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ORIGINAL ARTICLE

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Strategies to improve the postharvest management of flat oyster (*Ostrea edulis*) from aquaculture using the short-term storage and package in an innovative closed-circuit system

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Abstract: This study aimed to improve postharvest management of flat oysters reared in a longline system in the mid Adriatic Sea, using short-term storage and package in an innovative closed-circuit system. For the trial, 870 oysters were employed, divided into three experimental groups (A, B, and C), $N = 270$ oysters each group, whereas the remaining 60 oysters were used for the 2 controls. Each group differed in relation to the time spent in the depuration tank and the time of packaging: group A was packed and immediately transferred to the cell; group B was depurated in a tank for 48 h, then packed and transferred to the cell; group C was depurated in a tank for 48 h and then packed, depurated for another 24 h and transferred to a cell. Samples of each group were sampled at different times of permanence in cell ($t0$) up until 12 days ($t12$) for biomorphometric, sensorial, nutritional, and microbiological analysis. Although the nutritional and sensorial quality of the oysters was more pronounced in group A, B and C groups also showed good results. In these two groups, thanks to the use of the modern water recirculation system the quality and safety of oysters was improved by reducing the presence of sludge and eliminating fecal contaminants completely than A treatment and seawater control. These results were also confirmed by the tank control, where a more extended depuration period positively influenced the same parameters emphasizing the importance of the adequate depuration processes in oyster production.

KEYWORDS
depuration system, microbiological quality, nutritional quality of seafood, oyster product valorization, sensorial analysis

1 | INTRODUCTION

In marine food production it is becoming increasingly important to meet the growing global demand for animal protein within the global population. Among various types of aquatic organisms, the macroalgae, fish, crustaceans, and molluscs are the most important. In particular, marine bivalves constitute of approximately 18.4% of the total aquaculture production in the world (Food and Agriculture Organization [FAO], 2020) and Italy is among the major producing countries (61.6%). Along the Italian coasts of the mid Adriatic Sea, the cupped oyster (*Crassostrea gigas*) and flat oyster (*Ostrea edulis*) are usually cultivated in longline systems at 3 mi from the seashore. However, due to the highly perishable nature of bivalves and to environmental contaminants, their production requires proper handling and preservation to ensure food safety, quality, and nutritional benefits (Soares & Gonçalves, 2012), furthermore, to increase the reliability of marine bivalves as a healthy food source and to stimulate market demands (Wijsman et al., 2019). In this regard, many shellfish farmers do not have land-based facilities to select, package, and store products. This condition forces shellfish farmers to sell the product in bulk at a low price directly to wholesalers, without exploiting diversification in relation to size, quality, or packaging for retail sale. Moreover, the increasingly frequent strong tidal excursions due to climate change cause negative effects on the quality of oysters that are cultivated within the longline system, resulting in lower shell resistance (especially for *C. gigas*) and a reduced ability to remain closed and retain intervalval water (especially for *O. edulis*). Furthermore, even though the product does not come into direct contact with the seabed, the presence of sediment is often observed in the intertidal cavity water, especially for those living in eutrophic waters with a high level of organic enrichment. In these conditions, the shells of bivalve molluscs, and in particular those of oysters, serve as an underlayer for innumerable species of benthic organisms which live either on the surface of the shells or perforate them and colonize the molluscs from the interior. Worm infestations (*Polydora* sp.) affect the inner shell presentation and reduce the commercial value of oysters, creating unsightly mud blisters that are unappealing to consumers (Martinelli et al., 2020).

Shellfish are also known to be filter organisms able to concentrate a high level of pathogens naturally present in growing areas contaminated with polluted water, making the bivalves a high-risk food group (Bunruk et al., 2013; Chen et al., 2014; López Hernández et al., 2018; Pardío-Sedas, 2015).

