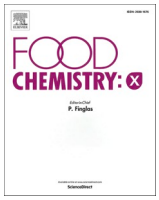
Food Chemistry: X 27 (2025) 102398

Contents lists available at ScienceDirect

Food Chemistry: X

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



Effects of anhydrous low-temperature storage on the post-capture survival, bacterial counts and meat quality of Hong Kong oyster *Crassostrea hongkongensis*

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ARTICLE INFO

Keywords:
Oyster
Freshness
Anhydrous low-temperature storage
Foodborne pathogens
Nutritional quality
Flavor compounds

ABSTRACT

This study comprehensively evaluated the freshness and quality changes of Hong Kong oyster (*Crassostrea hongkongensis*) during anhydrous low-temperature storage through a multi-indicator method. The results revealed that TVB-N and MDA levels showed significant increase after nine days of storage, while pH and surface color remained stable. The counts of *Escherichia coli*, *Vibrio parahaemolyticus*, and *V. vulnificus* peaked on day three before declining, contrasting with the continuous proliferation of anaerobic lactic acid bacteria. The crude protein, total lipid, phospholipid and fatty acid contents decreased significantly with prolonged storage, while glycogen content exhibited a biphasic trend, and triglyceride levels consistently increased. Most non-volatile compounds peaked at three to six days of storage, whereas lactic acid levels progressively increased. Among volatile compounds, aldehydes were the most abundant, with hexanal levels remaining stable and 2,4-heptadienal significantly increasing by day six. In conclusion, the optimal shelf life for Hong Kong oysters under anhydrous low-temperature storage conditions should be not exceed six days.

1. Introduction

As one of the most important aquatic species globally, the annual production of oyster has surpassed 7 million tons, with China contributing approximately 82.3 % of this total output (FAO, 2024). The Hong Kong oyster (*Crassostrea hongkongensis*), a species adapted to low-salinity estuarine environments, is predominantly cultured along the coastal regions of southern China, including Fujian, Guangdong, Guangxi and Hainan provinces. Due to its delicious taste and high economic value, this oyster species has been one of the largest coastal industries in the area, and the annual production amounts to 1.6 million tons. In China, the long-standing tradition of consuming aquatic products emphasizes activity and freshness as the criteria for assessing their quality and the primary considerations when purchasing them (Jiang et al., 2024). Prior to market release, oysters undergo long-time air exposure stress, resulting in gradual nutrient depletion, vitality decline, and ultimately, mortality. To address this issue, the technology of anhydrous transportation and preservation at low temperatures (0–4 °C) has emerged as an economically viable and practical solution for extending the shelf life of aquatic products. This method effectively inhibits microbial growth, reduces enzymatic activity, and preserves both the taste and nutritional value of seafood (Jiang et al., 2024). Consequently, it has found widespread application in the preservation of shrimp, crab, mollusks, and other aquatic species (Jiang et al., 2024; Lorentzen et al., 2016). However, there remains a significant gap in the literature regarding the impact of the cold storage duration on the quality of oysters.

From the consumer' s perspective, the characteristics of fresh seafood encompass sensory evaluation, substantial nutritional content, and original flavor quality. In terms of sensory evaluation, Liu et al. (2022) assessed the color difference (Lab values) between untreated and high hydrostatic pressure (HHP) -treated Suminoe oyster (*C. ariakensis*) under different storage temperatures. Additionally, Jiang et al. (2024) measured the Lab values of *Patinopecten yessoensis* to ascertain its viability and quality after short time storage at ice temperature. Regarding the nutritional content, Atayeter and Ercoşkun (2011) evaluated the fatty acid composition of the European squid (*Loligo vulgaris*) across various frozen storage temperatures. Their findings revealed an increase in saturated fatty acid (SFA) content, accompanied by a decrease in both monounsaturated fatty acids (MUFAs) and

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https://doi.org/10.1016/j.fochx.2025.102398
Received 20 February 2025; Received in revised form 15 March 2025; Accepted 18 March 2025
Available online 19 March 2025
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polyunsaturated fatty acids (PUFAs). Liu et al. (2021) investigated the impact of HHP and steaming on the proximate composition, amino acid contents, and fatty acid profiles of Hong Kong oysters, with results showing that slighter changes of biochemical compositions in HHP oysters compared to untreated oysters. As for the original flavor quality, free amino acids (FAAs), 5′-nucleotides, and organic acids are three main kinds of non-volatile compounds that significantly contribute to flavors such as umami, sweetness and sourness (Liu et al., 2022; Shi et al., 2025). Additionally, volatile organic compounds, which are closely associated with the characteristic aroma of oysters, also play a crucial role in determining their quality and freshness (Zhang et al., 2020).

Due to their filter-feeding behavior, mollusks accumulate higher concentrations of pathogens from their aquatic habits, thereby posing potential health risks to humans (Diner et al., 2023). Among these pathogens, *Vibrio* species and *Escherichia coli* are globally acknowledged as significant contributors to shellfish-associated gastroenteritis (Schets et al., 2010). Consequently, rigorous controls are enforced to restrict the presence of these pathogenic bacteria in seafood products. Furthermore, during the storage of aquatic products, the proliferation of both aerobic and anaerobic spoilage bacteria serves as a crucial indicator of both freshness and food safety (Odeyemi et al., 2018). For instance, Goulas et al. (2005) investigated the total viable count (TVC), *Pseudomonas* spp., lactic acid bacteria and H_2S-producing bacteria in mussels (*Mytilus galloprovincialis*) stored under modified atmosphere packaging conditions. Similarly, Ulusoy and Özden (2011) studied the effects of modified atmosphere packaging on the total viable aerobic bacterial count, psychrophilic count and total anaerobic bacteria in *M. galloprovincialis*. In addition to bacterial assessments, total volatile basic nitrogen (TVB-N) is the most prevalent chemical indicator used to evaluate the spoilage of marine foods (Dhaouadi et al., 2007).

Currently, a major challenge in oyster consumption is the accurate assessment of freshness, which extends beyond simply confirming their viability. The absence of systematic research has impeded the development of reliable methods for predicting shelf-life quality during cold storage, underscoring the urgent need to refine traditional quality evaluation systems. In this study, we investigate the shelf-life changes in color appearance, tissue structure, nutritional and flavor profiles, as well as different pathogen levels in live Hong Kong oysters. Our objective is to establish a comprehensive theoretical framework that elucidates the mechanisms underlying oyster deterioration under anhydrous low-temperature storage conditions.

2. Materials and methods

2.1. Oyster and pretreatment

In January 2024, a total of 300 Hong Kong oysters, purchasing from a commercial oyster farm in Haikou, Hainan Province, China, were acquired. These oysters were ~ 2 years old, with a shell height of 114.72 ± 16.06 mm, a wet body weight of 234.91 ± 34.81 g, and a soft tissue weight of 21.20 ± 4.57 g. The oysters were transported to the Mollusk Cultivation and Genetic Breeding Laboratory at Hainan University at a temperature of 15 °C and arrived within 3 h. To emulate the commercial cleaning process, all epibionts were carefully removed using a brush and a high-pressure water gun, utilizing filtered natural seawater with a salinity of approximately 35. Following this, the oysters were evenly distributed into ten foam boxes and subsequently stored in a refrigerator maintained at 4 °C for freshness determination. Sampling was conducted at 0, 3, 6 and 9 days based on the survival rate. Immediately after sampling, a comprehensive of analyses were performed, including assessments of color, histological structure, TVB-N content, pH levels, and microbiological measurements. For the analysis of proximate composition, fatty acids, free amino acids (FAA), 5′-nucleotides, and organic acids, the whole soft tissue of the oysters was stored at −80 °C.

2.2. Survival rate

Ninety oysters in three foam boxes were selected for assessing survival rates. Daily mortality checks were conducted at 9:00 AM and 9:00 PM. An oyster was deemed death if its shell exhibited a slight gap and there was no response from the mantle upon needle stimulation. The formula utilized for calculating the survival rate is outlined below:

$$\text{Survival rate}\ (\%) = \frac{\text{Survival oyster number}}{\text{Total detected oyster number}} \times 100$$

2.3. Color measurement and histological structure of adductor muscle and gonad

The color measurement of oysters was assessed utilizing the L^*, a^* and b^* parameters, following the method by Liu et al. (2022). As the gonads occupied most part of the soft tissues, the color change detection of oyster focused on gonads in this experiment. Specifically, oysters subjected to different storage durations were placed in an image acquisition apparatus and photographed (EOS850D, Canon, Oita, Japan) using a shutter speed of 1/125, an aperture size of 2.0 and an ISO setting of 800. For each sampling time point, five oysters were analyzed.

The photographs were then loaded into an Image Chroma Detection and Analysis Systemon (CSE-1 Imaging and Color-Measuring System, the National Color Science and Engineering Laboratory, Beijing, China). The brightness value L^*, redness values a^* and yellowness value b^* values were randomly measured three times in each of the five-set locations for each oyster. The total color difference (ΔE) was calculated as follows:

$$\Delta E = \sqrt[2]{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences in the L^*, a^*, and b^* values between each sampled group and oysters at 0 d.

After color measurement, the adductor muscle and gonad of each oyster were sampled and fixed in Bouin's solution for 24 h. The fixed tissues were dehydrated through a series of increasing ethanol concentrations (75 %, 85 %, 95 %, and 100 %). These tissues were then transferred to xylene for transparency and embedded in paraffin. Utilizing a rotary microtome (Leica RM2016, Wetzlar, Germany), sections of 5 μm in thickness were meticulously prepared. These sections were stained with hematoxylin and eosin for enhanced visualization. Microscopic fields of each sample were observed and photographed using an optical microscope (Olympus CX-23, Tokyo, Japan) equipped with an Olympus EP50 digital camera.

