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Packaging atmosphere and confinement as key factors in shelf-life extension of live clams

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ABSTRACT

Live bivalve molluscs are highly valued for their nutritional, gastronomic, and commercial qualities. However, they are extremely perishable, which increases production losses and waste in the supply chain. This study aimed to evaluate the shelf-life and physiological quality of live *Venerupis corrugata* clams stored at 3 ± 1°C in different packaging systems evaluating the effect of high-oxygen modified atmosphere packaging versus ambient air, and of physical confinement in net bags versus loose storage. Survival percentage, gases concentration, volatile organic compounds, pH, glycogen content, and biogenic amines were monitored throughout storage. Results showed that survival and physiological quality were strong influenced by the combined effects of oxygen availability and confinement. High-oxygen MAP delayed mortality by sustaining aerobic metabolism for longer, thereby reducing the respiration quotient. Physical confinement was critical in maintaining intravalvular liquid and preserving clam viability. Nevertheless, the modelled survivability extension was limited to only 1 day under optimal MAP conditions and confinement, highlighting the intrinsic sensitivity of this species.

1. Introduction

The search for sustainable protein sources is a key trend in the global food industry. Bivalve molluscs such as clams, mussels, and oysters are increasingly valued by both consumers and producers due to their nutritional, gastronomic, and commercial significance. Among them, the native European clam *Venerupis corrugata,* commonly known as pullet carpet shell, is considered a premium product, often priced three times higher than other invasive clam species due to its limited yield and high market demand (Anacleto et al., 2014a; European Parliament, 2016). The global clam market exceeds EUR 6.3 billion (FAO, 2018), yet challenges remain across the value chain, including limitations in large-scale production, packaging, and distribution. Innovative technologies are required to improve shelf-life, reduce waste, and reach broader markets in a sustainable manner.

V. corrugata is a bivalve species of the Veneridae family, commonly found in the northeastern Atlantic and Mediterranean coastal areas. It inhabits shallow sandy or muddy substrates and is distinguishable by its fused siphons, which perform respiration through water filtration (FAO, 2009; Ministério da Agricultura, do Desenvolvimento Rural e das Pescas, 2011). Unlike mussels, which can tolerate longer periods out of water due to their robust attachment to rocks and well-oxygenated habitats, clams are more vulnerable to oxygen depletion after harvesting, given their constant immersion in their natural environment (Gosling, 2003). As a result, post-harvest respiration and metabolic stress can compromise the viability of the clam. Several biological and environmental factors including species, size, temperature and ecological conditions further influence the respiration rate and overall resilience of bivalves (Khripounoff et al., 2017).

Fresh *V. corrugata* clams typically retain good quality for three or four days under refrigeration. According to the Codex Alimentarius Standard for live bivalve molluscs (CXS 292-2008), products must be sold alive, with no more than 5% dead specimens (FAO & WHO, 2008). Some commercial practices adopt less stringent viability thresholds, e.g., ≥80% live clams (Bernárdez & Pastoriza, 2011; Ratnawati et al., 2023).

Clams are often packed in polyethylene (PE), polypropylene (PP), or

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polyamide (PA) net bags, produced by extrusion, which allows lightweight and versatility. The appropriate packaging design and shell closure can preserve integrity and avoid exudate losses (Carneiro et al., 2025).

Modified Atmosphere Packaging (MAP) has been widely studied in seafood preservation, offering benefits, such as microbial inhibition and slow down the respiration rate, while maintaining the aerobic mechanism of live products for longer (Ho et al., 1997; Ratnawati et al., 2023). Its effectiveness depends on variables such as gas composition, packaging material, headspace volume, and storage temperature (Odeyemi et al., 2023; Pastoriza & Bernárdez, 2012; Sivertsvik et al., 2002). Most MAP studies regarding shellfish focus on mussels and oysters (Bernárdez & Pastoriza, 2011; Ratnawati et al., 2023; Rodezno et al., 2023; Tian & Liu, 2023), with fewer data available for clams, particularly *V. corrugata*. Studies have shown that high-oxygen MAP (60% O_2: 40% CO_2) can extend mussels *Mytilus edulis* shelf-life for 7-9 days under refrigerated storage considering the limit of 20% of mortality (Ratnawati et al., 2023). High levels of oxygen have also been reported as beneficial for clams, although literature is very scarce for this product. A comparison between *Ruditapes decussatus* clams packed in net bag under air and in MAP with 70% O_2: 30% N_2 for 6 days at 6.1 ± 0.7 °C did not show a significant difference between the percentage of mortality, with 10.9% ± 3.6% and 12.3% ± 2.6% respectively. However, the storage under MAP showed a beneficial effect in the sensorial quality of the clams and lower microorganisms' growth (Gonçalves et al., 2009). A recent study with *V. corrugata* analyzed the percentage of survival over time and reported only a 1 – 2 days extension using MAP with >70% O_2 at 3 ± 1 °C (Goes et al., 2025). These findings indicate potential but also highlight the need for species-specific studies.

The quality and safety of the shellfish are strongly connected to aroma and volatile organic compounds (VOCs) directly influencing consumer perception of freshness (Fratini et al., 2012). Fresh flavours and off-flavour such as aldehydes, alcohols, ketones and acids can be changed by microorganisms, enzymatic or auto-oxidative reactions before visible tissue degradation and during the decomposition (Fratini et al., 2012; Zhang et al., 2010). Biogenic amines (BAs) such as histamine, tyramine, putrescine, and cadaverine are examples of amines formed by amino acid decarboxylation and are widely recognized as spoilage indicators. While regulatory European limits exist primarily for histamine in fishery products (mean value ≤ 100 mg/kg or max. value < 200 mg/kg), their presence provides valuable insights into product quality and microbial activity (Arulkumar et al., 2023; Visciano et al., 2020).

From a physiological perspective, the metabolic state of live bivalves directly influences the sensory properties of their edible meat (Fu et al., 2024). Glycogen reserves in the adductor muscle contribute to organoleptic attributes and are also linked to post-harvest stress and survivability (Anacleto et al., 2014b; Bi et al., 2023; Gonçalves et al., 2009). Storage and transport conditions significantly influence glycogen retention. In *Ruditapes philippinarum*, refrigerated (4°C) semi-dry transport preserved higher glycogen levels than warmer or wet conditions (Bi et al., 2023). Regardless of the environmental conditions to which they are exposed, differences between species have already been reported. Anacleto et al. (2013) reported that *V. corrugata* has shown higher glycogen content than *R. philippinarum* under comparable scenarios. However, there is still a lack of studies on this species in modified atmosphere environments, which limits our ability to make critical comparisons.

Given the ecological and economic importance of *V. corrugata*, extending its post-harvest shelf-life is essential for promoting aquaculture, minimizing waste, and expanding market access. This study aimed to evaluate the effect of two parameters, that the packaging system can control on the shelf-life and physiological quality of live *V. corrugata* clams stored at 3 ± 1°C. The packaging systems were compared by examining clams in: (i) high-oxygen MAP versus ambient air, and (ii) in physical confinement in commercial net bags versus loose storage. Survival percentage, volatile organic compounds (VOCs), pH, glycogen content and biogenic amines were monitored over time.

2. Material and Methods

2.1. Clam samples and experimental set-up

Specimens of *V. corrugata* (shell length: 3.8 – 4.4 cm; body weight: 14.3 ± 1.9 g) were sourced from a commercial aquaculture facility in Ria de Aveiro (RIAV), Portugal, in January 2025. After 24 – 48 hours of depuration in a local installation (Falcamar – Comércio de Mariscos Lda.), the clams were refrigerated at 3 ± 1 °C and transported to the research facility for analysis. During depuration and storage, the clams were not fed. Empty and/or damaged shells were discarded; the remaining live clams were weighed, counted and divided into groups of 24 clams (average weight: 323.5 ± 1.9 g). Half of the samples were packed in plastic PE net bags commercially used by Falcamar (mesh size: 6.6 ± 0.7 mm square (SQ) and 10.1 ± 0.8 mm stretched (STR); thread thickness: 0.13 ± 0.04 mm) and sealed using a plastic clamp, thus replicating supplier conditions.