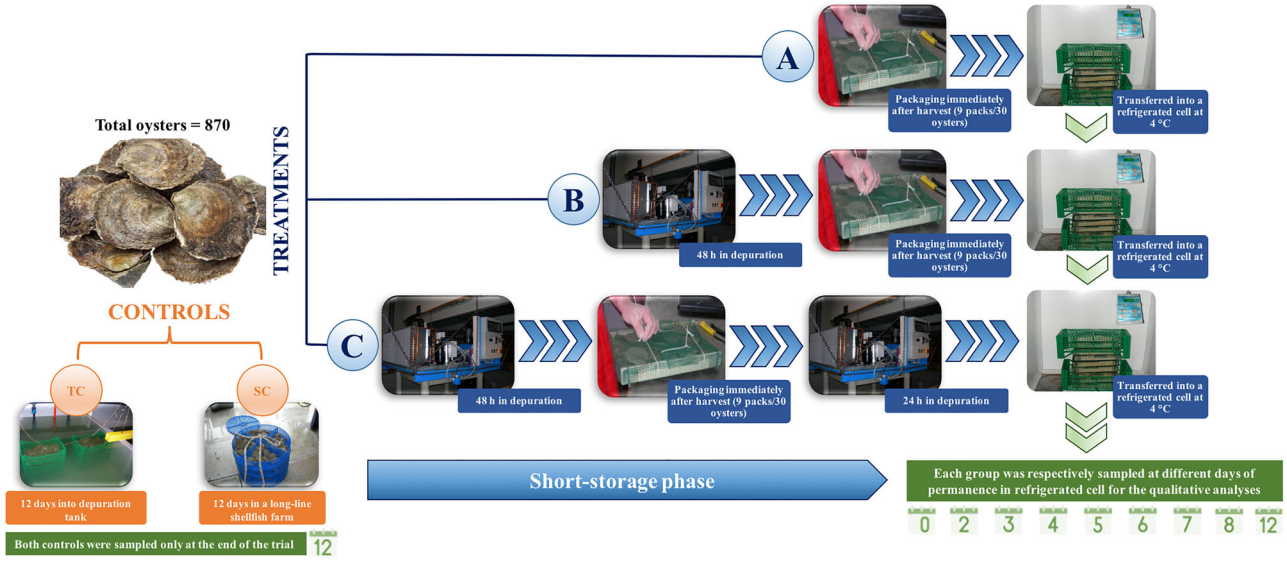
In this context, the finishing period or postharvest processes of bivalves can be useful in improving their microbiological safety (Baker, 2016) and to differentiate farmed oysters from the low-price of unpackaged harvested bivalves, providing added value to aquaculture products (Fratini et al., 2013) able to boost internal demand and exportation (Rong et al., 2010).

Among the different existing postharvest processing for seafood preservation, short storage in land-based tanks working in recirculating water system is a method that offers the ability to maintain oyster viability, freshness, and microbiological safety in a controlled environment, whereas they filter clean salt water that either continuously flows through a holding tank or is recirculated and replenished periodically (Lee et al., 2008). In most depuration systems, out flow water typically undergoes disinfection treatment before returning to the depuration tank through physical (UV irradiation) and/or chemical (chlorine and/or ozone) treatments to eliminate bacteria and other pathogens and spoilage microorganisms (Campbell et al., 2022; López Hernández et al., 2018). However, despite the advantages offered by this type of system, the depuration process up until now has only ever been deeply discussed and studied for shellfish coming from classified waters, according to the extent of microbial fecal contamination as zones B and C (Regulation (CE) 853/2004).

Several studies (Chen et al., 2014; Manousaridis et al., 2005; Ronholm et al., 2016) have also reported the ability of ozone to extend shelf-life especially when combined with refrigerated storage (temperature ranging between 1 and 5°C). Bivalves, in fact, tolerate being out of the water for some time without losing quality if they are not submitted to "trompage," used in oyster culture to reinforce the action of the adductor muscle to maintain the oyster closed and alive longer, during marketing when it is expose to air (Marteil, 1976). Indeed, the conditions in which they find themselves, such as the humidity and temperature when they are out of their natural habitat, may directly affect the nutritional and microbial qualities of seafood (Olafsdóttir et al., 1997).

The lack of a good packaging can cause an inability for oysters to maintain their valves closed and to then retain intervalval water, leading to a reduction in the quality of the product and its shelf life (Bordignon et al., 2020).

Based on these considerations, a study was performed in order to assess the effects of a recirculating water system for different lengths of time (0, 48, and 72 h) on the biomorphometric parameters, the microbiological status, sensorial, and nutritional quality of properly packaged flat oysters (*O. edulis* L.). For the aim, a modern, compact device, equipped with filtration UV and ozone treatment was used. The effects of this oyster management were also monitored for quality and sensory after 12 days in a refrigerated cell (4°C).



