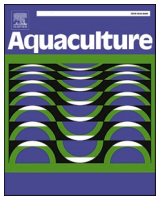
Aquaculture 599 (2025) 742066

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Effects of cooling rate on the physiological metabolism and flavor of thick-shell mussel (*Mytilus coruscus*) during low-temperature semi-anhydrous living-preservation

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ARTICLE INFO

Keywords:
Mytilus coruscus
Cooling rate
Semi-anhydrous living-preservation
Physiological metabolism
Flavor

ABSTRACT

Temperature has a significant effect on the quality of aquatic products during semi-anhydrous living-preservation. This study examined the effects of various cooling rates (1 °C/h, 4 °C/h, 8 °C/h, and 16 °C/h) on the physiological metabolism and flavor of the marine mussel *Mytilus coruscus* after low-temperature semi-anhydrous living-preservation. The survival rate in the gradient cooling group (1 °C/h, 4 °C/h, and 8 °C/h) surpassed that in the 16 °C/h group. Specifically, the 4 °C/h group maintained a 100 % survival rate after 2 days of preservation, with the percentage remaining the highest at 7 days. Respiratory metabolic indicators such as the oxygen consumption rate, ammonia excretion rate, 5′-nucleotide level, and adenosine energy charge (A.E.C.) were evaluated. The 4 °C/h group presented a greater ATP content and A.E.C., which was associated with a superior survival status. Flavor analysis indicated that the 4 °C/h group presented the lowest concentration of bitter amino acids after 2 days of semi-anhydrous living-preservation. The gas chromatography-ion mobility spectrometry (GC-IMS) results suggested that gradient cooling could effectively slow the loss of aroma compounds in mussels. In conclusion, a cooling rate of 4 °C/h maintained a high survival rate and quality of *Mytilus coruscus*, providing a theoretical basis for aquatic product processing and transportation in the food industry.

1. Introduction

Mussels are popular among consumers because of their tender texture, delicious flavor and high nutritional value (Ma et al., 2022). Fresh aquatic products are easily perishable and have a short shelf life, and when this shelf life is exceeded, the quality of the products significantly deteriorates. Currently, mussels are preserved primarily through methods such as freezing, drying, and salting and are also utilized in the production of health products to prolong their storage period and increase their value (Bongiorno et al., 2018; Qiao et al., 2024; Ratnawati et al., 2023). Although the aforementioned methods can extend the storage period of mussels, their taste and quality may decrease compared with those of fresh mussels. Therefore, ensuring the freshness of mussels from the point of fishing to transportation and ultimately to sale has become an urgent concern.

Semi-anhydrous living-preservation, a common and cost-effective method, involves inducing a semi-dormant or dormant state in aquatic products through low temperature or anesthesia. This process weakens respiratory function and metabolic levels, reduces mortality rates, and extends the shelf life of mussels by removing water carriers (Chen et al., 2015). Temperature plays an important role in maintaining the freshness and survival of aquatic products (Mridul et al., 2024). High temperatures can cause the death of aquatic products from oxygen deficiency, whereas excessively low temperatures can cause loss of vitality at the ecological ice point. Controlling temperature effectively not only extends the survival time of aquatic products but also slows nutrient loss (Nie et al., 2024). Current research has focused primarily on preserving aquatic products under semi-anhydrous or anhydrous

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https://doi.org/10.1016/j.aquaculture.2024.742066
Received 16 August 2024; Received in revised form 26 November 2024; Accepted 19 December 2024
Available online 24 December 2024

conditions at varying temperatures. For example, Anacleto et al. (2013) examined the transportation of two clam species at 4 °C and 22 °C and reported that 4 °C was optimal for maintaining high quality under semi-dry conditions. Similarly, Bi et al. (2023a) reported that clams are best transported at 4 °C. While the above studies focused mainly on the effects of direct cooling on shellfish, there is limited research on the impact of slow cooling. Our previous studies on oysters revealed that gradient cooling improved the survival rate, reduced stress, and improved the quality of oysters (Bi et al., 2023b). With respect to the effect of temperature on mussels, a study by Li et al. (2020) demonstrated that elevated temperatures resulted in a decrease in the number of newly secreted byssus in mussels (*Mytilus coruscus*), as well as a reduction in byssal polyphenol oxidase activity and foot protein abundance. Azizan et al. (2024) suggested that mussels may be more susceptible to bacterial pathogens under conditions of increased temperature. Furthermore, Zhang et al. (2024) reported that the impact of temperature on the filtration rate of golden mussels (*Limnoperna fortunei*) followed a skewed unimodal pattern, with an optimal temperature of approximately 20 °C. Notably, golden mussels can filter and survive at low water temperatures, even when they approach the freezing point. However, the effects of different cooling rates on mussels remain unexplored.

Physiological metabolism, including respiratory metabolism and energy metabolism, can reflect changes in important indicators of aquatic products induced by environmental changes (Chen et al., 2022a). The respiratory metabolism level of aquatic products can be reflected by the oxygen consumption rate and ammonia excretion rate, whereas ATP-related compounds can reflect changes in energy metabolism. Flavor and nutritional composition play crucial roles in influencing consumer purchasing decisions and serve as indicators of the quality of aquatic products (Li et al., 2022a). Gas chromatography-ion mobility spectrometry (GC-IMS) was used to determine the levels of volatile organic compounds (VOCs) in food, as they effectively reflect changes in the flavor of aquatic products (Chen et al., 2024c).

Therefore, this study simulated the transportation process of *Mytilus coruscus* (*M. coruscus*) and investigated the effects of gradient cooling on the flavor of mussels after they were harvested under semi-anhydrous conditions. The effect of the cooling rate on mussel respiratory conditions was evaluated by measuring the survival rate, ammonia excretion rate, oxygen consumption rate, and ATP-related compounds. The taste of *M. coruscus* was measured via an amino acid analyzer and an electronic tongue, and GC-IMS was used to analyze the odor of *M. coruscus* after gradient cooling treatment under semi-anhydrous living-preservation. This study provides new ideas for the cold-chain transportation of mussels in the food industry.

2. Materials and methods

2.1. Sample collection and acclimation

Fresh *M. coruscus* weighing 45.17 ± 9.33 g were purchased from a local mussel farm in Lianyungang city, Jiangsu Province, China. Following harvest, they were promptly delivered to the laboratory in a foam box with ice within approximately 3 h.

After receiving the mussel, 100 intact and lively *M. coruscus* individuals were randomly selected and bagged into four bags (25 individuals per bag) as control groups. Eight hundred live mussels were immediately cleaned to remove external contaminants and then randomly placed in four seawater tanks for acclimatization (200 individuals per tank). The temperature was maintained at 20 ± 1 °C by temperature-regulating devices.

