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Quality parameters and storage stability of the Norwegian red sea cucumber (*Parastichopus tremulus*)

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ABSTRACT

Quality parameters and storage stability were evaluated through biochemical, textural, and microbial analyses of the Norwegian red sea cucumber *Parastichopus tremulus* at two different storage temperatures (0 °C and 4 °C) over 17 days. During storage, the pH decreased from 7 to 6.3, the texture became softer decreasing from 47.3 to 0.3 N, while K-value increased from 4 to 52 %, microbial counts increased from 2 to 8 log cfu/g and formation of volatiles increased. After storage for 7 days at 0 °C and 5 days at 4 °C, the microbial counts exceeded the acceptability for consumption (7 log cfu/g). Microorganisms such as *Flavobacterium* and *Psychrobacter* were the main specific spoilage organisms contributing to the deterioration. Prolonged storage favored the *Polaribacter* and *Marinomonas* proliferation regardless of storage temperatures. The free amino acid concentration increased from 24 to 86 mg/100 g at both storage temperatures, probably resulting from the autolysis degradation. The pH decrease could be attributed to the production and dissolution of volatile compounds during storage. Odor-active compounds such as aldehydes (decanal, 2,4-decadienal, nonanal), ketones (1-octen-3-one, 1-penten-3-one), alcohols (1-octen-3-ol, 2-heptanol), aromatics (p-cresol and phenol), indoles (indole) and sulfides (dimethyl disulfide and trisulfide) showed increasing levels with storage time, with the highest levels found in the samples stored at 4 °C. This is the first study that demonstrate the quality changes on molecular and microbial level occurring during cold storage of sea cucumber *P. tremulus*. These results can be used for evaluating the shelf-life limiting factors of such species.

1. Introduction

The current global food system must change to become more sustainable and efficient to meet the needs of the growing population and enhance food security (UN, 2024). There is a growing interest in utilizing a broader range of species for food, such as sea cucumbers, which could provide essential nutrients (Ciriminna et al., 2024; Mondragon Portocarrero et al., 2023). Of the 900 sea cucumber species, about 80 are edible (Purcell et al., 2023). Sea cucumbers have highly nutritional value, containing essential amino acids, vitamins and minerals, and constitute high-value components exhibiting anti-inflammatory and antioxidant properties (Bordbar et al., 2011; Hossain et al., 2020). Sea cucumbers are mainly utilized in Asia and are often sold as luxury food products (fried, cooked or as bêche-de-mer) and as medical foods (Purcell et al., 2023). In Europe, the consumption is rare, and most bycaught sea cucumbers are processed into dried or frozen products before being exported to the Asian markets (Purcell et al., 2023).

Sea cucumbers are highly susceptible to autolysis during postmortem storage (Fan et al., 2024). It has been reported that typical post-mortem changes in sea cucumbers *Stichopus japonicus* (Xiong et al., 2020) and *Apostichopus japonicus* (Li et al., 2022) include texture and pH alterations, along with protein and nucleotide degradation. It is also known that other degradation processes such as lipid oxidation (Cramer et al., 2005) could occur during storage, and are accelerated by the exposure to UV light and temperature changes in the sea cucumbers (Purcell et al., 2016; Wu et al., 2013; Yan et al., 2014). These metabolic changes stimulate microbial growth, and the dominance of specific spoilage organisms (SSOs) (Odeyemi et al., 2018).

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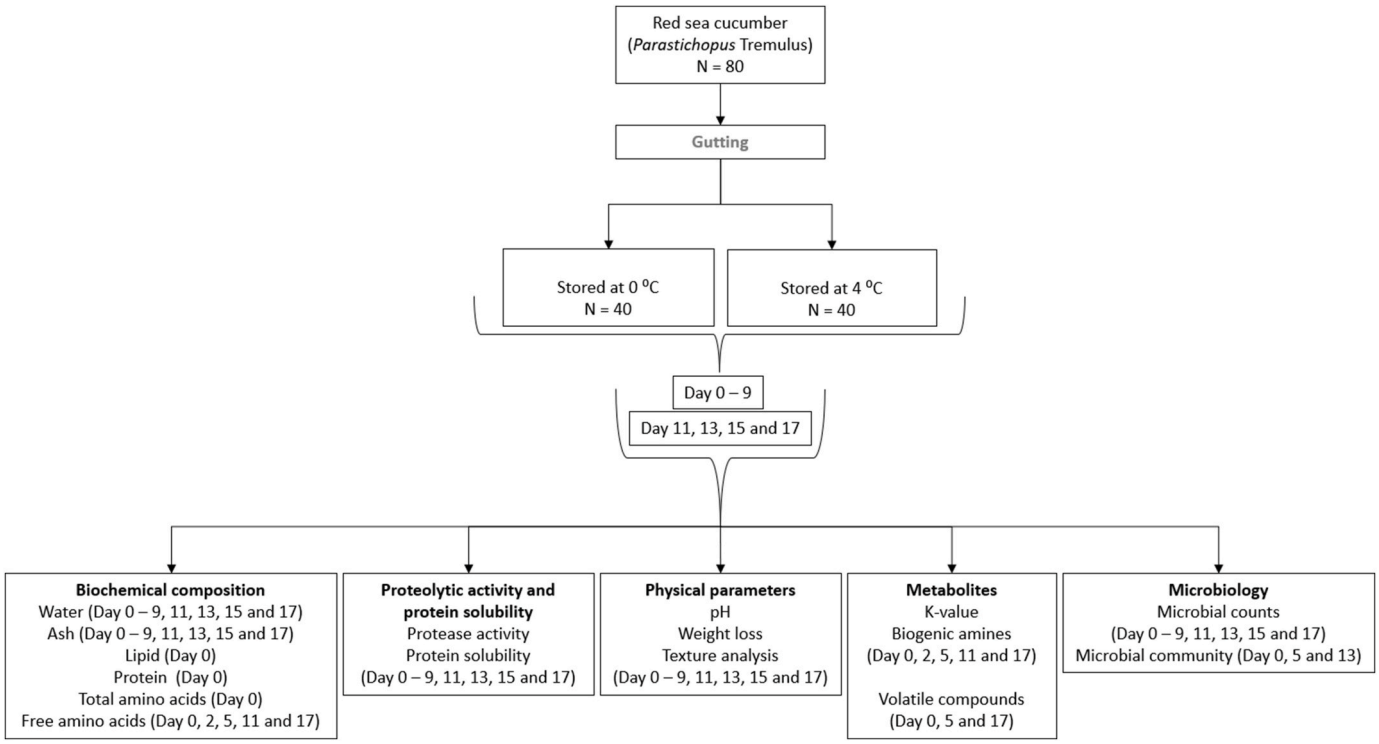

Fig. 1. Experimental design and overview of analyses conducted to investigate changes in biochemical composition, enzyme activity, physical parameters, metabolites, and microbiology changes during storage of red sea cucumber (*Parastichopus tremulus*) for 17 days at 0 °C and 4 °C.

SSOs are usually gram-negative bacteria which produce metabolic compounds responsible for odor-active compounds and off-flavors (Odeyemi et al., 2018). The dominance of SSOs is mainly determined by the initial microbial community composition, and physicochemical factors (a_w, nutrient availability) (Parlapani, 2021), post-capture contamination, storage conditions (packaging, temperature) and type of product (marinated, raw). Microorganisms are the major cause of seafood spoilage, which generally occurs when microbial plate counts reach between 6 and 9 log colony-forming unit (cfu/g). (Mikś-Krajnik et al., 2016).

The global sea cucumber stocks are declining due to over-exploitation, especially in the West- and Indo-Pacific regions (Conand, 2018). In Europe, the consumption of sea cucumbers is rare (Purcell et al., 2023), and more research is needed to assess their quality before potential utilization. The transferability of post-mortem biochemical changes from previous studies of sea cucumber to *P. tremulus* remains uncertain, and post-mortem changes of *P. tremulus* during storage is not well documented. Thus, this study focused on investigating the quality parameters and storage stability of *P. tremulus* stored under two cold storage conditions (0 °C and 4 °C) over 17 days. Specifically, the research aimed at: a) exploring the microbiota profile succession and microbial counts evolution; b) assessing the physical and biochemical changes, including texture, proximate composition, and protein profile; and c) analyzing the production of spoilage metabolites and volatile compounds throughout storage at both temperatures. *P. tremulus* was chosen due to its high abundance along the Norwegian coast (Kjerstad, Ringvold, Søvik, Knott, & Thangstad, 2015). This study could enhance knowledge of molecular, microbial and the physical changes of *P. tremulus* during cold storage. This knowledge could be used to retain quality, providing better guidelines for consumption and processing of *P. tremulus* during cold storage.

2. Material and methods

2.1. Experimental design and sampling plan

P. tremulus ($n = 80$) (75 ± 36 g) were caught using a crayfish trap cage along the coast of Frøya, Norway (63°45.017 North 8°53.292 East) at between 250 and 300 m depth on the ocean floor in March 2022. The samples were then transported to the laboratory in Trondheim and stored in tanks with oxygenated seawater and seaweed (17 h) until slaughtering. The slaughtering process included a cut through from one end to another of the sea cucumber's body, and the removal of inside water and intestines. The sea cucumbers were individually packed in tight zip lock bags and stored at 0 °C covered in ice ($n = 40$) or at 4 ± 0.5 °C ($n = 40$) in a refrigerated room.

Samples were taken daily from day 0 to day 9, and every second day from day 9 to day 17. Water, ash and lipid content, pH, weight loss, texture, and microbial analyses were conducted immediately after each sampling. The samples were thereafter stored at −80 °C until further analyses for chemical composition, protease activity, metabolites, and microbial community composition (Fig. 1). Lipid, protein, and total amino acids (TAAs) content were analyzed on day 0. Weight loss, pH, texture, water and ash content, protease activity, and microbial counts were assessed on day 0–9, day 11, 13, 15, and 17. Free amino acids (FAAs), freshness indicator (K-value) and biogenic amines were measured on day 0, 2, 5, 11 and 17. Microbial community composition and volatile compounds were analyzed at the start (day 0), in the middle (day 5) and at the end (day 17) of the storage trial.

2.2. Microbial counts and microbial community composition

The psychrotrophic and aerobic plate counts (PC and APC, respectively) and H_2S-producing bacteria were determined as described by NMKL (2006). Sea cucumber samples (10 g) were aseptically placed into a sterile stomacher bag and homogenized with 90 mL of sterile peptone water (1.0 g bacteriological peptone and 8.5 g/L sodium chloride (NaCl)

Table 1
Proximate composition (wet weight %), the sum of total amino acids (TAAs) (mg/g wet weight) and the sum of free amino acids (FAAs) (mg/100 g wet weight) of fresh red sea cucumber (*Parastichopus tremulus*) (day 0). The result is presented as mean ± standard deviation ($n = 3$).

