores of each treatment group gradually decrease, indicating a decline in the quality of fish products and a decrease in consumer acceptance. On Day 0, the initial evaluation score for each treatment group was 9.55, indicating that the purchased fish was very fresh. During the storage process, the samples treated with 0.5% ULP exhibited significantly higher scores, and their rate of decrease was relatively slow, similar to the effect seen in the PS group (Fig. 3E). This demonstrates that ULP effectively inhibits the accumulation of harmful metabolites.

The whiteness of perch fillets frequently changes during storage due to the oxidation of lipid, the whiteness of perch decreased with the prolongation of the storage time (Rostami, Abbaszadeh, & Shokri, 2017). The whiteness of the NC group decreased the fastest, while 0.5% ULP treatment delayed the decrease of whiteness, and the effect was the similar to the PS treatment (Fig. 3F). This might be attributed to the

antioxidant effect of the 0.5% ULP treatment group, which slowed the oxidation of lipid.

With the increasing storage time, and the carbonyl content of the ULP-treated group increased at a lower rate than that of the PS group and NC group. After Day 8 of storage, the carbonyl content increased by 23.43%, 30.27%, and 49.52%, respectively. The carbonyl content in the ULP-treated groups was significantly ($p < 0.05$) different from that in the PS and NC group (Fig. 3G). This was due to the ability of ULP to reduce the production of carbonyl groups and retard the degradation of myofibrillar protein in perch (Jannat-Alipour et al., 2019).

Sulfhydryl groups, present in cysteine, and the sulfhydryl content in myogenic fibers of perch fillets showed a decreasing trend with the prolongation of cold storage time (Shcherbatykh, Chernov'yants, & Popov, 2022). ULP group had the best inhibitory effect on sulfhydryl degradation, even exceeding PS treatment group. ULP inhibited the degradation of sulfhydryl content to a certain extent, and the inhibitory effect was twice that of NC group (Fig. 3H). This may be due to the change of spatial structure in the process of protein oxidation, which leads to the exposure of sulfhydryl groups, and exists in the form of disulfide bonds between or within polypeptide molecules after oxidation, which is further delayed by the preservation effect of ULP.

The decrease in Ca^{2+}-ATPase activity was the main cause of myosin hydrolysis (Koohmaraie, 1992). During the cold storage period, the faster the decrease in Ca^{2+}-ATPase activity, the greater the change in myosin properties and the higher the degree of denaturation. The Ca^{2+}-ATPase activity decreased to different degrees after continuous refrigeration at 4 °C for 8 days, the ULP treatment group decreased to 2.52 umoL Pi/ mg Prot/h, the PS group decreased to 2.26 umoL Pi/ mg Prot/h, the NC group decreased to 1.22 umoL Pi/ mg Prot/h (Fig. 3I), which showed that the inhibition of Ca^{2+}-ATPase activity by ULP had a significant effect.

The outcomes of SDS–PAGE analysis revealed that there were no notable disparities in the whole proteins (**Fig. S1A, B**), salt-soluble proteins (**Fig. S1C, D**), and water-soluble proteins (**Fig. S1E, F**) between the control treatment group and the ULP-treated group of perch during the refrigeration period at 4 °C. Remarkably, ULP application uniquely impeded the degradation of functional properties inherent in myofibrillar proteins of perch throughout the refrigeration process at 4 °C. Moreover, it significantly prolonged the preservation of enzymatic activity within the C^{a2+}-ATPase of the perch specimens. Importantly, it was also indicated to effectively suppress the deterioration of functional properties present in the myofibrillar proteins of perch undergoing refrigeration at a temperature of 4 °C, thereby effectively delaying the denaturation of the flesh proteins contained within the perch.