2.2. Experimental design

After a 4 h acclimatization period (RW4), with the exception of 100 mussels that were acclimatized for 24 h (RW24), the remaining mussels were bagged, transferred to four 20 °C incubators and gradually cooled to 4 °C. The cooling rates were set as 1 °C/h, 4 °C/h, 8 °C/h, and 16 °C/h for the groups labeled T-1, T-4, T-8, and T-16, respectively. The *M. coruscus* in each group was subsequently stored in a low-temperature incubator at 4 ± 1 °C for 7 days. The sampling groups included the control group, RW4, RW24, cooling to 4 °C (4-T-1, 4-T-4, and 4-T-8), 2 days of storage at 4 °C (2d-T-1, 2d-T-4, 2d-T-8, and 2d-T-16), and 4 °C storage for 7 days (7d-T-1, 7d-T-4, 7d-T-8, and 7d-T-16). For each sampling, intact and lively mussels were selected. The mussel shells were opened, the soft parts were removed, and the samples were frozen in liquid nitrogen. The samples were stored in a − 40 °C freezer for subsequent analysis.

2.3. Survival rate

At the above sampling points, 40 mussels were randomly selected from each group. The samples were placed in seawater at 20 °C and observed after 30 min. Once the mussels had been left open for a long time and could not be closed by pressing lightly with the hand, they were considered dead, and the number of survivors in each group was recorded.

$$\text{Survival rate}\,(\%) = \text{number of surviving mussels}/\text{total number of mussels} \times 100$$

2.4. Oxygen consumption rate (OCR)

At the sampling points, 8 mussels were randomly selected from each group and placed in a 1 L breathing bottle. Before being placed in the bottle, 1 L of seawater was added. The control group was an empty bottle. After the mussels had adapted to the breathing bottle for 0.5 h, the first test was performed via a dissolved oxygen analyzer (HQ30d, Hach Company, Loveland, CO, USA), and the results were recorded. Immediately after the test, the bottle was filled with seawater, and the gate of the breathing bottle was closed. After 1.5 h, the second test was performed, and the second result was recorded. The oxygen consumption rate was then calculated via the following formula (Chen et al., 2022b):

$$\mathrm{OCR} = (C_t - C_0) \bullet V \bullet T^{-1} \bullet W^{-1}$$

where OCR represents the oxygen consumption rate of *M. coruscus* (unit: $mg\bullet g^{-1}\bullet h^{-1}$), C_0 (in $mg\bullet L^{-1}$) represents the oxygen concentration at the end of the experiment, C_t (in $mg\bullet L^{-1}$) represents the oxygen concentration at the beginning of the experiment, V (in liters) represents the volume of seawater added, T (in hours) represents the time of the experiment, and W (in grams) represents the dry weight of the mussel muscle.

2.5. Ammonia excretion rate (AER)

Immediately after the second test, the seawater in the breathing bottle was sampled and stored in a − 20 °C freezer. The ammonia excretion rate was determined via the indigo blue spectrophotometric method, and the calculation formula was as follows (Chen et al., 2022a):

$$\mathrm{AER} = (N_t - N_0) \bullet V \bullet T^{-1} \bullet W^{-1}$$

where AER represents the ammonia excretion rate of *Mytilus coruscus* (unit: $\mu g\bullet g^{-1}\bullet h^{-1}$), N_t (in $mg\bullet mL^{-1}$) represents the ammonia nitrogen concentration after the end of the experiment, N_0 represents the ammonia nitrogen concentration after the end of the blank bottle (in $\mu g\bullet mL^{-1}$), V (in milliliters) represents the volume of seawater added, T (in hours) represents the duration of the experiment, and W (in grams) represents the dry weight of the mussel muscle.

2.6. 5'-nucleotide analysis

The analysis of 5'-Nucleotide in mussels was performed according to the methods of Chen et al. (2022a), with some modifications. Mussel samples were weighed (5.00 g), homogenized in 15.00 mL of 10 % cold perchloric acid, and then centrifuged at 4 °C for 10 min. This process was repeated, and the resulting supernatant was collected. The obtained supernatant was neutralized to pH 6.70 with KOH, filtered through a 0.22 μm membrane, and subsequently subjected to high-performance liquid chromatography (HPLC) using a CAPCELLPAK C18 SG column (4.60 mm × 150 mm, Shiseido Co., Ltd., Tokyo, Japan) equilibrated with a mixture of was 20 mmol/L phosphoric acid, 20 mmol/L citric acid, and 40 mmol/L triethylamine. The flow rate was set at 1.00 mL/min, with detection at a wavelength of 260 nm. A sample volume of 10 μL was injected, and nucleotides were identified and quantified by comparing the retention time and peak area with those of a standardized compound.

2.7. Adenosine energy charge (A.E.C.)

The following equation was used to calculate the A.E.C. (Chen et al., 2022a):

$$\text{A.E.C.}(\%) = ([\text{ATP}] + 1/2\,[\text{ADP}])/([\text{ATP}] + [\text{ADP}] + [\text{AMP}]) \times 100$$

2.8. Fatty acid analysis

The fatty acid analysis was performed according to the methods of Wang et al. (2022). A total of 1.50 g of homogenized mussel tissue sample was placed in a 50 mL centrifuge tube. Thirty milliliters of chloroform:methanol (2:1, *v*/v) was added to the centrifuge tube, which was subsequently homogenized and ultrasonicated for 10 min. Then, 10 mL of ultrapure water was added to the centrifuge tube, and the mixture was vortexed and centrifuged at 3000 r/min (4 °C) for 10 min. The chloroform layer was collected, 10 mL of chloroform was added to the remaining tube, and the extraction was repeated twice. The combined chloroform layer was concentrated via rotary evaporation, and the dried total lipid sample was redissolved in a chloroform-methanol solution. The solution was passed through a 0.22 μm filter membrane. The crude fat was methylated with a hydrochloric acid-methanol solution for 3 h in a metal bath at 90 °C to obtain fatty acid methyl ester. Subsequently, n-hexane was added to the mixture to dissolve the fatty acid methyl ester, and an equal amount of the n-hexane layer was injected into the inlet of the 6980 N gas chromatograph for analysis. The following parameters were used: Agilent J&W HP-88, 100 m long, 0.25 mm inner diameter, 0.20 mm film thickness; column temperature, 180–220 °C (3 °C/min); hold for 52 min; and carrier gas, nitrogen. The fatty acids were identified by comparing the retention times with those of the standard fatty acid methyl ester mixture from Sigma (Liu et al., 2024).

2.9. Electronic tongue analysis

A total of 8.00 g of mussel tissue sample was accurately weighed, 40 °C distilled water was added at a ratio of 1:5, the sample was homogenized thoroughly, and the sample was centrifuged at 3000 r/min for 10 min. The supernatant was collected and tested in an electronic tongue cup. Each group had 3 samples, and each sample was measured 4 times. The data of the last 3 measurements are taken. A mixture of potassium chloride and tartaric acid was used as a reference solution for comparison (Chen et al., 2022b).

2.10. Free amino acid analysis

A total of 2.00 g of mussel tissue sample was accurately weighed into a 50 mL centrifuge tube, 15 mL of 0.02 mol/L HCl was added, the sample was homogenized thoroughly, sonicated for 5 min, and centrifuged at 5000 r/min (4 °C) for 10 min. The remaining residue was added to 10 mL of 0.02 mol/L HCl, homogenized thoroughly, and centrifuged at 5000 r/min (4 °C) for 5 min. Then, the supernatant was diluted to 25 mL. Subsequently, 2.00 mL of the diluent was combined with 2.00 mL of 5 % sulfosalicylic acid, and the mixture was centrifuged at 10,000 r/min (4 °C) for 10 min. The supernatant was passed through a 0.22 μm filter membrane and analyzed in a liquid chromatography vial using an automatic amino acid analyzer (L-8900, Tokyo, Japan) (Chen et al., 2024b).