Each group was randomly assigned to one of four experimental conditions:

- **C1:** Loose clams stored under air;
- **C2:** Loose clams stored under MAP (96% O_2);
- **C3:** Clams packed in net bags under air;
- **C4:** Clams packed in net bags under MAP (96% O_2).

The four experimental conditions were tested using jars (0.7 L) designed for controlled storage and gas monitoring. The headspace of each monitored jar was determined as 482.4 ± 1.8 mL. This experimental setup allowed precise control of environmental variables to simulate and monitor each condition effectively (Fig. 1).

All the jars were closed with a hermetic metal lid. The jars used for the MAP condition and for headspace monitoring had two inlets to gas addition and a rubber septum for headspace sampling (Fig. 1). Eight jars were prepared for each group/condition; five to assess clam survival and three for measuring changes in the gas composition (non-destructive

Fig. 1. Jars sample with loose and confined clams in plastic net bag.

sampling) overtime. For the MAP conditions, two additional jars were used as gas composition control (without clams). The jar system was tested, and the loss of oxygen was lower than 2% after one week. For creating the modified atmosphere, oxygen gas (100% O_2, Gasin, Air Products Group - Spain) was flushed in the hermetically closed jars to generate a modified atmosphere with an oxygen concentration greater than 90% and no CO_2, according to previous studies reported in the literature (Bernardéz & Pastoriza, 2011; Goes et al., 2025; Ratnawati et al., 2023). The final value obtained after flushing was 96 ± 1% O_2 and 0% CO_2. All samples were stored in a refrigerated incubator at 3 ± 1°C (MIR253, Sanyo) and evaluated in six storage time sampling points (0, 3, 4, 5, 6 and 7 days).

2.2. Clams' quality monitoring

2.2.1. Survivability, opened clams, exudation

Clam survival was assessed by opening one of the five jars of each condition and assessing all the clams in that jar. In the last sampling day (7^{th}), the three jars used to measure gas concentration over time were also evaluated. Opened clams and sensorial aspects were registered and clams were classified as alive or dead based on valve closure upon tactile response. The percentage of live clams was calculated by observing the state of the valves, as well as by stimulating the clams (Bi et al., 2023; Wang et al., 2024). A clam was considered alive during the evaluation if, upon stimulation, the shell valve closed or opened, or if body movement was observed. The survival percentage was calculated according to Equation 1:

$$Live\ clams\ (\%) = \frac{No.\ live\ individuals}{No.\ total\ clams\ evaluated}\ x\ 100 \quad (1)$$

The exudated liquid collected in the jar was weighed and expressed in grams.

For each condition and group, the clams were prepared for chemical analysis as follows. A subsample of 10 whole clams (n= 10) was separated, and the adductor muscles were extracted and stored at -80°C for glycogen quantification. Death and alive clams were distinguished. The remaining edible meat of the same 10 clams was extracted for analysis of pH and biogenic amines. For identification of volatile compounds via gas chromatography mass spectrometry (GC–MS), three whole clams were stored separately. These samples were collected and stored at -20°C for simultaneous analysis.

2.2.2. Gas composition and estimation of respiration rate

The gas composition (% CO_2 and % O_2) inside the jars was measured over time with a headspace gas analyzer (CheckMate 3®, Dansensor, MOCON Europe A/S, Denmark).

The oxygen consumption and carbon dioxide production rate were calculated through equation 2 for each sampling point:

$$Rate\left(mg_{gas}\ g_{clams}{}^{-1} day^{-1}\right) = \frac{P\ V_{hs}\ \left|\Delta\%_{gas}\right|\ M_{gas}\ \times\ 1000}{R\ T\ m_{clams}\ \Delta t} \quad (2)$$

where P = pressure (atm), V_{hs} = headspace volume (L), $\Delta\%_{gas}$ = variation in gas concentration (percentage points), M_{gas}= molar mass (O_2 = 32.0 g mol^{-1}; CO_2 = 44.0 g mol^{-1}), R = 0.0821 L atm mol^{-1} K^{-1}, T = temperature (K), m_{clams} = edible meat of clams (g), and Δt = time difference (days).

The respiratory quotient (RQ) was calculated as the ratio of CO_2 production rate to O_2 consumption rate according to equation 3:

$$RQ = \frac{CO_2 rate}{O_2 rate} \quad (3)$$

The estimative method was based on Ho et al. (1997) with minor modifications. Changes in the gases were assumed to be caused exclusively by the clams, with the microflora being ignored. To calculate the volume of headspace and the mass of the clams without shells, previous results for average clam density (1.48 ± 0.03 g/mL) and edible meat percentage (49.83 ± 1.49%) were considered. Both data were obtained from fresh clams of the same species and size.

2.2.3. VOCs (GC–MS)

The clam's meat was prepared for analysis in accordance with Zhang et al. (2010). Whole clams' meat was mixed in a 1:1 ratio with NaCl (0.06 g mL^{-1}), following homogenisation in a T25 ULTRA-TURRAX® (IKA, Germany) and splitting into aliquots for analysis (duplicate). The analytical method was in accordance with Wang et al. (2024), with certain adaptations. GC–MS analysis was performed in a system Bruker® EVOQ 456 TQ GC/MS, with the following operating conditions: vector gas Helium at 1 mL min^{-1}; injection temperature 250°C and column DBWAX, 30 m × 250 µm × 0.25 µm. Temperature was isothermal at 40°C for 3 min, ramped to 90°C at a rate of 5°C/min, then ramped to 230°C at a rate of 8°C/min, and held at 230°C for 10 min, in splitless time (1 mL min^{-1}). The mass spectrometer is operated in the electron impact mode with the electron energy set at 70 eV. The ion source temperature was set at 230°C, and mass range was set at: 20–350 amu (m/z). Samples were extracted using a SPME fibre of 50/30 mm divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) at room temperature, with 5 min pre-incubation period and 40 min extraction period, with constant stirring at 250 rpm.

The identification of the volatile substances was carried out by comparing the retention indexes and mass spectra data with those from the NIST Tandem Mass Digital Library (Version 2.3, build May 4, 2017). Semi-quantitative analysis was carried out using an internal standard (deuterated dodecane, CAS No. 16416-30-1, concentration of 5.32 mg L^{-1}) from Sigma-Aldrich (Germany), prepared beforehand and used at room temperature.

2.2.4. Glycogen content

Glycogen was quantified in the adductor muscles of clams, in triplicate, applying a colorimetric detection method using anthrone-sulfuric acid and following the protocol of a commercial CheKine™ Micro Glycogen Assay Kit (Abbkine Scientific Co., Ltd; Georgia, USA). Briefly, 0.1 g of tissue was mixed with extraction buffer, boiled for 20 min (covered to prevent water loss), diluted in deionized water and centrifuged (8000 g for 10 min). The samples were incubated at 95°C for 10 min after the chromogenic reagent was added. The absorbance was measured at 620 nm with a Microplate reader Agilent BioTek Synergy H1 (Agilent Technologies, Santa Clara, CA, USA). A calibration curve was prepared using a glucose standard. The kit detection range and sensitivity are 0.003125-0.25 mg mL^{-1} and 0.003125 mg mL^{-1} respectively. The results were expressed as mg per g wet weight (WW).

2.2.5. pH

The pH was measured, in duplicate, at 20 ± 2°C using a pH meter (Crison GLP 22+, Barcelona, Spain) and a combined pH electrode (Crison 50 14T) in a sample of 10 clams edible meat mixed in a T25 ULTRA-TURRAX® homogenizer (IKA, Germany).