Composition	*P. tremulus*
Water	88.9 ± 1.0
Ash	7.7 ± 0.9
Lipid	1.0 ± 0.6
Totalt protein	4.9 ± 0.3
∑TAAs	45.3 ± 0.3
∑FAAs	23.8 ± 12.5

using a Stomacher 400 Lab Blender (Seward Medical Ltd., UK). Appropriate serial dilutions were made in sterile peptone water and plated onto agar media. The PC was enumerated using Long and Hammer (LH) agar supplemented with NaCl (1 %). Plates were incubated at 15 °C for 6 days. APC and H_2S-producing bacteria were quantified using iron agar (Oxoid) supplemented with L-cysteine (0.04 %, Sigma-Aldrich, Oslo, Norway) and incubated at 22 °C for 3 days.

The homogenized samples suspension was used to extract the genomic DNA for microbial community analysis. A volume of 2.5 ml suspension was aseptically transferred to separate sterile centrifuge tubes, and centrifuged at 150× *g* (2 min, 20 °C) to remove sea cucumber particles. The supernatant was transferred to another sterile centrifuge tube, and centrifuged at 4000× *g* (10 min, 20 °C). The pellet was resuspended using sterile peptone water (1 mL), transferred to sterile Eppendorf tubes, and centrifuged at 12 500× *g* (5 min, 20 °C). The supernatant was discarded, and the DNA extraction was performed on the pellet using PowerFood® DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA, USA) following the supplier's instructions. The DNA extraction was performed in 3 parallels ($n = 3$) on day 0, 5 and 13. The extracted DNA from each parallel of the same sample was pooled to increase the DNA concentration and reliability as well as reduce the experimental variability (Parlapani et al., 2018). The pooled DNA samples were stored at −80 °C until use. DNA integrity and concentration, metabarcoding analysis and bioinformatics were performed as described by Tsoukalas, Hoel, Lerfall, Valdramidis, et al. (2023).

2.3. Physical parameters

All individual sea cucumbers were weighed before slaughter, after gutting and when sampled to calculate weight loss. The pH of the samples was measured after gutting and at the time of sample collection using a portable pH-meter testo 206 (Testo AG, Titisee-Neustadt, Germany) directly on the sea cucumber's body wall. The texture was measured at sample collection and was analyzed as described by Tsoukalas et al. (2022). About 4 g, approximately 2 cm × 2 cm from the sea cucumber's body wall was cut, and the texture hardness (N) was measured by puncturing the sample using a Texture Profile Analysis (TPA) with a 5 kg load cell on a Texture Analyzer instrument (TA-XT Plus, Stable Micro Systems, UK). Textural measurement was performed with a constant speed of 2 mm/s. The results were processed using Exponent Lite software, version 6.1.16.0 (Stable Micro Systems Ltd.).

2.4. Biochemical composition of P. tremulus

Water and ash content were determined gravimetrically (AOAC., 2005). About 4 g of sample were dried at 105 °C for 24 h to assess water content, followed by incineration at 550 °C for 24 h in a muffle furnace to determine ash content.

Protein content was determined using the Kjeldahl method (Büchi, 2013). About 1.5 g of sample was added to tubes. Titanium tablets (2 tablets, Büchi Labortechnik AG, Flawil, Switzerland) and 15 mL sulfuric acid (98 %, 7664-93-9, Merck KGaA, Darmstadt, Germany) was added before being placed in a KjelDigester K-449 with a Scrubber K-415. The tubes were digested for approximately 2.5 h, then transferred to a KjelMaster K-375 for protein content determination, with a protein conversion factor of 6.25. Glycine (0.2 g, 56-40-6, Merch Life Science AS, Darmstadt, Germany) was used as reference. The protein content was measured twice on each individual sea cucumber.

Lipid content was determined gravimetrically after extraction as described by Bligh and Dyer (1959). Sample (2 g) was mixed with distilled water (2 mL), chloroform (4 mL, <99%, CAS: 67-66-3, VWR, Leuven, Belgium), and cold methanol (8 mL, <99.9%, CAS: 67-56-1, Merck Life Science AS, Darmstadt, Germany). The mixture was homogenized for 2 min at 4000× *g*. An additional 4 mL of chloroform and 4 mL distilled water were then added and homogenized for 30 s. The mixture was centrifuged at 11 800× *g* for 10 min. The chloroform phase was transferred to pre-weighed Kimax glass tubes, weighed, and placed in an evaporation unit at 60 °C for 1 h with N_2 streaming. The sample tubes were weighed again after evaporation to determine the lipid content. The lipid content was measured twice on each individual sea cucumber. System parameters, chemicals and instrument analysis were performed as previously described in Vu et al. (2024).

The composition of TAAs was analyzed based on the method by Blackburn (1978). About 50 mg of sample was added to Kimax tubes along with 1 mL of 6 M HCl (CAS: 7647-01-0, Sigma-Aldrich, Darmstadt, Germany) and hydrolyzed for 22 h at 105 °C. The Kimax tubes were then neutralized to a pH between 6.5 and 7.5, filtered using a Whatman glass microfibre filter, diluted (1:500), filtered again using a 0.22 μm filter, and transferred to HPLC vials for analysis. The TAAs were measured three times on each individual sea cucumber.

The composition of FAAs was analyzed based on the method by Osnes and Mohr (1985). About 5 g of sample was added to distilled water (10 mL), homogenized for 1 min at 10 000× g, and centrifuged for 15 min at 6000× *g*. The water-soluble protein extract (1 mL) was transferred to Eppendorf tubes, mixed with 0.25 mL of 10% sulfosalicylic acid (≥99%, CAS: 5965-83-3, Merck Life Science AS, Darmstadt, Germany), and left in the cold room at 4 °C for 30 min. The Eppendorf tubes were then centrifuged for 10 min at 13 000× *g*. The supernatant was diluted with distilled water, filtered using a 0.22 μm filter, and transferred to HPLC vials for analysis. The FAAs were measured twice on each individual sea cucumber.

Both TAAs and FAAs were analyzed using a high-performance liquid chromatography (HPLC) system (Dionex, Thermo Fisher Scientific, CA, USA). The HPLC system constituted a TSP P400 pump, an ultimate 3000WP injector, a fluorescence detector RF2000, and a Nova-Pak C18 column with a particle size of 4 μm (3.9 mm × 150 mm, WAT086344, Waters Corporation, MA, USA).

2.5. The determination of protein solubility and proteolytic activity

The concentration of protein solubility was determined as described by Lowry et al. (1951). The proteolytic activity was conducted as outlined in Barrett (1972) with modifications made by Stoknes et al. (1993). For measurement of proteolytic activity, samples were homogenized with distilled water (1:5, w/v). The proteolytic activity was performed at pH 7 for 1 h and was stopped using trichloroacetic acid (TCA) (2 mL, 5 % w/v). The analysis was performed three times on each individual sea cucumber.

2.6. Trichloroacetic acid extraction and Nuclear Magnetic Resonance Spectroscopy

The water-soluble polar metabolites were extracted as described by Ciampa et al. (2012). The extracted solution was centrifuged (20 000× *g* for 5 min) and the supernatant (2 mL) was stored at −80 °C until analysis by Nuclear Magnetic Resonance Spectroscopy (NMR) as described by Shumilina et al. (2015), followed by the determination of 5'-adenosine

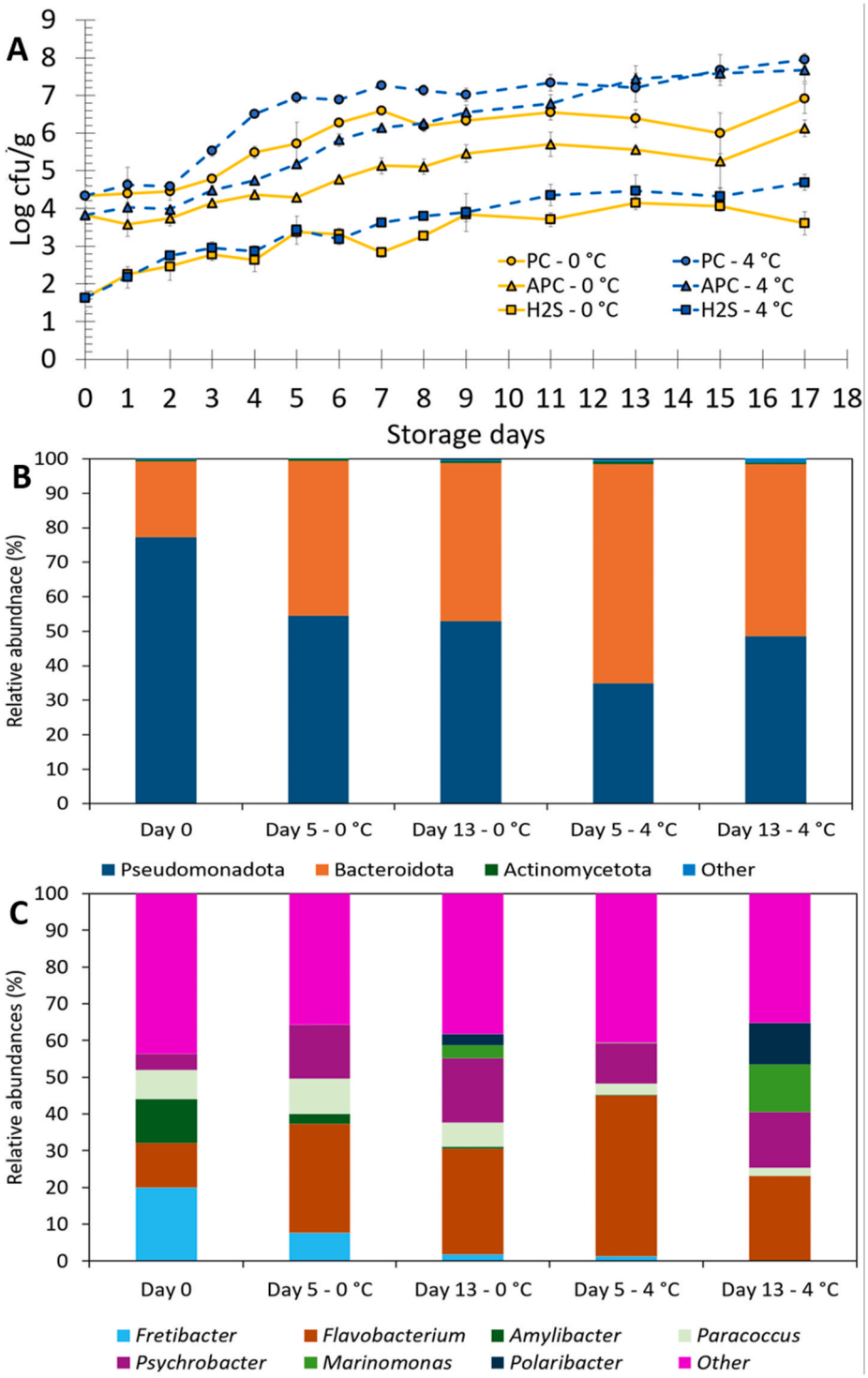

Fig. 2. Evolution of psychrotrophic aerobic plate counts (PC), aerobic plate counts (APC) and H_2S-producing bacteria of red sea cucumber (*Parastichopus tremulus*) during storage at 0 °C and 4 °C for 17 days (**A**). The results are presented as mean ± standard deviation ($n = 3$). Community barplot graphs of relative abundance of the top 3 phylum-level taxa (>5.0 % relative abundance) (**B**) and top 7 genus-level taxa (>5.0 % relative abundance) (**C**) of red sea cucumber (*Parastichopus tremulus*) during storage at 0 °C and 4 °C for 17 days.

triphosphate (ATP) and biogenic amines. The extraction and the analysis of NMR was performed once on each individual sea cucumber.

