Food Chemistry: X 21 (2024) 101210

Contents lists available at ScienceDirect

Food Chemistry: X

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

Changes in the physicochemical characteristics and microbial community compositions of the abdomen and cheliped muscles in swimming crab (*Portunus trituberculatus*) during frozen storage

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ARTICLE INFO

Keywords:
Swimming crab
Frozen storage
Quality properties
Physicochemical indexes
High-throughput sequencing

ABSTRACT

The physicochemical indexes and microbial diversity were investigated to compare the altered quality properties of the abdomen and cheliped muscle in swimming crab (*Portunus trituberculatus*) during 100 days of frozen storage at −20°C. Over the extended duration of frozen storage, the sensory evaluation, moisture content, water activity (Aw), and water-holding capacity (WHC) in the abdomen and cheliped muscles of swimming crab decreased, while the pH, total volatile basic nitrogen (TVB-N), and trimethylamine (TMA) increased. The increase and decrease rates of these indicators were smaller in the abdomen than those in the cheliped muscle. High-throughput sequencing results indicated a reduction in the microbial richness and diversity in the abdomen and cheliped muscles of the swimming crab as frozen storage time extended. *Proteobacteria*, *Actinobacteriota*, and *Firmicutes*, *Achromobacter*, *Kocuria*, and *Staphylococcus* were the dominant phylum and genus in both muscle tissues, respectively. Furthermore, the correlation analysis between the composition of the microbiota and physiochemical properties revealed that the growths of *Kocuria*, *Vibrio*, *Staphylococcus,* and *Aliiroseovarius* were closely related to the physiochemical factors. The study provides a theoretical reference for quality deterioration and develops new products of different parts in the swimming crab during frozen storage.

1. Introduction

The swimming crab (*Portunus trituberculatus*), a commercially significant coastal species, exhibits a broad distribution in the marine regions of Asian countries, including China, Japan and South Korea (Song, Shi, Meng, Wu, & Zhang, 2020). The production volume of swimming crabs was 442,000 tons in 2020 (FAO, 2022). Swimming crab has gained recognition as a unique culinary offering in coastal regions due to its delightful taste, nutritional richness, and distinctive flavor (Yang, Hu, Takaki, Yu, & Yuan, 2021). Due to the high salt and oxygen in the oceanic environment, the survival rate of the swimming crab is significantly diminished after capture (Liu, Chen, Chen, Wang, Li, & Mao, 2023). The quality of the untreated swimming crab undergoes swift deterioration after death due to biochemical, microbiological and physical changes. At present, frozen storage is commonly used for swimming crab preservation. However, some quality spoilage, such as decreased freshness, unacceptable qualities, and microbial growth, still occurs during frozen storage (Zhou, Ding, Benjakul, Shui, & Zhang, 2023).

Several studies have reported notable alterations in the physicochemical attributes of crab muscle during low-temperature storage, encompassing a reduction in the salt extractable protein and Ca^{2+}-ATPase activity; a gradual increase in the histamine, total viable count, pH, total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) (Sun, Zhao, Ling, Yu, Shang, & Liu, 2017; Guo, et al., 2023; Anupama, Laly, Kumar, Sankar, & Ninan, 2018). These changes caused the deterioration of the flavor, texture, and nutritional content of the crab. However, these reports focused on a single part of the crab muscle. Comprehensive systematic investigations were currently lacking to compare the quality attributes of the abdomen and cheliped muscles in

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https://doi.org/10.1016/j.fochx.2024.101210
Received 21 December 2023; Received in revised form 23 January 2024; Accepted 5 February 2024
Available online 10 February 2024

the swimming crab during frozen storage.

High-throughput sequencing (HTS) methods based on 16S rRNA are efficient molecular biological technology to discern alterations in microbial community structure of aquatic products, which could furnish precise information on microbial biodiversity (Chen et al., 2022). HTS enables the simultaneous determination of millions of DNA molecular sequences, breaking the constraints of traditional microbiology and enabling the concurrent identification of dominant flora in a given sample (Li, Zhao, Li, Zhang, Sun, & Li, 2023). Wang et al. (2022) used HTS to observe the fluctuations in microbial diversity in tilapia fillets subjected to high voltage atmospheric cold plasma as storage time extended, and the long treatment time exhibited a significant reduction in bacterial quantities. Zhang et al. (2023) observed a substantial reduction in microbial community diversity in large yellow croaker during storage when employing a low-voltage electrostatic field treatment combined with partial freezing, as assessed through HTS. However, research has yet to report on using HTS to analyze the microbial changes and composition of the swimming crab muscle during frozen storage.

Therefore, the present study used physicochemical indicators such as sensory score, moisture content, water activity (Aw), water-holding capacity (WHC), pH, TVB-N, and TMA, as well as an analysis of microbiota composition to evaluate the quality of the abdomen and cheliped muscles in swimming crab during 100 days of frozen storage (-20℃). This study established a foundation for quality changes and spoilage bacteria growth of different part muscles in the swimming crab.

2. Materials and methods

2.1. Chemical reagents

Sodium chloride (NaCl), boric acid, magnesium oxide (MgO), hydrochloric acid, methyl red, bromocresol green, and ethanol were procured from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). A TMA assay kit was obtained from Jiangsu Jingmei Biological Technology Co., Ltd. (Yancheng, China).