2.2.6. Biogenic amines (BAs)

The flesh of 10 clams' individuals was pooled to obtain a homogeneous and representative composite sample. Briefly, a 10 g portion of the composite was weighed into a 50 mL test tube and mixed with 35 mL of 10% (w/v) trichloroacetic acid (TCA) using a T25 ULTRA-TURRAX® homogenizer (IKA, Germany). The homogenate was then diluted to a final volume of 50 mL with ultrapure water and filtered through a 0.45 µm membrane filter. Biogenic amines were quantified by high-performance liquid chromatography (HPLC) after derivatization with *ortho*-phthalaldehyde (OPA), based on the method described by Komprda et al. (2004). The analysis was performed using a Beckman HPLC system (Beckman Coulter, USA) equipped with a fluorescence detector (excitation wavelength: 350 nm; emission wavelength: 445 nm). A Chromolith® Performance RP-18 column (100 mm × 3 mm;

Merck, Germany) was used for the separation. The following amines were determined: histamine*, tyramine*, isoamylamine*, ethylamine*, putrescine**, cadaverine**, 2-phenylethylamine** and methylamine**. The method has limits of detection and quantification (LOD/LOQ): * < 0.6 / 2.0 and **<0.9 / 3.0.

2.2.7. Statistical analysis and data handling

One-way and two-way analysis of variance (ANOVA) was performed to compare results between conditions and to compare conditions x time, respectively. A statistically significant result was considered when *p-value* was lower than 0.05. Statistical analysis was performed using IBM SPSS STATISTICS, 29.0.2.0 Package (IBM Corporation, New York, USA, 2024).

The experimental data for the survival percentages were fitted to a Boltzmann sigmoidal function according to equation 4:

$$y = \beta + \frac{(\alpha - \beta)}{\left(1 + \exp\left(\frac{x-\varphi}{\tau}\right)\right)} \quad (4)$$

where y is the % live clams and x is the time (days). The model parameters, α (initial value), β (final value), φ (centre) and τ (time constant), were estimated by non-linear regression analysis using the Levenberg-Marquardt algorithm to minimise the sum of the squares of the differences between the predicted and experimental values. The precision of the parameters was evaluated by their 95% confidence intervals, and the regression quality assessed by residuals analysis and the coefficient of determination R^2.

Principal Component Analysis (PCA) was performed using survival percentage, % open shells, exudate (g), gases concentration (O_2 % and CO_2 %), pH and glycogen content in Origin Pro (Version 2024 by OriginLab, USA).

3. Results and Discussion

3.1. Survival percentage, open clams and exudation

The results are presented in Fig. 2 showing a decrease in percentage of live clams (the survival percentage) over the 7 days of refrigerated storage period, as expected.

The 5% mortality threshold established by the Codex Alimentarius proved to be a stringent benchmark. Based on experimental data, the time to reach 95% live clams was as follows: C1 (loose clams, air) – 5.0 days; C4 and C3 (net bag, MAP and air) – 4.6 days; and C2 (loose clams, MAP) – 3.6 days. Beyond day 5, condition C4 (net bag, MAP) began to outperform the others in maintaining clam viability, showing a significantly higher percentage of live clams compared to conditions C1 (loose, air) and C3 (net bag, air). Condition C2, under MAP, shows an intermediate improvement, although no significant difference from the other conditions. On day 7, the percentage of live clams in C4 was 24.3 ± 12.0%; in C2, 21.0 ± 10.6%; in C3, 4.3 ± 6.1% and in C1, 3.3 ± 6.5%.

Table 1 presents the model parameters obtained for each packaging condition. This model should be seen as indicative only because of the low number of sampling points compared with the number of the parameters of the fitting equation. Additionally, the sampling period does not allow to define with precision β (final value) for all experimental conditions. According to the model (Fig. 2), there is an increase in time of survivability within the Codex limit when packaging confinement and high-oxygen MAP (C4) is applied, as compared to storage of loose clams in air (C1). However, the difference in time was less than one day in this study. Once the death process begins, the rate of decline in the number of alive clams appears to be similar among the conditions tested, as denoted by the slope of the model between days 5 and 7.

Shell closure in bivalves is controlled by the adductor muscle, which contracts to keep the valves shut. Upon death, muscle relaxation prevents valve closure, resulting in shell opening and exposure of the intravalvular fluid, ultimately leading to flesh desiccation (Fratini et al., 2013). However, in experiments conducted in closed system such as the jar, no drying of the flesh could be observed. Although the clams remained succulent in sensory appearance, a loss of vitality was noticed from day 3 onwards, with the shells opening and exudate being released (Fig. 3). Up to day 5, no signs of spoilage were detected, but after that, an

Table 1
Parameters obtained from Boltzmann sigmoidal model fitting of survival curves under conditions (C1 - C4) overtime.

Parameters	C1	C2	C3	C4
α	100.05 ± 0.05	97.40 ± 4.32	100.08 ± 0.08	98.00 ± 4.58
β	0.94 ± 0.11	12.00 ± 8.38	3.41 ± 0.16	7.50 ± 132.2
φ	5.90 ± 0.00	5.80 ± 0.15	5.65 ± 0.00	6.37 ± 1.90
τ	0.29 ± 0.00	0.28 ± 0.16	0.28 ± 0.00	0.28 ± 1.07
Reduced Chi-sqr	0.01	53.75	0.20	60.47
R^2	1.00	0.98	1.00	0.97
Adj R^2	1.00	0.95	1.00	0.93

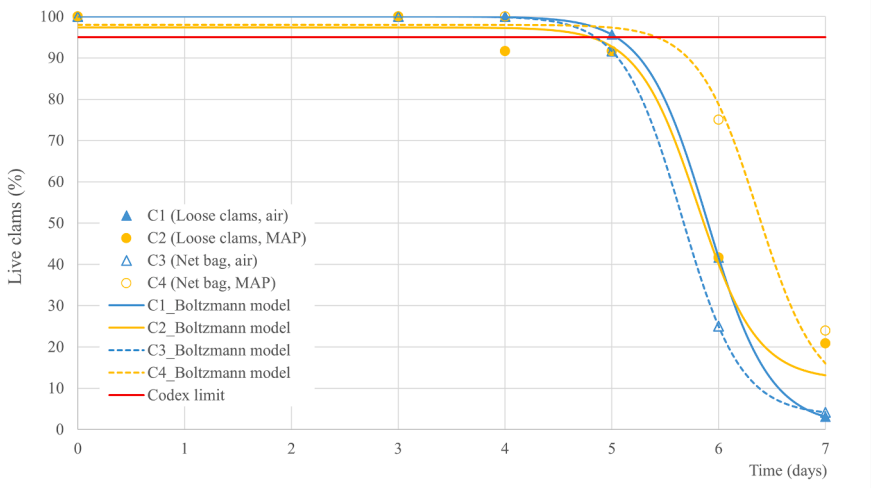

Fig. 2. Survival percentage (%) of clams stored at conditions (C1 - C4) over time in scatter markers. Boltzmann model for each condition represented in lines. Red line represents the CODEX limit of 5% of non-live clams.