Each spectrum was processed with MestReNova version 12.0.0–20080 (Mestrelab research SL, Spain) by manually adjusting baseline and phase, and applying a line broadening factor of 0.5 Hz. The NMR data was conducted using the literature data (Fan, 1996; Fan & Lane, 2008) for the reference standards (BMRB, HMDB, YMDB, ECMDB databases) and the published experimental data from Shumilina et al. (2015). The range of the NMR spectra was limited to between 0.0 ppm and 12.0 ppm, the metabolites proton signals were integrated and normalized to the resonance of the TSP signal.

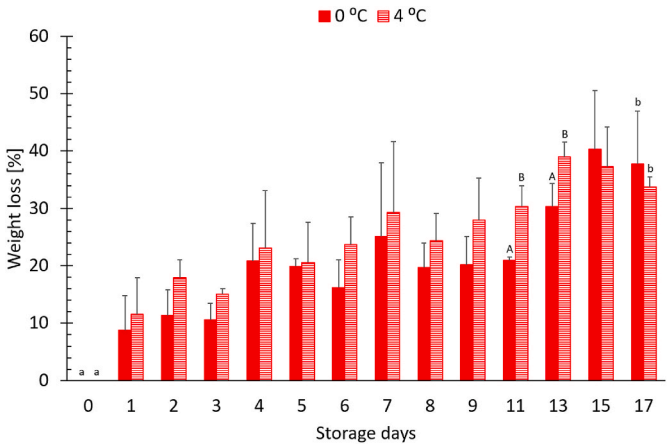

Fig. 3. Weight loss (wet weight %) of red sea cucumber (*Parastichopus tremulus*) stored at 0 °C and 4 °C. The results are presented as mean ± standard deviation ($n = 3$). Full and shaded color indicate storage temperatures at 0 °C and 4 °C, respectively. (a–b) Different superscript letters within the same storage temperature indicate significant differences ($p < 0.05$) throughout the storage trial. (A–B) Different superscript letters within the same sampling day indicate significant differences ($p < 0.05$) between storage temperatures.

2.7. K-value

Supernatants from TCA extraction were analyzed for ATP and ATP degradation products (5'-adenosine diphosphate (ADP), 5'-adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR) and hypoxanthine (Hx)) based on the method by Lerfall et al. (2018) as described in Tsoukalas et al. (2022). The supernatants were filtered through a syringe filter (0.22 μm, VWR, Radnor, PA, USA) and transferred to HPLC vials (VWR®, 548–3253) for analysis. Standards of ATP (Roche Diagnostics gmbh, Mannheim, Germany), and standards of ADP (≥95 % CAS: 20398-34-9), AMP (≥95 %, CAS: 149022-20-8), IMP (≥99 %, CAS: 352195-40-5), HxR (≥99 %, CAS: 58-63-9) and Hx (≥99 %, CAS: 68-94-0), all from Steinheim, Germany, were diluted to 0.001 M in dH_2O ranging from 0 to 250 μmol/L. The retention times for standard solutions and samples were compared. In addition, samples were spiked with standards for verification. The K-value was measured three times for each individual sea cucumber.

The K-value were calculated using the equations presented by Hong et al. (2017);

$$\text{K-value (\%)} = [(\text{HxR} + \text{Hx})/(\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx})] \times 100$$

2.8. Biogenic amines

The determination of the biogenic amines was performed as described by Lerfall et al. (2018). Briefly, the supernatant from the TCA extraction was filtered (0.22 μm, VWR, Radnor, PA, USA), added sodium hydroxide (1 mL, KOH, 2 M, CAS: 1310-73-2, Merch, Darmstadt, Germany) and derived with 2 % benzoyl chloride (1 mL, 99 %, CAS: 98-88-4 Sigma-Aldrich, Steinheim, Germany) in acetonitrile (≥99.9 %, CAS: 75-05-8, Steinheim, Germany). The benzyl-amine samples were filtered using Polytetrafluoroethylene (PTFE) filter media (Cyntiva, 6874-1302, Buckinghamshire, UK) and analyzed using an ultra-high performance liquid chromatography (UHPLC) system. The UHPLC-system constituted of an Agilent 1290 chromatograph (Agilent technologies, Paolo Alto, CA, USA), an UHPLC column YMC triart PFP (1.9 μm, 100 × 2 mm) and an Agilent 1260 diode array UV–VIS detector. The samples were detected at 254 nm using a mixture of acetonitrile and water (90:10) as mobile phase (isocratic, flow 1.0 mL/min). Standards of tryptamine (tryptamine hydrochloride, 99 %, CAS: 343-94-2, Sigma-Aldrich, Steinheim, Germany), cadaverine (cadaverine dihydrochloride, ~98 %, CAS: 1476-39-7, Sigma-Aldrich, Steinheim, Germany), spermidine (spermidine trihydrochloride, ≥99 %, CAS: 334-50-9, Steinheim, Germany), spermine (spermine tetrahydrochloride, ≥99.5 %, CAS: 306-67-2, Steinheim, Germany) and tyramine (tyramine hydrochloride, ≥98 %, CAS: 60-19-5, Steinheim, Germany) were prepared according to Özogul et al. (2002), and ranged between 0 and 50 μg/mL. The retention times of standards were compared with samples to identify the peaks. The biogenic amines were analyzed three times for each individual sea cucumber.

2.9. Volatile organic compounds (VOC)

Volatile organic compounds were analyzed using an automated dynamic headspace purge and trap system with a thermal desorption unit (Gerstel DHS-TDU-MPS., Gerstel GmbH & Co. KG, Mühlheim, Germany) interfaced with a gas chromatograph (Agilent 6890 Palo Alto, CA, USA) coupled with a mass spectrometer (Agilent 2977B), headspace gas chromatography mass spectroscopy (HS-GC/MS) system. Samples (1 g) were transferred to a 20 mL sample vial, flushed with nitrogen, corked with a screw cap with Teflon septum and transferred to the sample exchanger of the HS-GC/MS instrument. Ethyl heptanoate in methanol (>99 %, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was automatically added as an internal standard (0.4 μg) to the sample vial. The sample vials were incubated (70 °C) with shaking in 5 min. Volatile compounds were dynamically extracted and trapped on the adsorbent tube with active charcoal (Tenax GR, particle size 60/80 mesh, Alltech Associates Inc., Deerfield, IL, USA) for 2 min at 100 mL/min, total 200 mL gas volume. Then the adsorbent sample tubes were back-flushed with nitrogen (100 mL/min) for 20 min for the removal of water. The volatile compounds are transferred into the gas chromatograph by thermal desorption. The compounds were separated on a polar DBWAXetr gas chromatographic column (J & W Scientific/Agilent, 0.25 mm i.d., 0.5 μm film, 30 m). Helium (99.9999 %) was used as carrier gas. The following temperature program was used: 30 °C (10 min) – 1 °C/min to 40 °C (0 min) – 3 °C/min (0 min) to 70 °C (0 min) – 6.5 °C/min to 230 °C (5 min). Mass spectra were recorded in positive scan mode (m/z = 33–500) after electron impact ionization at 70 eV. The integration of peak areas was performed with the Mass Hunter software (Version 10.0, Agilent Technologies). The identification of compounds was confirmed by comparison of the measured mass spectra of the GC peaks with the pure standards according to the NIST14L mass spectral library (version 2.2, 2014, US Commerce/Agilent). The analysis of volatile compounds was measured once on each individual sea cucumber.

The odor activity values (OAVs) have been calculated based on Giri et al. (2011) with the following equation:

$$\text{OAVs} = \text{Concentration of volatile components in sample (μg/100g)/ Odor threshold in water (μg/100g)/}$$

2.10. Statistical analysis

Three individual sea cucumbers were analyzed on each sample collection, unless otherwise stated. All results are presented as average ± standard deviation. IBM SPSS Statistics version 29.0.1.0 (IBM, New York, USA) was used to evaluate the significance of differences. Univariate analysis of variance (ANOVA) was conducted with Tukey's post hoc test ($p < 0.05$) and independent-sample *t*-test ($p < 0.05$). Pearson correlation analysis was performed to explore the dependency between parameters.

Fig. 4. The fresh sea cucumber before storage (Day 0), sample variability before the storage trial, and the autolysis process at the end of the storage trial stored at 4 °C (Day 17).

Table 2
Distribution of free amino acids (mg/100 g wet weight sea cucumber) in the red sea cucumber (*Parastichopus tremulus*) stored at 0 °C and 4 °C over 17 days of storage. The results are presented as mean ± standard deviation ($n = 3$). (a-d) Different superscript letters within the same storage temperature indicate significant differences ($p < 0.05$) throughout the storage trial. (A-B) Different superscript letters within the same sampling day indicate significant differences ($p < 0.05$) between storage temperatures. *p*-value corresponds to comparison between days within the same storage temperature.