2.2. Swimming crab samples and treatments

Sixty live swimming crabs (200 ± 50 g) were procured from a local aquatic market in Zhoushan, Zhejiang Province, China. Swimming crabs were carefully placed in an oxygenated sealed bag and then promptly transferred to an incubator filled with ice, which was immediately transported to the laboratory within 40 min. The live swimming crabs underwent a meticulous washing procedure using distilled water upon arrival and were subsequently euthanized through rapid cooling. The swimming crab samples were stored at −20℃ for 100 days, and ten crab samples were randomly selected every 20 days (0, 20, 40, 60, 80, and 100 days) for further analyses. The abdomen and cheliped muscles of swimming crabs were derived from the body and first claws, respectively, which were used to detect subsequent indexes.

2.3. Sensory analysis

A sensory evaluation was conducted using quantitative descriptive analysis (QDA) following the methodology outlined by Yang et al. (Yang et al., 2021). After multiple rounds of testing, training and sensory experiments, eight preferred evaluators (three men and five women) were finally selected to form a sensory evaluator group. Eight sensory description words and definitions of swimming crab were determined by the evaluators (Table S1). The swimming crab muscle with different storage times were assessed by the trained sensory panel. The panelists individually scored the samples on a scale of 0 to 10, where 0 represented none, 4 indicated the acceptability limit, and 10 signified the highest intensity, referencing the criteria outlined in Table S1.

2.4. Moisture content and water activity (Aw) analyses

The moisture content of swimming crab muscle during frozen storage was quantified through a direct drying method following the previously reported method with slight modifications (Ding, Zhou, Liao, Lin, Deng, Zhang, 2022).

The Aw of the swimming crab muscle was determined using the diffusion method for the water activity meter (JH-HD, Qingdao Juchuang Environmental Co., Ltd., Qingdao, China) in accordance with the methodology outlined by Yu et al. (Yu et al., 2023).

2.5. Water-holding capacity (WHC) and pH analyses

The WHC was measured following the previously reported method with minor adjustments (Yang, Ma, Bian, Mei, & Xie, 2023). Muscle samples (3.00 g, M_1) were accurately weighed and wrapped in filter paper and then put into centrifuge tubes with cotton. Following centrifugation at 4℃ (3000 × g for 10 min; H1750R, Hunan Xiangyi Laboratory Instrument Development Co., Ltd., Changsha, China), the samples were removed from the centrifuge tubes and then reweighed (M_2). The WHC in the abdomen and cheliped muscles of the swimming crab was computed using the formula: $WHC = M_2 / M_1 \times 100$ %.

The pH value of swimming crab muscle during frozen storage was conducted following methodology outlined by Wang et al. with slight adjustments (Wang, Wang, Xu, & Sun, 2022). Saline (18 mL) was added to the crab muscles (2.00 g), homogenized for 1 min and bathed at 4℃ for 20 min. Subsequently, the homogenized mixture underwent centrifugation at 3000 × g under 4℃ for 10 min. The resulting supernatant was then measured for pH value utilizing a pH meter (FE28, Mettler Toledo Instrument (Shanghai) Co., Ltd., Shanghai, China).

2.6. Total volatile basic nitrogen (TVB-N) content and trimethylamine (TMA) content analyses

The determination of TVB-N content in swimming crab muscle during frozen storage was conducted using an automatic Kjeldahl apparatus (KDN-520, Shanghai Bangyi Precision Measuring Instrument CO., LTD., Shanghai, China). In brief, 10.0 g of swimming crab sample was precisely weighted and placed into a distillation tube. Subsequently, 75 mL of distilled water was added to ensure thorough mixing and allowed to soak for 30 min. Then, 1 g of MgO powder was introduced into the mixture and promptly transferred to the automatic Kjeldahl apparatus to commence measurement in accordance with the operational manual of the instrument. The resulting measurements were reported as mg of TVB-N per 100 g of swimming crab sample.

TMA content was quantified utilizing a TMA assay kit (Jiangsu Jingmei Biological Technology Co., Ltd., Yancheng, China). In brief, the muscle samples from the swimming crab were homogenized in a saline solution at a ratio of 9 volumes. Subsequently, the mixture was centrifuged at 3000 × g under 4℃ for 10 min. The resulting supernatant was collected and analyzed in accordance with the instructions provided by the kit. The final results were the TMA content in swimming crab muscle and expressed as μg/g.

2.7. High throughput sequence analysis

The total microbial genomic DNA extraction from swimming crab muscle was carried out following the instructions of the FastDNA® Spin Kit (MP Biomedicals, Santa Ana, CA, USA). The quality of the isolated genomic DNA was assessed through 1 % agarose gel electrophoresis, while the DNA concentration and purity were determined using NanoDrop® ND-2000 spectrophotometer (Thermo Scientific Inc., USA). The hypervariable region V3-V4 of the 16S rRNA gene was PCR amplified using universal primers 341F and 806R to analyze the bacterial community (Liu et al., 2016). Each sample underwent processing in triplicate. The PCR products from the same sample were pooled, and gel extraction of the PCR products was performed using 2 % agarose gel. The recovered products were purified with the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), followed by detection through 2 % agarose gel electrophoresis.

The sequence analysis used Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA), following standardized protocols provided by Majorbio Bio-Pharm Technology CO. Ltd. (Shanghai, China). The raw sequencing reads have been deposited into the NCBI Sequence Read Archive database. The data processing and bioinformatic analyses of the microbiota were conducted utilizing the Majorbio cloud platform.