SCHEME 1 Experimental design: A–C—three different treatments. SC, seawater control; TC, tank control.

2 | MATERIALS AND METHODS

2.1 | Oysters and experimental design

During the winter (February), after a 3-month finishing period, wild flat oysters (*O. edulis*) were harvested in a longline shellfish farm, located in the middle Adriatic Sea (temperature seawater = 8.5°C), and graded by size on board of the vessel. Then, the oysters were kept in portable coolers and transported to the plant.

In total, 870 flat oysters with an average total weight (TW) of 68.8 ± 17.3 g were employed and divided into three groups/treatments (A, B, and C) of 270 oysters each in turn subdivided into $N = 9$ packs; the remaining 60 oysters were used for the two controls. Each group differed in relation to the time spent in the depuration tank and the time of packaging as reported in the experimental design (Scheme 1).

The packs were made of wooden frames, wrapped in a PVC square mesh net (4 × 4 mm^2), the size of the packs was 33 l × 20 w × 5 h cm. Attention was paid in placing oysters horizontally with the lower valve located at the bottom. In order to keep all the animals in this position and to avoid opening the closure of each pack, straps and lanyards were placed.

The cold cell was exclusively dedicated to host the groups of oysters of the trial.

For the measurement of biomorphometric parameters each group was respectively sampled at the transfer to the cell on the first day ($t0$) and during the following days of permanence in refrigerated cell (from $t2$ to $t12$). For the total antioxidant status (TAS), each group was respectively sampled immediately postharvest and during different days of permanence in refrigerated cell (from $t2$ to $t12$). For biochemical composition and the sterols, vitamin E and fatty acid content in each group were respectively sampled immediately postharvest and at the end of the experiment ($t12$).

2.2 | Short-storage phase using an innovative and compact depuration device

The trials were carried out at an experimental hatchery located 100 m from the coastline. For the short-storage time in depuration, we used a 9 m^3 tank (water level cm 204 l × 428 w × 128 h). Twenty days before starting the trial, the tank was filled with seawater obtained through a sub-sand PVC pipeline system, running 3 m below the sand of the shoreline. This pipeline system transported and filtered water through gravity to fill a 100 m^3 storage tank located near an outdoor area of the facility.

The tank was connected to a recirculating filtration system provided by Acqua&Co (Cadelbosco Sopra, RE, Italy) which assembled all the technology components taking into consideration the minimum space to occupy. In this way, the recirculating filtration system was placed over the same tank, positioned on a platform (cm 88 l × 115 w × 68 h) that was crafted to maintain the system at a safe distance from the water.

The recirculating filtration system consisted of a pump (maximum flow rate 16,000 L/h), a Venturi's tube as an air injector, a system of heating and cooling which controlled and regulated the temperature of the water (set to 9°C), a protein skimmer (0.03 m^3), a UV sterilizing lamp (Helix Max 11 w), and an ozone system (250 mL/h, Steril

250, Innovaqua). A control panel was used to set water temperature and check the regular working of the different components of the circuit.

2.3 | Biomorphometric parameters

Each sampling day, oysters were randomly sampled ($n = 30$). For each sample, the TW, the weight of the top and bottom valves, from which the total shell weight (SW) was derived, and the fresh flesh weight (FW) after opening and draining on absorbent paper were measured on a balance (WLC 20/A2, ±0.1 g, RADWAG). The intervalval water weight (IWW) was calculated by formula 1.

$$IWW = TW - (SW + FW) \quad (1)$$

The condition index (CI) is a tool that is used to estimate the effect that different environmental factors have on oyster meat quality (Rheault & Rice, 1996; Van Dolah et al., 1992). In the present study, it was calculated according to Formula (2) of Baird (1958) as follows:

$$CI = \frac{FW}{(TW - SW)} \times 100 \quad (2)$$

The estimation of some qualitative parameters, such as intervalval water index (IWI), the "presence" or "absence" of sludge in the intervalval cavity, and the opening state of the oysters, was based on the visual observation of the samples and established according to qualitative scales (see Figures 1–3 for the legend of qualitative scales used to classify the samples within each parameter). For each parameter the proportion of individuals that fell within a given quality level was established on a sample of 30 oysters taken from the refrigerated cell.