Amino Acids	Day 0	Day 2 0 °C	Day 5 0 °C	Day 11 0 °C	Day 17 0 °C	*p*-value 0 °C	Day 2 4 °C	Day 5 4 °C	Day 11 4 °C	Day 17 4 °C	*p*-value 4 °C
Alanine	3.4 ± 1.3	4.6 ± 1.8	4.2 ± 1.7	9.4 ± 8.3	10.2 ± 2.4	0.19	6.6 ± 1.6	4.9 ± 2.3	8.4 ± 7.5	10.6 ± 3.6	0.36
Asparagine	0.0 ± 0.0^{a}	0.1 ± 0.1^{a}	0.21 ± 0.1^{a}	0.8 ± 0.6^{a}	1.2 ± 0.4^{b}	<0.01	0.1 ± 0.0^{ab}	0.2 ± 0.1^{ab}	0.3 ± 0.1^{ab}	0.6 ± 0.6^{b}	0.05
Aspartic acid	0.4 ± 0.2^{a}	2.0 ± 0.2^{a}	1.2 ± 0.3^{a}	9.90 ± 1.3^{b}	13.4 ± 3.5^{b}	<0.01	2.0 ± 0.1^{a}	1.6 ± 1.2^{a}	8.3 ± 0.5^{b}	11.5 ± 1.7^{c}	<0.01
Glutamic acid	7.0 ± 3.3^{a}	17.6 ± 3.8^{ab}	19.4 ± 2.4^{ab}	20.4 ± 14.0^{ab}	34.1 ± 9.8^{b}	0.03	17.2 ± 3.8	16.2 ± 4.3	27.2 ± 18.9	24.3 ± 7.0	0.20
Glutamine	0.6 ± 0.2	0.8 ± 0.2	1.2 ± 0.2	2.2 ± 2.7	2.2 ± 0.6	0.41	1.1 ± 0.1	1.0 ± 0.4	2.2 ± 1.9	2.8 ± 0.4	0.13
Arginine/ Glycine	10.5 ± 6.6	12.0 ± 6.0	18.1 ± 5.1	16.1 ± 10.7	26.1 ± 6.9	0.12	11.4 ± 6.7	11.3 ± 2.4	24.4 ± 19.7	16.1 ± 7.9	0.48
Histidine	0.1 ± 0.1^{a}	0.2 ± 0.1^{ab}	0.4 ± 0.1^{ab}	0.6 ± 0.7^{ab}	1.1 ± 0.3^{b}	0.04	0.2 ± 0.2^{a}	0.3 ± 0.1^{a}	0.3 ± 0.3^{a}	0.8 ± 0.1^{b}	0.01
Isoleucine	0.1 ± 0.0^{a}	0.1 ± 0.0^{A,a}	0.3 ± 0.1^{ab}	1.2 ± 1.1^{ab}	1.8 ± 0.8^{b}	0.02	0.2 ± 0.0^{B,ab}	0.5 ± 0.2^{bc}	0.6 ± 0.2^{c}	1.6 ± 0.1^{d}	<0.01
Leucine	0.1 ± 0.1^{a}	0.3 ± 0.0^{A,a}	0.7 ± 0.2^{ab}	2.3 ± 2.0^{ab}	3.3 ± 1.3^{b}	0.02	0.4 ± 0.0^{B,a}	1.1 ± 0.5^{ab}	1.5 ± 0.5^{c}	3.0 ± 0.2^{d}	<0.01
Lysine	0.8 ± 0.3^{a}	0.8 ± 0.1	1.7 ± 0.6	4.1 ± 3.8	5.3 ± 1.8	0.05	1.3 ± 0.4^{a}	1.6 ± 0.4^{a}	2.7 ± 1.3^{a}	5.2 ± 0.7^{b}	<0.01
Methionine	<0.01	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.3	0.1 ± 0.1	0.40	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.6 ± 0.6	0.10
Phenylalanine	0.1 ± 0.0^{a}	0.1 ± 0.0	0.4 ± 0.1	1.4 ± 1.5	1.7 ± 0.8	0.07	0.3 ± 0.1^{a}	0.5 ± 0.2^{a}	0.8 ± 0.3^{a}	2.0 ± 0.6^{b}	<0.00
Serine	0.4 ± 0.2^{a}	0.6 ± 0.2	0.9 ± 0.1	2.3 ± 2.2	3.4 ± 1.5	0.05	0.5 ± 0.2^{a}	1.00 ± 0.4^{a}	1.2 ± 0.7^{ab}	2.5 ± 0.5^{b}	<0.01
Threonine	0.2 ± 0.0^{a}	0.4 ± 0.1	0.5 ± 0.1	1.3 ± 1.2	1.7 ± 0.6	0.05	0.2 ± 0.0^{a}	0.4 ± 0.1^{a}	0.9 ± 0.7^{ab}	1.5 ± 0.4^{b}	<0.01
Tyrosine	0.1 ± 0.0^{a}	0.1 ± 0.0^{A}	0.3 ± 0.1	1.1 ± 1.2	1.8 ± 0.9	0.04	0.2 ± 0.0^{B,a}	0.4 ± 0.2^{a}	0.6 ± 0.3^{a}	1.8 ± 0.7^{b}	<0.00
Valine	0.1 ± 0.1^{a}	0.2 ± 0.0^{A,ab}	0.4 ± 0.1^{ab}	1.30 ± 1.2^{ab}	1.9 ± 0.8^{b}	0.03	0.3 ± 0.0^{B,a}	0.6 ± 0.3^{a}	0.7 ± 0.2^{a}	2.0 ± 0.5^{b}	<0.00
∑Free amino acids	23.8 ± 12.3^{a}	40.0 ± 6.3^{ab}	49.8 ± 8.2^{ab}	74.4 ± 52.4^{ab}	109.3 ± 29.7^{b}	0.03	41.9 ± 6.7	41.5 ± 10.4	80.1 ± 52.7	86.8 ± 10.2	0.09

Tryptophan was destroyed during acid hydrolysis and was thus not detected. Cysteine, proline, and taurine were not detected in this method. Arginine/Glycine could not be separated in this method. Asparagine and glutamine were deaminated during acid hydrolysis and were therefore included in the aspartic acid and glutamic acid.

3. Results and discussion

This study has investigated the quality parameters and the storage ability of *P. tremulus*. It is important to acknowledge that before any increase utilization of this species, it is essential to implement sustainable harvesting practices to prevent the overexploitation of sea cucumber stocks (Ciriminna et al., 2024). Sea cucumbers play a crucial role in processing organic matter within the food chain, highlighting their importance in maintaining ecological balance (Ciriminna et al., 2024). Their absence from natural habitats could significantly disrupt the ecosystems, and lead to consequences for the benthic communities (Ciriminna et al., 2024). By understanding the post-mortem changes, quality parameters, and storage stability of *P. tremulus*, we can develop better guidelines for their sustainable use, ensuring that their populations remain healthy, and their ecological functions are preserved.

3.1. Biochemical composition of fresh sea cucumber

The proximate composition of fresh *P. tremulus* (Table 1), consisting of water (90 %), protein (5 %), lipid (1 %) and ash (8 %) was comparable to previously published data (Vu et al., 2024), although with higher ash content. The higher ash content could be due to differences in the mineral deposits (Al Azad et al., 2017). The TAAs (Supplementary Table 1) and FAAs composition of the sea cucumbers were also comparable to previous studies of *P. tremulus* (Ringvold & Kjerstad, 2018; Vu et al., 2024).

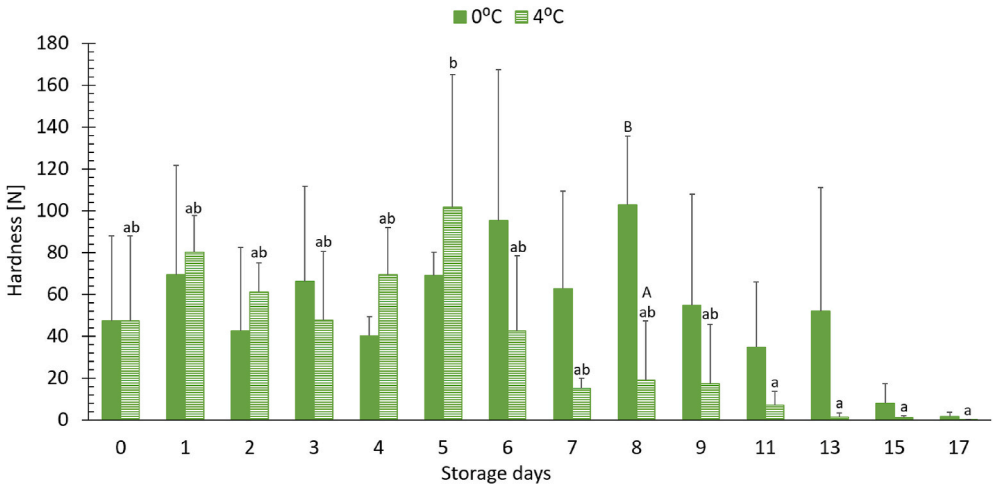

Fig. 5. The textural hardness (Newton, N) of red sea cucumber (*Parastichopus tremulus*) stored at 0 °C and at 4 °C over the course of 17 days. Full and shaded color indicate storage temperatures at 0 °C and 4 °C, respectively. The results are presented in mean ± standard deviation ($n = 3$). (a–b) Different superscript letters within the same storage temperature indicate significant differences ($p < 0.05$) throughout the storage trial. (A–B) Different superscript letters within the same sampling day indicate significant differences ($p < 0.05$) between storage temperatures.

Table 3
Water- and acid-soluble proteins (wet weight %) of red sea cucumber (*Parastichopus tremulus*) stored at 0 °C and 4 °C during a storage trial of 17 days. The results are presented as mean ± standard deviation ($n = 3$). (a-b) Different superscript letters within the same storage temperature indicate significant differences ($p < 0.05$) throughout the storage trial. (A-B) Different superscript letters within the same sampling day indicate significant differences ($p < 0.05$) between sample stored at 0 °C and 4 °C. *p*-value corresponds to comparison of days between the two storage temperatures.

Storage days	Water-soluble proteins		*p*-value	Acid-soluble proteins		*p*-value
	0 °C	4 °C		0 °C	4 °C	
0	1.04 ± 0.06^{a}	1.04 ± 0.06^{a}	1.00	0.08 ± 0.03^{a}	0.08 ± 0.03^{a}	1.00
1	1.21 ± 0.17	1.22 ± 0.55	0.98	0.07 ± 0.03	0.07 ± 0.03	0.99
2	1.31 ± 0.47	1.22 ± 0.21	0.61	0.07 ± 0.03	0.08 ± 0.04	0.80
3	1.20 ± 0.17^{B}	0.89 ± 0.10^{A}	<0.01	0.05 ± 0.03	0.07 ± 0.03	0.46
4	0.66 ± 0.16^{A}	1.22 ± 0.38^{B}	<0.01	0.05 ± 0.01	0.06 ± 0.01	0.09
5	0.87 ± 0.14^{B}	0.68 ± 0.20^{A}	0.04	0.05 ± 0.01	0.05 ± 0.02	0.69
6	1.02 ± 0.19	1.29 ± 0.55	0.18	0.05 ± 0.01	0.05 ± 0.02	0.45
7	0.90 ± 0.21^{A}	1.12 ± 0.18^{B}	0.03	0.06 ± 0.02	0.06 ± 0.03	0.81
8	1.13 ± 0.13	1.44 ± 0.52	0.10	0.09 ± 0.05^{B}	0.05 ± 0.02^{A}	0.03
9	1.14 ± 0.41	1.59 ± 0.29	0.02	0.07 ± 0.04	0.08 ± 0.04	0.73
11	0.95 ± 0.36^{A}	1.36 ± 0.10^{B}	<0.01	0.06 ± 0.03	0.06 ± 0.02	0.72
13	1.52 ± 0.42^{A}	2.06 ± 0.53^{B}	0.03	0.07 ± 0.02	0.08 ± 0.04	0.58
15	1.67 ± 0.41	1.48 ± 0.38	0.33	0.08 ± 0.03	0.07 ± 0.03	0.28
17	1.59 ± 0.22^{b}	1.97 ± 0.21^{b}	<0.01	0.08 ± 0.03^{a}	0.08 ± 0.03^{a}	0.11

3.2. Quality changes of sea cucumbers during storage

3.2.1. Microbial counts and microbial community composition

The microbial quality of the samples was assessed by quantifying the PC, APC, and H_2S-producing bacteria (Fig. 2A). The mean initial PC, APC and H_2S-producing bacteria counts were 4.3 ± 0.3, 3.8 ± 0.1 and 1.6 ± 0.4 log cfu/g, respectively. These values are lower than those reported in other sea cucumber species (Boziaris et al., 2023). The counts of PC, APC, and H_2S-producing bacteria were found to be significantly affected by the storage temperature and time ($p < 0.001$).