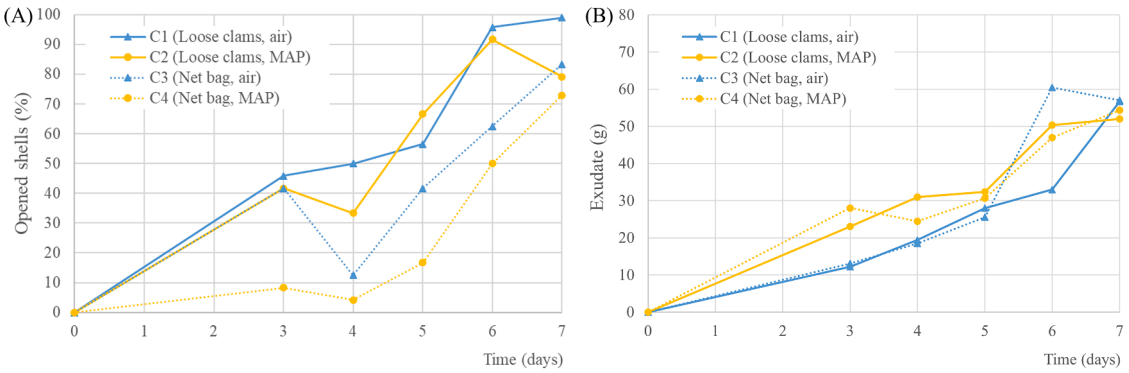

Fig. 3. Percentage of opened clam shells (A) and mass of exudate (B) in samples stored at different conditions (C1 - C4).

unpleasant odour was easily detected when the jars were opened. On the 6th day, the clams under condition C2 had a strong bad odour; those under conditions C1 and C3 had a bad odour less intense; and those under condition C4 were without spoilage notes, appearing in this sample condition only on day 7.

A progressive increase in number of open shells (Fig. 3A) and amount of exudate release (Fig. 3B) was observed over time across all storage conditions. Fig. 3A shows a trend for the clams confined in net bags (C3, C4) with high number of closed shells reflecting the effect of the confinement process. Until days 4 – 5, when a high number of clams were alive in all conditions, some clams were naturally opened at the time of evaluation possibly as result of search for nutrients and oxygen. However, after this period, the proportion of opened clams showed to increase steadily, suggesting a relationship with the rising number of dead clams shown in Fig. 2. On the day 7, the % opened clams were higher for the samples under air condition with C1 (99.0 ± 2.1%), C3 (83.3 ± 5.9%) compared to MAP conditions C2 (79.2 ± 0.0%) and C4 (72.9 ± 8.7%).

Fig. 3B shows the amount of exudate released by the clams. The values increased over time for all conditions, and there was a tendency for lower values in the air sample conditions (C1, C3). Although with no statistically significant difference between the conditions tested, the results show a tendency for samples with MAP to have higher amount of exudate compared to air. The results may be affected by a possible difference in the amount of initial liquid inside the clams (including water) that could not be controlled. The subsequent loss of this liquid would account as exudate in the conditions of test. It is suggested in the literature that the continuous aerobic metabolic process in clams with MAP, with or without the use of glycogen, produces metabolic water that is retained in a closed environment. After the fifth day, with an increase in dead clams mainly in C1 - C3, an increase in the exudate rate of clams in these conditions was noted. In addition to the loss of intravalvular fluid, the death process releases intracellular fluid and haemolymph (Bejaoui et al., 2020).

3.2. Changes in gases concentration and respiration rate

The results for the concentration of gases (O_2 and CO_2) in the headspace over time, for all conditions tested, are presented in Fig. 4.

Fig. 4A illustrates the progressive decline in O_2 concentration across all conditions throughout the storage period. The conditions under air (C1, C3) started with 20.5 ± 0.0% and 20.7 ± 0.1% respectively and, after 7 days were 4.6 ± 3.6% and 6.9 ± 2.2% of oxygen, respectively. The conditions under MAP (C2, C4) initiated with 96.9 ± 0.1% and 95.4 ± 1.6% and, in the 7th day of evaluation were 39.9 ± 6.4% and 55.6 ± 7.4%, respectively. Although no statistically significant differences were observed between conditions C1 and C3 or between C2 and C4, two consistent trends emerged: the higher rate of O_2 consumption under MAP conditions than in air and the slower decrease in O_2 levels in confined clams compared to loosen ones. This trend was particularly pronounced in the MAP groups after day 4 of storage.

The O_2 consumption and CO_2 rates were calculated for the periods between sampling point times (Table 2). The conditions under MAP (C2, C4) showed an O_2 consumption rate four times higher than the samples under air (C1, C3). After the 4th day, the rates at conditions C1, C3 and C4 apparently stabilized and the rate at condition C2 decrease until the 7th day. The oxygen rate for each condition is plotted in Figure S1A (Supplementary material).

The carbon dioxide concentration increased overtime for all conditions (Fig. 4B), and no statistical differences were found among the samples. The results showed a trend towards higher CO_2 production in samples under MAP conditions (C2, C4) until day 5-6, with a reversal of the scenario from this point onwards. The CO_2 concentration after 7 days was C1 (13.1 ± 1.8%), C3 (12.1 ± 1.2%), C4 (11.9 ± 0.4%) and C2 (11.3 ± 0.5%). The carbon dioxide production rate increased over time for all samples (Fig. S1B). Initially, the samples under air (C1, C3)

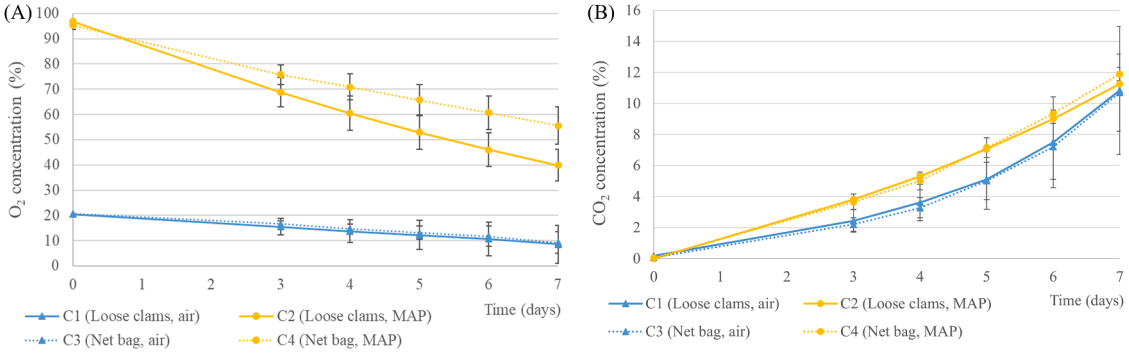

Fig. 4. Gas concentration (average ± standard deviation) in headspace: (A) percentage oxygen and (B) percentage carbon dioxide of each condition (C1 - C4) over time.

Table 2
Oxygen consumption rate, carbon dioxide production rate and respiration quotient over time for each condition (C1 - C4).

	Day	C1	C2	C3	C4
Oxygen consumption rate (mg O_2 g_{clam}^{-1} day^{-1})	*Initial (0-3)*	4.80 ± 0.75	19.66 ± 4.17	3.19 ± 0.83	13.70 ± 3.84
	4	5.08 ± 1.54	17.45 ± 2.13	4.21 ± 1.17	10.17 ± 2.51
	5	4.55 ± 1.68	15.98 ± 0.05	4.74 ± 0.72	10.91 ± 2.36
	6	4.19 ± 1.74	14.40 ± 0.40	4.62 ± 0.26	10.59 ± 1.03
	7	5.31 ± 0.47	12.93 ± 0.41	5.88 ± 0.39	10.59 ± 1.32
Carbon dioxide production rate (mg CO_2 g_{clam}^{-1} day^{-1})	*Initial (0-3)*	2.55 ± 0.21	3.66 ± 0.01	2.17 ± 0.47	3.46 ± 0.55
	4	4.05 ± 0.82	4.34 ± 0.40	3.33 ± 0.60	3.89 ± 0.21
	5	5.49 ± 1.23	5.06 ± 0.22	5.94 ± 1.00	6.20 ± 0.21
	6	8.38 ± 2.05	5.64 ± 1.04	7.96 ± 0.58	6.35 ± 1.22
	7	11.70 ± 0.63	6.50 ± 0.63	10.86 ± 0.26	7.36 ± 1.83
Respiration quotient	*Initial (0-3)*	0.54 ± 0.04	0.19 ± 0.04	0.68 ± 0.03	0.27 ± 0.12
	4	0.81 ± 0.08	0.25 ± 0.05	0.80 ± 0.08	0.40 ± 0.12
	5	1.24 ± 0.19	0.32 ± 0.01	1.25 ± 0.02	0.58 ± 0.15
	6	2.08 ± 0.37	0.39 ± 0.08	1.72 ± 0.03	0.60 ± 0.06
	7	2.21 ± 0.08	0.50 ± 0.06	1.85 ± 0.08	0.69 ± 0.09

Data are average ± standard deviation (n=2).

produced less carbon dioxide than the MAP samples (C2, C4), but after days 5-6, the rates increased. This behaviour aligns with the increase in the number of dead clams in conditions C1 and C3 (Fig. 2).