2.4. TVB-N, pH and MDA measurement

At each sampling time, nine oysters were selected for the measurement of TVB-N and pH values; these were subsequently grouped into three sets, with each set consisting of three oysters. The determination of TVB-N was conducted using the method of Wang et al. (2023) and expressed as mg TVB-N per 100 g soft tissue. Specifically, 10 g of chopped oyster was placed in a distillation tube and mixed with 75 mL distilled water at room temperature for 30 min. Subsequently, 1 g MgO was added to the tube and connected to the distiller immediately. A 30-mL solution of boric acid (20 g/L) was used as the receiving solution. Ten drops of a mixed indicator solution, consisting of a 5:1 ratio of bromocresol green ethanol solution to methyl red ethanol solution, were added, and the distillation process was carried out for 3 min. The receiving solution was then titrated using a 0.1 M HCl standard titrant.

For the measurement of pH, a homogenate was prepared by blending 2 g of soft tissue with 18 mL of distilled water for 30 s. Subsequently, the mixture was centrifuged at 4 °C for 10 min at 5000 r/min, and the supernatant was obtained for pH determination with a pH meter (Ohaus ST2100-F, USA).

For the measurement of malondialdehyde (MDA) content, 0.2 g of the sample was homogenized (8000 rpm, 30 s) using 1.8 mL pre-cooled and sterilized 0.9 % normal saline. The homogenate was then centrifuged at 4000 rpm for 10 min at 4 °C, and the supernatant was collected for MDA analysis. MDA content was quantified using the thiobarbituric acid (TBA) method according to the manufacturer's instructions (Jiancheng Biological Engineering Institute, Nanjing, China).

2.5. Microbiological analysis

A total of 15 oysters were randomly selected to estimate the counts of total aerobic bacteria, *E. coli*, *V. vulnificus*, *V. parahaemolyticus*, total anaerobic bacteria and anaerobic lactic acid bacteria at 0, 3, 6 and 9 d, respectively. The mantle, adductor muscle, digestive gland, gill and gonad of three oyster were sampled, respectively, and placed in sterile plastic bags and homogenized for 3 min using a stomacher (XHF-DY, Ningbo New Biotechnology Co., Ltd., China). Then, 3 g of homogenized samples were weighed and mixed with 27 mL sterile peptone buffer. Following homogenizing, a decimal dilution series was prepared for microbiological analysis. Each dilution (0.1 mL) of oyster homogenates was pipetted onto different culture media for plate count.

For total aerobic bacteria, plate count agar (PCA) prepared according to the manufacturer's instructions (Qingdao Hope Bio-Technology, China), was used. Eosin methylene blue agar (EMB, Qingdao Hope Bio-Technology, China) was used for isolating *E. coli*, with colonies exhibiting a violet-black hue, with or without a metallic sheen, being counted. Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS, Qingdao Hope Bio-Technology, China) agar was utilized for isolating *V. parahaemolyticus*, characterized by translucent and smooth-surfaced green colonies. Modified Colistin-Polymyxin B-Cellobiose (mCPC, Qingdao Hope Bio-Technology, China) agar was employed for isolating *V. vulnificus*, which presented as colonies that were either transparent or had transparent edges, ranging in color from yellow to orange. All media were incubated at 36 °C for 48 h. After incubation, colonies on the plates were counted and the data were converted to CFU per g of oyster tissue. To confirm the presence of *V. parahaemolyticus* and *V. vulnificus*, 20 colonies (or all colonies, if fewer than 20 were present) from each tissue at each sampling time were identified using the primers described by Tarr et al. (2007).

EG Medium (Solarbio, China) and MRS Medium (Qingdao Hope Biotechnology, China) were prepared according to the manufacturer's instructions and utilized for the isolation of total anaerobic bacteria and anaerobic lactic acid bacteria, respectively. Both media were placed in anaerobic bags and incubated at 36 °C for 48 h. In the MRS medium, colonies exhibiting medium size and regular edges, with a white appearance, were identified as lactic acid bacteria.

2.6. Proximate composition analysis

The moisture of oyster soft tissues was determined by drying it at 60 °C until a constant weight was attained. The crude protein content was determined using the Kjeldahl method, which involved the use of a fully automated Kjeldahl nitrogen/protein analyzer (FOSS-Soxtec 2050, Sweden). Prior to analysis, acid digestion was performed using a Tecator digestor 2020. The extraction of crude lipids was carried out using petroleum ether, as described previously by Liu et al. (2021). The triglyceride content was measured by the GPO-PAP enzymatic method (Jiancheng Biological Engineering Institute, Nanjing, China). The glycogen content was quantified using a Glycogen Content Kit following the manufacturer's instructions (Jiancheng Biological Engineering Institute, Nanjing, China). The ash content was determined gravimetrically through incineration at 550 °C. All samples were analyzed in triplicate.

The total phospholipid content was determined using an acid digestion and colorimetric method based on the formation of a phosphomolybdate complex. Briefly, approximately 150 μg of crude lipid was digested with 0.5 mL of perchloric acid at 170 °C for 20 min. After cooling to room temperature, 3.5 mL of distilled water, 0.5 mL of ascorbic acid solution, and 1 mL of ammonium molybdate solution were sequentially added to the mixture. The reaction mixture was then heated in a boiling water bath for 7 min. The absorbance of the resulting solution was measured at 810 nm using a spectrophotometer.

2.7. Fatty acid analysis

Fatty acids were extracted from freeze-dried soft tissues of oysters using a chloroform-methanol mixture in a 2:1 volume ratio. Subsequently, fatty acid methyl esters (FAME) were prepared through esterification with 0.4 M KOH in methanol, followed by analysis using gas chromatograph/mass spectrometer (GC/MS), as described by Sun et al. (2023). Detection of FAME peaks was facilitated using either a flame ionization detector or a mass spectrometry detector. The obtained data were analyzed using Chemstation software (version B04.02) or Mass Hunter software (version B.05.00). The identities of the peaks were confirmed by comparing their retention times with those of known FAME standards (Supelco 37 Component FAME Mix, Bellefonte, PA, USA) under the same conditions. The fatty acid content was quantified in μg/g of soft tissues. All measurements were performed in triplicate.

2.8. Free amino acid (FAA) assay

The assessment of FAAs was conducted using the method of Liu et al. (2022). Briefly, 2.5 g of frozen soft tissue was homogenized in three volumes of 10 % trichloroacetic acid. Following centrifugation at 10,000 ×*g* for 15 min at 4 °C, supernatants were obtained and aliquoted into 25 μL portions. These supernatants were then analyzed for FAAs using high-performance liquid chromatography (HPLC) on a Waters 2996 system (Waters Corporation, Milford, MA, USA), equipped with a Waters Pico-Tag-C18 column (3.9 mm × 150 mm). All analyses were performed in triplicate. For the qualitative and quantitative determination of each amino acid, comparisons were made with the retention times and peak areas of respective amino acid standards sourced from Sigma-Aldrich (St. Louis, MO, USA).

2.9. 5′-nucleotide assay

The extraction and analysis of 5′-nucleotides of oyster were conducted according to the methodology described in our previous study (Liu et al., 2022). The HPLC conditions employed for the analysis of 5′-nucleotides were as follows: injection volumes of 20 μL, a mobile phase consisting of methanol and 0.05 % phosphoric acid, a flow rate of 1.0 mL/min, a column temperature maintained at 30 °C, and a detector wavelength set at 260 nm. Each sample was analyzed in triplicate to ensure accuracy. The identification and quantification of nucleotides were achieved by comparing the retention times and peak areas of the samples to those of nucleotide standards obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.10. Organic acid assay

The concentrations of lactic acid, malic acid, citric acid, and succinic acid were extracted and analyzed using a previously established method (Liu et al., 2022). Briefly, 2 g of oyster soft tissue was homogenized in 10 mL of purified water for 5 min. The supernatants were then centrifuged at 10,000 ×*g* for 20 min at 4 °C and filtered through a 0.45-μm cellulose membrane (Whatman GmbH, Dassel, Germany) prior to HPLC analysis. The HPLC conditions employed were similar to those described in Section 2.9 for the analysis of 5′-nucleotide, with the exception of the detection wavelength, which was adjusted to 215 nm. All analytical procedures were performed in triplicate to ensure reproducibility.

2.11. Volatile compounds analysis

The volatile compounds in the oysters were analyzed using headspace monolithic material sorptive extraction coupled with gas chromatography–mass spectrometry (HS-MMSE-GC–MS). A mixture of sample (2 g) and NaCl (1.125 g) was placed in a glass vial along with a magnetic stirrer and heated at 70 °C for 40 min. Volatile compounds were collected using a solid-phase microextraction (SPME) fiber coated with DVB/CAR/PDMS (Supelco Inc., Bellefonte, USA) in the vial's headspace. The absorbed compounds were then desorbed in a thermal desorption unit and introduced into a GC–MS system (7890 A-5975C; Agilent, Santa Clara, USA) *via* a cooled injection system. The GC–MS conditions included an HP-5MS column (30 m × 250 μm × 0.25 μm, Agilent), helium carrier gas (99.999 %) at a flow rate of 3.0 mL/min, a detector interface temperature of 280 °C, and an ion source temperature of 230 °C. The ionization energy was set to 70 eV. The oven temperature program started at 40 °C, increased to 160 °C at 3 °C/min, then to 230 °C at 15 °C/min, and was held for 2 min. Mass spectra were acquired in total ion chromatogram (TIC) mode, scanning from 50 to 550 amu for compound identification. All analytical procedures were performed in triplicate to ensure reproducibility.

2.12. Statistical analysis

The data were presented as mean ± standard deviation (SD). The statistical significance of differences among various treatments was assessed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test. All statistical analyses were conducted using the Data Processing System (DPS) statistical software. A *P*-value of less than 0.05 was deemed statistically significant.