3.3. The maximum tolerated dose of mice

The in vitro animal experiments were carried out by the MTD method. Mice received oral administration (gavage) of the maximum dosage of 5000 mg/kg ULP for 14 days. The results showed that mice administered ULP showed no signs of toxicity and survived the treatment period. For female mice, the average weight was 25.98 g in the NC (negative control) group and 25.61 g in the ULP group (Fig. 4A). In male mice, the average weight was 34.41 g in the NC group and 34.28 g in the ULP group (Fig. 4B). There was no significant difference ($p > 0.05$) in body weight changes between the NC and ULP groups. The organ weight percentage is a sensitive, effective and economical index, and the organ weight percentage can reflect the degree of damage to some organs by the subject to some extent (Jannat-Alipour et al., 2019). No significant difference ($p > 0.05$) was observed in the indices of the major organs (heart, liver, spleen, lungs, and kidneys) of the mice (Fig. 4 **C & 4D**). Liver function-related indices (total protein, TP; serum albumin, ALB; globulin, GLB; alanine aminotransferase, ALT; aspartate transaminase, AST), lipid-related indices (total cholesterol, TCHO; triglyceride, TG), and renal function-related indices (creatinine, CREA; blood urea nitrogen, BUN) in mice showed no significant differences ($p > 0.05$) compared to the control group (Table 1). This further proves the security

Table 1
Changes in blood chemistry in mice.

Project	Female		Male	
	NC	ULP	NC	ULP
ALB (U/L)	33.67 ± 0.06	34.92 ± 0.92	32.64 ± 0.32	32.16 ± 0.44
ALT (U/L)	35.29 ± 0.27	36.71 ± 0.18	33.86 ± 0.10	36.00 ± 0.60
AST (U/L)	202.43 ± 0.18	200.50 ± 0.29	227.86 ± 0.02	225.40 ± 0.02
BUN (mmol/L)	7.65 ± 0.89	7.64 ± 0.28	9.03 ± 0.86	8.95 ± 0.29
CREA (μmol/L)	20.37 ± 0.72	21.08 ± 0.50	18.07 ± 0.73	19.24 ± 0.19
GLB (g/L)	24.58 ± 0.33	24.53 ± 0.08	25.76 ± 0.10	25.34 ± 0.70
TG (mmol/L)	1.03 ± 0.44	0.95 ± 0.14	0.97 ± 0.14	0.91 ± 0.31
TP (g/L)	59.19 ± 0.35	59.46 ± 0.96	57.24 ± 0.21	57.50 ± 0.22
TCHO (mmol/L)	2.71 ± 0.24	2.74 ± 0.40	3.78 ± 0.53	3.79 ± 0.44