Among the microbial counts, PC had the highest average levels throughout the storage, followed by APC and H_2S-producing bacteria at both storage temperatures (Fig. 2A). PC reached a plateau of 7.3 ± 0.2 log cfu/g after day 5 of storage at 4 °C, while at 0 °C, PC plateaued at 6.6 ± 0.3 log cfu/g after 7 days. Notably, APC in samples stored at 4 °C showed a steady increase up to day 13, achieving a maximum population density of 7.6 ± 0.3 log cfu/g, whereas APC at 0 °C plateaued on day 11 with a maximum density of 5.7 ± 0.3 log cfu/g. Similar to PC and APC, H_2S-producing bacteria counts were consistently lower at 0 °C compared to 4 °C. Consistent with our findings, previous studies on echinoderms and fish from the cold waters of the Norwegian Sea also reported higher PC than APC levels (Tsoukalas et al., 2023b, 2024), suggesting that PC may serve as a more reliable indicator of microbial spoilage in seafood from cold environments. The lower microbial counts observed at 0 °C compared to 4 °C are consistent with other studies on various marine species (Boziaris et al., 2011; Gornik et al., 2011; Ólafsdóttir et al., 2006), underscoring the inhibitory effect of lower storage temperatures on microbial proliferation.

The microbial community analysis revealed that the microbiota profile of fresh sample predominantly composed of the bacterial phyla Pseudomonadota (77 %) and Bacteroidota (22 %) (Fig. 2B). These phyla commonly dominate the microbial communities of marine species from the cold temperate waters of the North Atlantic and the North Sea (Boziaris & Parlapani, 2017). Additionally, Pseudomonadota and Bacteroidota have been identified in the microbiota profiles of both the flesh and in the intestine of sea cucumbers (Boziaris et al., 2023; Kim et al., 2017; Pagán-Jiménez et al., 2019).

At the genus level, metabarcoding analysis identified *Fretibacter*, *Flavobacterium*, *Amylibacter*, *Paracoccus*, and *Psychrobacter* as the primary constituents of the microbial communities in fresh samples (Fig. 2C). These genera are typically found in aquatic environments and are part of the indigenous microbiota of seafood (Boziaris et al., 2023;

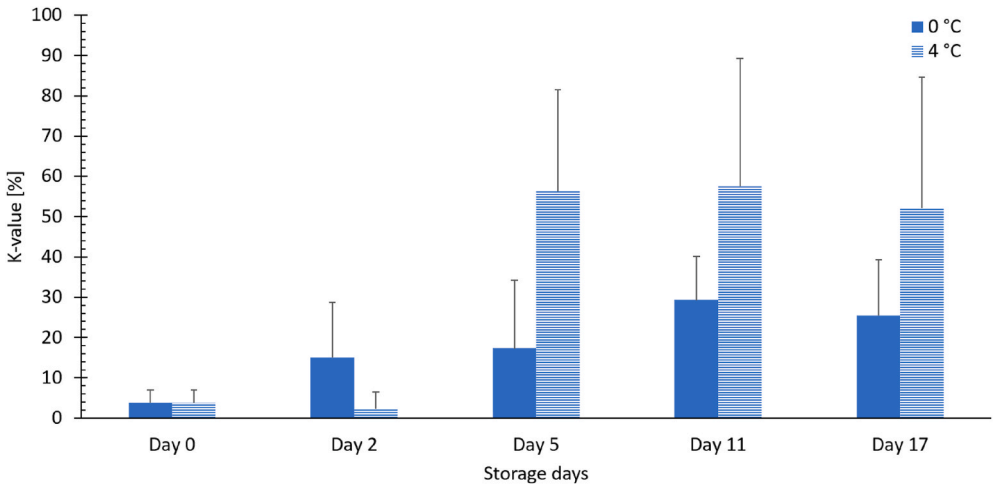

Fig. 6. K-value as a freshness indicator is based on the sum of inosine and hypoxanthine over the sum of 5'-adenosine triphosphate, 5'-adenosine diphosphate, 5'-adenosine monophosphate, inosine monophosphate, inosine and hypoxanthine content, in the Norwegian red sea cucumber (*Parastichopus tremulus*) stored at 0 °C and 4 °C during 17 days of storage. Full and shaded color indicate storage temperatures at 0 °C and 4 °C, respectively. The results are presented as mean ± standard deviation ($n = 3$).

Teramoto & Nishijima, 2014). During storage, *Amylibacter* and *Fretibacter* were progressively eliminated due to competitive exclusion, as storage conditions selectively favored more resilient species, driving a microbial succession in which only the most ecologically adaptable organisms persisted. Regardless of storage temperature, *Flavobacterium* and *Psychrobacter* were the dominant genera and considered SSOs. Previous studies have shown that both genera produce volatile compounds during cold storage of seafood (Anagnostopoulos et al., 2022; Broekaert et al., 2012) and were reported among the dominant taxa of aerobically stored seafood (Reynisson et al., 2010; Tsoukalas et al., 2024). *Psychrobacter* are aerobic and psychrotrophic bacteria which produces off-flavors due to the ability to hydrolyze amino acids and break down lipids (Odeyemi et al., 2018). *Flavobacterium* are gram-negative and strictly aerobic bacteria (Waśkiewicz & Irzykowska, 2014) which produces amine compounds (Lakshmanan et al., 2002).

Furthermore, *Marinomonas* and *Polaribacter* represented significant fractions of the microbiota in spoiled samples after 13 days of storage, with samples stored at 0 °C exhibiting lower relative abundances of these genera compared to those stored at 4 °C. Our findings indicate that prolonged storage and elevated temperatures favor the *Marinomonas* and *Polaribacter* proliferation.

3.2.2. Water content, ash content and weight loss

A common way to evaluate quality during storage is to measure drip loss (Suyani et al., 2019). The water content did not change during the 17 days storage, ranging between 88 and 90 % (Supplementary Fig. 1). Neither water nor ash content changed significantly during the storage period, and were similar at both storage temperatures (Supplementary Fig. 1). The water content is comparable to previous studies of different sea cucumber species (Vu et al., 2023, 2024). The ash content (7.4–8.7%) was found to be higher compared to previous study on *P. tremulus* (Vu et al., 2024). This variation might be due to differing mineral deposits found in sea cucumbers (Al Azad et al., 2017).

The sea cucumbers lost weight during storage. Although high individual variations, an overall increasing weight loss was observed (Fig. 3) in sea cucumber stored at both storage temperatures. Since the water content remained the same throughout the storage period, the observed weight loss may result from the autolytic process in the samples (Fig. 4) (Wu et al., 2013), leading to protein (Li et al., 2022), lipid (Zheng et al., 2012) and nucleotide degradations (Tavares et al., 2021). Consequently, this process contributes to the production of slime (Jiang & Xue, 2023), which could explain the high variations within the same sample group.

3.2.3. Free amino acids

The FAAs profile of the sea cucumbers was assessed at both storage temperatures. Apart from alanine, glutamine, arginine/glycine, and methionine, the concentration of all the other FAAs significantly increased ($p < 0.05$) during storage independent of temperature (Table 2). The increase of FAAs during storage could be explained by the autolysis process in the sea cucumbers (Wu et al., 2013), causing the degradation of nucleotides (Tavares et al., 2021) and proteins (Li et al., 2022). Previous studies have documented that the degradation of proteins is caused by internal enzymes such as different types of cathepsins in the sea cucumber body wall (Fan et al., 2024), resulting in the proteolysis of sea cucumber (Dong et al., 2018). This could explain the increasing FAAs profile of the sample during storage. The high standard deviation observed at both storage temperatures can be attributed to initial individual variations arising from different life stages within the sample groups (Elvevoll et al., 2022).

3.2.4. pH

Despite the pH of *P. tremulus* were fluctuating throughout the storage trial (6.6–7.0), a significantly decreased ($p < 0.05$) of pH were found at the end of storage compared to the beginning of storage at both storage temperatures (Supplementary Fig. 2). The pH fluctuations observed during storage are comparable to a previous study on the sea cucumber *Stichopus japonicus* (Xiong et al., 2020). This suggests that pH alone cannot adequately reflect changes in the quality *of P. tremulus* during storage. The pH decrease can be attributed to the accumulation of octopine, a lactic acid analog (Hockachka et al., 1977), derived from alanine and arginine, as found in squid (Ollivaux et al., 2014). Additionally, the decrease in pH may also result from the dissolution and production of volatile compounds (Xiong et al., 2020).

3.2.5. Texture

The textural attributes of seafood are known to degrade during storage (Liu et al., 2022). The hardness of *P. tremulus* (Fig. 5) fluctuated, probably due to individual variations, but showed a decreasing trend after approximately one week of storage at both storage temperatures. These results are comparable to previous studies on *Stichopus japonicus* (Dong et al., 2018; Xiong et al., 2020). The decrease of textural hardness could be linked to the autolysis process during storage (Kontominas et al., 2021), caused by proteolytic activities such as cathepsins, degrading the proteins in the samples (Xiong et al., 2020). Furthermore, an inverse correlation ($p < 0.05$) was found between texture and weight loss in samples stored at 0 °C ($r = -0.52$) and 4 °C ($r = -0.35$),

Table 4
Development of volatile compounds (μg/100 g wet weight) during storage of red sea cucumber (*Parastichopus tremulus*) at 0 °C and 4 °C. "n.d" indicates 'not detected'. The compounds and the reactions are based on the studies by Parlapani et al. (2014, 2023), Poveda (2021), Banerjee et al. (2010), Huang et al. (2021), Poirier et al. (2023), Wen et al. (2022), Celeiro et al. (2020), Nollet and Toldrá (2009), Grebenteuch et al. (2021) and Chu et al. (2023). The analysis of volatile compounds was measured only once on each sample.