2.11. Analysis of VOCs

In accordance with Teng et al. (2023), the volatile organic compounds in mussel tissue samples were identified via GC-IMS (FlavourSpec®, Gesellschaft für Analytische Sensorsysteme mbH, Germany) with minor adjustments. A total of 2.00 g of mussel tissue sample was placed in a 20 mL headspace bottle. The sample was then incubated at 60 °C for 10 min while stirring at 500 rpm with pure nitrogen as the carrier gas. The nitrogen flow program was as follows: 2 mL/min for 5 min, 100 mL/min for 5 min, and 150 mL/min for 7 min. For qualitative evaluation of each compound, the retention time (RT) and drift time (DT) were compared with those of the NIST 2014 and IMS databases.

2.12. Data processing and statistical analysis

The data were processed and analyzed using SPSS 27.0 software (SPSS Inc., USA) and subjected to one-way analysis of variance with Duncan's multiple comparison method, and the results were expressed as the means ± standard deviations. $P < 0.05$ indicated a significant difference. Origin 2022 was used for drawing the figures.

3. Results and discussion

3.1. Survival rate

The impact of different cooling rates on the survival rate of fresh mussels at 20 °C when cooled to 4 °C was investigated. As shown in Fig. 1, the T-4 and T-8 groups had no mortality, whereas the T-1 group had some mortality, deviating from the results of the other groups. This difference in survival rate was attributed to the prolonged exposure to high temperatures due to slower cooling rates in the T-1 group. On the other hand, the T-4 and T-8 groups presented shorter exposure times to high temperatures, allowing the mussels to enter a semi-dormant or dormant state more quickly, leading to higher survival rates. The T-4 group presented the highest survival rate after two days of storage at 4 °C, with no improvement observed. In contrast, the T-1, T-8, and T-16 groups presented a decrease in survival rates, with the T-16 group having the lowest survival rate. This suggested that excessively rapid cooling rates can also negatively impact mussel survival. After seven days of storage, all the experimental groups experienced a decrease in survival rates, but the T-4 group maintained the highest survival rate. The results highlighted that mussel struggled to survive long-term under semi-anhydrous conditions, leading to a decline in survival rates over time.

3.2. Oxygen consumption rate

The oxygen consumption rate is a crucial indicator for assessing respiratory excretion and can provide insights into the survival of mussels. As shown in Fig. 2-A, lowering the temperature to 4 °C led to a significant decrease in the OCR across all groups. Specifically, the T-4 group presented the highest rate, followed by the T-8 and T-16 groups, with the T-1 group showing the lowest rate. After two days of storage at 4 °C, a further decrease in the OCR was observed, with the T-4 group maintaining the highest rate. This decline might be attributed to the rapid cooling of the T-8 and T-16 groups, which induced stress and

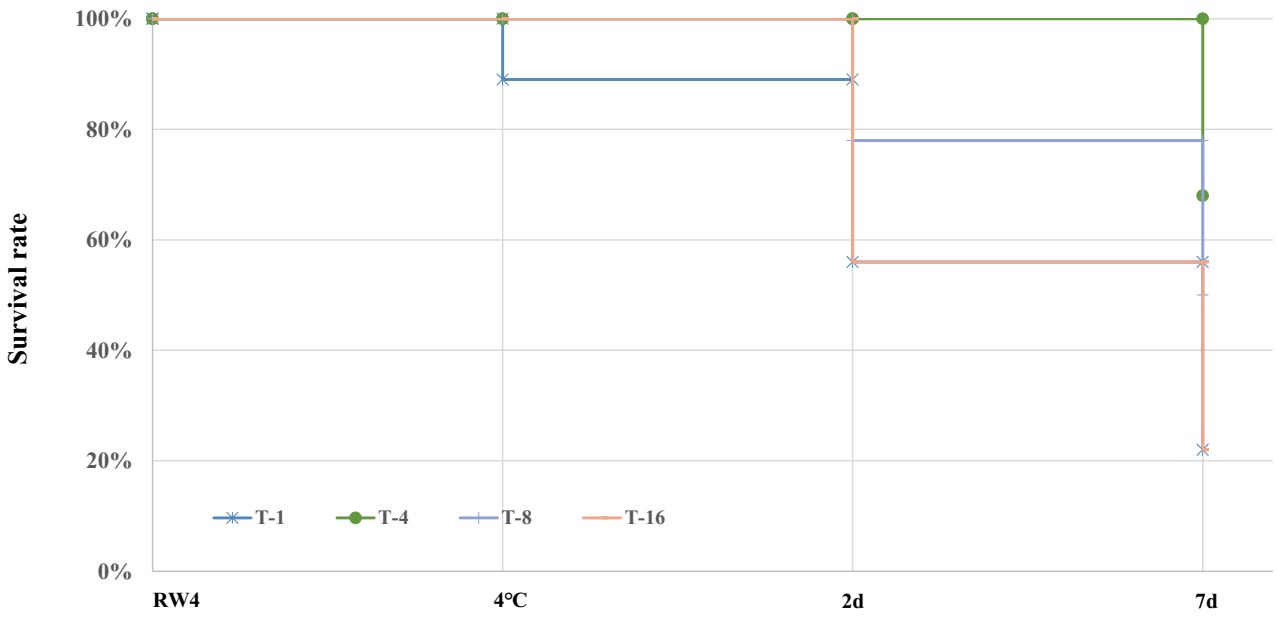

Fig. 1. Survival rates of *M. coruscus* during the semi-anhydrous living-preservation at different cooling rates.

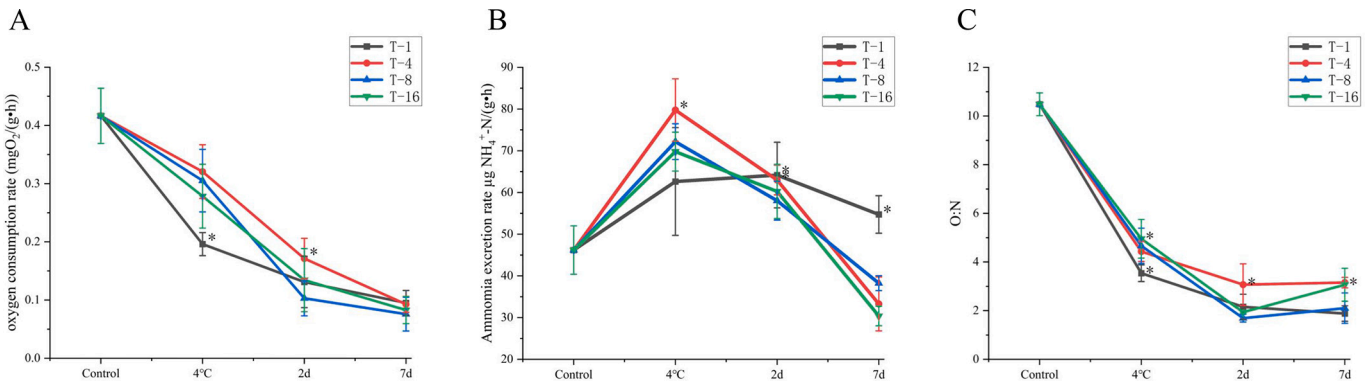

Fig. 2. Respiration of *M. coruscus* during the semi-anhydrous living-preservation at different cooling rates. A: Oxygen consumption rate; B: Ammonia excretion rate; C: Oxygen-nitrogen ratio.

reduced viability. Conversely, the slow cooling rate of the T-1 group prolonged the exposure time to high temperatures, delaying entry into a dormant state and increasing energy consumption. After 7 days of storage, all the groups presented significantly low OCRs without notable differences ($P > 0.05$), indicating that all the mussels had entered a dormant state. This decrease in the OCR might be because mussels are unable to obtain new nutrients for a long time and need to change their metabolic capacity to reduce energy consumption (Chen et al., 2024a). Most mussels showed signs of weakness, with some mortality observed, leading to a decrease in overall survival rates, which aligns with the above results concerning the survival rate of mussels.