2.8. Statistical analysis

All experiments in this study were replicated in three replications (n = 3). The obtained measurement results underwent statistical analysis employing SPSS 27.0 software (SPSS Inc., Chicago, IL, USA). A comparative analysis of the treatment average was conducted using Duncan's test, and a *P*-value < 0.05 indicated significance in difference. Pearson correlation analysis between physiochemical indexes and microbiota composition was conducted utilizing Origin 2021 (Origin - Lab Corp, Northampton, MA, USA).

3. Results and discussions

3.1. Sensory property analysis

Sensory assessment serves as a prevalent and intuitive measure for gauging the freshness and quality attributes of marine products (Du, Chen, Jiang, &Zhang, 2022). According to the sensory description vocabulary in Table S1, the muscle quality in the abdomen and cheliped muscles of the swimming crab were scored and shown in Fig. 1. The scores of fresh swimming crab (0 days) have no significant difference between the abdomen and cheliped muscles. However, two parts of the muscle gradually loosed, and the color of the muscle changed from white to beige during 100 days of frozen storage. An irritating and putrefactive odor gradually replaced the typical crab fragrance. The liquid exudation gradually increased, and the muscle stickiness was enhanced. These results suggested that the quality of crabs had deteriorated under cold stress. Compared with the abdomen, the cheliped muscle presented a greater degree of relaxation, stronger viscosity, irritant and ammonia odor, and more liquid exudation, indicating that the deterioration rate of the cheliped muscle was faster than that of the abdomen muscle.

3.2. Moisture content and water activity (Aw) analyses

The moisture content plays a significant role in determining the storage stability of aquatic products, which direct or indirect impacts their overall quality (Yu et al., 2023). The moisture content in the abdomen and cheliped muscles of the swimming crab gradually decreased throughout the frozen storage period, as depicted in Fig. 2A. Previous studies have indicated that when the storage temperature of perch fell below freezing, a fraction of the water within the fish would undergo freezing. The sublimation effect subsequently occurred, which caused the loss of water from the product (Shi et al., 2018). The moisture content in the abdomen muscle was notably greater than the cheliped muscle ($p < 0.05$); this finding aligned with the results reported in the muscle tissues of Chinese mitten crab by Zhang et al. (Zhang, Wang, Zhou, Zheng, & Wang, 2020).

The Aw has an important influence on the proliferation of microorganisms and the tissue structure of aquatic products (Xie et al., 2023). Sustaining a heightened water activity has been recognized as a pivotal factor in ensuring optimal quality for aquatic products (Farouk, Wieliczko, & Merts, 2004). The Aw values in the abdomen and cheliped muscles of the swimming crab showed similar decreasing trends during frozen storage (Fig. 2B). The initial Aw value in the abdomen and cheliped muscles of swimming crab was 0.986 and 0.981, which significantly decreased to 0.806 and 0.805 ($P < 0.05$), respectively, after 100 days. This decline in Aw could be ascribed to ice formation involving approximately 80 % of the water in the swimming crab muscle and the concurrent decrease in the moisture content during the frozen storage process at −20℃.

3.3. Water-holding capacity (WHC) and pH analyses

The WHC pertains to the capability of muscle to retain moisture during postmortem storage, which is primarily influenced by the extent of protein denaturation and proteolysis (Zhang, Zhao, Chen, Zhang, & Wei, 2019). Fig. 3A showed that the WHC in the abdomen and cheliped muscles of the swimming crab continuously decreased during frozen storage ($p < 0.05$). The variation tendency of WHC was consistent with the moisture content and Aw. Some previous studies suggested that the creation of big ice crystals due to slow freezing in refrigerators could lead to detrimental effects on muscle tissue and myocytes, thereby facilitating the release of cell contents and intercellular drip loss (Zhou, et al., 2023; Nikoo, Benjakul, Ahmadi Gavlighi, Xu, & Regenstein, 2019). The abdomen muscle showed higher WHC than the cheliped muscle after 40 days ($p < 0.05$), revealing that the abdomen muscle exhibited significantly better quality than the cheliped muscle at the end

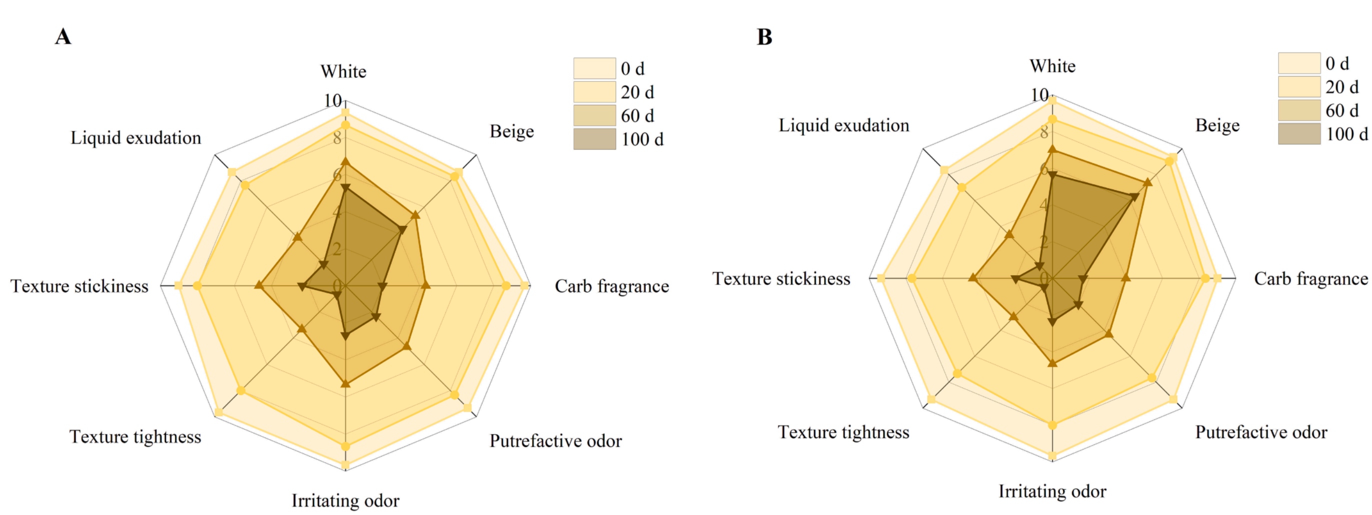

Fig. 1. Quantitative descriptive analysis in the abdomen (A) and cheliped (B) muscles of swimming crab during frozen storage.