3. Results and discussion

3.1. Effects of anhydrous low-temperature storage on the survival and freshness indicators

3.1.1. Survival

The survival rate of oysters decreased progressively with the extension of storage time (Fig. 1). The survival rate remained at 100 % for the first three days post-removal from water. Under anhydrous storage at 4 °C, a relatively higher survival rate of over 94.44 % was maintained until the sixth day. However, a significant increase in mortality was observed on the seventh day, and by the twelfth day, the survival rate had dropped to 44.44 %. Oysters, inhabitants of the intertidal zone, are renowned for their remarkable tolerance to air exposure (Guo et al., 2015). Pacific oysters have been documented to exhibit a high survival rate even after ten days of air exposure at 4 °C (Kawabe et al., 2010). However, in the present experiment, a notable decline in the survival rate of oysters was observed on the seventh day. This decline can be attributed to two primary factors: firstly, Hong Kong oysters, which belong to tropical species, exhibit a relatively limited tolerance to low temperatures. Secondly, commercial oysters often undergo physical treatments, such as high-pressure water jetting, to remove barnacles, algae, and other adherent organisms from their shells. These treatments adversely affect the physiological performance of oysters, subsequently impairing their resistance to air exposure.

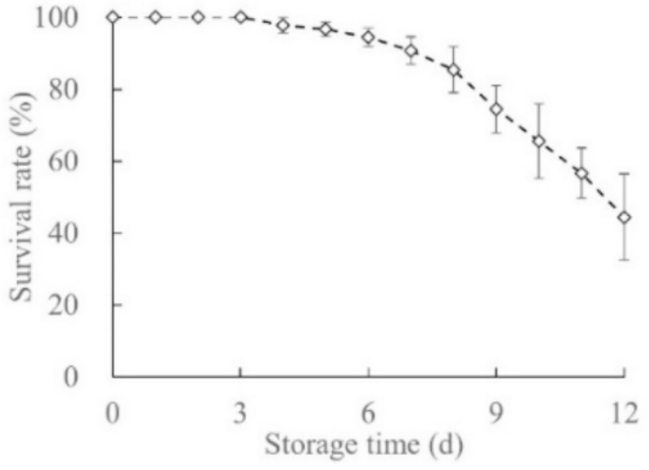

Fig. 1. Survival rate of Hong Kong oysters during anhydrous low-temperature storage.

3.1.2. TVB-N, pH, MDA and surface color

TVB-N, pH and MDA are three key indicators for assessing the biochemical status and quality of fresh aquatic products during their shelf life. As shown in Fig. 2A, the TVB-N content increased progressively with extended storage time, with statistically significant differences observed between oysters stored for 0, 3, and 6 days compared to those stored for 9 days ($P < 0.05$). TVB-N, comprising a suite of biogenic amines resulting from the decomposition of proteins and other nitrogenous compounds, served as a critical indicator of seafood quality (Dhaouadi et al., 2007). It is widely recognized as the most prevalent chemical marker indicative of seafood spoilage, including fish, oyster, shrimp and crab (Kim et al., 2020; Odeyemi et al., 2018; Wang et al., 2023). In our study, the TVB-N content ranged from 13.33 to 16.19 mg/100 g, which aligned with values reported for other bivalve species, such as Akoya pearl oyster (*Pinctada fucata*) (Chung et al., 2021). Furthermore, the significant increase in the TVB-N content on the sixth day indicated a certain degree of spoilage under the given condition.

In this study, the pH values of oysters exhibited a slight upward trend, ranging between 6.05 and 6.31, as storage duration increased (Fig. 2B). Generally, prolonged storage leads to the production of alkaline or acidic volatile organic compounds, particularly during the later stages. For example, Wang et al. (2023) observed significant pH variations in Fujian oysters stored at 4 °C for more than four days using pH indicator dyes. Previous research conducted by Zhang et al. (2009) revealed that spoiled oysters generated a greater variety of organic acids and trimethylamine, absent in fresh oysters but detectable in deteriorated samples (Zhang et al., 2009). Consequently, the combined presence of alkaline nitrogen compounds and organic acids influences the pH values. The comparable pH values (despite a slight increase) among oysters stored for different durations suggested that the production of alkaline nitrogen compounds, such as trimethylamine, counterbalanced the changes in organic acids, resulting in insignificant pH variations in the examined oysters. Similarly, López-Caballero et al. (2000) reported a slight increase in pH values in *Ostraea edulis* subjected to HHP treatment as storage duration increased.

As shown in Fig. 2C, MDA content progressively increased with extended storage duration, rising from 7.77 to 13.11 nmol/mg prot. Significant differences were observed between oysters stored for 0 days and those stored for 9 days ($P < 0.05$). MDA content serves as a crucial indicator of lipid oxidation. These lipid oxidation products are known to pose risks to human health, often being linked to membrane damage, heart disease, and cancer (Bejaoui et al., 2021). Our results indicate that, although there was a modest increase in MDA content on the sixth day of storage at 4 °C, this difference was not statistically significant, implying a relative degree of safety under these conditions.

The effects of storage duration on the surface color of oysters are shown in Fig. 2D. In terms of color parameters, the L^*, b^* and ΔE values exhibited an initial increase followed by a subsequent decrease, with the peak values occurring at 6, 3 and 3 days, respectively. The a^* value demonstrated a consistent increase throughout the entire storage period. As reported by Lekjing and Venkatachalam (2018), the soft tissues of oysters initially exhibit a cream-colored appearance, which undergoes sequential transformations during storage (initially turning yellow, then brown, and ultimately green). These color alterations during low-

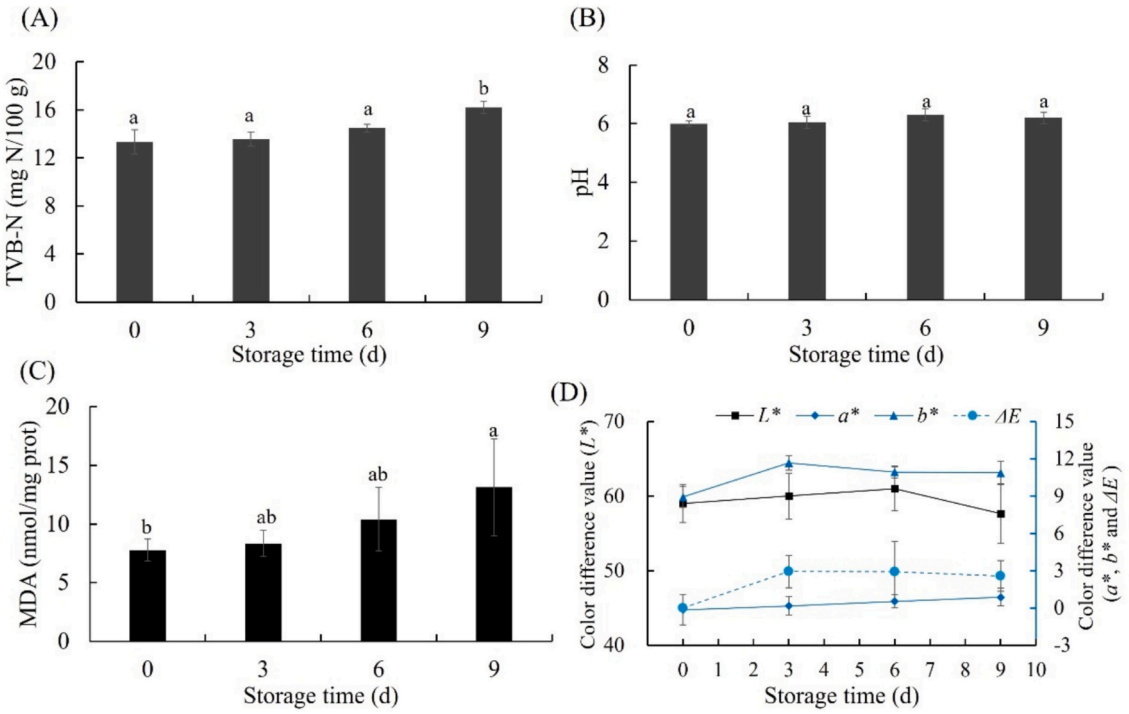

Fig. 2. TVB-N content (A), pH (B), MDA content (C) and color difference (D) changes of Hong Kong oysters during anhydrous low-temperature storage. Data represent means ± SD ($n \geq 3$). Different letters indicate significant differences among treatments ($P < 0.05$).

temperature storage are often attributed to the formation of yellow pigments resulting from protein denaturation, lipid oxidation, and the Maillard reaction (Rodezno et al., 2023). In our study, despite the oysters remaining alive, their protein, lipid, and moisture contents progressively decreased with storage duration, potentially contributing to the observed color changes. Notably, the color variation between the initial oysters and other storage-treated groups was relatively minor, indicating that the chemical and microbial deterioration of the oysters remained limited (Lekjing & Venkatachalam, 2018). Similar findings have been reported in other seafood studies, such as pasteurized oysters (*C. belcheri*) and scallop (Jiang et al., 2024; Lekjing & Venkatachalam, 2018).

3.1.3. Histological structure

Significant differences were observed in both the adductor muscle and gonad of oysters across various storage durations (Fig. S1). Specifically, in the adductor muscle, no obvious changes were observed between freshly harvested oysters and those stored for three days. However, by the sixth day of storage, mild muscle degeneration became evident, which further intensified on the ninth day, accompanied by a considerable widening of muscle fiber interstices. Similarly, the gonad exhibited vacuolation as early as the third day of storage, with this phenomenon progressively intensifying over the extended storage period.

Aquatic animals subjected to anhydrous storage often endure hypoxic or anoxic stress, which impacts their antioxidant and immune functions and stimulates the production of reactive oxygen species (ROS) (Zhang et al., 2022). When ROS levels exceed the antioxidant system's scavenging capacity, oxidative damage and cellular apoptosis in tissues occur (Lu et al., 2021). Such tissue alterations have been extensively reported in aquatic animals such as oysters, clams, and shrimp subjected to anhydrous transportation (Lu et al., 2021; Zhang et al., 2022). Furthermore, from an energy budget perspective, the increased energy and nutritional consumption, coupled with the inability to replenish energy through feeding, leads to a gradual depletion of nutrients in the adductor muscle and gonad of oysters over storage time, ultimately resulting in the tissue changes.