Compounds	Reactions	Day 0	Day 5 (0 °C)	Day 17 (0 °C)	Day 5 (4 °C)	Day 17 (4 °C)
Aldehydes						
(E,E)-2,4-Heptadienal	Lipid oxidation	n.d	0.27	1.41	0.95	0.64
(E,E)-2,4-Nonadienal	Microbial metabolites	n.d	n.d	n.d	n.d	0.19
(E,Z)-2,4-Heptadienal	Lipid oxidation	1.14	n.d	n.d	n.d	n.d
(E,Z)-2,6-Nonadienal	Lipid oxidation	n.d	n.d	0.36	n.d	n.d
10-Undecenal	Lipid oxidation	0.35	n.d	n.d	0.06	0.07
13-Octadecenal	Lipid oxidation	0.17	n.d	n.d	0.27	1.03
2,4-Decadienal	Lipid oxidation and Microbial metabolites	0.67	n.d	0.58	0.56	0.73
2,4-Dimethyl-Pentanal	Lipid oxidation	n.d	0.05	n.d	n.d	n.d
2-Decenal	Lipid oxidation and Microbial metabolites	0.19	n.d	0.27	0.31	0.40
2-Heptenal	Lipid oxidation	0.12	n.d	n.d	0.15	0.19
2-Hexenal	Lipid oxidation and Microbial metabolites	0.28	0.07	0.38	0.23	0.30
2-Methyl-Butanal	Protein degradation	0.05	0.05	0.14	n.d	9.98
2-Methyl-Pentanal	Lipid oxidation	n.d	n.d	n.d	n.d	0.00
2-Methyl-Undecanal	Lipid oxidation	0.09	n.d	n.d	0.07	0.33
2-Nonenal	Lipid oxidation and Microbial metabolites	n.d	n.d	0.00	n.d	n.d
2-Octenal	Lipid oxidation and Microbial metabolites	0.39	0.12	0.42	0.42	0.54
2-Pentenal	Lipid oxidation and Microbial metabolites	0.08	n.d	0.08	0.07	0.09
3-Methyl-2-Butenal	Lipid oxidation	0.11	n.d	n.d	0.09	0.12
3-Methyl-Butanal	Protein degradation	0.07	0.11	0.47	0.15	0.71
4-Heptenal	Lipid oxidation and Microbial metabolites	n.d	n.d	n.d	n.d	0.08
4-Pentenal	Lipid oxidation	n.d	n.d	n.d	0.03	n.d
Benzaldehyde	Lipid oxidation and Microbial metabolites	n.d	0.43	0.34	n.d	n.d
Butanal	Lipid oxidation	n.d	n.d	0.07	n.d	n.d
Decanal	Lipid oxidation and Microbial metabolites	0.08	0.84	n.d	6.93	8.99
Dodecanal	Lipid oxidation and Microbial metabolites	0.04	n.d	0.02	0.48	0.52
Heptanal	Lipid oxidation and Microbial metabolites	0.08	0.20	0.65	0.93	1.21
Hexanal	Lipid oxidation and Microbial metabolites	2.27	0.76	2.46	2.54	1.71
Nonanal	Lipid oxidation and Microbial metabolites	0.05	0.64	0.89	4.77	7.36
Octanal	Lipid oxidation and Microbial metabolites	0.30	0.00	1.08	2.52	3.26
Pentanal	Lipid oxidation and Microbial metabolites	0.38	0.13	0.34	0.32	0.60
Undecanal	Lipid oxidation and Microbial metabolites	0.62	0.05	n.d	0.51	0.67
Alcohols						
(R)-2-Octanol	Microbial metabolites	n.d	0.01	n.d	n.d	n.d
(S)-(+)-1,2-Propanediol	Microbial metabolites	n.d	0.03	n.d	n.d	n.d
(S)-2-Octanol	Microbial metabolites	n.d	0.18	n.d	n.d	n.d
1-Butoxy-2-Propanol	Microbial metabolites	n.d	0.08	n.d	n.d	n.d
1-Hexanol	Lipid oxidation and Microbial metabolites	n.d	0.17	0.77	n.d	1.41
1-Nonanol	Microbial metabolites	n.d	n.d	0.02	n.d	0.73
1-Octen-3-ol	Lipid oxidation and Microbial metabolites	0.97	0.48	1.76	1.09	2.24
1-Pentanol	Lipid oxidation and Microbial metabolites	n.d	0.14	0.43	n.d	0.87
1-Penten-3-ol	Lipid oxidation and Microbial metabolites	1.38	0.71	2.54	1.14	2.76
2-Butyl-1-Octanol	Microbial metabolites	n.d	n.d	0.04	n.d	n.d
2-Decanol	Microbial metabolites	n.d	0.03	n.d	n.d	n.d
2-Ethyl-1-Hexanol	Lipid oxidation and Microbial metabolites	0.26	n.d	n.d	0.21	0.44
2-Ethyl-2-Propyl-1-Hexanol	Microbial metabolites	n.d	0.04	n.d	n.d	n.d
2-Heptanol	Microbial metabolites	n.d	n.d	n.d	0.24	0.31
2-Methyl-1-Propanol	Lipid oxidation and microbial metabolites	n.d	n.d	n.d	n.d	0.21
2-Methyl-3-Pentanol	Microbial metabolites	n.d	n.d	n.d	0.01	n.d
2-Nonanol	Microbial metabolites	0.08	n.d	n.d	0.07	0.09
2-Octen-1-ol	Lipid oxidation and Microbial metabolites	0.29	0.18	0.48	0.24	0.32
2-Penten-1-ol	Lipid oxidation	0.39	0.16	0.87	0.32	0.75
3-Methyl-1-Butanol	Lipid oxidation and Microbial metabolites	0.39	n.d	0.26	0.32	10.39
Benzeneethanol, αβ-Dimethyl-	Microbial metabolites	n.d	n.d	n.d	0.52	n.d
Ethanol	Microbial metabolites	n.d	0.48	n.d	n.d	n.d
Isopropyl Alcohol	Microbial metabolites	n.d	n.d	0.32	n.d	n.d
Phenylethyl Alcohol	Microbial metabolites	n.d	n.d	n.d	n.d	0.89
Ketones						
1-Octen-3-one	Lipid oxidation and Microbial metabolites	0.23	0.08	0.28	0.19	0.19
1-Penten-3-one	Lipid oxidation	0.47	0.15	0.62	0.29	0.40
2,3-Pentanedione	Microbial metabolites	0.14	0.02	0.24	0.04	0.25
2-Butanone	Lipid oxidation and microbial metabolites	n.d	0.14	0.11	0.10	0.20

(*continued on next page*)

Table 4 (*continued*)

Compounds	Reactions	Day 0	Day 5 (0 °C)	Day 17 (0 °C)	Day 5 (4 °C)	Day 17 (4 °C)
2-Nonanone	Microbial metabolites	n.d	0.03	0.10	0.06	0.08
3-Octanone	Microbial metabolites	n.d	n.d	0.07	n.d	0.44
6-Methyl-5-Hepten-2-one	Lipid oxidation and Microbial metabolites	0.45	n.d	n.d	0.49	0.74
Acetone	Microbial metabolites	0.12	0.07	0.16	0.23	0.18
Acetophenone	Lipid oxidation and Microbial metabolites	0.23	0.21	0.42	0.19	0.14
Acids						
2-Methyl-Hexanoic acid	Microbial metabolites	n.d	n.d	n.d	n.d	19.17
2-Methyl-Propanoic acid	Microbial metabolites	n.d	n.d	n.d	n.d	4.49
3-Hydroxy-Dodecanoic acid	Microbial metabolites	0.13	n.d	0.24	0.04	0.64
3-Methyl-Decanoic acid	Microbial metabolites	n.d	n.d	n.d	n.d	0.11
Acetic acid	Microbial metabolites	n.d	n.d	n.d	n.d	19.54
Butanoic acid	Microbial metabolites	n.d	n.d	n.d	n.d	2.41
Hexanoic acid, anhydride	Microbial metabolites	n.d	n.d	n.d	0.35	0.46
Palmitoleic acid	Microbial metabolites	0.04	n.d	n.d	0.04	0.05
Propanoic acid	Microbial metabolites	n.d	n.d	n.d	n.d	31.28
Esters						
2,2,4-Trimethyl-3-Carboxyisopropyl Pentanoic acid, Isobutyl ester	Microbial metabolites	0.10	n.d	n.d	0.08	0.10
2,2-Dimethyl-Propanoic acid, Octyl ester	Microbial metabolites	n.d	n.d	n.d	n.d	0.04
3-Hydroxy-Dodecanoic acid, Ethyl ester	Microbial metabolites	0.51	n.d	n.d	0.42	0.55
4-Hexadecyl-Hexanoic acid, Ester	Microbial metabolites	0.06	n.d	n.d	0.05	0.06
5-Methyl-Nonanoic acid, Ethyl ester	Microbial metabolites	0.18	n.d	n.d	0.15	0.02
E−11-Hexadecenoic acid, Ethyl ester	Microbial metabolites	n.d	0.45	n.d	n.d	0.96
Ethyl Acetate	Microbial metabolites	0.14	n.d	n.d	0.12	0.15
Ethylbenzene	Microbial metabolites	n.d	0.04	0.05	0.13	0.07
Heptanoic acid, Methyl ester	Microbial metabolites	n.d	n.d	n.d	3.57	7.04
Hexadecanoic acid, Ethyl ester	Microbial metabolites	3.25	0.72	0.30	2.69	1.77
Hexadecanoic acid, Methyl ester	Microbial metabolites	0.07	n.d	n.d	n.d	0.08
Hexanoic acid, ethyl ester	Microbial metabolites	0.07	0.03	0.05	0.06	0.04
Undec-10-ynoic acid, Pentyl ester	Microbial metabolites	n.d	n.d	n.d	0.07	0.09
Hydrocarbons						
Dodecane	Microbial metabolites	0.10	0.08	0.44	0.08	0.22
Nonane	Microbial metabolites	0.15	n.d	0.05	0.22	0.34
Octane	Microbial metabolites	n.d	n.d	n.d	0.15	0.15
Pentadecane	Microbial metabolites	0.70	0.14	n.d	0.99	0.75
p-Xylene	Microbial metabolites	0.28	n.d	n.d	0.23	0.83
Styrene	Microbial metabolites	n.d	0.02	0.10	n.d	n.d
Tetradecane	Microbial metabolites	0.09	n.d	n.d	0.08	0.10
Toluene	Microbial metabolites	n.d	n.d	0.03	n.d	n.d
Undecane	Microbial metabolites	0.08	0.33	0.54	0.07	0.09
Other						
2-Ethyl-Furan	Lipid oxidation	n.d	n.d	0.11	n.d	0.08
2-Pentyl-Furan	Lipid oxidation	0.09	0.04	n.d	0.08	n.d
Dibromo-Methane	Marine Algae	0.03	0.02	n.d	0.01	0.02
Dichloronitro-Methane	Marine Algae	n.d	0.08	n.d	0.02	0.03
Dimethyl Disulfide	Microbial metabolites	n.d	n.d	n.d	n.d	2.28
Dimethyl Trisulfide	Microbial metabolites	n.d	n.d	n.d	n.d	0.93
Di-Tert-Dodecyl Disulfide	Microbial metabolites	n.d	n.d	n.d	n.d	0.08
Ethyl (S)-(−)-Lactate	Microbial metabolites	n.d	0.06	n.d	n.d	n.d
Indole	Microbial metabolites	n.d	n.d	n.d	n.d	6.73
Limonene Oxide, Trans-	Microbial metabolites	n.d	n.d	n.d	0.03	0.04
p-Cresol	Microbial metabolites (Intestine and fermentation product)	0.41	n.d	n.d	0.34	22.87
Tribromo-Methane	Marine Algae	1.91	1.67	n.d	1.59	2.06
Phenol	Microbial metabolites (Intestine and fermentation product)	0.73	n.d	n.d	0.61	10.16

indicating that texture decreases as weight loss increases, consistent with the finding of a previous study (Yu et al., 2024).