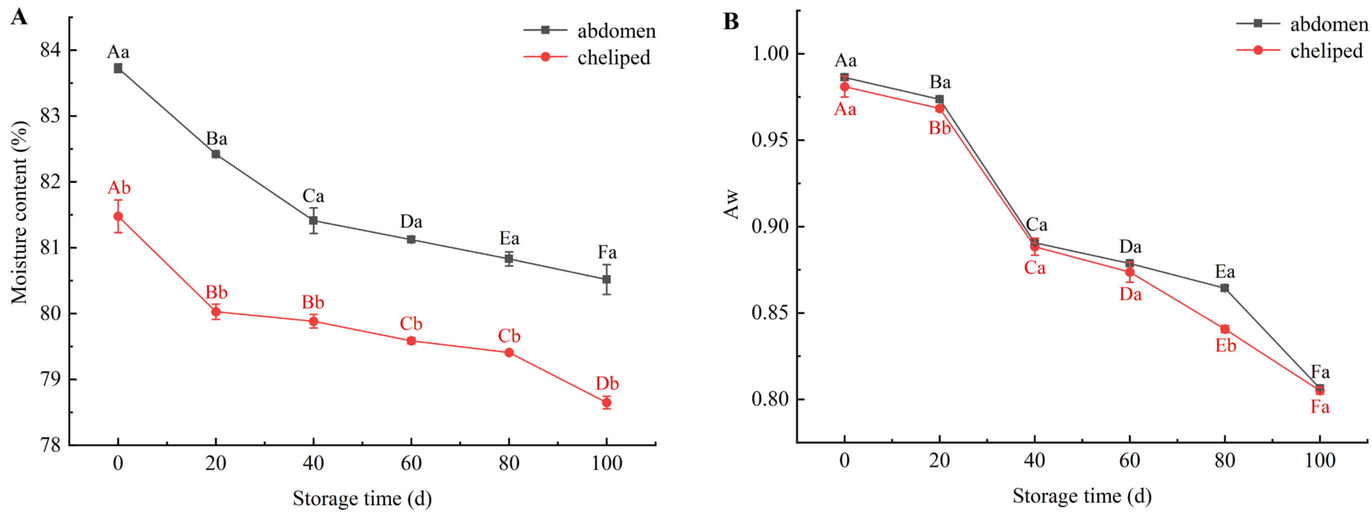

Fig. 2. Changes in the moisture content (A) and water activity (B) in the abdomen and cheliped muscles of swimming crab during frozen storage. Distinct uppercase letters indicate statistically significant differences in the same part of swimming crab under different storage times ($p < 0.05$). Dissimilar lowercase letters denote significant differences between the abdomen and cheliped muscles of swimming crab under the same storage time ($p < 0.05$).

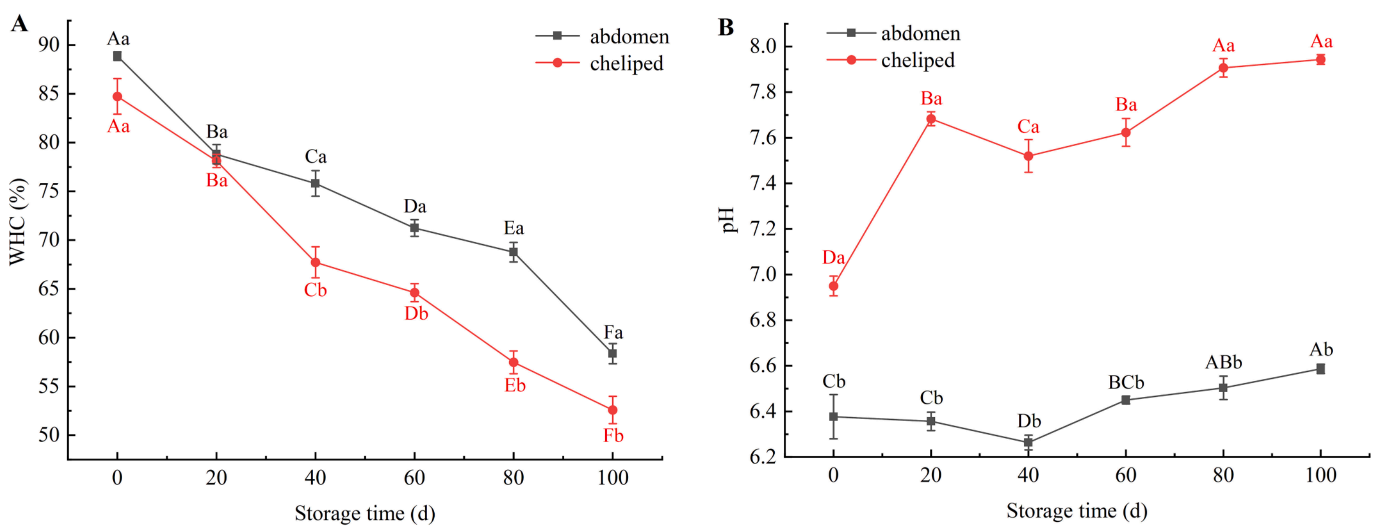

Fig. 3. Changes in the water-holding capacity (A) and pH (B) in the abdomen and cheliped muscles of swimming crab during frozen storage. Distinct uppercase letters indicate statistically significant differences in the same part of swimming crab under different storage time ($p < 0.05$). Dissimilar lowercase letters denote significant differences between the abdomen and cheliped muscles of swimming crab under the same storage time ($p < 0.05$).

of frozen storage time.