3.2. Effects of anhydrous low-temperature storage on bacterial counts

As shown in Fig. 3A-D, the total aerobic bacteria, *E. coli*, *V. parahaemolyticus* and *V. vulnificus* counts in oysters exhibited an increase followed by a decline during anhydrous low-temperature storage, with peak counts observed at 6, 3, 3 and 6 days, respectively. At the initial time point (0 days), the concentrations of total aerobic bacteria ranged from 1.77×10^6 to 3.90×10^6 CFU/g, *E. coli* from 0.03×10^3 to 8.59×10^3 CFU/g, *V. parahaemolyticus* from 0.83×10^3 to 4.00×10^3 CFU/g. Notably, the highest bacterial loads were recorded in the mantle tissue, whereas the gonadal tissue exhibited the lowest counts. Additionally, the initial counts of *V. vulnificus* in various tissues ranged from 1.08×10^3 to 6.25×10^3 CFU/g, with the gill tissue demonstrating the highest levels. Furthermore, the population of total anaerobic bacteria displayed an initial decline, followed by an ascending trend (Fig. 3E), while the count of anaerobic lactic acid bacteria showed a modest upward trend (Fig. 3F). At the initial point, the highest bacterial concentrations of total anaerobic bacteria and anaerobic lactic acid bacteria were observed in the gill (6.23×10^5 CFU/g) and digestive gland (3.39×10^5 CFU/g), respectively. After nine days of storage at 4 °C, significant increase in total anaerobic bacteria were observed in the mantle and gonad tissues ($P < 0.05$), and similar trends were noted for anaerobic lactic acid bacteria in the mantle, adductor muscle, and gonad tissues ($P < 0.05$).

Due to their filter-feeding habits, bivalves accumulate various contaminants, including human pathogens, marine toxins, and coastal pollutants, posing potential health risks to consumers (Diner et al., 2023). Current monitoring protocols for bacterial pathogens in seafood, particularly raw-consumed oysters, primarily target specific infectious agents such as *E. coli*, *V. vulnificus* and *V. parahaemolyticus* (Liu et al., 2022). In our investigation, the bacterial concentrations of *E. coli*, *V. vulnificus* and *V. parahaemolyticus* in Hong Kong oyster ranged from 31 to 8586 CFU/g, 833 to 4000 CFU/g, and 2990 to 6250 CFU/g, respectively. These levels are consistent with previous studies, such as Froelich

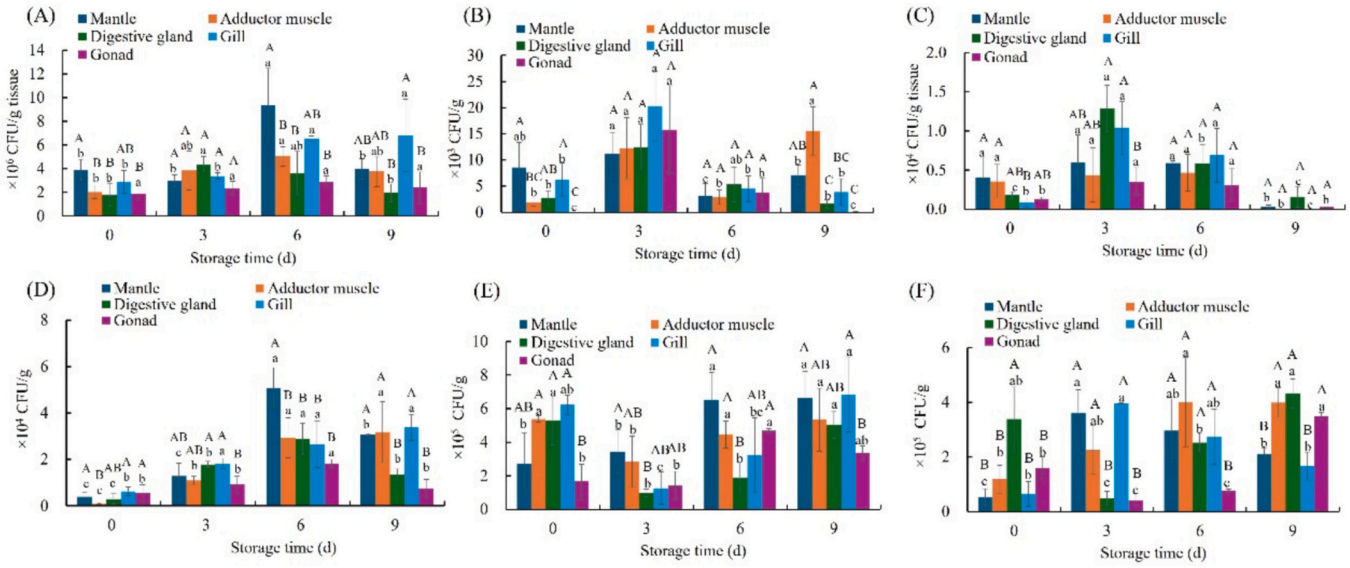

Fig. 3. Bacterial counts of total aerobic bacteria, *Escherichia coli*, *Vibrio parahaemolyticus*, *V. parahaemolyticus*, total anaerobic bacteria and anaerobic lactic acid bacteria in Hong Kong oysters during anhydrous low-temperature storage. Data represent means ± SD ($n = 5$). Values with different upper- and lower-case letters indicated significant differences among different tissues at the same time point, or the same tissue at different time points, respectively ($P < 0.05$).

et al. (2017), who reported *V. vulnificus* counts in *C. virginica* oysters ranging from 30 to 10,000 CFU/g during the sampled period from April to October. However, the *V. parahaemolyticus* levels in our study were higher than those reported in Eastern North Carolina (10 to 5000 CFU/g). Additionally, Diner et al. (2023) observed lower bacterial counts in Pacific oysters (*E. coli*: ~100 CFU/g, *V. vulnificus*: ~1000 CFU/g, *V. parahaemolyticus*: <10 CFU/g) after exposing indoor-purified oysters to the natural environment. Given the stringent standards set by regulatory bodies such as the United States Food and Drug Administration (FDA), the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN), and the Chinese government for *Vibrio* species in raw shellfish, the elevated counts of *Vibrio* species in our study suggest that the oysters may not be suitable for direct raw consumption.

The proliferation dynamics of pathogenic microorganisms and spoilage bacteria are crucial determinants of the freshness and shelf life of aquatic products (Goulas et al., 2005; Yu et al., 2021). In this study, the initial increase in total aerobic bacteria and foodborne pathogens can be attributed to the gradual weakening of the oyster's vitality during storage, reducing its resistance to bacterial colonization. However, as storage duration increased, the internal microenvironment shifted from aerobic to anaerobic metabolism, leading to a decline in aerobic bacteria and a rise in anaerobic bacteria populations. A similar phenomenon has been reported in the microbiological analysis of raw and cooked snow crab stored at 0 and 4 °C (Lorentzen et al., 2016). Furthermore, the gradual increase in lactic acid bacteria, consistent with the observed rise in lactic acid levels, indicates progressive spoilage.

Mollusks, with their open circulatory systems, harbor diverse bacterial communities that vary across tissues due to unique microenvironments (Lokmer et al., 2016; Vezzulli et al., 2017). In our study, all detected bacterial species were present in all five examined oyster tissues, with the mantle and gill tissues exhibiting higher total aerobic bacterial and foodborne pathogen counts compared to other tissues. This is consistent with previous findings that tissues directly exposed to the external environment, such as the mantle and gill, provide distinct niche spaces for bacterial colonization (Chung et al., 2021). For instance, variations in bacterial communities have been documented in the hemolymph, digestive gland, and gills of pearl oysters, oysters, and mussels (Chung et al., 2021; Vezzulli et al., 2017). These tissue-specific differences highlight the importance of considering microbial dynamics in different tissues when assessing oyster quality and safety.

3.3. Effects of anhydrous low-temperature storage on nutrient composition

As shown in Table 1, there was a significant decrease in moisture, crude protein, lipid and total phospholipid contents with the prolongation of storage duration($P < 0.05$). Conversely, the triglyceride content exhibited an increasing trend. The glycogen content initially increased and then decreased, peaking at the 3-d storage point. Additionally, the ash content demonstrated a consistent increase throughout the entire storage period, with statistically significant differences observed among the 0- and 3-d storage groups *vs.* 6-d storage group *vs.* 9-d storage group ($P < 0.05$).

The fatty acid profiles of oysters stored for different durations are presented in Table 2. In this study, all detected fatty acid contents exhibited a significant decreasing trend throughout the storage period ($P < 0.05$). Among the fatty acids in the oyster, C22:6n3, C16:0 and C20:5n3 were the most abundant. The concentrations of PUFA, SFA and MUFA ranged from 869.81 to 1327.56, 565.30 to 847.81, 286.29 to 456.52 μg/g of wet soft tissue, respectively. Notably, most fatty acids demonstrated a sharp decrease after 3 d of storage, including C22:6n3

Table 1
Proximate composition of oysters during anhydrous low-temperature storage.

Items	Storage time			
	0 d	3 d	6 d	9 d
Moisture (% wet weight)	83.84 ± 0.53a	83.02 ± 0.32ab	82.91 ± 0.47bc	82.33 ± 0.90c
Crude protein (% wet weight)	9.12 ± 0.01a	9.01 ± 0.02b	8.69 ± 0.00c	8.37 ± 0.04d
Crude lipid (% wet weight)	2.82 ± 0.16a	2.52 ± 0.07b	2.39 ± 0.15bc	2.17 ± 0.13c
Triglyceride (mmol/g prot)	0.14 ± 0.01c	0.31 ± 0.03bc	0.46 ± 0.08ab	0.64 ± 0.11a
Total phospholipid (% wet weight)	1.30 ± 0.06a	1.18 ± 0.01b	1.03 ± 0.06c	0.76 ± 0.07d
Glycogen (% wet weight)	2.17 ± 0.12bc	3.07 ± 0.11a	2.34 ± 0.15b	1.97 ± 0.05c
Ash (% wet weight)	2.04 ± 0.13c	2.31 ± 0.22c	3.22 ± 0.14b	3.99 ± 0.12a

Data represent means ± SD (n = 3). Values in a row with different letters indicate significant differences among treatments ($P < 0.05$).