3.3. Ammonia excretion rate

Ammonia nitrogen (NH_4^+-N) is a crucial parameter for assessing respiratory excretion in mussels. Like oxygen consumption, it can effectively reflect the metabolic and survival status of mussels. The results demonstrated that as the temperature decreased from 20 °C to 4 °C at varying rates, the AER of the mussels gradually increased (Fig. 2-B). This might be attributed to alterations in the external environmental conditions of the mussels, from water to semi-anhydrous conditions, from the optimal temperature range to the ecological ice temperature range, and from large temperature fluctuations to accelerated physiological changes in individual mussels. These changes increase the metabolic levels of mussels, leading to increased production of ammonia and urea, thereby increasing the AER (Jiang et al., 2024; Zheng et al., 2025). A significant difference was observed in the AER of mussels among the different cooling rate groups ($P < 0.05$). The T-4 group presented the highest AER, whereas the T-1 group presented the lowest.

The AER tended to decrease during storage at 4 °C. Low-temperature stress caused physiological disorders in mussels, which weakened their vitality, reduced the activity of their enzymes and slows their metabolism, resulting in a downward trend in ammonia excretion (Li et al., 2024). After 7 days of storage, the T-1 group had a greater AER, whereas the other three groups did not significantly differ.

3.4. Oxygen:Nitrogen (O:N) ratio

The O:N ratio can be used to assess the utilization of metabolic substrates by organisms. A higher ratio suggested a reliance on fat and carbohydrates for energy, whereas a ratio of approximately 20 indicated a mix of protein and fat as energy sources. A ratio of less than 7 signifies that a protein is the main energy source (Barber and Blake, 1985). The results (Fig. 2-C) revealed that when the temperature was reduced to 4 °C at various rates, the T-1 group presented significantly lower values than the other groups did, but the differences were not significant. This

difference could be linked to insufficient edible food and carbohydrates to sustain metabolic levels. Some studies have reported that lower O:N values indicate an increase in protein breakdown to maintain normal metabolism (Pousse et al., 2020). After 2 days of storage at 4 °C, the 2d-T-4 group presented the highest O:N ratio, indicating lower protein dependency than the other groups, which were not significantly different.

3.5. Analysis of ATP-related compounds

In this study, the levels of ATP-related compounds in mussels were measured. Fig. 3-A and B show that the levels of ATP and ADP slightly increased after the mussels were cleaned and placed in seawater at 20 °C for four hours, indicating the recovery of the mussels. When the temperature was gradually lowered to 4 °C, the ATP and ADP levels were lower than those in RW4. Since mussels are ectotherms, they can regulate their body functions in response to changes in environment temperature by behavioral and physiological plasticity, resulting in decreases in ATP and ADP contents (Abdelsaleheen et al., 2024). There was no significant difference in the ADP content among the T-1, T-4 and T-8 groups ($P > 0.05$), and the ADP content in all three groups was greater than that in the RW24 group. In contrast, the AMP content tended to increase. This may be caused by the fact that the degradation and synthesis of ATP occurred simultaneously, maintaining a dynamic equilibrium within the organism. After 2 days of storage at 4 °C, a significant decrease in ATP and ADP contents was observed, whereas the AMP content increased significantly. In the absence of external energy sources under low-temperature and semi-anhydrous conditions, mussels are compelled to consume their inherent energy to maintain bodily functions, resulting in a notable decline in energy reserves. This phenomenon was most evident in group T-16. After 7 days, long-term low-temperature storage completely inhibited the aerobic metabolism of mussels and reduced energy biosynthesis (Yin et al., 2023), and the ATP and ADP contents of all the groups markedly decreased, although notable differences remained between the groups. Compared with the other three groups, the T-4 group presented a relatively higher ATP content, whereas the T-16 group presented the lowest ATP content. In addition, AMP, GMP, and IMP are the main components of umami flavor (Chu et al., 2024). As shown in Fig. 3-C, D and E, the fresh mussels had the highest concentration of AMP, followed by IMP and GMP, which is consistent with the results reported in the study of Ma et al. (2022).

The A.E.C. value, which represents the adenosine energy charge, reflects the influence of natural or anthropogenic stressors (Bi et al., 2023a). An increased A.E.C. suggested reduced ATP production and increased ATP consumption (Anacleto et al., 2013). As shown in Fig. 3-F, the A.E.C. value exceeded 60 % after four hours of acclimation, indicating the recovery of mussels during the acclimation process. Following the completion of the cooling process, the A.E.C. values of all the groups were lower than those of RW4. However, except for RW24, the A.E.C. values of the other three groups were all above 50 %. During storage, a notable decrease in the A.E.C. value was observed, reflecting the mussels' response to the transition from the hydrous to semi-anhydrous state and from high to low temperatures. The low A.E.C. value observed after two days of storage at 4 °C could be attributed to the combined effects of stress from low temperatures approaching the ecological ice point stress induced by starvation. After 7 days of storage, the A.E.C. values of each group continued to decrease, with the 7d-T-16 group showing the lowest A.E.C. value. This suggested that a gradual cooling process is crucial for the semi-anhydrous living-preservation of *M. coruscus*.