The pH, serving as a crucial indicator of aquatic product quality, is intricately connected to the chemical reactions that contribute to the deterioration of aquatic products (Diao, Cheng, Wang, & Xia, 2021). The pH variations in the swimming crab muscle were presented in Fig. 3B. Initially, the pH values for the fresh abdomen and cheliped muscles were 6.38 and 6.95, respectively. The pH of the fresh swimming crab detected by Ling et al. was 6.50 (2021), similar to the present result. On day 40, the pH of both muscles tended to decrease, potentially attributed to postmortem muscle glycolysis. As storage time increased, the pH value of the abdomen and cheliped muscles showed increasing trends and reached final values of 6.59 and 7.94 at the conclusion of the storage period. Throughout the entire storage duration, the pH of the cheliped muscle was significantly higher than that of the abdomen muscle. During the frozen storage process, endogenous and microbial enzymes were pivotal in facilitating protein degradation. This degradation process resulted in the release of secondary alkaline compounds, subsequently causing an increase in the pH value of the muscle tissue (Bu, Han, Tan, Zhu, Li, & Li, 2022). In addition, the critical acceptability margin of pH in crustaceans was 7.8–7.95 (Laly, Anupama, Ashok Kumar, Sankar, & Ninan, 2021), suggesting that the cheliped muscle of the swimming crab was prone to spoilage, although the final pH was within the acceptable range.

3.4. Total volatile basic nitrogen (TVB-N) content and trimethylamine (TMA) content analyses

The rise in TVB-N content in aquatic products is associated with bacterial degradation and the activities of endogenous enzymes (Lan, Lang, Zhou, & Xie, 2021). Fig. 4A illustrates a noteworthy and statistically significant rise in TVB-N content observed in the abdomen and cheliped muscles throughout storage duration ($p < 0.05$). Initially, the TVB-N content in two muscles of fresh swimming crab measured 9.04 mg/100 g and 10.85 mg/100 g, which escalated to 19.46 mg/100 g and 23.23 mg/100 g on 100 d, respectively. The elevation in TVB-N content could be ascribed to increased endogenous enzyme activity, the proliferation of spoilage bacteria, and subsequent biochemical reactions resulting in the accumulation of degradation products from microbial

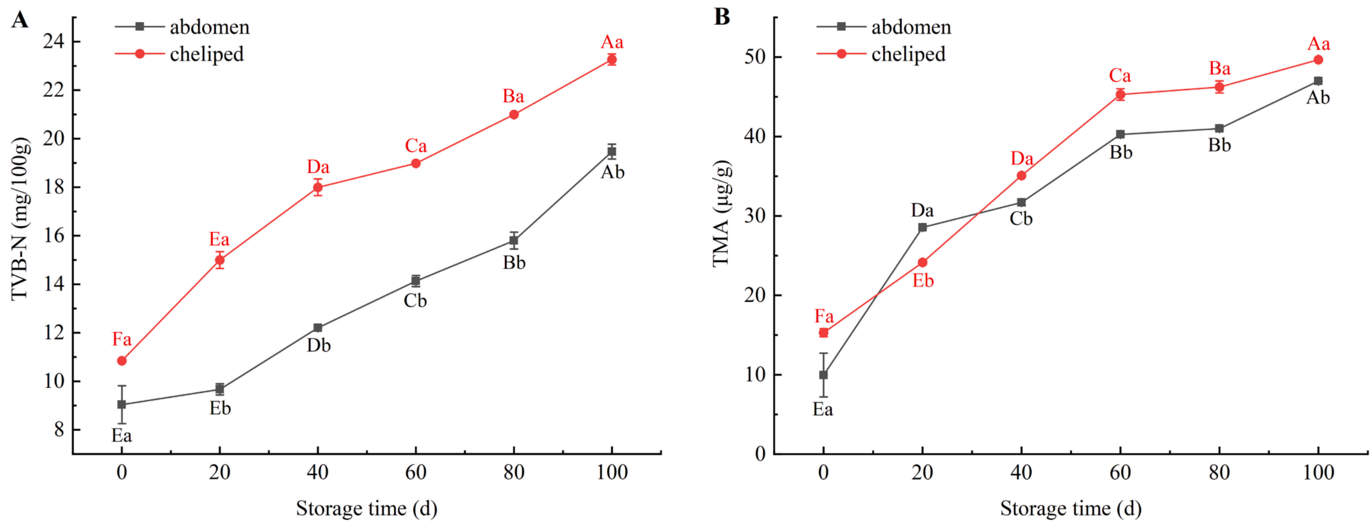

Fig. 4. Changes in the total volatile basic nitrogen content (A) and trimethylamine content (B) in the abdomen and cheliped muscles of swimming crab during frozen storage. Distinct uppercase letters indicate statistically significant differences in the same part in swimming crab under different storage time ($p < 0.05$). Dissimilar lowercase letters denote significant differences between the abdomen and cheliped muscles of swimming crab under the same storage time ($p < 0.05$).

activity (Lan, Sun, Liu, Guan, Zhu, & Xie, 2022). The permissible TVB-N content in sea crab is < 25 mg/100 g (Yang, Hu, Takaki, Yu, & Yuan, 2021). In this study, the TVB-N content in the cheliped muscle approached the standard range after 100 days of frozen storage, notably surpassing those observed in the abdomen muscle. The result suggested that the quality deterioration of the cheliped muscle was more severe than the abdomen muscle.