Although the decrease in O_2 levels and increase in CO_2 levels throughout the storage period suggest that this is a result of bivalve respiration (Gonçalves et al., 2009), the higher availability of O_2 MAP conditions may improve oxygen diffusion in the clam tissues and in the intravalvular liquid, allowing aerobic respiration to continue for longer. This may also favour the auto-oxidation of lipids, proteins and pigments, which are reactions that consume oxygen without producing CO_2. Additionally, microbial activity may also consume oxygen and store some of the carbon as biomass. For long storage, and especially after the death of the clams, the microbial load has a major influence on gas changes as well as other parameters, such as VOCs (Goes et al., 2025; Ho et al., 1997). However, due to the short lifespan in the present study, this effect was no considered.

The respiratory quotient (RQ) is an indicator of a ratio between the carbon dioxide evolution rate to oxygen consumption rate overtime is presented in Fig. 5.

RQ values increased over time for all conditions tested (Table 2). However, for samples under MAP, values remained below 1, with a tendency to be higher in C4 than in C2 (Fig. 5). For samples under air, values started between 0.5-0.7, but between days 4 and 5 they crossed the reference line for metabolism based on aerobic oxidation of carbohydrates and continued to increase until day 7. Information on the respiratory quotient of bivalve molluscs under air or MAP is lacking in the literature, particularly for the *V. corrugata* clam species. Ho et al. (1997) found RQ values ranging from 1.4 to 1.9 for *M. mercenaria* (hard clams) when individuals were exposed to different MAP conditions (6% O_2: 16% CO_2; 11% O_2: 10% CO_2; and 16% O_2: 4% CO_2). A study conducted by Khripounoff et al. (2017) with submerged *Christineconcha regab* clams and *Bathymodiolus azoricus* mussels obtained RQ values ranging from 0.8 to 1.5. Similar oxygen consumption between mussels and clams was noted, but clams showed higher carbon dioxide production attributed to anaerobic metabolism.

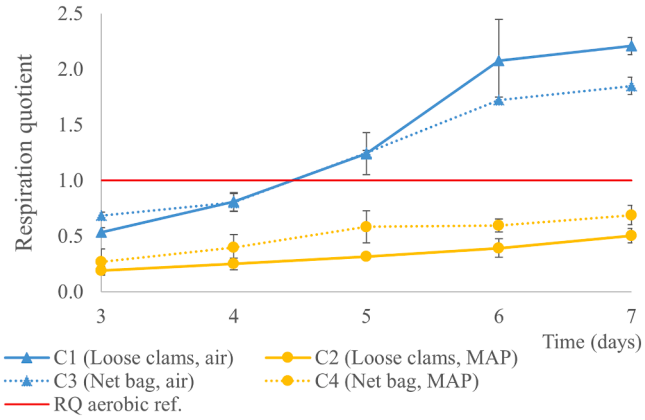

Fig. 5. Respiratory quotient (RQ) for *V. corrugata* clams stored at different conditions (C1 - C4) over time. Data are average ± standard deviation (*n*=2).

3.3. Identification of Volatile Compounds Via GC-MS

To monitor the progression of spoilage and aroma compound formation, volatile organic compounds (VOCs) were analysed using GC-MS. Table 3 shows the VOCs profile of the clams stored at 3 ± 1°C under conditions C1 - C4 for 0, 4 and 6 days. Four groups of chemical compounds were detected in all conditions and sample points, i.e. alcohols, aldehydes, ketones and carboxylic acids with a varying number of compounds in each group. A plot showing the VOCs profile for each condition and time is presented in Supplementary material (Figure S2).

Among the detected VOCs, alcohols were predominant (87 – 92% of total volatiles), followed by aldehydes (4 – 9%), ketones (2 – 4%) and carboxylic acids (<2%). The predominant alcohol in all conditions tested was 1,5-octadien-3-ol. Notwithstanding the high intensity, there was no significant difference in the concentration among the conditions tested for the period analysed. This compound is characterized by a fresh mushroom or moss-like odor (Kawai, 1996) and is commonly found in oysters such as *Ostrea edulis* (Fratini et al., 2012; van Houcke et al., 2016) and *Crassostrea gigas* (van Houcke et al., 2016), as well as in *Cerastoderma edule* (Fratini et al., 2012), *Pollicipes cornucopia* and in *V. corrugata* (Fratini et al., 2012).

1-penten-3-ol, is known to result from the lipoxygenases on PUFAn-3 (German et al., 1991; Hu & Pan, 2000), was one of the few volatiles in which a significant difference was observed between the conditions tested (Table 3). It has been detected in various bivalves, including *M. galloprovinciallis, V. corrugata* (Fratini et al., 2012), and oysters (Fratini et al., 2013; van Houcke et al., 2016) and is associated with a grassy odor (Hu & Pan, 2000). Results indicate a much higher concentration for this alcohol at day 4 compared to the other sampling points, for conditions C1, C2, and C4 (Fig. 6). Both 1-penten-3-ol and 1-octen-3-ol have also been identified as volatile organic compounds produced by seafood spoilage bacteria (Odeyemi et al., 2018). 1-Octen-3-ol has been cited as a marker of lipid oxidation in horse mackerel muscle (Iglesias & Medina, 2008) and was also detected in *E. ensis* (Fratini et al., 2012) and it is associated with a plant-like odor (Hu & Pan, 2000). Additionally, 1-pentanol, which is associated with a desirable apple-like aroma (Hu & Pan, 2000), was detected in *V. corrugata, M. galloprovinciallis* (Fratini et al., 2012) and oysters (Fratini et al., 2013). Both 1-penten-3-ol and 1-pentanol were significantly more abundant in *V. corrugata* compared to other shellfish species (Fratini et al., 2012). Both these compounds showed significant differences between time and the interaction condition x time (Table 3).

Aldehydes are the second largest group (4 – 9%) of VOCs detected in the samples under the analytical conditions used. Lilac aldehyde was well represented in this group, but with fluctuations in values over time

Table 3
Volatile organic compounds (VOCs) profile of *V. corrugata* clam under different conditions of gas composition and confinement during storage at 3 ± 1°C. Values are expressed as chromatographic peak /I.S. area.