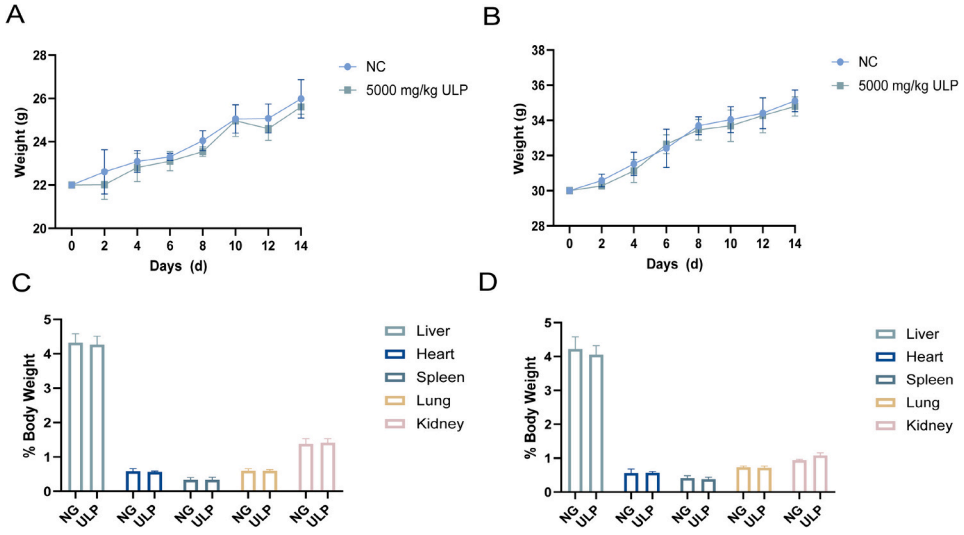

Fig. 4. Changes in body weight and organs in mice. (A) Body weight changes of the NC group and ULP group in female mice. (B) Body weight changes of the NC group and ULP group in male mice. (C) Organ weight percentage changes of the NC group and ULP group in female mice. (B) Organ weight percentage changes of the NC group and ULP group in male mice.

of ULP.

3.4. Analysis of the microbiota diversity

After homogenizating of all samples, the sequences were divided into OTUs based on different similarity levels. The results of the OTUs that