3.6. Fatty acid analysis

A total of 18 fatty acids were identified, 11 of which were unsaturated, representing 61.1 % of the total fatty acid content. Additionally, there were 4 monounsaturated fatty acids (MUFAs) and 7 polyunsaturated fatty acids (PUFAs). Saturated fatty acids (SFAs) made up

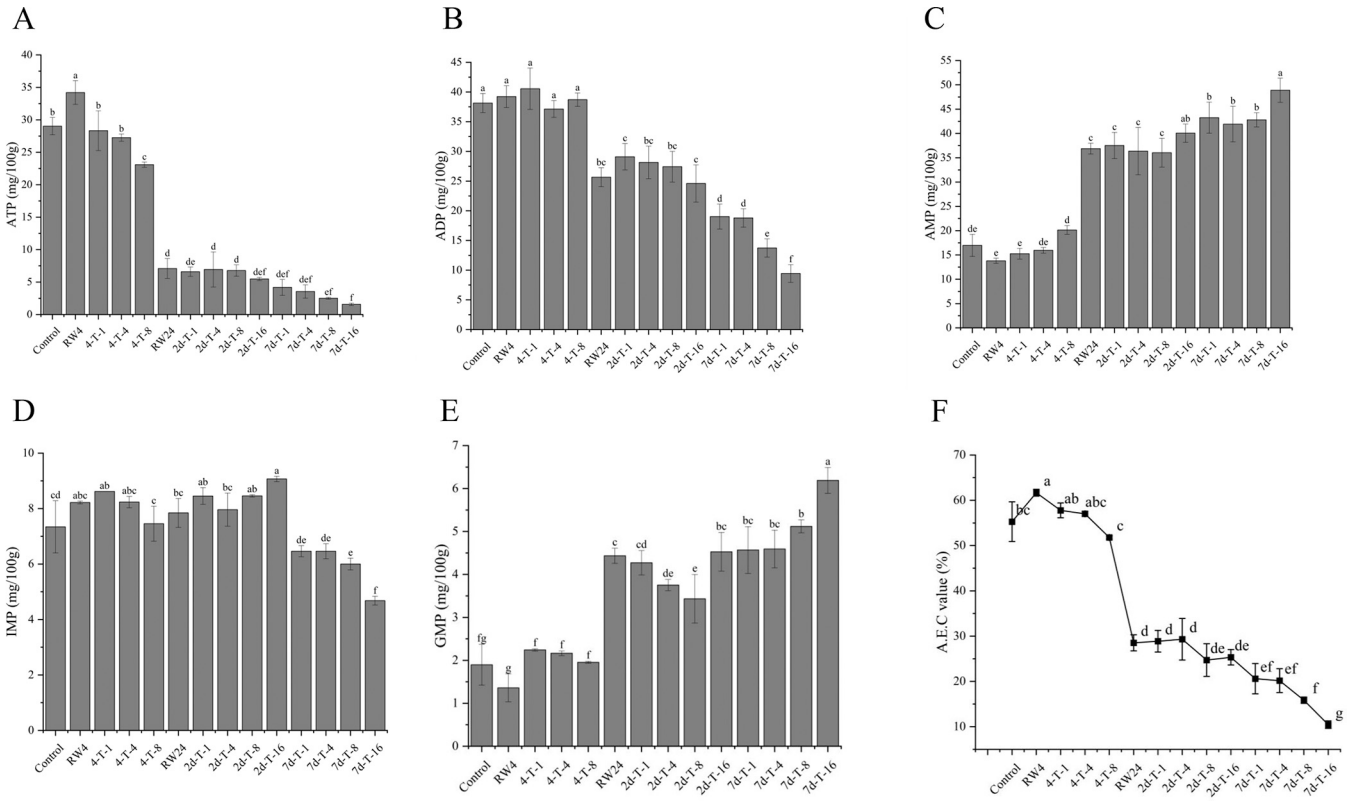

Fig. 3. The contents of ATP-related compounds (mg/100 g) in *M. coruscus* during the semi-anhydrous living-preservation at different cooling rates. A: ATP content; B: ADP content; C: AMP content; D: IMP content; E: GMP content; F: A.E.C. value.

38.9 % of the total fatty acids, with C16:0 being the most prevalent at approximately 23 %, followed by docosahexaenoic acid (DHA) at 16 % and eicosapentaenoic acid (EPA) at 11 %. *M. coruscus* was found to be rich in DHA and EPA, which are essential for human intake, as supported by previous research (Reksten et al., 2024). These findings confirm the prominence of DHA and EPA as primary lipids in mussels.

The results presented in Table 1 demonstrated that different cooling rates had varying effects on the composition of SFAs and UFAs. Specifically, the C17:0 content decreased significantly ($P < 0.05$) after the second day of semi-anhydrous living-preservation compared with that during the cooling process, and the 2d-T-16 group presented a lower C17:0 content than did the other three groups did ($P < 0.05$). Despite this decrease, the total saturated fatty acid content did not significantly differ among these groups ($P > 0.05$). UFAs are composed of MUFAs and PUFAs. For MUFAs, the T-16 group displayed a decreasing trend after gradient cooling, with a significant decrease observed after the second day of semi-anhydrous living-preservation. This suggested that temperature stress during the cooling process led to the utilization of MUFAs for energy. Similarly, PUFAs exhibited a similar trend. Specifically, on the second day of semi-anhydrous living-preservation, the EPA content in the T-16 group significantly decreased ($P < 0.05$), whereas the DHA content tended to decrease without a significant difference (Nie et al., 2023).

This study revealed that the T-1, T-8, and T-16 groups presented notable reductions in the fatty acid levels of the mussels, with the T-16 group having the most pronounced impact. The T-4 group and the associated semi-anhydrous living-preservation group presented the highest levels of DHA and EPA. This phenomenon could be attributed to the adjustment in fatty acid composition under conditions of low-temperature stress to increase the fluidity of the cell membrane (Fadhlaoui and Couture, 2016).

3.7. Free amino acid analysis

Free amino acids are considered to be the main component of mussel flavor (Ma et al., 2022) and can be categorized into umami, sweet, bitter and tasteless amino acids. The umami taste of mussel meat is associated with the presence of aspartic acid (Asp) and glutamic acid (Glu), while the sweetness is linked to alanine (Ala), proline (Pro), threonine (Thr), serine (Ser), and glycine (Gly). Bitterness is attributed to amino acids such as arginine (Arg), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), lysine (Lys), and histidine (His). Tasteless amino acids include valine (Val), tyrosine (Tyr), and methionine (Met) (Yin et al., 2022).

The free amino acid contents of the mussels after different treatments were tested. The results depicted in Fig. 4-C revealed that glutamic acid, glycine, and alanine were the predominant free amino acids in mussels,

Table 1
Fatty acid composition of *Mytilus coruscus* during semi-anhydrous living-preservation at different cooling rates (as a percentage of total fatty acids).