VOCs		C1		C2		C3		C4		Condition	Time	Conditionx Time
Days of storage:	0[a]	4[a]	6[b]	4[a]	6[b]	4[a]	6[ab]	4[a]	6[ab]		*p value*	
Alcohols												
1-Penten-3-ol	0.37 ±0.30	2.49 ±0.83	0.37 ±0.05	0.42 ±0.17	0.31 ±0.11	0.97 ±0.44	1.02 ±0.55	1.74 ±0.27	0.34 ±0.16	0.044*	<0.001*	0.010*
1-Pentanol	0.02 ±0.02	0.08 ±0.03	0.03 ±0.00	0.02 ±0.01	0.04 ±0.02	0.04 ±0.02	0.16 ±0.09	0.04 ±0.01	0.02 ±0.01	0.061	0.048*	0.030*
1,5-Octadien-3-ol	3.87 ±3.07	7.47 ±2.28	4.70 ±0.57	2.48 ±0.97	3.90 ±1.59	6.07 ±2.90	8.63 ±4.82	5.70 ±0.66	3.19 ±2.06	0.325	0.479	0.555
1-Octen-3-ol	0.28 ±0.22	0.67 ±0.20	0.28 ±0.04	0.22 ±0.08	0.27 ±0.11	0.41 ±0.19	0.68 ±0.39	0.53 ±0.05	0.25 ±0.17	0.400	0.249	0.281
Aldehydes												
2,4-Heptadienal	0.00 ±0.00	0.02 ±0.01	0.01 ±0.00	0.00 ±0.00	0.01 ±0.00	0.02 ±0.01	0.01 ±0.01	0.01 ±0.00	0.00 ±0.00	0.125	0.042*	0.347
Lilac aldehyde D	0.36 ±0.38	0.69 ±0.14	0.38 ±0.10	0.19 ±0.12	0.37 ±0.19	0.47 ±0.23	1.42 ±1.04	0.35 ±0.10	0.44 ±0.32	0.286	0.324	0.348
Ketones												
2-Nonanone	0.04 ±0.04	0.10 ±0.04	0.05 ±0.00	0.05 ±0.03	0.04 ±0.02	0.14 ±0.08	0.10 ±0.07	0.08 ±0.02	0.04 ±0.02	0.239	0.109	0.770
3,5-Octadien-2-one	0.04 ±0.03	0.28 ±0.07	0.07 ±0.01	0.04 ±0.00	0.07 ±0.02	0.27 ±0.11	0.20 ±0.09	0.16 ±0.02	0.07 ±0.03	<0.001*	<0.001*	<0.001*
2-Undecanone	0.00 ±0.00	0.01 ±0.00	0.01 ±0.00	0.00 ±0.00	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00	1.000	0.391	0.889
Carboxylic acids												
Octanoic acid	0.01 ±0.01	0.02 ±0.01	0.01 ±0.00	0.01 ±0.00	0.01 ±0.00	0.01 ±0.01	0.03 ±0.03	0.02 ±0.02	0.02 ±0.01	0.489	0.801	0.443
Nonanoic acid	0.03 ±0.02	0.04 ±0.01	0.04 ±0.00	0.01 ±0.00	0.03 ±0.01	0.14 ±0.06	0.06 ±0.04	0.04 ±0.03	0.04 ±0.03	0.026*	0.312	0.089*

Data are average ± standard deviation (n=2).
Superscript letter in day means a = live clams, b = dead clams, ab = relative proportion value of live and dead clams.
* Means significant difference ($p<0.05$)

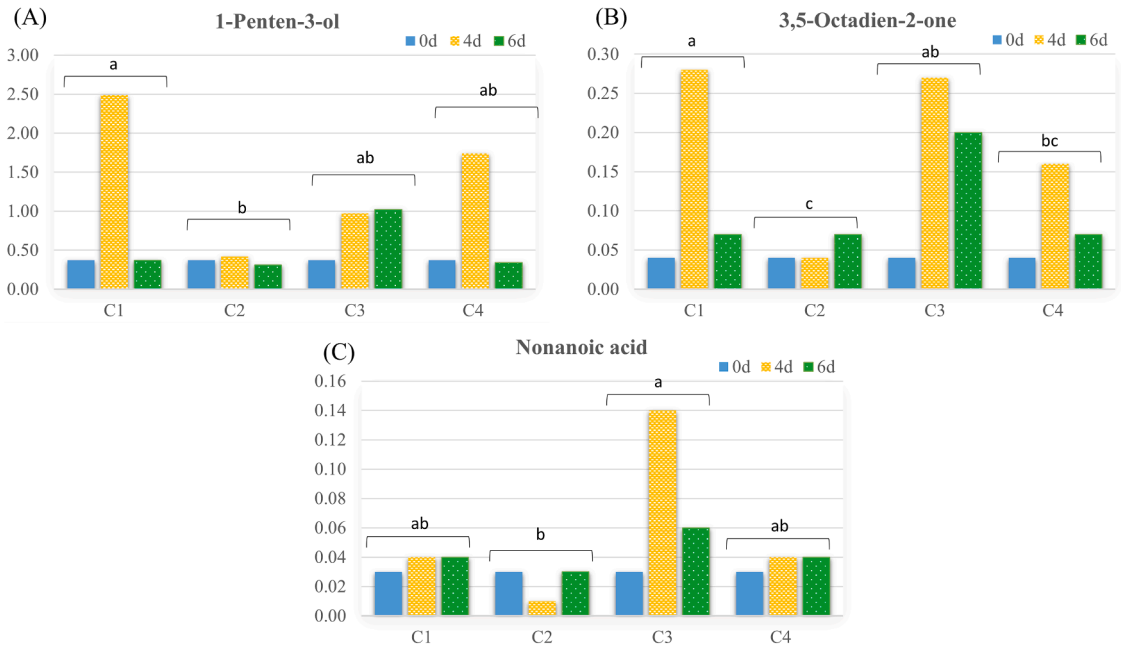

Fig. 6. Changes in concentration of volatile compounds (A) 1-penten-3-ol, (B) 3,5-octadien-2-one and (C) nonanoic acid in *V. corrugata* clams stored at different conditions (C1 - C4) overtime. The values are expressed chromatographic peak /I.S. area. Different letters indicate statistical differences among conditions ($p<0.05$).

and no significant differences among conditions (Table 3). It is characterized by a floral lilac odor (Acree & Arn, 2008; Fratini et al., 2012; van Houcke et al., 2016) was detected in *V. corrugata, O. edulis* (Fratini et al., 2012; Fratini et al., 2013; van Houcke et al., 2016), *C. gigas* (Fratini et al., 2013; van Houcke et al., 2016), *E. ensis* and *P. cornucopia* species (Fratini et al., 2012). In *C. ariakensis* fresh oyster this compound also dominated the aldehyde group (Fu et al., 2024). 2,4-Heptadienal was identified as the main product of EPA degradation through lipid autoxidation (Kawai, 1996) and described as having an extremely rancid odor (Hu & Pan, 2000). Other authors have described this compound as having a mushroom, moss or green odor (Fratini et al., 2012). This compound had low representation among VOCs, but a significant

difference was noted over time (Table 3).

The most representative ketone (2 - 4% in total) was 3,5 octadien-2-one. It showed a large increase in values on day 4 and a decrease on day 6 for conditions C1, C3 and C4. This volatile compound contributes to the fatty fruity odor (Fratini et al., 2012; Hu & Pan, 2000; Kawai, 1996) and has been detected in *C. gigas* (Fratini et al., 2013; van Houcke et al., 2016), *O. edulis* (van Houcke et al., 2016), *E. ensis* and *P. cornucopia* (Fratini et al., 2012). The other ketone 2-nonanone was detected in *V. corrugata, E. ensis, P. cornucopia* (Fratini et al., 2012) as well as in *C. gigas* (Fratini et al., 2013). The values found for 2-nonanone showed the same behaviour of rising on the fourth day and falling on the sixth day for all conditions tested. 2-Undecanone, ketone with lower representation, showed slight variations over time between conditions. This compound has been found in the *E. ensis* and *P. cornucopia* species (Fratini et al., 2012).

Two carboxylic acids (0 - 2%) were identified in all samples under the analytical experimental conditions applied. No reference was found for these compounds in bivalve molluscs, but they were reported as off-odours in fish species in aquaculture (Noguera et al., 2024). Nonanoic acid (pelargonic acid), the most representative within this group, showed significant different values among condition tested (Table 3), with C3 condition (net bag, air) standing out. This compound was found in fish collagen peptides and characterised as having a fatty, musty odor (Mahmoud & Buettner, 2017). Octanoic acid (caprylic acid), although there were no statistical differences among the conditions tested, it also showed the highest values for C3 on days 4 and 6. It is a saturated fatty acid found in Atlantic salmon, aquafeed and rainbow trout and is characterized as fruity-acidic, irritating, musty, coriander-like, fresh and roasty odor (Wang et al., 2023).