Table 2
Fatty acid profiles of oysters during anhydrous low-temperature storage.

Fatty acid (μg/g)	Storage time			
	0 d	3 d	6 d	9 d
C14:0	30.43 ± 0.13a	24.22 ± 0.27b	23.05 ± 1.25bc	22.71 ± 0.32c
C15:0	20.71 ± 0.22a	15.28 ± 0.61b	14.49 ± 0.97b	14.15 ± 0.53b
C16:0	502.25 ± 1.02a	390.70 ± 4.7b	353.97 ± 5.49c	338.93 ± 12.79d
C17:0	41.03 ± 0.47a	30.61 ± 0.30b	26.36 ± 1.76c	24.57 ± 0.14d
C18:0	253.38 ± 0.19a	202.25 ± 2.47b	177.21 ± 1.17c	164.94 ± 7.09d
SFA	**847.81 ± 2.03a**	**663.06 ± 6.67b**	**595.08 ± 9.63c**	**565.30 ± 14.87d**
C16:1n5	5.80 ± 0.07a	4.21 ± 0.02c	4.65 ± 0.17b	4.04 ± 0.15c
C16:1n7	24.80 ± 0.14a	19.59 ± 0.39b	18.00 ± 0.34c	18.23 ± 0.14c
C16:1n10	5.80 ± 0.12a	4.99 ± 0.04b	4.81 ± 0.01c	4.93 ± 0.07bc
C18:1n9Z	47.68 ± 0.37a	34.30 ± 0.63b	27.60 ± 0.79c	26.10 ± 0.61d
C18:1n9E	152.64 ± 1.26a	116.48 ± 1.37b	83.50 ± 5.19c	82.71 ± 1.09c
C20:1n9	83.87 ± 0.25a	73.46 ± 0.12b	64.79 ± 0.53c	60.89 ± 1.05d
C20:1n7	131.87 ± 0.13a	91.43 ± 4.10b	79.91 ± 0.68c	76.43 ± 2.46c
C22:1n9	4.05 ± 0.14b	4.00 ± 0.12b	3.02 ± 0.12c	29.65 ± 0.20a
MUFA	**456.52 ± 2.47a**	**348.46 ± 5.78b**	**286.29 ± 5.83c**	**302.98 ± 5.47c**
C18:2n6	39.12 ± 0.38a	28.76 ± 0.58b	21.87 ± 1.95d	25.19 ± 0.53c
C18:3n6	4.42 ± 0.11a	4.45 ± 0.07a	3.87 ± 0.24b	3.97 ± 0.10b
C18:3n3	63.52 ± 0.41a	46.87 ± 0.39b	40.01 ± 1.80c	40.94 ± 0.40c
C18:4n3Z	56.69 ± 1.23a	42.31 ± 0.32b	35.27 ± 1.32d	38.35 ± 0.41c
C20:2n6	6.67 ± 0.01a	5.52 ± 0.03b	4.77 ± 0.37c	5.02 ± 0.09c
C20:3n6	6.87 ± 0.05a	5.30 ± 0.08b	4.18 ± 0.21d	4.88 ± 0.03c
C20:4n6	147.25 ± 0.35a	114.37 ± 1.32b	93.97 ± 4.51c	93.76 ± 2.57c
C20:3n6	5.31 ± 0.05a	4.90 ± 0.07b	4.22 ± 0.08c	4.29 ± 0.09c
C20:5n3	420.10 ± 3.54a	321.48 ± 3.18b	303.06 ± 2.35c	269.22 ± 1.82d
C22:4n6	9.46 ± 0.18a	8.07 ± 0.13b	7.33 ± 0.04c	6.68 ± 0.01d
C22:5n6	59.48 ± 1.70a	50.90 ± 0.69b	37.13 ± 3.00c	42.92 ± 1.49c
C22:6n3	508.68 ± 2.17a	374.64 ± 4.89b	332.14 ± 14.77c	334.58 ± 6.20c
PUFA	**1327.57 ± 10.17a**	**1007.58 ± 10.75b**	**887.83 ± 27.64c**	**869.81 ± 14.74c**
TFA	**2631.89 ± 8.00a**	**2019.10 ± 20.88b**	**1769.20 ± 27.24c**	**1738.09 ± 31.83c**
SFA/TFA	0.32	0.33	0.34	0.33
MUFA/TFA	0.17	0.17	0.16	0.17
PUFA/TFA	0.50	0.50	0.50	0.50

Data represent means ± SD (n = 3). Values in a row with different letters indicate significant differences among treatments ($P < 0.05$). SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; TFA: total fatty acid.

(from 508.68 to 374.64 μg/g), C16:0 (from 502.25 to 390.70 μg/g), C20:5n3 (from 420.10 to 321.48 μg/g), and Σn-3 (from 1049.00 to 785.31 μg/g). However, the rate of degradation slowed on the sixth and ninth days of storage. Furthermore, in this study, the relative contents of SFA, MUFA and PUFA remained virtually unchanged with variations in the storage duration of oysters.

Oysters are the most cultivated marine mollusk species globally, renowned for their high-quality proteins and omega-3 long-chain polyunsaturated fatty acids (n-3 PUFA). In this study, the crude protein content of raw oyster was found to be 9.12 %, exceeding that of *C. belcheri* sourced from Bandon Bay, Thailand (8.3 %) (Songsaeng et al., 2010), *C. hongkongensis* from Guangxi Province, China (8.43 %) (Liu et al., 2021), and *C. gigas* from Porto Recanati, Italy (8.85 %) (Felici et al., 2020). Furthermore, the fatty acid composition of our oyster samples, particularly C22:6n-3 (508.68 μg/g, accounting for 19.33 % of total fatty acids) and C20:5n-3 (420.10 μg/g, representing 15.96 % of total fatty acids), was comparable to previous findings in *C. ariakensis* (where C20:5n-3 and C22:6n-3 were 17.80 % and 11.20 %, respectively) and *C. gigas* (where C22:6n-3 and C20:5n3 were 21.39 % and 17.53 %, respectively). However, these values were notably higher than those reported in *C. hongkongensis* from Guangxi Province, China (where C20:5n3 and C22:6n3 were 6.62 % and 6.60 %, respectively). These variations in protein and fatty acid composition in oysters might be attributed to multiple factors, including species-specific traits, genetic characteristics, seasonal changes, and cultural environments (Felici et al., 2020; Gao et al., 2021; Pogoda et al., 2013; Songsaeng et al., 2010).

When compared to storage at freezing temperatures (typically ranging from −20 °C to −80 °C), mollusks preserved at 4 °C retained a certain level of vitality, leading to a gradual, albeit minimal, consumption of material energy over an extended period (Bi et al., 2023; Songsaeng et al., 2010). Additionally, anhydrous storage conditions also caused a notable loss of moisture within the mollusks' bodies (Jiang et al., 2024). Generally, organisms tend to prioritize the utilization of stored fats over proteins when exposed to environmental stressors, such as low temperatures (Chen et al., 2024). Consequently, in this experiment, the total lipid content in oysters underwent a pronounced decrease as the duration of storage increased. Specifically, both phospholipids and fatty acids exhibited a downward trend, whereas the content of non-polar triglycerides increased. This finding suggests that polar lipids are preferentially metabolized during low-temperature storage. In contrast, proteins, which were vital for maintaining functions like the opening and closing of bivalve shells and constituted the primary building blocks of striated and smooth muscles in mollusks and, exhibited a lesser reduction over time compared to lipids (Liu et al., 2021). The initial elevation in glycogen levels observed in oysters could be attributed to the early degradation of lipids, which were then subsequently converted into more readily utilizable glycogen through the tricarboxylic acid cycle (TCA) as a coping mechanism against environmental stressors (Bi et al., 2023).

3.4. Effects of anhydrous low-temperature storage on non-volatile taste active compounds

3.4.1. FAAs and 5′-nucleotides

In this study, we identified a total of twenty free amino acids (FAAs) in oysters (Table 3). Among these FAAs, alanine, glycine and taurine were the most predominant, exhibiting concentrations of 2.16, 181, and 1.66 mg/g of wet weight, respectively. At the initial point (0 days), the concentrations of MSG-like, sweet and bitter FAAs were 1.13, 5.68 and 2.02 mg/g wet weight, respectively. Over the storage period, the levels of these FAAs exhibited a trend of initial increase followed by a decrease, with the peak concentrations observed at either 3 or 6 days of storage. Conversely, the taurine content in oysters demonstrated a decreasing trend with prolonged storage time. Additionally, the concentrations of 5′-nucleotides were measured in oysters (Table 4). In the control oysters (0 d of storage), IMP was the primary nucleotide component (61.62 μg/g of wet weight), followed by GMP (40.48 μg/g of wet weight) and CMP (30.73 μg/g of wet weight). The concentrations of AMP and UMP were the lowest, at 17.44 and 13.22 μg/g of wet weight, respectively. The contents of all 5′-nucleotides exhibited an initial increase followed by a decrease, with the highest values observed at 6 d of storage, except for CMP and UMP, which peaked at 3 d of storage.

The concentration of FAAs and 5′-nucleotides are crucial to the taste of seafood. This is because most FAAs contribute components of umami, sweetness, sourness, and bitterness, thereby directly influencing the

Table 3
The contents (mg/g wet weight) and taste attributes (+pleasant, − unpleasant) of free amino acids (FAA) of oyster during anhydrous low-temperature storage.