2.4 | Biochemical composition and fatty acid profile

For each experimental group, the oyster meat was collected from a pool of five specimens, homogenized, and subjected to proximate analysis (protein, lipid, and ash content). The protein content was determined using the Kjeldahl method, and the ash content analysis followed the guidelines of the Association of Official Analytical Chemists (AOAC) procedures (AOAC, 1990).

Total lipid content was measured using the procedure described by Folch et al. (1957). After determining total lipid content, fatty acids were converted into methyl esters following the method described by Christopherson and Glass (1969). The separation of fatty acids was carried out by using a GC 3800 gas chromatograph (Varian Strumentazione) with a WP-4 Shimadzu integration system (Shimadzu Corporation), which was equipped with a Supelco SPTM—2340 capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness; Supelco) and a flame ionization detector. The operating conditions of the gas chromatograph were as follows: The oven temperature was maintained at 170°C for 15 min, increased to 190°C at a rate of 1°C/min, then increased to 220°C at a rate of 5°C/min, and maintained at this temperature for 17 min. The temperature of the injector was 270°C, whereas that of the detector was 300°C. Helium was used as the carrier gas at a constant flow of 1.7 mL/min. The identification of individual fatty acids was accomplished by comparing the retention times to fatty methyl esters of standard mixtures (37 FAME Mix and C22:5*n*3, Supelco).

2.5 | Determination of sterol and vitamin E contents

The sterols content was determined using a gas chromatography method according to the UNI EN ISO 12228-1 July 2014.

Vitamin E content was estimated using 10 g of flesh, according to the ISTISAN 96/34 method. Briefly, after alkaline saponification of the sample and exhaustive extraction of the nonsaponifiables with dichloroethane, the dichloroethane phase was filtered on anhydrous sodium sulfate. An aliquot was evaporated by a rotary evaporator and dried under nitrogen. The extract residue was dissolved in methyl alcohol and a standard solution of vitamin E acetate (Sigma Aldrich 59-02-9), filtered through a 0.45 μm pore size filter, and an aliquot was directly injected into the chromatograph (Agilent), equipped with a db5 (60 m × 0.25 mm) with helium (1 mL/min) as a carrier gas at a constant flow of 1.0 mL/min. Vitamin E was determined by reversed-phase high performance liquid chromatography (RP-HPLC), using a C18 column (5 μm particle size, l × i.d. 25 cm × 4.6 mm, Supelco) kept at 30°C. The mobile phase included methanol–water (98:2, v/v) and the elution was performed at a flow rate of 1.2 mL/min. After separation on an RP-HPLC column, Vitamin E was detected fluorometrically (excitation 292 nm/emission 330 nm).

2.6 | Determination of total antioxidant status

The TAS was measured using the Trolox equivalent antioxidant capacity method with the Randox kit NX2332 (Laboratoires Randox, Montpellier, France), according to the manufactory protocol. Briefly, five muscle samples per group (10 g) were homogenized in a Waring