TMA has frequently been recognized as a biochemical marker for evaluating the freshness of aquatic products, which was the decomposition products of proteins (Du, Lan, & Xie, 2023; Zhang, Ma, Deng, Xie, & Qiu, 2015). The higher the TMA content, the lower the freshness value of seafood and the more severe the degree of corruption. The initial TMA content in the abdomen and cheliped muscles of fresh swimming crab was 9.96 μg/g and 15.29 μg/g ($p > 0.05$), respectively (Fig. 4B). However, over the storage duration, there was a notable elevation in the content of TMA ($p < 0.05$). Towards the conclusion of the storage period, the TMA content in the cheliped muscle notably surpassed that in the abdomen muscle, similar to the result observed in TVB-N content. This may be an important reason why the pungent and ammonia odor of the cheliped muscle was stronger than that of the abdomen muscle at the later stage of storage. The high levels of TVB-N and TMA content observed in all fresh samples may be attributed to the abundant presence of free amino acids and nitrogenous compounds in crustacean meat that make the muscle tissues prone to rapid degradation by spoilage microorganisms (Anacleto, Teixeira, Marques, Pedro, Nunes, & Marques, 2011).

3.5. Characteristics of sample sequence and alpha-diversity analyses

The alpha diversity index was obtained through HTS analysis to investigate the alterations in bacterial richness and diversity within swimming crab samples during frozen storage. The sequencing coverage rate serves as an indicator that reflects whether the sequencing results can accurately depict the microbial composition within the samples. The accuracy of the sequencing results is directly proportional to both the detection value and probability of the sample sequence, with higher values indicating increased precision (Zhong et al., 2023). All samples exhibited sequencing coverage rates surpassed 0.999, signifying that ample bacteria could be detected in swimming crab samples (Table S2). Based on a 97 % similarity threshold, 612 and 638 valid sequence clustering OTUs were obtained from the abdominal and cheliped muscles, respectively. The Ace and Chao indices were commonly employed as indicators of bacterial community richness. In contrast, the Shannon and Simpson indices were typically utilized to gauge the species diversity within bacterial communities (Zhou et al., 2023). The Ace, Chao, and Shannon indices had downward trends varying degrees at the abdomen muscle with storage period, revealing that the bacterial diversity and abundance of abdomen muscle decreased with increasing storage time. The results meant that only a limited number of species were linked to food spoilage towards the conclusion of the storage period. Additionally, the Ace, Chao, and Shannon values of the cheliped muscle exhibited an initial increase followed by a decrease over extended storage periods. The cheliped sample presented the highest Shannon and the lowest Simpson indices on day 60, suggesting decreased microbial community diversity and richness with increased storage time.

3.6. Composition of bacterial community analysis

Changes in the microbial community composition of swimming crab muscle were analyzed on 0, 40, and 80 days of frozen storage. At the phylum level, the predominant microbial composition in all samples was characterized by *Proteobacteria*, *Actinobacteriota*, and *Firmicutes* (Fig. 5A), which were consistent with the found in largemouth bass fillets under salt solution treatment during refrigerated storage reported by Ling et al. (2023). *Proteobacteria* and *Actinobacteriota* in fresh abdomen muscle accounted for 58.03 % and 31.84 %, respectively. Throughout the storage period, there was an initial rise followed by a subsequent decline in the relative of *Proteobacteria*, while *Actinobacteriota* decreased gradually. After an 80-day storage period, the major phyla identified in the abdomen muscle were *Proteobacteria* and *Firmicutes*, accounting for 56.28 % and 36.66 %, respectively. *Proteobacteria* played a dominant role in fresh cheliped muscle, with a value of 95.74 %, significantly decreasing to 34.45 % on 80 days of storage. In contrast, *Actinobacteriota* and *Firmicutes* displayed a noteworthy increase as the storage time progressed, indicating the importance of which in the quality and shelf life of swimming crabs. Parlapani et al. (2019) reported that *Proteobacteria*, *Firmicutes*, and *Actinobacteriota* as the predominant bacterial phyla in the blue crab, similar to our results.

Fig. 5B depicted the composition and relative abundance of microbiota at the genus level among all samples. The three genera with the highest relative abundance in swimming crab samples, except fresh abdomen (*Achromobacter*, *Kocuria*, and *Aliiroseovarius*) and cheliped muscle (*Vibrio*, *Achromobacter*, and *Kocuria*), were *Achromobacter*,