3.2.6. *Protein solubility and proteolytic activity*

Seafood quality deteriorates during storage due to the activity of endogenous enzymes (Fuente-Betancourt et al. 2008). Changes in protein solubility in seafood may be reflected in the autolysis process during storage (Lin & Park, 1996). The development of water- and acid-soluble proteins fluctuated (Table 3), but with an increasing trend. The protein solubility at both storage temperatures was significantly higher ($p < 0.05$) at the end compared to the beginning of storage. The results were comparable to a previous study on squid stored on ice at 0 °C (Fuente-Betancourt et al. 2008). The increasing protein solubility could be a result of the activity of endogenous enzymes (Xiong et al., 2020) degrading nucleotides (Tavares et al., 2021) and proteins (Li et al., 2022) in the samples. Significant differences ($p < 0.05$) were found between the two storage temperatures regarding water-soluble proteins.

The protease activity was analyzed at pH 7 and 50 °C, but no activity was found. The digestive tract was absent or removed during gutting of the sea cucumbers prior to the experimental storage, which could explain the absence of protease activity. Additionally, the selected pH and temperature prior to analysis may have affected the protease activity, as previously reported (Haard & Simpson, 2000). A previous study reported high proteolytic activity in the digestive tract of sea

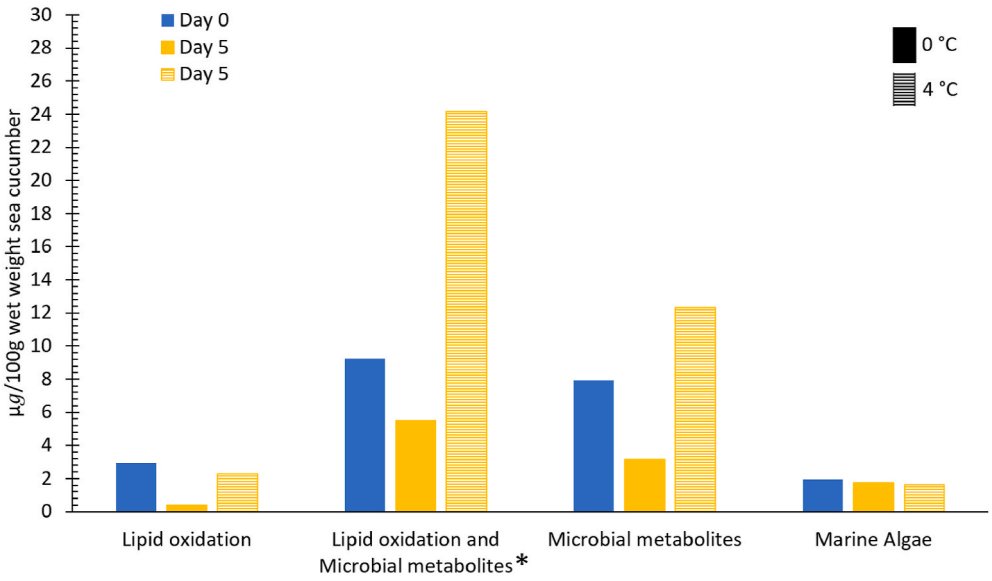

Fig. 7. Volatile compounds (µg/100 g sea cucumber wet weight) related to lipid oxidation, both lipid oxidation and microbial metabolites, microbial metabolites, and marine algae detected in red sea cucumber (*Parastichopus tremulus*) stored 5 days at 0 °C or 4 °C. Full and shaded color indicate storage temperatures at 0 °C and 4 °C, respectively. The results are presented as mean ($n = 3$). *Indicate volatile components which could be derived both from lipid oxidation and microbial growth. The analysis of volatile compounds was measured only once on each sample.

cucumber *Stichopus japonicus* (Fu et al., 2006). Factors such as gender maturation, species differences, and seasonal influences affect the protease composition in the digestive tract of sea cucumbers (Fu et al., 2006; Haard & Simpson, 2000). A recent study found that various endogenous enzymes in the intestine (including different cathepsin enzymes and serine protease) and the internal body wall (such as cathepsin L-like proteinase and acid phosphatase) cause autolysis of the sea cucumber *Apostichopus japonicus* (Fan et al., 2024).

3.2.7. K-value

The K-value has commonly been used to evaluate the freshness of seafood (Hong et al., 2017). In the present study, a gradual increase in K-value was observed until day 11 at 0 °C and day 5 at 4 °C, before plateauing (Fig. 6). Nevertheless, no significant differences ($p > 0.05$) were found between the two temperatures and storage time. However, samples stored at 4 °C showed a higher, though not significant ($p > 0.05$), K-value compared to samples stored at 0 °C. A previous study on the sea cucumber *Stichopus japonicus* (Xiong et al., 2020) reported an increase in K-value during storage at 4 °C on ice for 8 days. Other studies have reported that the K-value of whiteleg shrimp (*Litopenaeus vannamei*) plateaus after day 5 at 4 °C (Wang et al., 2022) or at 2 °C (Liu et al., 2023). The high variation of K-value in this study could be associated with the autolysis process, resulting in the leakage of ATP-degradation products as a part of weight loss (Xiong et al., 2020). The K-value also depends on handling conditions, seasons and species, which affect the initial concentration of ATP and ATP degradation products in the samples (Olafsdottir et al., 1997).

A K-value of 20 % is a general threshold for raw fish consumption (ElMasry et al., 2015), while a K-value of 60 % is the threshold limit of acceptability (Li et al., 2016). Based on the K-value of 20 %, samples stored at 0 °C can be consumed raw for up to 5 days, while samples stored at 4 °C can be consumed raw for up to 4 days. Sea cucumbers have comparable glycogen content to other fish species (Tanikawa & Yoshitani, 1955), and the synthesis of ATP during post-mortem storage of sea cucumbers may be affected by the amount of glycogen present in the muscle (Hong et al., 2017).

3.2.8. NMR

Samples stored at 0 °C and 4 °C were first analyzed by NMR as a pre-screening test before the analysis of volatile compounds using HS/GC-MS. Screening of the 1D ^{1}H NMR spectra (Supplementary Figs. 3 and 4) showed that samples stored at 4 °C had a higher, though not significant ($p > 0.05$) content of acetic acid after 17 days of storage compared to samples stored at 0 °C, except on day 2 (Supplementary Fig. 5). The increase, with high variation in acetic acid concentration could be due to microbial metabolic production (Guettler et al., 1999; Parlapani et al., 2014) in the sea cucumber during storage. It is well known that lower storage temperature inhibits the development of spoilage compounds such as acetic acid in various food products (Taormina, 2021).

3.2.9. Volatile organic components and biogenic amines

The composition of volatile organic compounds was characterized, in order, by aldehydes, alcohols, ketones, acids, esters, and other volatile compounds (Table 4). They represent typical secondary lipid oxidation products, bacterial metabolites, and spoilage compounds. The highest concentrations were found for propanoic acid (31.2 µg/100g), p-cresol (22.9 µg/100g), acetic acid (19.5 µg/100g), 2-methyl-hexanoic acid (19.2 µg/100g), 3-methyl-1-butanol (10.4 µg/100g) and phenol (10.2 µg/100g), all representing microbial metabolites.

Samples stored at 4 °C (Table 4) resulted in a higher formation of volatile secondary lipid oxidation products and microbial metabolites compared to sample stored at 0 °C. Specifically, levels of aldehydes (2-methyl-butanal, decanal, nonanal and octanal), alcohols (1-hexanol, 1-octen-3-ol, 1-penten-3-ol and 3-methyl-1-butanol), acids (2-methyl-hexanoic acid, 2-methyl-propanoic acid, acetic acid and propanoic acid), esters (heptanoic methyl ester), indole, p-cresol and phenol showed a significant increase at the end of storage in samples stored at 4 °C compared to samples stored at 0 °C. As expected, increasing temperature results in increased development of volatiles compounds during storage (Bosse et al., 2021).

The halogenated compounds dibromo-methane, tribromo-methane (Goodwin et al., 1997), and dichloronitro-methane (König & Wright, 1997) found in *P. tremulus* are derived from marine algae and seaweed beds (Al-Adilah et al., 2022), which *P. tremulus* feeds on (Pasquini et al., 2023). Additionally, certain compounds such 3-methyl-butanal, 3-methyl-1-butanol, 2,3-pentadione, acetic acid, phenol and p-cresol found in the samples during storage, could be related to protein degradation products (Xiong et al., 2020) and microbial metabolites (Boziaris

Table 5
Odor threshold in water (μg/100 g), and odor activity values ≥ 1 (μg/100 g wet weight sea cucumber) calculated from odor-active compounds (OACs) in red sea cucumber (*Parastichopus tremulus*) during storage at 0 °C and 4 °C. "n.d" indicates 'not detected'. The odor threshold in water was obtained from Associates (1990), Zhang et al. (2018) and ChemicalBook (2024).

Odor-active compounds	Odor threshold in water (μg/100 g)	Odor activity values (μg/100 g wet weight)				
		Day 0	Day 5 (0 °C)	Day 17 (0 °C)	Day 5 (4 °C)	Day 17 (4 °C)
Aldehydes						
(E,E)-2,4-Heptadienal	1.5	n.d	≤1	≤1	≤1	≤1
2,4-Nonadienal	0.009	n.d	n.d	n.d	n.d	21.1
2,6-Nonadienal	0.001	n.d	n.d	360	n.d	n.d
2,4-Decadienal	0.007	96	n.d	83	80	104
2-Hexenal	0.2	≤1	≤1	≤1	≤1	≤1
2-Nonenal	n.d	n.d	≤1	n.d	n.d	n.d
2-Decenal	0.03	6	n.d	9	10	13
2-Octenal	0.3	1	≤1	1	1	2
2-Methyl-Butanal	0.44	≤1	≤1	≤1	n.d	23
3-Methyl-Butanal	0.12	≤1	1	4	1	6
4-Heptenal	0.08	n.d	n.d	n.d	n.d	1
Decanal	0.01	8	84	n.d	693	899
Dodecanal	0.2	≤1	n.d	≤1	2	3
Heptanal	0.3	≤1	≤1	2	3	4
Hexanal	0.45	5	2	6	6	4
Nonanal	0.1	≤1	6	9	48	74
Octanal	0.07	4	≤1	15	36	47
Pentanal	1.2	≤1	≤1	≤1	≤1	≤1
Undecanal	0.5	1	≤1	n.d	1	1
Alcohols						
1-Octen-3-ol	0.1	9.7	4.8	17.6	10.9	22.4
1-Penten-3-ol	40	≤1	≤1	≤1	≤1	≤1
2-Heptanol	0.3	n.d	n.d	n.d	≤1	1
2-Octen-1-ol	7.5	≤1	≤1	≤1	≤1	≤1
2-Penten-1-ol	110	≤1	≤1	≤1	≤1	≤1
Ketones						
2,3-pentanedione	2	≤1	≤1	≤1	≤1	≤1
1-Octen-3-one	0.0005	460	160	560	380	380
1-Penten-3-one	0.1	5	2	6	3	4
6-Methyl-5-Hepten-2-one	5	≤1	n.d	n.d	≤1	≤1
Acids						
2-Methyl-Hexanoic acid	92	n.d	n.d	n.d	n.d	≤1
Acetic acid	2200	n.d	n.d	n.d	n.d	≤1
Propanoic acid	2000	n.d	n.d	n.d	n.d	≤1
Heptanoic acid, Methyl ester	300	n.d	n.d	n.d	≤1	≤1
Others						
Dimethyl Disulfide	0.012	n.d	n.d	n.d	n.d	190
Dimethyl Trisulfide	0.0005	n.d	n.d	n.d	n.d	1860
Indole	14	n.d	n.d	n.d	n.d	≤1
Phenol	590	≤1	n.d	n.d	≤1	≤1
p-Cresol	5.5	≤1	n.d	n.d	≤1	4

et al., 2023; Saito et al., 2018) (Fig. 7). Acetic acid is a microbial metabolic product (Guettler et al., 1999; Parlapani et al., 2014), and its development was probably inhibited by the lower temperature storage related to different food products (Taormina, 2021). The quantitative results showed variations in both concentrations and the composition of the volatile compounds, which is comparable to a previous study by Zhang et al. (2018) on different dried sea cucumber species.