Fatty acid composition	Control	RW4	4-T-1	4-T-4	4-T-8	RW24	2d-T-1	2d-T-4	2d-T-8	2d-T-16
C14:0	1.90 ± 0.02a	1.95 ± 0.04a	1.89 ± 0.05a	1.80 ± 0.02a	1.82 ± 0.10a	1.83 ± 0.14a	1.79 ± 0.12a	1.84 ± 0.09a	1.70 ± 0.04a	1.71 ± 0.16a
C15:0	0.71 ± 0.03a	0.69 ± 0.01a	0.71 ± 0.02a	0.68 ± 0.01a	0.68 ± 0.01a	0.69 ± 0.03a	0.68 ± 0.01a	0.69 ± 0.01a	0.66 ± 0.02a	0.66 ± 0.06a
C15:1	0.14 ± 0.01a	0.15 ± 0.03a	0.14 ± 0.01a	0.14 ± 0.01a	0.15 ± 0.01a	0.14 ± 0.01a	0.15 ± 0.01a	0.14 ± 0.01a	0.14 ± 0.01a	0.14 ± 0.01a
C16:0	22.54 ± 0.89a	22.53 ± 0.19a	23.62 ± 0.48a	22.45 ± 0.59a	22.77 ± 0.38a	22.48 ± 0.15a	23.00 ± 0.59a	22.67 ± 0.67a	22.10 ± 0.48a	22.18 ± 1.13a
C16:1	3.62 ± 0.14a	3.54 ± 0.07a	3.67 ± 0.17a	3.51 ± 0.10a	3.36 ± 0.09a	3.41 ± 0.38a	3.32 ± 0.19a	3.44 ± 0.25a	3.00 ± 0.22a	3.06 ± 0.47a
C17:0	0.92 ± 0.03ab	0.94 ± 0.04ab	1.03 ± 0.06a	1.02 ± 0.01a	1.01 ± 0.01a	0.95 ± 0.04ab	0.91 ± 0.07ab	0.90 ± 0.08ab	0.89 ± 0.02ab	0.84 ± 0.07b
C17:1	0.73 ± 0.16ab	0.72 ± 0.15ab	0.69 ± 0.20ab	0.85 ± 0.06ab	1.11 ± 0.05a	0.80 ± 0.02ab	0.66 ± 0.03ab	0.68 ± 0.08ab	0.66 ± 0.11ab	0.33 ± 0.10c
C18:0	4.48 ± 0.04a	4.43 ± 0.10a	4.75 ± 0.28a	4.48 ± 0.12a	4.27 ± 0.12a	4.49 ± 0.13a	4.55 ± 0.15a	4.27 ± 0.24a	4.28 ± 0.15a	4.40 ± 0.15a
C18:1	1.8 ± 0.07ab	1.92 ± 0.08a	1.81 ± 0.03ab	1.70 ± 0.01ab	1.71 ± 0.08ab	1.74 ± 0.16ab	1.64 ± 0.15b	1.82 ± 0.03b	1.59 ± 0.02ab	1.59 ± 0.12b
C18:2n-6	1.65 ± 0.10a	1.61 ± 0.05a	1.57 ± 0.06a	1.65 ± 0.01a	1.60 ± 0.03a	1.60 ± 0.08a	1.60 ± 0.14a	1.63 ± 0.05a	1.61 ± 0.05a	1.56 ± 0.12a
C18:3n-6	0.24 ± 0.01ab	0.23 ± 0.01ab	0.24 ± 0.01ab	0.23 ± 0.01ab	0.22 ± 0.01ab	0.21 ± 0.02ab	0.24 ± 0.01a	0.22 ± 0.01ab	0.20 ± 0.01b	0.21 ± 0.02ab
C18:3n-3	1.14 ± 0.03abc	1.11 ± 0.06abc	1.26 ± 0.13a	1.03 ± 0.01bc	0.93 ± 0.01c	1.02 ± 0.16bc	0.95 ± 0.04bc	0.98 ± 0.12bc	1.17 ± 0.03ab	0.93 ± 0.11c
C20:0	1.25 ± 0.01a	1.24 ± 0.05a	1.33 ± 0.12a	1.24 ± 0.04a	1.43 ± 0.04a	1.39 ± 0.16a	1.40 ± 0.14a	1.30 ± 0.09a	1.34 ± 0.06a	1.38 ± 0.15a
C20:1n-9	0.95 ± 0.01a	0.70 ± 0.27a	1.22 ± 0.04a	0.78 ± 0.39a	0.74 ± 0.35a	1.10 ± 0.02a	1.16 ± 0.05a	1.08 ± 0.02a	0.98 ± 0.09a	1.11 ± 0.03a
C20:2	0.24 ± 0.03a	0.26 ± 0.01a	0.27 ± 0.01a	0.26 ± 0.01a	0.21 ± 0.01a	0.24 ± 0.01a	0.21 ± 0.02a	0.25 ± 0.01a	0.25 ± 0.01a	0.26 ± 0.02a
C20:4n-6	2.62 ± 0.13ab	2.64 ± 0.06ab	2.70 ± 0.09ab	2.69 ± 0.14ab	2.85 ± 0.17ab	2.67 ± 0.12ab	2.80 ± 0.03ab	2.71 ± 0.16ab	2.53 ± 0.01b	2.98 ± 0.14a
EPA	11.31 ± 0.42ab	11.60 ± 0.27a	11.20 ± 0.24ab	11.24 ± 0.50ab	10.48 ± 0.28b	10.94 ± 0.55ab	11.17 ± 0.42ab	11.52 ± 0.11a	10.54 ± 0.23b	10.67 ± 0.50ab
DHA	15.79 ± 0.61a	16.19 ± 0.47a	16.19 ± 0.13a	16.30 ± 0.94a	15.15 ± 0.16a	15.18 ± 0.83a	15.71 ± 0.34a	16.41 ± 0.43a	15.22 ± 0.89a	15.00 ± 1.25a
∑SFA	31.80 ± 0.91a	31.78 ± 0.04a	33.34 ± 0.89a	31.65 ± 0.73a	31.98 ± 0.44a	31.83 ± 0.20a	32.31 ± 1.00a	31.67 ± 0.90a	30.97 ± 0.64a	31.18 ± 1.65a
∑MUFA	6.21 ± 0.20a	6.32 ± 0.04a	6.31 ± 0.09a	6.20 ± 0.14a	6.32 ± 0.22a	6.09 ± 0.51ab	5.78 ± 0.13a	6.08 ± 0.19a	5.40 ± 0.29bc	5.12 ± 0.40c
∑PUFA	33.94 ± 0.73a	34.34 ± 0.91a	34.65 ± 0.29a	34.18 ± 1.70a	32.18 ± 0.61a	32.96 ± 1.51a	33.84 ± 0.72a	34.80 ± 0.50a	32.49 ± 1.28a	32.71 ± 1.83a

The data are presented as the means ± standard deviations ($n = 3$). Different letters in the same row indicate significant differences ($P < 0.05$). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: C20:5n-3; DHA: C22:6n-3.