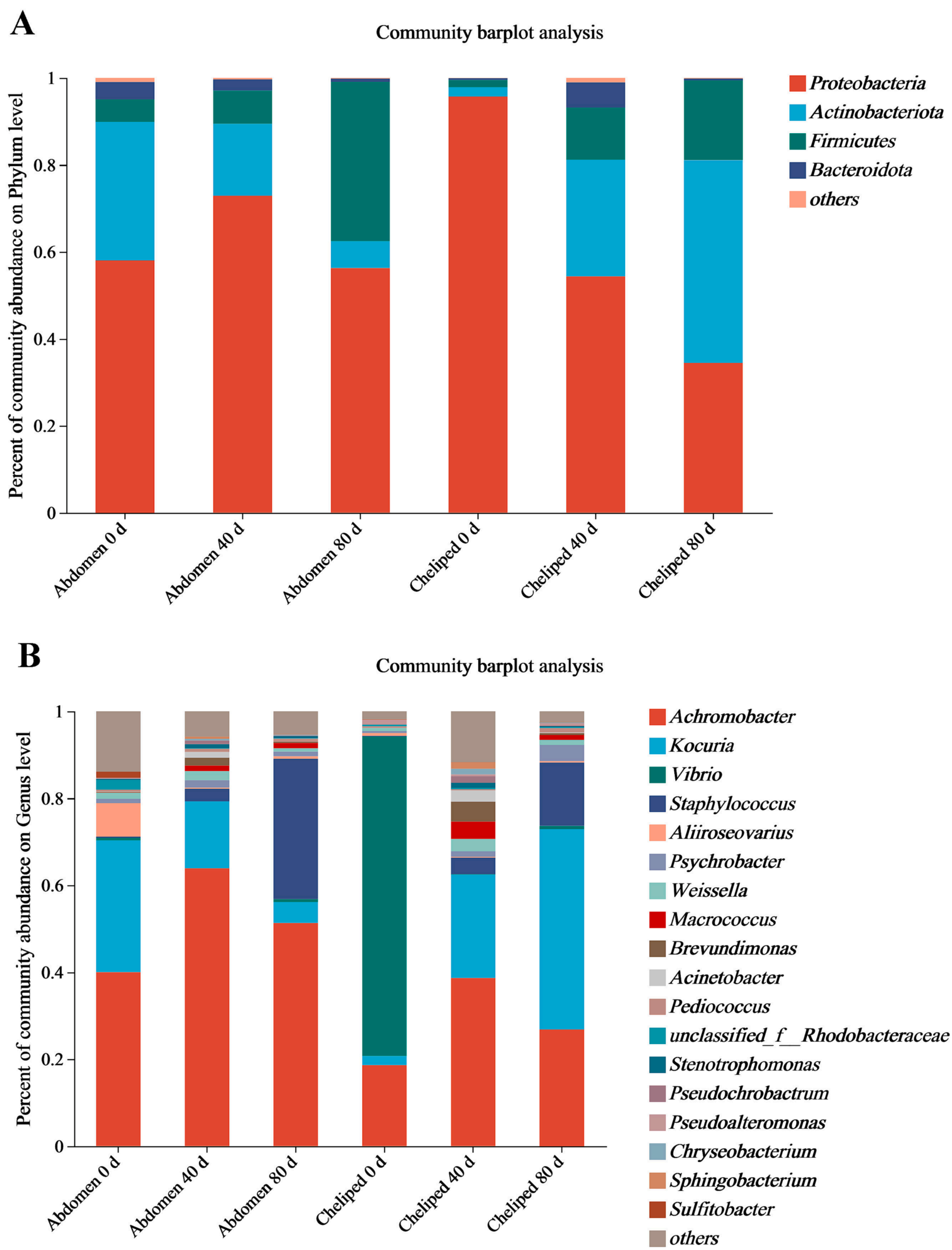

Fig. 5. Composition and relative abundance at the phylum level (A) and genus level (B) of microbiota in swimming crab muscle during frozen storage.

Kocuria, and *Staphylococcus*. With the progression of storage time, the relative abundance of *Achromobacter* in the abdomen and cheliped muscles increased first and then decreased, and the relative abundance in the abdomen muscle surpassed that in the cheliped muscle. In the abdomen muscle, the relative abundance of *Kocuria* experienced a decrease as the storage time extended. Whereas the opposite trend of *Kocuria* was found in the cheliped muscle. The relative abundance of *Staphylococcus* in the abdomen and cheliped muscles increased over storage time, which accounted for 32.25 % and 14.55 % at the end of storage, respectively. *Staphylococcus* genera belonged to *Firmicutes* (Zhou et al., 2023), the relative abundance that aligns with the result observed at the phylum level in the bacterial community. In addition, *Aliiroseovarius* was one of the main genera in fresh abdomen muscle. *Vibrio* dominated the initial microbiota of the cheliped muscle, accounting for 73.53 %. *Vibrio* naturally resides in the aquatic environment, which is commonly present in marine seafood products (Li, Lei, Tan, Zhang, Hong, & Luo, 2022).

3.7. Correlation analysis between physiochemical changes and microbiota composition

The heatmap (Fig. 6) depicted the correlation analysis between specific bacterial strains and environmental factors, showcasing positive correlations in red and negative correlations in blue. In the abdomen muscle (Fig. 6A), the *Kocuria* and *Aliiroseovarius* exhibited positive correlations with moisture content, Aw and WHC, but displayed negative correlations with TVB-N and TMA. *Staphylococcus* has a significant positive correlation to TVB-N and a negative correlation to WHC, suggesting that *Staphylococcus* played a crucial role in the quality deterioration of the abdomen muscles. In addition to the *Acinetobacter* being negatively correlated with pH, most bacterial strains had no significant correlation with pH value.