The major odor-active compounds (OACs), along with their odor-thresholds in water and calculated odor-activity values (OAVs), are shown in Table 5. Among the identified OACs in the samples from the storage trial, the majority of compounds contributing to odor were in decreasing order sulfides (dimethyl trisulfide and dimethyl disulfide), aldehydes (decanal, 2,4-decadienal, nonanal, octanal, 2-methyl-butanal, 2,4-nonadienal and 2-decenal), ketones (1-octen-3-one and 1-penten-3-one), alcohols (1-octen-3-ol). Dimethyl trisulfide and dimethyl disulfide have a malodorous sulfur and garlic like smell (Parker, 2015), 1-octen-3-one has a mushroom like odor (Glindemann et al., 2006) and decanal has a sweet floral like odor (NIH, 2024). These compounds increased at both storage temperatures, and are comparable to previous storage studies of oyster (Kawabe, Murakami, & Usui, 2019) and raw sardines (Prost et al., 2004). Odor-active values (OAVs) ≥ 1 contribute more to odor (Ma et al., 2020), and samples stored at 4 °C developed higher OAVs than samples stored at 0 °C. 2-pentyl-furan, acetophenone, benzaldehyde, styrene, p-xylene, toluene, 1-octen-3-ol, nonanal, octanal, heptanal, hexanal, pentanal, 2-methyl-butanal and 3-methyl-butanal have previously been mentioned as OACs in dried sea cucumbers (Zhang et al., 2018). In another study by Lee et al. (2014), octanal, heptanal, hexanal and pentanal were found in dried sea cucumber (*Stichopus japonicus*) as OACs. The aldehydes hexanal and octanal derived from lipid oxidation, have been associated with fishy odor in freeze-dried sea cucumber powder (Wei et al., 2024). It is important to note that the interaction of volatile compounds under different storage conditions, such as fresh or dried products, influences the overall odor development in seafood products (Zhu et al., 2020).

Biogenic amines in the samples were analyzed at both storage temperatures using HPLC and NMR, and no concentrations were detected. This suggests that only a limited concentration of biogenic amines may have formed and were undetectable, or other degradation processes may have dominated during the storage stability experiment. The development of biogenic amines during storage depends on the type of species, the microbial population and their metabolic capacity (the synthesis of proteolytic enzymes and amino acid decarboxylase), the availability of FAAs, and the environmental storage conditions of the seafood (Arulkumar et al., 2023; Ding & Li, 2024). This could maybe explain the absence of biogenic amines in the samples. More study is needed to verify the biogenic amines content during storage of sea cucumbers.

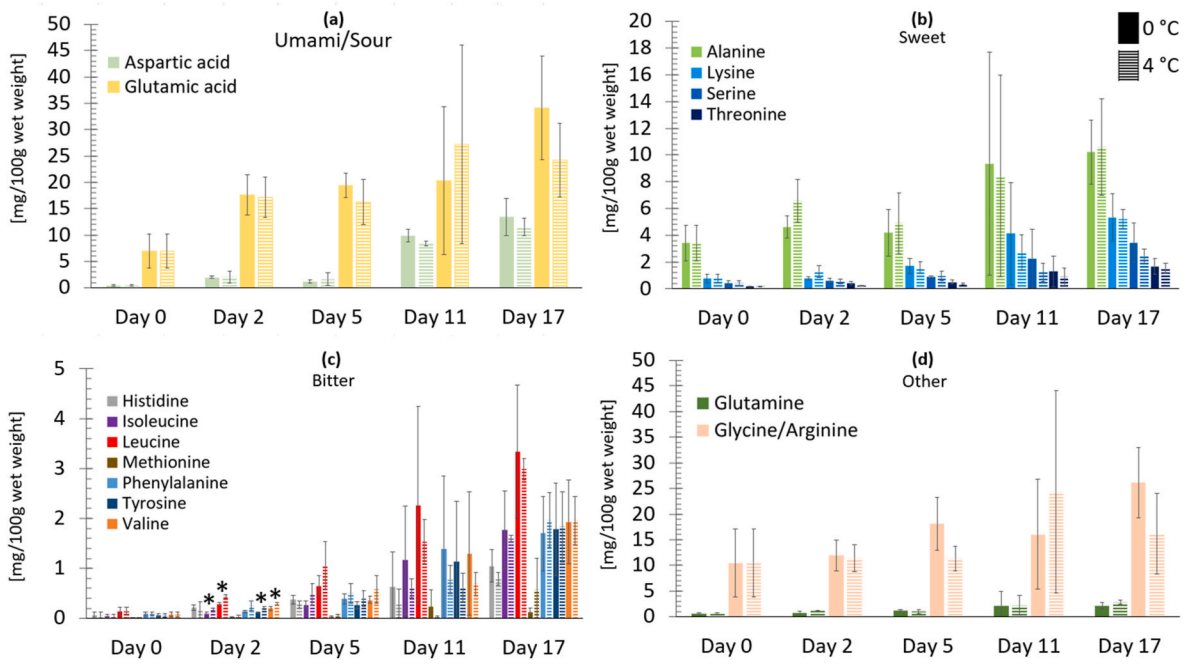

Fig. 8. Distribution of free amino acids (FAAs) (mg/100 g wet weight) in red sea cucumber (*Parastichopus tremulus*) throughout a storage trial for 17 days at 0 °C and 4 °C. Full and shaded color indicate storage temperatures at 0 °C and 4 °C, respectively. The amino acids are grouped after their respective tastes umami/acidic (a), sweet (b), bitter (c) and other (d) The results are presented as mean ± standard deviation ($n = 3$). *Indicate significant differences ($p < 0.05$) within the same sampling day) between storage temperature.

3.2.10. Taste components

FAA can influence various tastes, including sweet, bitter, acidic, and umami. These contributions are categorized according to the respective tastes, as shown in Fig. 8 (Vu et al., 2024). High concentrations of glutamic acid and glycine/arginine were found in all samples throughout storage. It is known that aspartic acid and glutamic acid have an acidic taste, and these two amino acids could contribute to the umami taste in the presence as sodium salts, such as monosodium glutamate (MSG) (Kendler et al., 2023; Yoshida & Ninomiya, 2024).

Compounds related to umami/acidic taste showed the highest concentration compared to other taste components in this study. Previous studies have shown that tyrosine and phenylalanine could have the ability to improve the umami taste in foods (Chen et al., 2021; Lin et al., 2023; Sarower et al., 2012). The two different storage temperatures did not significantly ($p > 0.05$) affect the concentration of bitter FAAs, except isoleucine, leucine, tyrosine, and valine on day 2 for samples stored at 4 °C. A previous study found that the rejection threshold limit for bitter taste varies with the type of amino acid, such as tryptophan (153 mg/100g), phenylalanine (173 mg/100g), leucine (605 mg/100g) and isoleucine (575 mg/100g) (Mura et al., 2018). The intensity of bitter taste in this current study is lower compared to the previous result (Mura et al., 2018). This may indicate that the consumption of sea cucumber will not result in rejection due to the content of bitter compounds.

In this study, it was not possible to separate glycine/arginine, and these were grouped as 'other' tastes. However, glycine is associated with a sweet taste and arginine is linked to a bitter taste (Kirimura et al., 1969). Glutamine has been ascribed to both umami and sweet taste, however, the tastes are weak in intensity compared to other amino acids (Kirimura et al., 1969). The taste of sea cucumber is dependent on the species, the biochemical composition, the environment and the processing methods (Sarower et al., 2012). It is worth noting that the sensory quality was not investigated in this study. Further research is needed to explore the sensory quality of *P. tremulus*.

The increase of taste components during storage could be attributed to the autolysis process in the sea cucumber (Wu et al., 2013), resulting in the degradation of proteins (Li et al., 2022) and nucleotides (Tavares et al., 2021). High standard deviations were observed a both storage temperatures related to the taste components. This variation could be explained by the initial individual variations at different life stages within the sample group (Elvevoll et al., 2022). An increasing trend of the taste components were observed, and significant differences ($p < 0.05$) were found at the end of storage compared to the beginning of storage for the different taste components (Fig. 8). Based on the K-value of 20 % as a general threshold for raw fish consumption (ElMasry et al., 2015), this suggests that sea cucumbers could maintain their favorable flavor profile for up to 5 days when stored at 0 °C, and for less than 5 days when stored at 4 °C.

4. Conclusion

This study presents new insights into the biochemical and microbial changes affecting the quality of *P. tremulus* during postmortem storage at two different cold temperatures. An observed decrease in texture, along with an increase in free amino acids and protein solubility, was linked to the autolysis process. The reduction in pH is likely due to the production and dissolution of volatile compounds. Notably, volatile compounds such as 2-methyl-butanal, 3-methyl-1-butanol, 2-methyl-hexanoic acid, heptanoic methyl ester, indole, p-cresol, and phenol were found at higher levels at 4 °C compared to 0 °C. Microbial growth emerged as the primary factor limiting quality during cold storage, stabilizing at a plateau (>6 log cfu/g of PC) after 7 days at 0 °C or 5 days at 4 °C. The primary specific spoilage organisms identified were *Flavobacterium* and *Psychrobacter*, with prolonged storage favoring *Polaribacter* and *Marinomonas*. These findings are valuable for future commercial applications, offering detailed insights into the extent and nature of changes occurring in the raw materials.

CRediT authorship contribution statement

Dat Trong Vu: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dionysios Tsoukalas:** Writing – review & editing, Writing –

original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anita Nordeng Jakobsen:** Writing – review & editing, Visualization, Conceptualization. **Eva Falch:** Writing – review & editing, Visualization, Supervision, Conceptualization. **John-Erik Haugen:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Ida-Johanne Jensen:** Writing – review & editing, Visualization, Supervision, Conceptualization.

Research data

The research data are available upon request.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2025.105846.

Data availability

Data will be made available on request.

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