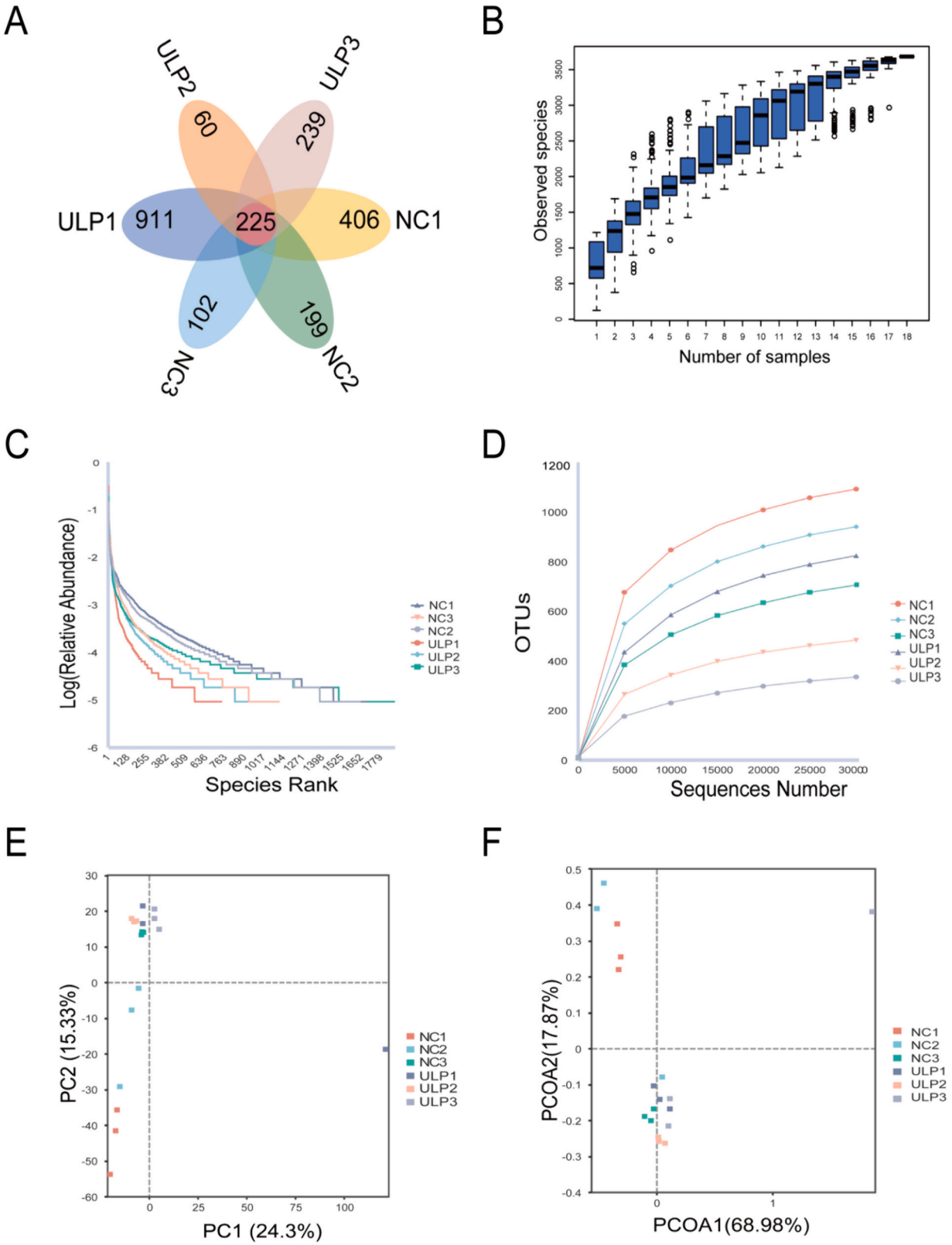

Fig. 5. Analysis of bacterial diversity. (A) Venn diagram compares OTUs among the groups, (B) Box plot of species accumulation, (C) Species dilution curve, (D) Rank Abundance curve, (E)Score plot of PCA, (F) Score plot of PCOA.

were common and unique among the groups were obtained from the clustering (Fig. 5A). The box plot of species accumulation is shown at the beginning of the analysis, as the sample capacity increased, the box plot showed a rapid growth trend, indicated that the number of species was higher in this period. The box plot tended to flatten out, indicating that the number of species did not rise with increasing sample size at the later stage of the analysis. This curve indicated adequate sampling and illustrated the richness of the sample flora (Fig. 5B). The analysis revealed distinct numbers of OTUs among the different groups. Specifically, the NC1 group had 406 unique OTUs, the NC2 group had 199 unique OTUs, and the NC3 group had 102 unique OTUs. On the other hand, the ULP1 group exhibited 911 unique OTUs, the ULP2 group had 60 OTUs, and the ULP3 group had 239 OTUs, a combined total of 225 OTUs across the six groups. Furthermore, results from the dilution curve indicated a substantial increase in species abundance with larger sample sizes (Fig. 5C). In terms of species richness, the hierarchical clustering curve demonstrated a reduction following the ULP intervention. The curve's vertical orientation represented species homogeneity within the samples, with a smoother curve indicating a more uniform distribution (Fig. 5D). Principal Component Analysis (PCA) showed that PC1 accounted for 24.1% of the observed variation, while PC2 explained 15.3%. The varying richness of flora across samples further confirmed the high quality of the samples (Fig. 5E). Additionally, PCOA highlighted the distinct separation between the ULP and NC groups, indicating dissimilar species composition and further analysis (Fig. 5F).

3.5. *Effect of ULP on the microbiota in perch fillets during the cold storage period*

At the genus level, the phylogenetic relationships of the top 100 genus-level species, the colony structure, and relative abundance of each group obtained by multiple sequence comparisons are shown in the genus-level evolutionary tree. The top 100 genera were mainly concentrated in Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria (Fig. 6A). The overall community structure of microflora at the phylum level during cold storage of perch fillets was consistent with the results of species evolution at the genus level, in which Proteobacteria, Firmicutes, and Bacteroidetes were the dominant phyla throughout the storage process. The ULP-treated group on Day 0 and Day 4 was mainly composed of Proteobacteria, Firmicutes, and Bacteroidetes, and the