The volatile compounds with statistically significant differences among conditions (C1- C4) are showed in Fig. 6.

According to previous results, the largest number of clams died earlier under air conditions. Considering this, the formation and modification of volatiles should also occur at slightly different times. Fig. 6 shows higher values for the compounds evaluated in samples under air conditions (C1, C3) compared with MAP (C2, C4) in addition to a tendency for most compounds to decrease after the death of the clam. Further studies are necessary to understand the evolution of these compounds over time and what impact they have, particularly on the consumer experience with clams in a closed modified atmosphere packaging.

3.4. Glycogen quantification and pH

Glycogen is a principal energy reserve in bivalves and serves as a key physiological marker of stress response and survival under refrigerated storage (Anacleto et al., 2013; Liu et al., 2020). In addition, glycogen also contributes to organoleptic properties. Therefore, the measure of its content is relevant (Gonçalves et al., 2009). The content of glycogen and pH values over time are reported in Fig. 7. The full line represents the values obtained in clams alive, while the dotted line represents the values obtained for dead clams (storage times of 6 and 7 days).

The initial glycogen content of live clams was 25.5 ± 0.6 mg g^{-1} WW (Fig. 7A). Anacleto et al. (2013) reported values of about 17 mg g^{-1} WW for the same species. The values of glycogen decreased overtime particularly between days 0 and 3, with a more severe drop observed in samples under MAP conditions (C2, C4) than in samples under air conditions (C1, C3). Between day 3 and day 7, the values oscillated and, on day 7, it was observed to be significantly lower in value for C2 condition (4.7 ± 0.04 mg g^{-1} WW) in relation to the other conditions tested C1 and C4 (11.6 ± 0.2 mg g^{-1} WW), C3 (9.9 ± 0.2 mg g^{-1} WW). The individual results for glycogen are plotted in Figure S3 (Supplementary material).

Anacleto et al. (2013) demonstrated that *V. corrugata* maintained the metabolic reserves when kept at 4°C in contrast to clams transported at 22°C. Furthermore, when compared to *R. philippinarum*, the native clam showed higher glycogen content than the exotic clam. Bi et al. (2023) studied the species *R. phillippinarum* under different temperatures and modes of transport and found a decrease in glycogen content over time, which was explained by the consequence of environmental stress caused by a rapid change in temperature. Under stressful conditions, clams need to consume more polysaccharides, which leads to a decrease in glycogen content to maintain needs. A correlation has also been suggested with the formation of lactic acid explained by the degradation of glycogen via glycolysis, also mentioned by Chen et al., 2021.

On days 6 and 7 of storage, the glycogen content was quantified in both live and dead clams. On day 6, live clams in condition C1 showed 10.1 ± 0.1 mg g-1 WW while dead clams showed 9.7 ± 0.4 mg g-1 WW. Live clams in condition C2 showed 9.1 ± 0.3 mg g-1 WW, while dead clams showed 6.0 ± 0.0 mg g-1 WW. On day 7, for condition C4, live clams presented 13.0 ± 0.1 mg g-1 WW and dead clams presented 10.1 ± 0.3 mg g-1 WW. The results showed a tendency for lower glycogen values in dead clams than in live clams (Figure S3).

At the beginning of the experiment, the pH of live clams was 6.22 ± 0.01 (Fig. 7B). This value showed a slight increase by day 3 across all conditions. For samples stored in air (C1, C3), a gradual decrease in pH was observed over time, reaching 6.14 ± 0.01 and 6.01 ± 0.01 on day 7, respectively. In contrast, samples stored under MAP conditions (C2, C4) exhibited a more stable pH trend, with values of 6.49 ± 0.05 and 6.32 ± 0.00 on day 7. These findings align with Gonçalves et al. (2009), who studied live *R. decussatus* clams conditioned in MAP (70% O_2: 30% N_2) compared to clams stored in air within net bags at 6.1 ± 0.7°C for 6 days. Their results also showed that clams stored in air had lower pH values than those in high-oxygen conditions. The authors attributed this to the higher oxygen availability allowing the clams to maintain predominantly aerobic metabolism and confirmed that six days of storage were not sufficient to detect signals of oxidative stress, using malondialdehyde (MDA) determination. In contrast, oxygen-restricted conditions induced anaerobic metabolism, leading to acid production and a consequent pH decline (Gonçalves et al., 2009).

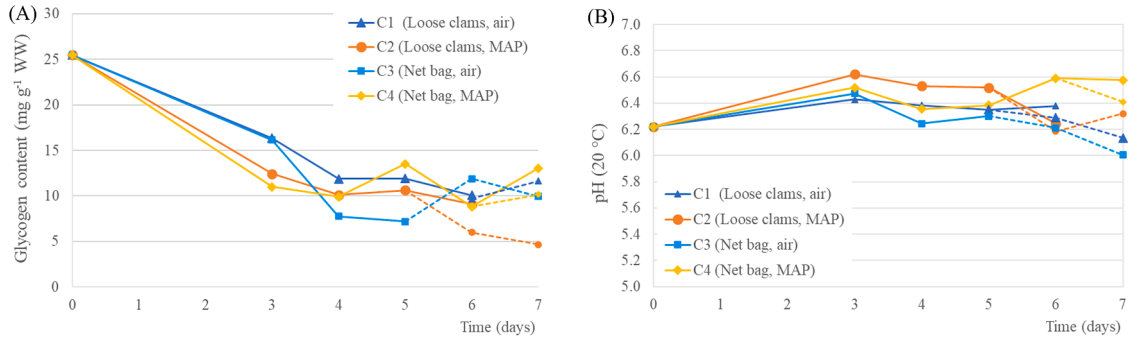

Fig. 7. (A) Glycogen content (mg g-1 WW) and (B) pH of the clams stored at different conditions (C1-C4) overtime. Full line: results for live clams and dotted line: results for dead clams. Average ± standard deviation.

3.5. Biogenic amines

Biogenic amines were evaluated, and results are shown in Supplementary material (Figure S4). The amount of 2-phenylethylamine, isoamylamine and cadaverine showed values below the LOD in the samples evaluated. The results showed that the BAs formation is very low until day 5. Tyramine, an aromatic monoamine originated from tyrosine decarboxylation, was detected only on day 6 and increased the values in day 7 for all conditions. The condition C3 (net bag, air) had the higher value 17.8 mg/kg and the condition C4 the lower value 6.6 mg/kg. In the literature, for crustacean and mollusc species, the highest amount of tyramine recorded was 29.0 mg/kg in sea squirt samples, with an average value of 15.2 mg/kg. It is suggested that the maximum average value for fresh fish, cephalopods, crustaceans and molluscs should not exceed 100 mg/kg, as it has been reported that tyramine can be toxic if consumed at 100-800 mg/kg food (Arulkumar et al., 2023).

Putrescine, an aliphatic diamine originated from ornithine, was also detected on day 7 in conditions C1 and C3 with 3 mg/kg. Although not acutely toxic, putrescine can potentiate the effects of histamine and other BAs, as it favors its adsorption and interfere with the detoxication system (Visciano et al., 2020). In seafood, highest amounts of putrescine were registered for baby octopus' sample (190 mg/kg) and the highest mean value was Japanese mystery snail with 134.1 mg/kg (Arulkumar et al., 2023).