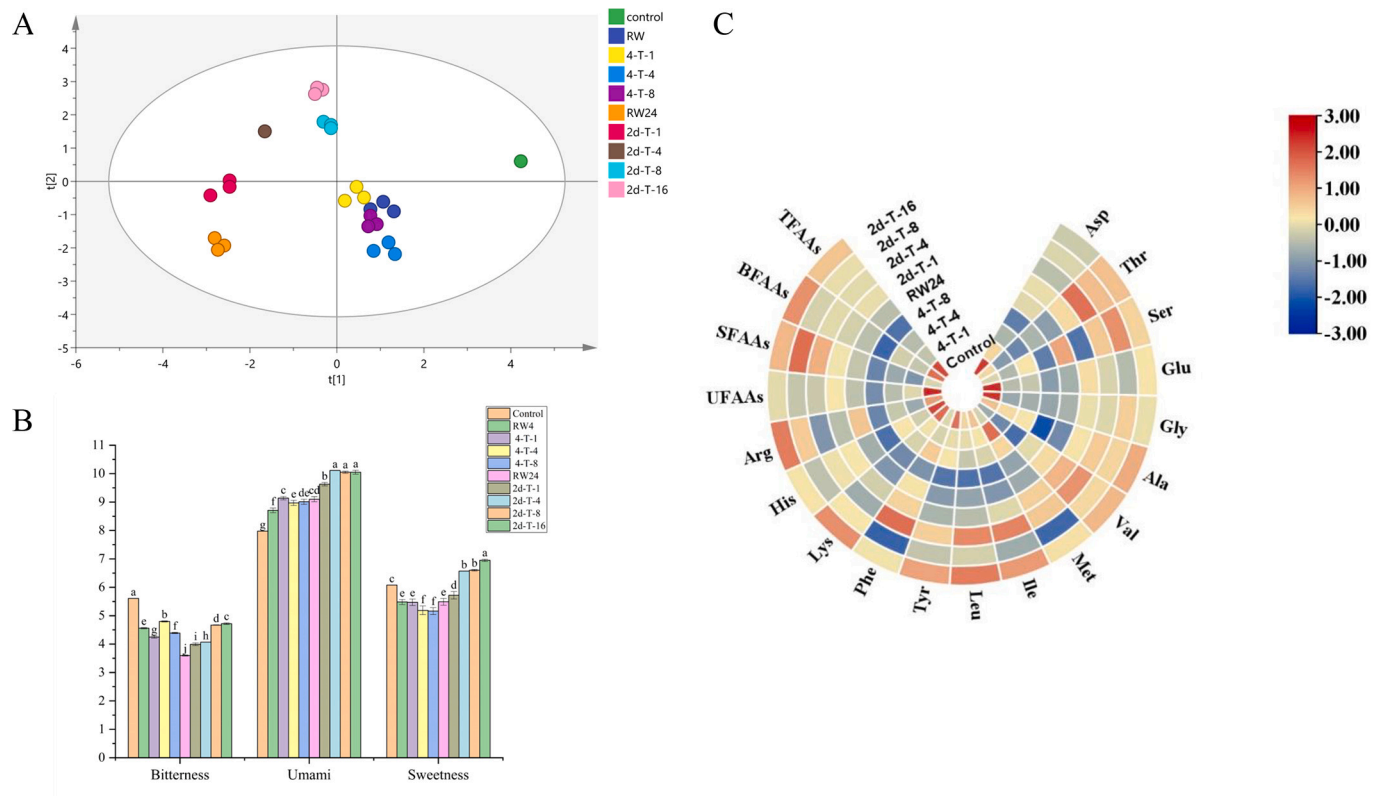

Fig. 4. Changes in the flavor of *M. coruscus* during the semi-anhydrous living-preservation at different cooling rates. A: Principal component analysis based on the results obtained by the electronic tongue; B: Results of umami, bitterness and sweetness response values obtained by the electronic tongue; C: Heatmap of free amino acids in each group of *M. coruscus*.

with Glu being the most abundant. These key amino acids contribute significantly to the fresh and sweet flavor of mussels. Conversely, amino acids such as tryptophan, cysteine, and ornithine were undetected because of their minimal quantities. Following the gradient cooling treatment, a decrease in the levels of certain free amino acids in mussels was observed due to temperature-induced stress. The combination of low temperatures, a lack of water, and the inability of mussels to feed led to a scenario in which they needed to utilize protein reserves to sustain normal physiological functions. When the temperature was decreased to 4 °C under semi-anhydrous conditions, the total free amino acid content of the T-8 group tended to decrease, reaching the lowest level. In contrast, the RW24 group and the T-1 and T-4 groups, which were temporarily stored in water, experienced a decrease, possibly attributed to rapid temperature fluctuations within a short timeframe leading to content reduction. After 2 days of preservation under semi-anhydrous conditions, the content of free amino acids tended to increase, with the most significant increase in the 2d-T-16 group, which was mainly due to an increase in the content of bitter amino acids.

Aspartic acid and glutamic acid, both umami amino acids, are crucial for the umami flavor of mussels (Liu et al., 2022). Compared with those in the control group, their levels decreased notably during cooling and storage ($P < 0.05$). The synthesis of glutamic acid relies on the catalysis of glutamic acid dehydrogenase (Luczkowska et al., 2024). Exposure to low temperatures led to a reduction in enzyme activity, which subsequently caused a significant decrease in glutamic acid content ($P < 0.05$) (Bruhns et al., 2023). Similarly, the levels of aspartic acid decreased because its production involves enzyme catalysis and energy consumption. Conversely, alanine and glycine levels remained relatively stable, as these amino acids presented enhanced sweetness properties at relatively low temperatures (Yu et al., 2024).

Except for the control group, no significant difference in His content was detected among the other groups ($P > 0.05$). Some studies have shown that arginine is the main amino acid that causes the bitter taste of mussels (Ma et al., 2022). Arginine was the most abundant bitter-tasting amino acid, accounting for approximately 25 % of the total bitter-tasting amino acids. The arginine content in the mussels in the gradient cooling treatment group was lower than that in the RW24 group, indicating that gradient cooling treatment slowed the increase in arginine and reduced the bitterness of the mussels. The results of the arginine content in mussels after two days of storage revealed that the differences in arginine content among the different groups were more significant, with the 2d-T-4 group having the lowest content and the 2d-T-16 group having the highest. These results showed that gradient cooling was better than direct cooling, with the taste of the T-4 group being better than that of the other three groups, and that gradient cooling could slow the generation of bitterness in mussels compared with direct cooling.

3.8. Electronic tongue analysis

The bitterness, sweetness, sourness, astringency, saltiness, bitterness aftertaste and astringency aftertaste of different groups of *M. coruscus* were measured, and the results revealed that the response values of umami, bitterness, sweetness, saltiness and richness were all positive, with umami, sweetness and bitterness exerting greater influences on taste. Conversely, the response values for sourness, astringency and aftertaste of *M. coruscus* were all below the tasteless point, indicating a negligible effect on taste. Principal component analysis was performed, as shown in Fig. 5-A. The different groups of *M. coruscus* exhibited a greater degree of divergence, indicating significant differences in the overall taste among the groups. The effects of freshness, sweetness and bitterness on the taste of *M. coruscus* are shown in Fig. 5-B. Compared with that of the control group, the amount of bitterness was lower at the end of the freezing process and during storage. Similarly, freshness and sweetness also tended to differ from those of the control, indicating that