In the cheliped muscle (Fig. 6B), the *Kocuria* and *Staphylococcus* exhibited a negative correlation with moisture content, Aw and WHC, but displayed a positive correlation with pH, TVB-N, and TMA. The results confirmed that *Kocuria* and *Staphylococcus* were the important causes of the cheliped muscle spoilage. The opposite correlation was found between *Vibrio* and the physiochemical indicators. The growth of spoilage microorganisms contributed to muscle K value, which was a potential optimal freshness indicator for mud crabs (Lin et al., 2022).

4. Conclusions

This investigation systematically evaluated the alterations in bacterial community dynamics and quality attributes within the abdomen and cheliped muscles of the swimming crab during −20℃ of frozen storage. The physicochemical properties, including the sensory evaluation, moisture content, Aw, and WHC, decreased in all samples, while the pH, TVB-N, and TMA content increased as storage time extended. The quality of the abdomen and cheliped muscles of the swimming crab deteriorated at the end of frozen storage. Moreover, high-throughput sequencing analysis indicated a progressive reduction in the diversity of the microbial community as the frozen storage period extended. After the period of frozen storage, *Proteobacteria*, *Actinobacteriota*, and *Firmicutes*, *Achromobacter*, *Kocuria*, and *Staphylococcus* were the main phyla and genera in the abdomen and cheliped muscle of swimming crab, respectively. Furthermore, the results of correlation analysis indicated that the *Kocuria*, *Vibrio*, *Staphylococcus* and *Aliiroseovarius* were significantly correlated with the physicochemical properties. This study will contribute valuable insights into the alterations in the quality and microbial composition analysis in different parts of swimming crab muscles during frozen storage. However, the current study on the protein quality of frozen crab is still lacking. Simultaneously, the mechanism of quality difference between the abdomen and cheliped muscles during frozen storage needs for an in-depth exploration.

Declaration of adherence to ethical and professional practices for sensory analysis

The experiments on the sensory analysis in this study have strictly adhered to the ethical and professional practices outlined in the *IFST (the Institute of Food Science & Technology, UK) Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods*. Authors confirm that the rights and privacy of all participants were protected during the execution of the research, e.g., no coercion to participate, full disclosure of study requirements and risks, written or verbal consent of participants, no release of participant data without their knowledge, ability to withdraw from the study at any time.

CRediT authorship contribution statement

Ruyi Dong: Validation, Methodology. **Yingru Wu:** Investigation,

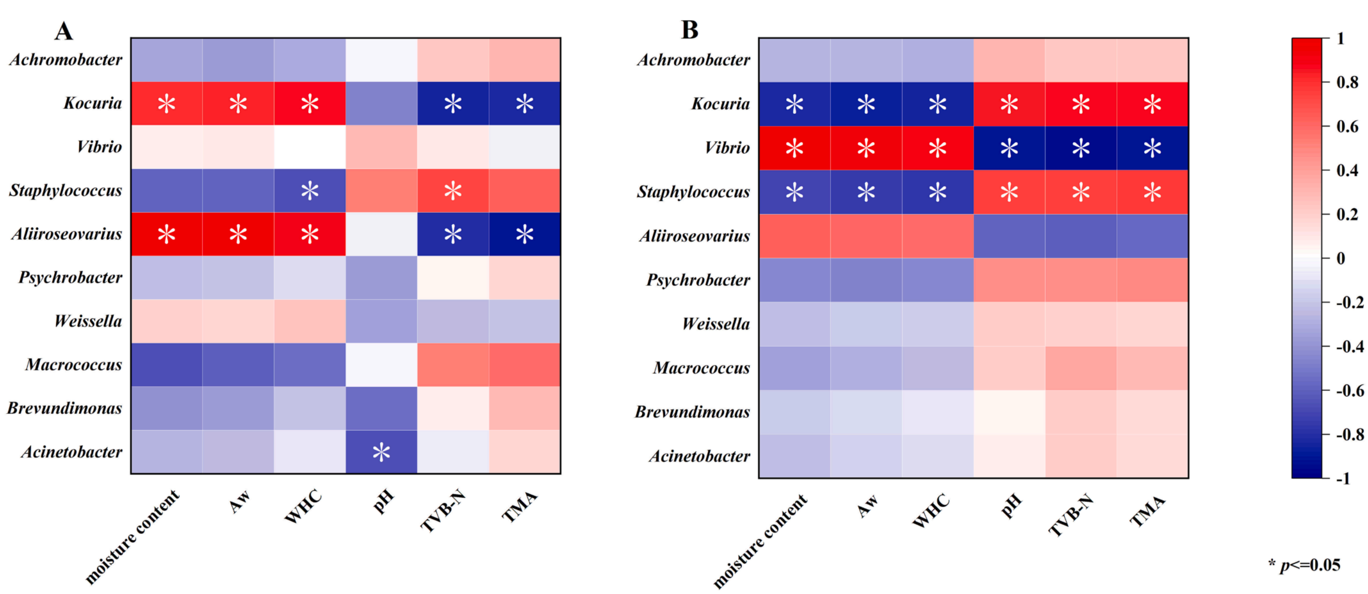

Fig. 6. Pearson correlation analysis between physiochemical indexes and microbiota composition in the abdomen (A) and cheliped (B) muscles of swimming crab during frozen storage.

Formal analysis. **Qi Du:** Software, Data curation. **Rui Lu:** Validation, Methodology. **Soottawat Benjakul:** Visualization, Validation, Supervision. **Bin Zhang:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Shanshan Shui:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

This study was funded by National Key R&D Program of China (Grant No. 2021YFD2100504), National Natural Science Foundation of China (Grant No. 32301972 and U23A20263), and Zhejiang Leading Training Program of China (No. 2020R52027).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101210.

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