Many authors refer the relation between BAs formation and microorganisms, temperature, NaCl, pH, oxygen availability and other factors (Arulkumar et al., 2023; Visciano et al., 2020). At low pH, microorganisms are more likely to produce decarboxylase as a protective mechanism against acidity. Conversely, conditions that lead to a reduced redox potential, enhance histamine formation. Some bacteria, such as *Pseudomonas,* produce low quantities of BAs when NaCl concentrations are 4-5%, even though decarboxylation reactions are still occurring (Visciano et al., 2020). In this study, microorganisms' counts were not evaluated but in Figure S4 it is possible to note that the higher values of BAs were found in condition C3 (net bag, air) with lower pH (6.01 ± 0.01) on day 7. MAP with different concentrations of gases may provide the optimal conditions for effectively retarding both microbiological and chemical processes, although in the present study this was not targeted, but rather the effect on keeping the clams alive. High O_2 concentrations in MAP (% CO_2/O_2 at 60/40 and 40/60) had an inhibiting effect on the production of BAs, including tyramine and putrescine, in chilled hake (*Merluccius merluccius L.*) stored for 33 days at 2 ± 1°C (Ruiz-Capillas & Moral, 2001). In our study, the condition C4 (net bag, MAP) had the lowest level of tyramine, however C2 (loose clams, MAP) had almost the double of C4 and, for putrescine, C4 and C2 were below the limit of quantification.

3.6. Multivariate analysis of storage and packaging conditions

Principal component analysis (PCA) was applied using all data (all replicates) of survival percentage (% live clams), number of open shells, exudate released by the clams, head space gas concentrations (% O_2 and % CO_2), pH and glycogen content (Fig. 8). The first two components explained 80.97% of the variance (PC1: 60.00%, PC2: 20.97%).

PC1 represented the main spoilage gradient, with positive loadings for % CO_2, amount of exudate and number of open shells, and negative loadings for % live clams. Movement towards positive PC1 values thus indicated progressive deterioration. Fresh samples (C1_0d, C2_0d, C3_0d, C4_0d) clustered at the negative side of PC1 with low variation between replicates and conditions, as expected before storage. Late air-stored samples (C1_7d, C3_7d) rest in the far positive side, reflecting advanced spoilage, related to the low number of live clams, high number of open shells and high % CO_2 produced in head space, aligned with Fig.s 2, Fig. 3A and Fig. 4B, respectively.

PC2 captured variability associated with changes in pH, where higher PC2 scores (e.g., C4_6d, C2_3d) reflected higher pH, and lower

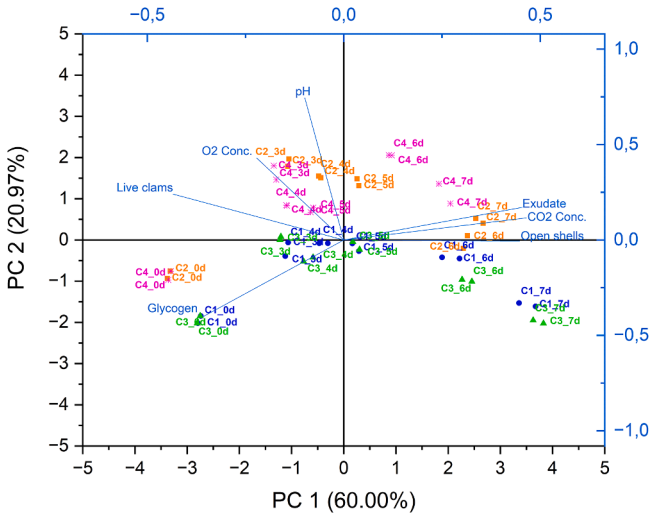

Fig. 8. Score plot of PC1 versus PC2 for parameters of *V. corrugata* samples stored at conditions (C1-C4) over time (0, 3, 4, 5, 6 and 7 days). Data points are shown as blue circles (C1), orange squares (C2), green triangles (C3) and pink stars (C4).

scores (e.g., C3_7d) corresponded to pH decline aligned with Fig. 7B. Glycogen content and % O_2 shared contributions to both components: their loading vectors were oriented partly along PC1 (spoilage gradient) and partly along PC2 (pH-related variability), indicating that these parameters influenced both factors. Samples stored under air (C1, C3) tended to align with negative PC2 scores, while MAP-stored samples (C2, C4) shifted toward positive PC2 values, reflecting the influence of MAP on pH and O_2 dynamics.

MAP-stored clams (C2, C4) also show attenuated spoilage progression along PC1 compared with air (C1, C3). For example, C2_7d scored at PC1 ≈ 2.8 while C1_7d reached ≈ 3.6–3.8. Packaging format further modulated outcomes: under MAP, net bag clams (C4) showed higher pH and O_2 than loose clams (C2) at later storage times.

Zhang et al. (2010) previously demonstrated that PCA effectively depicts transitions from fresh to deteriorated states in seafood through volatile. In the present study, VOCs were excluded from PCA because the other parameters already explained > 80% of the variance and clearly distinguished spoilage progression across conditions. While VOCs could provide additional biochemical detail insight, the selected parameters sufficiently captured the deterioration trends.

Overall, PCA revealed clear distinct differences between storage conditions. Air-stored clams deteriorated most rapidly, whereas MAP delayed spoilage. The effect of the confinement using net bag under MAP (condition C4) showing the greatest preservation.

4. Conclusions

This study evaluated the evolution of specific physiological characteristics of live *V. corrugata* clams during the shelf-life, highlighting the impact of shells confinement through packaging and modified atmosphere.

The analysis of survival rate, number of open clams, and exudate released confirmed the effectiveness of confinement. This extension of shelf-life was directly associated with the lower oxygen consumption rate. The effect of MAP is confirmed and associated with the CO_2 release and glycogen depletion.

The increase in clams' mortality was closely linked to the higher number of opened clams, higher exudate release, originated both from the metabolic water during life and from intravalvular fluid and tissue breakdown after death, together with pH decline and biogenic amine formation.

The effectiveness of packaging in high-oxygen MAP alone, i.e.

without confinement, seems to be not relevant, while the two factors synergically result in increased preservation. Shell confinement was crucial to preserve intravalvular liquid and ensure the beneficial effect of oxygen availability. Without confinement, liquid losses compromised preservation. These findings demonstrate that, while packaging conditions can improve clam survival and biochemical stability, the commercial application of MAP to this species remains challenging. The shelf-life extension by one only day, although corresponding to ca 25% of increase, is still too low, in absolute terms, to justify the investment required for MAP.

Overall, the intrinsic variability of this highly valued species, combined with the sensitivity, represents a major challenge for extending shelf-life. Ensuring that the clams reach the consumers fresh and alive is essential to safeguard product quality and minimize loss.

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Ethics statement

The study supporting the article "Packaging atmosphere and confinement as key factors in shelf-life extension of live clams", does not include experiments on human subjects or collecting and processing of any personal data.

CRediT authorship contribution statement

Cintia Borghetti Goes: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Tiago Monteiro Vieira:** Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Joel Pereira:** Writing – original draft, Methodology, Investigation, Formal analysis. **Ana Mota:** Writing – original draft, Formal analysis. **Andreia Cruz:** Writing – review & editing, Validation, Resources. **Morten Sivertsvik:** Writing – review & editing, Validation, Supervision, Conceptualization. **Fátima Poças:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors report there are no competing interests to declare.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2026.101679. Figure S1: (A) Oxygen consumption rate and (B) Carbon dioxide production rate for each condition (C1 - C4) over time. Average ± standard deviation. (n=2). Figure S2: Volatile organic compounds profile in *V. corrugata* clams at different conditions (C1 - C4) and refrigeration (3 ± 1°C) for 0, 4 and 6 days. The average values are expressed as chromatographic peak /I.S. area. Figure S3: Glycogen content (mg g^{-1} WW) and pH of the clams stored at different conditions (C1 - C4) over time. Within each plot site, different letters on the bars denote significant differences between days of storage ($p < 0.05$, n=10). Legend: blue solid-filled bar means that analysis was done with live clams and orange-dotted bars with dead clams. Figure S4: Biogenic amines (mg/kg) and % live clams stored at different conditions (C1 - C4) overtime. Limit of Quantification (LOQ): 2.0 mg/kg. (n=10).

Data availability

Data will be made available on request.

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