FAA	Taste attribute	Storage time			
		0 d	3 d	6 d	9 d
Aspartic acid	Umami (+)	0.31 ± 0.00b	0.31 ± 0.01b	0.42 ± 0.00a	0.28 ± 0.00c
Glutamic acid	Umami (+)	0.82 ± 0.01d	0.90 ± 0.02b	0.87 ± 0.01c	0.94 ± 0.01a
MSG-like FAA		**1.13 ± 0.01c**	**1.22 ± 0.03b**	**1.28 ± 0.01a**	**1.22 ± 0.01b**
Threonine	Sweet (+)	0.24 ± 0.00b	0.22 ± 0.01c	0.31 ± 0.01a	0.21 ± 0.00c
Serine	Sweet (+)	0.22 ± 0.00b	0.19 ± 0.01c	0.26 ± 0.00a	0.16 ± 0.00d
Glycine	Sweet (+)	1.81 ± 0.06c	2.73 ± 0.08a	1.01 ± 0.02d	2.08 ± 0.01b
Alanine	Sweet (+)	2.16 ± 0.03c	2.16 ± 0.06c	2.62 ± 0.03a	2.30 ± 0.02b
Arginine	Bitter/ sweet (−)	0.21 ± 0.00b	0.17 ± 0.00d	0.21 ± 0.01c	0.24 ± 0.00a
Proline	Sweet/ bitter (+)	1.04 ± 0.02c	1.25 ± 0.02a	1.20 ± 0.02b	1.21 ± 0.01ab
Sweet FAA		**5.68 ± 0.11c**	**6.72 ± 0.18a**	**5.62 ± 0.09c**	**6.21 ± 0.04b**
Valine	Bitter/ sweet (−)	0.09 ± 0.00b	0.09 ± 0.00c	0.14 ± 0.00a	0.09 ± 0.00c
Methionine	Bitter/ sweet/ sulfurous (−)	0.09 ± 0.00b	0.09 ± 0.00b	0.13 ± 0.00a	0.06 ± 0.00c
Isoleucine	Bitter (−)	0.05 ± 0.00b	0.05 ± 0.00d	0.08 ± 0.00a	0.05 ± 0.01c
Leucine	Bitter (−)	0.06 ± 0.00b	0.06 ± 0.00c	0.09 ± 0.00a	0.06 ± 0.01b
Tryptophan	Bitter (−)	0.14 ± 0.00c	0.15 ± 0.00b	0.17 ± 0.01a	0.14 ± 0.00c
Phenylalanine	Bitter (−)	0.14 ± 0.00b	0.15 ± 0.00b	0.18 ± 0.01a	0.18 ± 0.01a
Lysine	Bitter/ sweet (−)	0.32 ± 0.02b	0.38 ± 0.02a	0.09 ± 0.00c	0.09 ± 0.00c
Histidine	Bitter (−)	1.06 ± 0.02d	1.14 ± 0.02c	1.63 ± 0.01a	1.18 ± 0.01b
Tyrosine	Bitter (−)	0.07 ± 0.00c	0.06 ± 0.00d	0.10 ± 0.00a	0.08 ± 0.00b
Bitter FAA		**2.02 ± 0.05c**	**2.14 ± 0.02b**	**2.62 ± 0.02a**	**1.93 ± 0.03d**
Asparaginate	Tasteless	0.29 ± 0.00a	0.20 ± 0.01c	0.24 ± 0.01b	0.16 ± 0.01d
Glutamine	Tasteless	0.51 ± 0.01b	0.67 ± 0.02a	0.50 ± 0.01b	0.48 ± 0.00b
Taurine	Tasteless	1.66 ± 0.03a	1.42 ± 0.03b	1.43 ± 0.02b	1.37 ± 0.03c
Total FAA		**10.16 ± 0.19c**	**11.15 ± 0.24a**	**10.41 ± 0.14bc**	**10.55 ± 0.11b**

Data represent means ± SD (n = 3). Values in a row with different letters indicate significant differences among treatments ($P < 0.05$).

distinctive flavors of specific foods. Additionally, sweetness and umami FAAs exhibit synergistic effects with 5′-nucleotides. Oysters possess a rich content of free amino acids and flavor nucleotides, thus presenting a delightful taste. In our study, for instance, the concentrations of aspartic and glutamic acids (both umami-contributing FAAs) in *C. hongkongensis* were 0.31 and 0.81 mg/g of wet weight, respectively. These values were slightly higher than those reported by Liu et al. (2022) for *C. ariakensis* (0.26 and 0.73 mg/g) and comparable to glutamic acid levels in *C. gigas* reported by Gao et al. (2021) (0.81 mg/g of wet weight), but higher for its aspartic acid (0.10 mg/g of wet weight). Notably, they were also higher than the concentrations in *C. gigas* reported by Bi et al. (2023) (0.19 mg/g and 0.21 mg/g for aspartic and glutamic acids, respectively). Additionally, the taurine content in *C. hongkongensis* from our study was 1.66 mg/g, marginally exceeding that reported for *C. gigas* (1.43 mg/g) by Bi et al. (2023). As for the total 5′-nucleotides content in

Table 4
The concentrations of 5′-nucleotides of oyster during anhydrous low-temperature storage.

Nucleotides (μg/g)	Storage time			
	0 d	3 d	6 d	9 d
CMP	30.73 ± 3.66b	41.19 ± 5.89a	30.57 ± 1.01b	37.09 ± 2.53ab
UMP	13.22 ± 0.69a	14.27 ± 4.23a	13.70 ± 0.61a	18.49 ± 1.27a
GMP	40.84 ± 2.74c	49.89 ± 5.79b	69.41 ± 1.54a	32.28 ± 0.77d
IMP	61.62 ± 5.73b	62.33 ± 0.73b	82.84 ± 2.64a	56.56 ± 1.97b
AMP	17.44 ± 1.04c	23.30 ± 2.98b	33.51 ± 3.59a	21.91 ± 0.80bc
Total	163.85 ± 10.56c	190.98 ± 15.61b	230.04 ± 8.94a	166.34 ± 7.08c

Data represent means ± SD (n = 3). Values in a row with different letters indicate significant differences among treatments ($P < 0.05$).

C. hongkongensis, it was 163.85 μg/g, which was lower than the range reported for *C. gigas* (97.71–726.57 μg/g) and *C. ariakensis* (480 μg/g) (Bi et al., 2021; Liu et al., 2022). In our study, IMP was the most abundant 5′-nucleotides in *C. hongkongensis*, consistent with findings in *C. gigas*, while AMP was the highest in *C. ariakensis* (Bi et al., 2023; Liu et al., 2022).

FAAs and nucleotides are not only key flavor contributors but also essential energy-yielding metabolites (Gao et al., 2021). In aquatic animals, FAAs play roles in osmolality and oxidative stress response (Chalamaiah et al., 2012). Concurrently, nucleotides are the fundamental constituents of DNA and RNA. Their concentrations are correlated with species-specific characteristics and also fluctuate in response to changes in physiological states (Gao et al., 2021; Liu et al., 2022). In our research, we observed a trend of initial increase followed by a subsequent decrease in the concentrations of FAAs and 5′-nucleotides after anhydrous low-temperature storage. FAAs are derived from the hydrolysis of catalyzed by protein hydrolase (Chalamaiah et al., 2012). Nucleotides, such as IMP and AMP, play pervasive roles in the fundamental life activities of organisms and supply energy for physiological processes (Yang et al., 2016). During the initial storage period, the concentrations of FAAs and 5′-nucleotides increase through the degradation of endogenous proteins and ribose/deoxyribose, allowing the oyster to resist low-temperature stress. However, as storage duration extends, these compounds are continuously consumed internally, and their depletion is exacerbated by the proliferation of microorganisms within the oyster, ultimately leading to a reduction in their concentrations. Regarding taurine, a non-protein amino acid, it possesses various physiological functions in aquatic animals, such as antioxidation and immunomodulation, and maintaining homeostasis (Sun et al., 2023). Due to the absence of an exogenous source, its concentration consistently declines as it is consumed during storage.

3.4.2. Organic acid

In this study, four organic acids (lactic acid, citric acid, succinic acid and malic acid) were identified (Table 5). Among these, citric acid was the primary organic acid, with a concentration of 805.10 μg/g wet weight in the fresh oysters, followed by lactic acid and succinic acid. Notably, malic acid was absent from our experimental detection. Furthermore, as the storage duration increased, the concentrations of citric acid and succinic acid exhibited an initial rise followed by a rapid decline, reaching their peak values at 3 days and 6 days post-storage, respectively. In contrast, the lactic acid content demonstrated a gradual increase throughout the entire storage period.

Citric, lactic and succinic acids are recognized as flavor-enhancing acids that play pivotal roles in shaping the intricate and distinctive taste profiles of oysters. Specifically, citric acid contributes a mild crisp and acidic flavor, lactic acid enhances buffer capacity, and succinic acid

Table 5
The concentrations of organic acids of oyster during anhydrous low-temperature storage.

Organic acids (μg/g)	Storage time			
	0 d	3 d	6 d	9 d
Lactic acid	349.05 ± 27.94d	717.54 ± 6.48c	970.25 ± 34.01b	1066.69 ± 27.62a
Citric acid	805.10 ± 34.29c	1590.97 ± 14.42a	1072.56 ± 1.86b	355.76 ± 2.71d
Succinic acid	5.08 ± 1.89d	195.77 ± 7.34b	300.80 ± 19.27a	40.77 ± 9.18c
Total	1159.22 ± 59.35d	2504.28 ± 13.56a	2343.61 ± 55.14b	1463.21 ± 15.73c

Data represent means ± SD ($n = 3$). Values in a row with different letters indicate significant differences among treatments ($P < 0.05$).

adds a sour taste (Chen et al., 2022; Liu et al., 2022). Our research revealed that the total organic acid content in fresh *C. hongkongensis* was 1.16 mg/g, which was lower than that reported for *C. ariakensis* (7.92 mg/g, Liu et al., 2022), but higher than for *C. gigas* (0.24 mg/g, Chen et al., 2022). Citric and succinic acids are well known components of the TCA and have been demonstrated to possess potent antibacterial effects against significant foodborne pathogens. In this study, the concentrations of these two acids initially increased and then decreased, suggesting a gradual reduction in the oyster's resistance to foodborne pathogens as storage time increased. As for lactic acid, it can be produced through both the anaerobic respiration of oysters and the activity of anaerobic lactic acid bacteria. The progressive rise in lactic acid levels could be attributed to the increasing population of anaerobic lactic acid bacteria within the oysters.