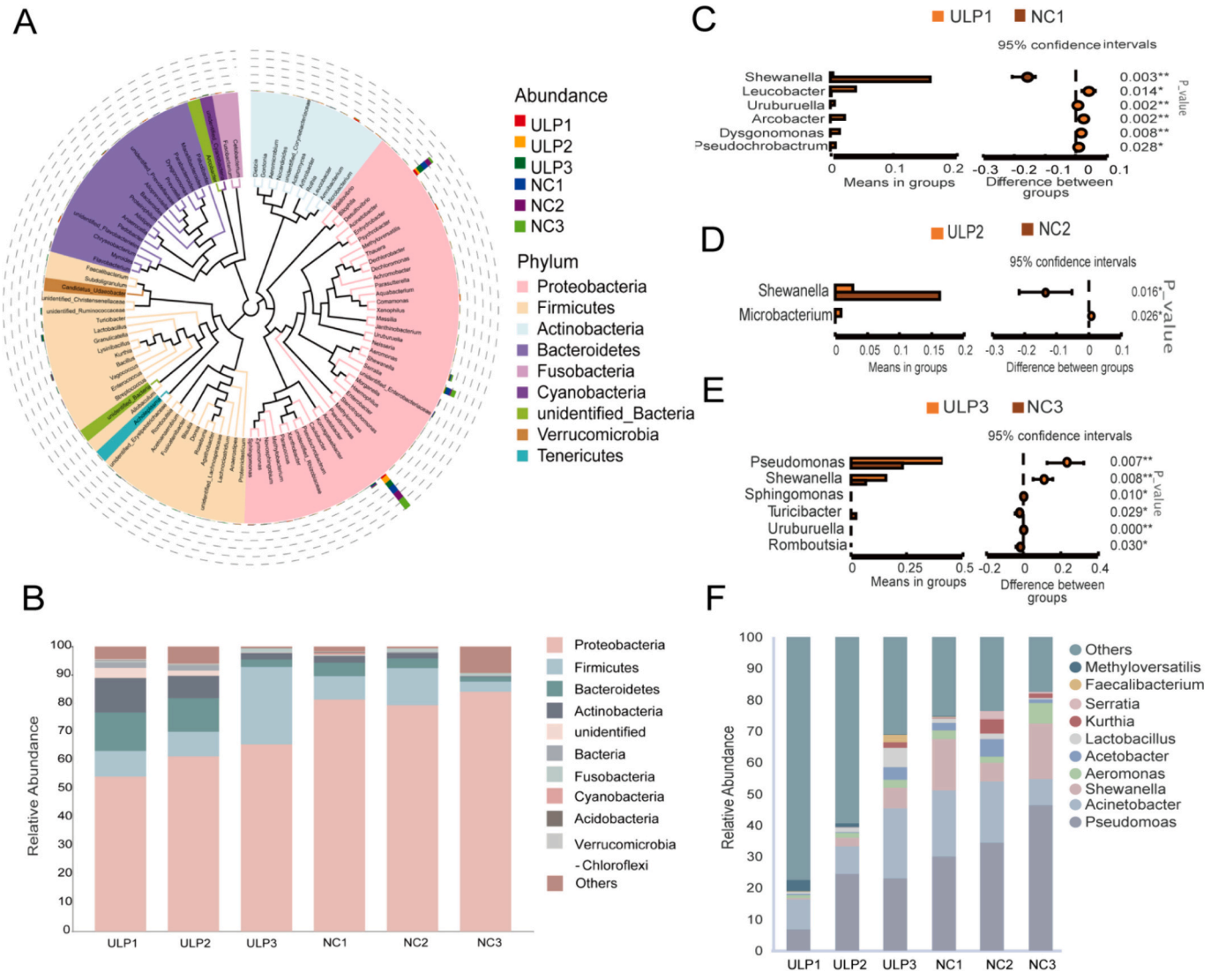

Fig. 6. Effect of ULP on the microbiota in perch fillet during the cold storage period. (A) Phylogenetic relationships of the top 100 species at the genus level, (B) Column diagram of species relative abundance at phylum level, (C) The *t*-test analysis inter-group differences at the genus level on day 0, (D) The t-test analysis inter-group differences at the genus level on day 4, (E)The t-test analysis inter-group differences at the genus level on day 8, (F) Column diagram of species relative abundance at genus level.