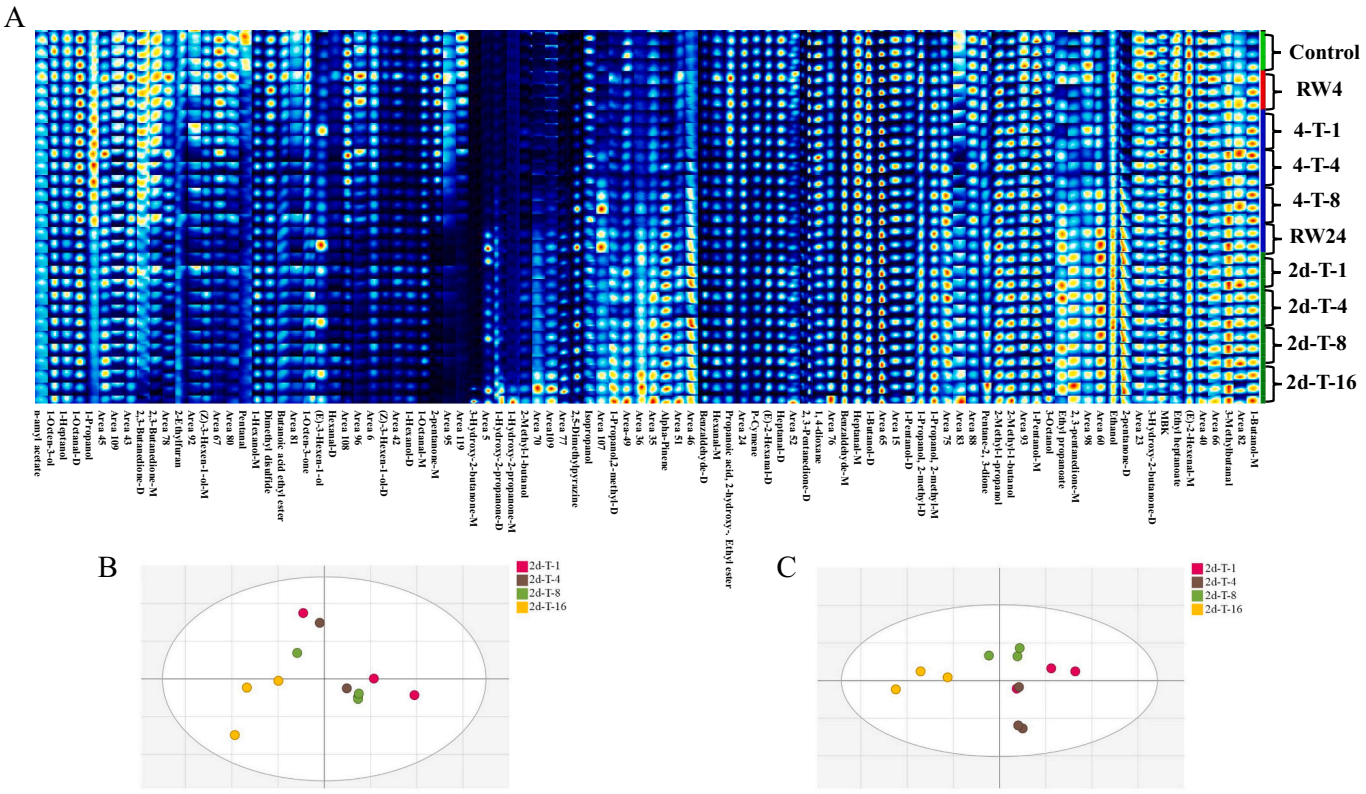

Fig. 5. Analysis of volatile organic compounds in *M. coruscus* during the semi-anhydrous living-preservation at different cooling rates. A: GC-IMS-based fingerprint; B: Principal component analysis (PCA) based on GC-IMS; C: Orthogonal partial least squares discriminant analysis (OPLS-DA) analysis based on GC-IMS.

different cooling rates had a certain degree of impact on taste. The electronic tongue analysis revealed that umami was the predominant flavor in mussels, which was similar to the results of free amino acids, with umami amino acids, such as Asp and Glu, being present in greater proportions than other amino acids.

3.9. Analysis of volatile organic compounds

GC-IMS was used to analyze changes in mussel flavor during storage after the mussels were cooled to 4 °C at different rates. As shown in Fig. 5, 57 volatile organic compounds, including 19 alcohols, 14 ketones, 12 aldehydes, 5 esters and other compounds, were identified, which was in line with previous research by Tuckey et al. (2013). The key flavor components, aldehydes and ketones, were produced mainly through the Maillard reaction, with ketones also produced from amino acid breakdown. Esters are derived from the degradation of amino acids and alcohols are derived from lipid degradation and play a significant role in fruity aromas (Li et al., 2022b). The fingerprint chart (Fig. 5-A) indicated that the mussels in the control groups contained relatively high concentrations of volatile compounds, such as (Z)-3-hexen-1-ol, (E)-2-hexenal, 1-octen-3-one, 1-octen-3-ol, and 1-octanal, which contribute fruity or milky flavors. The levels of pentanal, 2,3-butanedione, 1-heptanol, 1-hexanol, and others can reduce the pungent odor of fatty acids and enhance the overall flavor of mussels (Cheng et al., 2023) were found to be increased. Additionally, when the mussels were stored at 4 °C, the concentrations of these volatile organic compounds notably decreased, resulting in an overall reduction in mussel flavor.

The impact of alcohols on mussel flavor is negligible because of their high threshold (Le Guen et al., 2000). Compared with alcohols, aldehydes play a crucial role in the overall flavor of mussels (Deng et al., 2022). After 2 days of storage at 4 °C, a decrease in aldehydes such as 1-octanal and pentanal, which are known for their fruity or floral aromas, was observed compared with those in the control group. The 2d-T-16 group had the lowest concentration of 1-octanal, while the other three groups had comparable concentrations that were higher than those of the 2d-T-16 group, suggesting that gradient cooling could effectively slow the loss of aroma compounds in mussels. Moreover, the concentrations of 2-ethylfuran, 2-pentanone, 2,3-butanedione, and 1-octen-3-one tended to decrease compared with those in the control group. 2-Ethylfuran, which is abundant in shellfish, imparts a rich sweet and coffee flavor to mussels, with its concentration decreasing with decreasing temperature. Conversely, several compounds, such as ketones and alcohols, tended to increase, possibly due to the secretion and accumulation of various enzymes by microorganisms during semi-anhydrous living-preservation (Cheng et al., 2023). For example, methyl sulfide is produced through the reactions of amino acids, peptides, and other substances with enzymes generated by microbial metabolism. Studies have shown that methyl sulfide is a key compound in mussels responsible for their rot-sour odor (Ratnawati et al., 2023). Compared with the control group, the gradient cooling group was able to decrease the concentration of methyl sulfide, reducing the foul odor and positively impacting the overall aroma of the mussels.

4. Conclusion

This study investigated the effects of gradient cooling on the physiological metabolism and quality of mussels under semi-anhydrous conditions. Compared with T-16 (16 °C/h), gradient cooling improved the respiratory metabolism of the mussels, reduced their mortality, and improved their survival status. The oxygen consumption rate and ammonia excretion rate of mussels were greater than those of the 16 °C/h group, and the ATP-related compounds and A.E.C. values also showed similar results, among which the survival rate and survival status of the T-4 (4 °C/h) group were the best; on the other hand, the gradient cooling

group could effectively slow the taste quality of mussels and slow the increase in the content of bitter amino acids such as arginine. Among the groups, the 4 °C/h group presented the lowest content of bitter amino acids and a stable content of sweet amino acids, and the gradient cooling treatment slowed the loss of light-smelling volatile organic compounds. In conclusion, 4 °C/h had a positive effect on the quality of *M. coruscus* stored under low-temperature semi-anhydrous living-preservation, providing a reference for the processing and transportation of mussels.

CRediT authorship contribution statement

Shuai Wu: Writing – original draft, Methodology, Conceptualization. **Haoxiang Ma:** Methodology. **Yu Liu:** Writing – original draft, Methodology, Supervision. **Haohao Shi:** Visualization, Supervision. **Changhu Xue:** Supervision, Project administration. **Lipin Chen:** Writing – review & editing, Project administration, Supervision. **Zhaojie Li:** Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2023YFD2100201) and the Hainan University Research Fund Project (XJ2400012944).

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