3.5. Effects of anhydrous low-temperature storage on volatile compounds

The changes in volatile compounds in oyster stored for different storage durations are presented in Table S1. A total of thirty-six volatile compounds were identified, including seventeen aldehydes, eight alcohols, five ketones, and four alkenes. Among these, 1-penten-3-ol, 2,3-pentanedione, hexanal, 2,4-heptadienal and 2,5,5-trimethyl-2-hexene were identified as the dominant volatile compounds in Hong Kong oyster. Notably, the composition and content of volatile flavor compounds vary significantly among different oyster species reported in previous studies. These variations can be attributed to multiple factors, including species-specific characteristics, aquaculture environments, reproductive and developmental stages, post-harvest storage conditions, as well as the analytical conditions used for volatile compound determination (Kawabe et al., 2010; Liu et al., 2023).

In this experiment, the proportion of aldehydes increased significantly by the sixth day of storage, rising from 37.93 %–40.19 % to more than 50 %. In contrast, the proportions of other volatile compounds, such as alcohols, ketones, and alkenes, gradually decreased during the same period. The increasing trend of aldehydes has also been observed in cold stored Eastern oyster (*C. virginica*) and deteriorated Pacific oyster compared to fresh individuals (Zhang et al., 2009; Zhang et al., 2020). Hexanal is a volatile marker commonly used to distinguish the freshness of aquatic products. In our experiment, the proportion of hexanal did not exhibit significant changes, a phenomenon consistent with the results reported by Zhang et al. (2020) for raft-cultured oysters under low-temperature storage. In contrast, Kawabe et al. (2019) observed a gradual decline in hexanal content during 1–7 days of storage at 5 °C, although the differences among groups were not statistically significant. 2,4-Heptadienal is an important off-flavor compound and a marker for the loss of freshness, which is an autoxidation product of PUFAs (Zhang et al., 2020). In this study, the content of 2,4-heptadienal in oysters increased significantly from the 6th day of storage, a trend consistent with the decline in polyunsaturated fatty acids, indicating a reduction in oyster freshness. Furthermore, in this study, although the contents of 2,5,5-trimethyl-2-Hexene, 2,3-Pentanedione, and 1-Penten-3-ol varied compared to those reported in other oyster species, their proportions consistently decreased over storage time, suggesting their potential as markers for oyster freshness (Kawabe et al., 2019).

4. Conclusion

This study systematically investigated the effects of anhydrous low-temperature storage on the post-capture survival, microbial dynamics, and meat quality of Hong Kong oysters. Our findings demonstrate that storage at 4 °C maintains a high survival rate (>94.44 %) for up to six days, beyond which significant mortality occurs. Comprehensive analysis revealed that: (1) Progressive spoilage indicators, including TVB-N and MDA levels, showed marked increases after nine days, while pH and surface color remained stable; (2) Tissue-specific sensitivity was observed, with adductor muscle damage appearing after six days and gonad deterioration after three days; (3) Microbial dynamics exhibited an initial increase followed by a decline in total aerobic bacteria and pathogenic species (*E. coli*, *V. parahaemolyticus*, and *V. vulnificus*), contrasting with the continuous proliferation of anaerobic lactic acid bacteria; (4) Nutritional analysis revealed significant declines in crude protein, total lipid, phospholipid, and fatty acid contents, while glycogen displayed a biphasic trend and triglycerides consistently increased; (5) Biochemical analysis showed dynamic changes in non-volatile compounds, with most FAAs, 5′-nucleotides, and organic acids increasing initially before decreasing, while lactic acid progressively accumulated; (6) Volatile compound analysis identified aldehydes as the dominant group, with hexanal remaining stable and 2,4-heptadienal significantly increasing by day six. Based on these comprehensive findings, we recommend that the storage duration for Hong Kong oysters at 4 °C should not exceed six days to ensure optimal quality and safety.

CRediT authorship contribution statement

Chun-Sheng Liu: Writing – review & editing, Writing – original draft, Supervision, Software, Funding acquisition, Formal analysis, Conceptualization. **Qing-Song Hu:** Software, Methodology, Investigation, Conceptualization. **Ling-Xiang Bao:** Methodology, Investigation. **Xin Hong:** Methodology, Investigation. **Yi Yang:** Resources, Investigation, Data curation. **Ai-Min Wang:** 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.

Acknowledgements

This research was financially supported by the Key Research and Development Project of Hainan Province (ZDYF2023XDNY032, ZDYF2024SHFZ139).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102398.

Data availability

Data will be made available on request.

References

Atayeter, S., & Ercoşkun, H. (2011). Chemical composition of European squid and effects of different frozen storage temperatures on oxidative stability and fatty acid

composition. *Journal of Food Science and Technology, 48*(1), 83–89. https://doi.org/10.1007/s13197-010-0139-5

Bejaoui, S., Ghribi, F., Chetoui, I., Aouini, F., Bouaziz, M., Houas-Gharsallah, I., … Cafsi, M. E. (2021). Effect of storage temperature and time on the fatty acids and nutritional quality of the commercial mussel (*Mytilus galloprovincialis*). *Journal of Food Science and Technology, 58*(9), 3493–3503. https://doi.org/10.1007/s13197-021-05008-5

Bi, S., Chen, L., Sun, Z., Wen, Y., Xue, Q., Xue, C., Li, Z., Sun, C., Wei, Z., & Liu, H. (2021). Investigating influence of aquaculture seawater with different salinities on non-volatile taste-active compounds in Pacific oyster (Crassostrea gigas). *Journal of Food Measure and Characterization, 15*, 2078–2087. https://doi.org/10.1007/s11694-020-00807-4

Bi, S., Xue, C., Wen, Y., Du, X., Xue, Q., Li, Z., & Liu, H. (2023). Effects of cooling rates during depuration on the quality of Pacific oysters (*Crassostrea gigas*) at anhydrous preservation stage. *Food Chemistry: X, 17*, Article 100606. https://doi.org/10.1016/j.fochx.2023.100606

Chalamaiah, M., Dineshkumar, B., Hemalatha, R., & Jyothirmayi, T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chemistry, 135*, 3020–3038. https://doi.org/10.1016/j.foodchem.2012.06.100

Chen, L., Wang, W., Shi, H., Li, Z., Gao, C., Zhang, X., Xue, Y., & Zhang, H. (2024). Investigating comprehensive effects of depuration salinity and duration on posterior anhydrous living-preservation of Pacific oyster (*Crassostrea gigas*). *Food Chemistry, 435*, Article 137545. https://doi.org/10.1016/j.foodchem.2023.137545

Chen, L., Xue, Y., Zhang, H., Wang, Y., Shi, H., & Li, Z. (2022). The flavor profile changes of Pacific oysters (*Crassostrea gigas*) in response to salinity during depuration. *Food Chemistry: X, 16*, Article 100485. https://doi.org/10.1016/j.fochx.2022.100485

Chung, W. H., Howieson, J., & Chaklader, M. D. R. (2021). The ameliorative effects of low-temperature pasteurization on physicochemical and microbiological quality of raw Akoya pearl oyster (*Pinctada fucata*). *Food Control, 129*, Article 108241. https://doi.org/10.1016/j.foodcont.2021.108241

Dhaouadi, A., Monser, L., Sadok, S., & Adhoum, N. (2007). Validation of a flow-injection-gas diffusion method for total volatile basic nitrogen determination in seafood products. *Food Chemistry, 103*(3), 1049–1053. https://doi.org/10.1016/j.foodchem.2006.07.066

Diner, R. E., Zimmer-Faust, A., Cooksey, E., Allard, S., Kodera, S. M., Kunselman, E., … Gilbert, J. A. (2023). Host and water microbiota are differentially linked to potential human pathogen accumulation in oysters. *Applied and Environmental Microbiology, 89*, e00318–e00323. https://doi.org/10.1128/aem.00318-23

FAO. (2024). *The state of world fisheries and aquaculture 2024*. Rome: Blue Transformation in action. https://doi.org/10.4060/cd0683en

Felici, A., Vittori, S., Meligrana, M. C. T., & Roncarati, A. (2020). Quality traits of raw and cooked cupped oysters. *European Food Research and Technology, 246*, 349–353. https://doi.org/10.1007/s00217-019-03348-3

Froelich, B. A., Phippen, B., Fowler, P., Noble, R. T., & Oliver, J. D. (2017). Differences in abundances of total *Vibrio* spp., *V. Vulnificus*, and *V. Parahaemolyticus* in clams and oysters in North Carolina. *Applied and Environmental Microbiology, 83*. https://doi.org/10.1128/AEM.02265-16. e02265–16.