group on Day 8 mainly consisted of Proteobacteria (Fig. 6B). Test between groups identified species with significant differences ($p < 0.05$), and the ULP effectively reduced Shewanella, Leucobacter, Uruburella, Arcobacter, Dysgomonas, and Pseudochrobactrum on Day 0 (Fig. 6C). On Day 4, ULP effectively reduced Shewanella and *Microbacterium* (Fig. 6D). On Day 8, ULP effectively reduced *Pseudomonas*, *Shewanella*, *Sphingomonas*, *Turicibacter*, *Uruburuella*, and *Romboutsia* (Fig. 6E). At the genus level, the predominant spoilage bacteria in the NC groups were identified as *Pseudomonas*, *Actinetobacter*, and *Shewanella* (Fig. 6F). However, the application of ULP exhibited a certain inhibitory effect on the three spoilage bacteria. Under ULP treatment, microorganisms with strong spoilage ability were inhibited, and microorganisms with weak spoilage ability became the dominant bacteria (*Acetobacter, Lactobacillus*, and *Faecalibacterium*), which may be one of the factors by which ULP treatment can effectively extend the shelf life of perch fillets in cold storage. During cold storage, the application of chitosan-sodium alginate-nisin preservatives to *Penaeus vannamei* shrimp has shown remarkable efficacy in reducing the populations of dominant spoilage bacteria. This was achieved through the suppression of growth among *Psychrobacter*, *Vibrio*, *Acinetobacter*, and *Carnobacterium* (Cen et al., 2021). Similarly, an investigation by Yu et al. (2022) focused on the application of pomegranate peel extract and carboxymethyl chitosan coating through vacuum impregnation for fish quality retention during cold storage. The results revealed that the active coating exhibited inhibitory effects on the growth of spoilage bacteria, such as *Shewanella*.

4. Conclusion

The study demonstrated that ULP (7–10 kDa) effectively acted as a natural antioxidant in preserved perch fillets. Additionally, it is a potential bacteriostatic ingredient that significantly inhibits microbial growth and reduces undesirable changes in perch fillets. Among the various ULP concentrations tested, 0.5% ULP proved most effective at preserving the quality, inhibiting microbial growth, and ensuring the safety of the perch fillets during refrigeration. Specifically, 0.5% ULP resulted in a substantial reduction in pH levels, T*V*B-N content, TBA values, and the total bacterial count, respectively, compared to the control group. The reduction in these parameters highlights ULP's exceptional capability to prevent detrimental changes in perch fillets. Consequently, the whiteness, texture degradation, and protein oxidation typically observed in perch fillets are notably delayed under the protective effects of ULP. Acute oral administration studies definitively confirm the safety of ULP compounds. Utilizing 16S rRNA high-throughput sequencing technology further confirmed the impact of ULP solution on the microbial community structural diversity of perch fillet. The ULP solution inhibits microorganisms with strong spoilage capabilities, such as *Pseudomonas*, *Actinetobacter*, and *Shewanella*, while promoting the dominance of microorganisms with weak spoilage abilities such as *Acetobacter*, *Lactobacillus*, and *Faecalibacterium*. This exerts a discernible inhibitory effect on the spoilage process, further confirming the invaluable role of ULP in maintaining the quality of *Lateolabrax maculatus* fillet.

CRediT authorship contribution statement

Yajun Huang: Writing – original draft, Investigation, Formal analysis, Writing – review & editing. **Biying Luo:** Writing – original draft, Investigation, Formal analysis. **Shuo Shan:** Writing – review & editing. **Yijing Wu:** Writing – review & editing. **Haiyan Lin:** Writing – review & editing. **Feifei Wang:** Writing – review & editing. **Chuan Li:** Writing – review & editing. **Ruiyu Zhu:** Writing – review & editing, Writing – original draft, Conceptualization. **Chao Zhao:** Writing – review & editing, Writing – original draft, Conceptualization, Funding acquisition, Investigation, Supervision.

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.

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Appendix A. Supplementary data

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