Gao, J., Zhang, Y., Huang, X., Liu, R., Dong, X., Zhu, B., & Qin, L. (2021). Comparison of amino acid, 5′-nucleotide and lipid metabolism of oysters (*Crassostrea gigas* Thunberg) captured in different seasons. *Food Research International, 147*, Article 110560. https://doi.org/10.1016/j.foodres.2021.110560

Goulas, A. E., Chouliara, I., Nessi, E., Kontominas, M. G., & Savvaidis, I. N. (2005). Microbiological, biochemical and sensory assessment of mussels (*Mytilus galloprovincialis*) stored under modified atmosphere packaging. *Journal of Applied Microbiology, 98*(3), 752–760. https://doi.org/10.1111/j.1365-2672.2004.02512.x

Guo, X., He, Y., Zhang, L., Lelong, C., & Jouaux, A. (2015). Immune and stress responses in oysters with insights on adaptation. *Fish & Shellfish Immunology, 46*(1), 107–119. https://doi.org/10.1016/j.fsi.2015.05.018

Jiang, P., Qin, X., Fan, X., Zhang, C., & Chen, D. (2024). The impact of anhydrous storage and transportation at ice temperatures on the post-capture viability and quality of *Patinopecten yessoensis*. *Journal of Agriculture and Food Research, 18*, Article 101277. https://doi.org/10.1016/j.jafr.2024.101277

Kawabe, S., Takada, M., Shibuya, R., & Yokoyama, Y. (2010). Biochemical changes in oyster tissues and hemolymph during long-term air exposure. *Fisheries Science, 76*, 841–855. https://doi.org/10.1007/s12562-010-0263-1

Kim, S. H., Jung, E. J., Hong, D. L., Lee, S. E., Lee, Y. B., Cho, S., & Kim, S. B. (2020). Quality assessment and acceptability of whiteleg shrimp (*Litopenaeus vannamei*) using biochemical parameters. *Fisheries and Aquatic Sciences, 23*, 21. https://doi.org/10.1186/s41240-020-00167-6

Lekjing, S., & Venkatachalam, K. (2018). Effects of modified atmos pheric packaging conditions on the quality changes of pasteurized oyster (*Crassostrea belcheri*) meat during chilled storage. *Journal of Aquatic Food Product Technology, 27*(10), 1106–1119. https://doi.org/10.1080/10498850.2018.1534917

Liu, C., Gu, Z., Lin, X., Wang, Y., Wang, A., Sun, Y., & Shi, Y. (2022). Effects of high hydrostatic pressure (HHP) and storage temperature on bacterial counts, color change, fatty acids and non-volatile taste active compounds of oysters (*Crassostrea ariakensis*). *Food Chemistry, 372*, Article 131247. https://doi.org/10.1016/j.foodchem.2021.131247

Liu, C., Ji, W., Jiang, H., Shi, Y., He, L., Gu, Z., & Zhu, S. (2021). Comparison of biochemical composition and non-volatile taste active compounds in raw, high hydrostatic pressure-treated and steamed oysters *Crassostrea hongkongensis*. *Food Chemistry, 344*, Article 128632. https://doi.org/10.1016/j.foodchem.2020.128632

Liu, L., Zhao, Y., Lu, S., Liu, Y., Xu, X., & Zeng, M. (2023). Metabolomics investigation on the volatile and non-volatile composition in enzymatic hydrolysates of Pacific oyster (*Crassostrea gigas*). *Food Chemistry: X, 17*, Article 100569. https://doi.org/10.1016/j.fochx.2023.100569

Lokmer, A., Kuenzel, S., Baines, J. F., & Wegner, K. M. (2016). The role of tissue specific microbiota in initial establishment success of Pacific oysters. *Environmental Microbiology, 18*, 970–987. https://doi.org/10.1111/1462-2920.13163

López-Caballero, M. E., Pérez-Mateos, M., Montero, P., & Borderías, A. J. (2000). Oyster preservation by high-pressure treatment. *Journal of Food Protection, 63*(2), 196–201. https://doi.org/10.4315/0362-028X-63.2.196

Lorentzen, G., Rotabakk, B. T., Olsen, S. H., Skuland, A. V., & Siikavuopio, S. I. (2016). Shelf life of snow crab clusters (*Chionoecetes opilio*) stored at 0 and 4 °C. *Food Control, 59*, 454–460. https://doi.org/10.1016/j.foodcont.2015.06.019

Lu, A., Zhang, X., Zheng, P., Zhang, Z., Li, J., Wang, D., Xian, J., Wang, A., & Wang, L. (2021). Effects of air exposure on survival, histological structure, non-specific immunity and gene expression of red claw crayfish (*Cherax quadricarinatus*). *Aquaculture Reports, 21*, Article 100898. https://doi.org/10.1016/j.aqrep.2021.100898

Odeyemi, O. A., Burkea, C. M., Bolcha, C. C. J., & Stanley, R. (2018). Seafood spoilage microbiota and associated volatile organic compounds at different storage temperatures and packaging conditions. *International Journal of Food Microbiology, 280*, 87–99. https://doi.org/10.1016/j.ijfoodmicro.2017.12.029

Pogoda, B., Buck, B. H., Saborowski, R., & Hagen, W. (2013). Biochemical and elemental composition of the offshore-cultivated oysters *Ostrea edulis* and *Crassostrea gigas*. *Aquaculture, 400-401*, 53–60. https://doi.org/10.1016/j.aquaculture.2013.02.031

Rodezno, L. A. E., Bonilla, F., Reyes, V., Xu, Z., Bechtel, P. J., & Sathivel, S. (2023). Modified atmosphere packaging (MAP) on nutritional stability and quality retention of oyster meat (Crassostrea virginica) during frozen storage. *Journal of Package Technology and Research, 7*, 11–22. https://doi.org/10.1007/s41783-022-00145-4

Schets, F. M., Berg, H. H. J. L. V., Rutjes, S. A., & Husman, A. M. D. R. (2010). Pathogenic *Vibrio* species in Dutch shellfish destined for direct human consumption. *Journal of Food Protection, 73*, 734–738. https://doi.org/10.4315/0362-028X-73.4.734

Shi, Y., Qiu, Z., Yin, M., Zhang, L., Zhao, Y., Zhang, R., Shi, W., & Wang, X. (2025). Key non-volatile taste components and characteristics in abdominal muscle of *Eriocheir sinensis* under various thermal processing methods. *Food Chemistry, 464*, Article 141902. https://doi.org/10.1016/j.foodchem.2024.141902

Songsaeng, S., Sophanodora, P., Kaewsrithong, J., & Ohshima, T. (2010). Quality changes in oyster (*Crassostrea belcheri*) during frozen storage as affected by freezing and antioxidant. *Food Chemistry, 123*(2), 286–290. https://doi.org/10.1016/j.foodchem.2010.04.033

Sun, Y., Du, X., Yang, Y., Wang, A., Gu, Z., & Liu, C. (2023). Dietary taurine intake affects the growth performance, lipid composition, and antioxidant defense of juvenile ivory shell (*Babylonia areolata*). *Animals, 13*(16), 2592. https://doi.org/10.3390/ani13162592

Tarr, C. L., Patel, J. S., Puhr, N. D., Sowers, E. G., Bopp, C. A., & Strockbine, N. A. (2007). Identification of *Vibrio* isolates by a multiplex PCR assay and rpoB sequence determination. *Journal of Clinical Microbiology, 45*(1), 134–140. https://doi.org/10.1128/JCM.01544-06

Ulusoy, Ş., & Özden, Ö. (2011). Preservation of stuffed mussels at 4 °C in modified atmosphere packaging. *Journal of Aquatic Food Product Technology, 20*, 319–330. https://doi.org/10.1080/10498850.2011.573180

Vezzulli, L., Stagnaro, L., Grande, C., Tassistro, G., Canesi, L., & Pruzzo, C. (2017). Comparative 16SrDNA gene-based microbiota profiles of the Pacific oyster (*Crassostrea gigas*) and the Mediterranean mussel (*Mytilus galloprovincialis*) from a shellfish farm (Ligurian Sea, Italy). *Microbial Ecology, 75*, 495–504. https://doi.org/10.1007/s00248-017-1051-6

Wang, S., Huang, S., Han, Y., Wu, J., Jiao, T., Wei, J., Chen, X., Chen, Q., & Chen, Q. (2023). Applications of colorimetric sensors for non-destructive predicting total volatile basic nitrogen (TVB-N) content of Fujian oyster (*Crassostrea angulata*). *Food Control, 153*, Article 109914. https://doi.org/10.1016/j.foodcont.2023.109914

Yang, C. Y., Sierp, M. T., Abbott, C. A., Li, Y., & Qin, J. G. (2016). Responses to thermal and salinity stress in wild and farmed Pacific oysters *Crassostrea gigas*. *Comparative biochemistry and physiology. Part A, Molecular & Integrative Physiology, 201*, 22–29. https://doi.org/10.1016/j.cbpa.2016.06.024

Yu, M., Wang, X., & Yan, A. (2021). Microbial profiles of retail Pacific oysters (*Crassostrea gigas*) from Guangdong province, China. *Frontiers in Microbiology, 12*, Article 689520. https://doi.org/10.3389/fmicb.2021.689520

Zhang, J., Liu, S., Walton, W. C., & Wang, Y. (2020). Volatile organic compounds of eastern oyster (*Crassostrea virginica*) cultured by two treatments and their changes during cold storage. *Aquaculture Research, 52*, 1442–1452. https://doi.org/10.1111/are.14998

Zhang, T., Xu, D., Lv, G., Wang, A., & Wen, H. (2022). Histological, physiological, and transcriptomic responses of hepatopancreas to air exposure in Asian freshwater clam *Corbicula fluminea*. *Frontiers in Physiology, 13*, Article 952744. https://doi.org/10.3389/fphys.2022.952744

Zhang, Z. M., Li, T. L., Wang, D., Zhang, L., & Chen, G. N. (2009). Study on the volatile profile characteristics of oyster Crassostrea gigas during storage by a combination sampling method coupled with GC/MS. *Food Chemistry, 115*(3), 1150–1157. https://doi.org/10.1016/j.foodchem.2008.12.099

Further reading

King, W. L., Kaestli, M., Siboni, N., Padovan, A., Christian, K., Mills, D., … Gibb, K. (2021). Pearl oyster bacterial community structure is governed by location and tissue-type, but *Vibrio* species are shared among oyster tissues. *Frontiers in Microbiology, 12*, Article 723649. https://doi.org/10.3389/fmicb.2021.723649