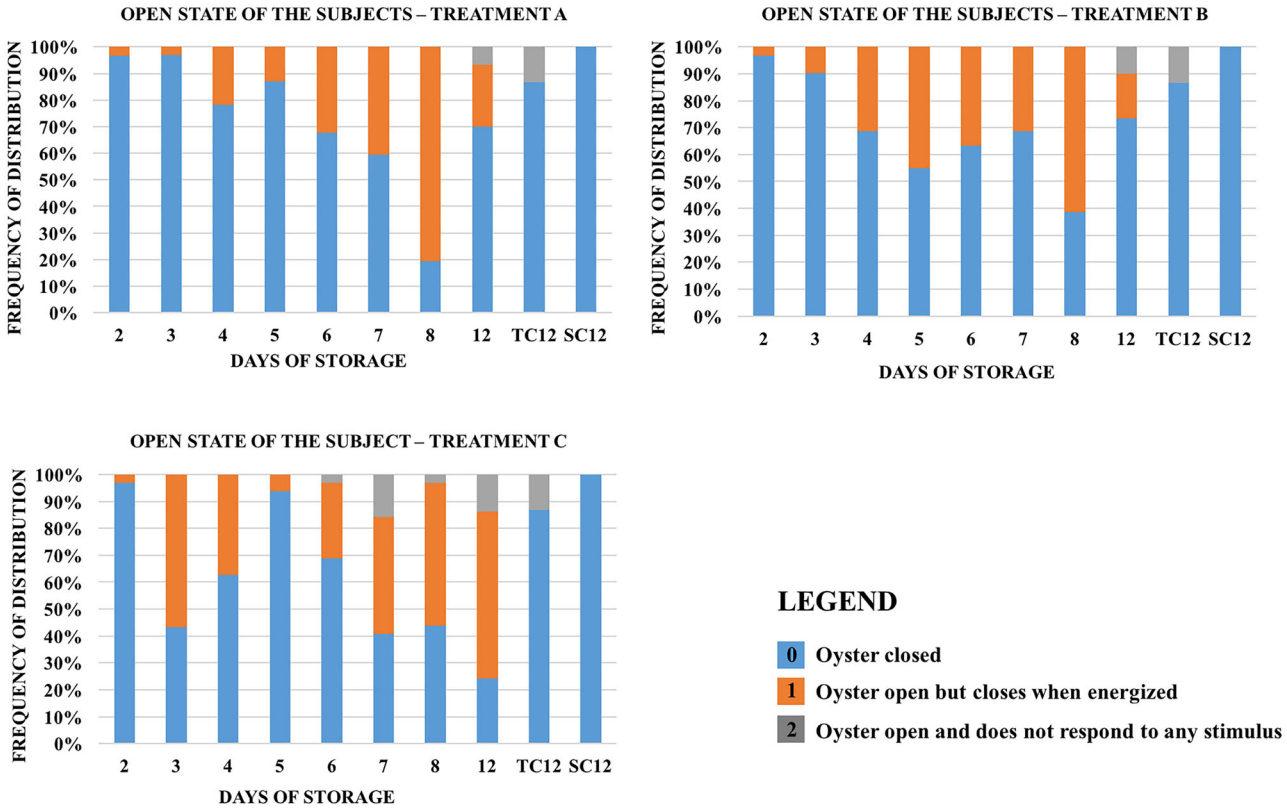


FIGURE 1 Variations in the opening status of oysters according to the treatment undergone (A–B–C) and the cold storage time (*t*2–*t*12) compared to the controls (TC 12—tank control at 12 days; SC 12- seawater control at 12 days). Level 0 (blue)—oyster closed; Level 1 (orange)—oyster open but closes when energized; Level 2 (gray)—oyster open and does not respond to any stimulus.

Blendor with 100 mL of 50 mM phosphate buffer (pH 7.0) and centrifuged at 1000 *g* for 15 min at 4°C (Gatellier et al., 2004). The, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) was incubated with metmyoglobin and hydrogen peroxide to produce ABTS radical cation. Twenty microliters of meat extract were added to the reaction medium, and reduction of ABTS radical cation formation was estimated by measuring the absorbance at 600 nm. Antioxidants present in the sample caused a reduction in absorption proportional to their concentration. The TAS value of the samples tested is expressed as an equivalent of the millimolar concentration of Trolox solution (Erel, 2004).

2.7 | Microbiological analysis

The microbiological analyses were carried out for flesh and intervalval water on a pool of 30 oysters sampled by each treatment at the beginning and at the end of cold storage, as well as by the 2 controls. Quantification of *Escherichia coli* was performed according to the Official International standard method ISO/TS 16649-3:2015 and expressed as most probable number (MPN)/100 g (International Organization for Standardization, 2015). The presence/absence of *Salmonella* spp. in 25 g was carried out according to the method of the Association of French Normalization Organization Regulation AFNOR BIO 16/12-09/05. All microbiological analyses were carried out by the laboratory of the ASUR Marche, Area Vasta 3, Macerata. The evaluation of bacteria involved in fecal contamination was followed through as required by European law [Commission Regulation (EC) no. 2073/2005 and Commission Implementing Regulation (EU) 2019/627] in order to ensure consumer safety.

2.8 | Sensory analysis

At the end of experiment, sensory analysis was voluntarily conducted by a group of untrained panelists (five women and five men), represented by different categories related to the shellfish chain, such as fisherman, shellfish farmer, cooks, and restaurateurs with experience in oyster products. For the trial, each panelist received n. 3 raw oysters/treatments, provided in three different cups with ice in opened shells. Each panelist had to evaluate descriptors of appreciation of edible parts appearance

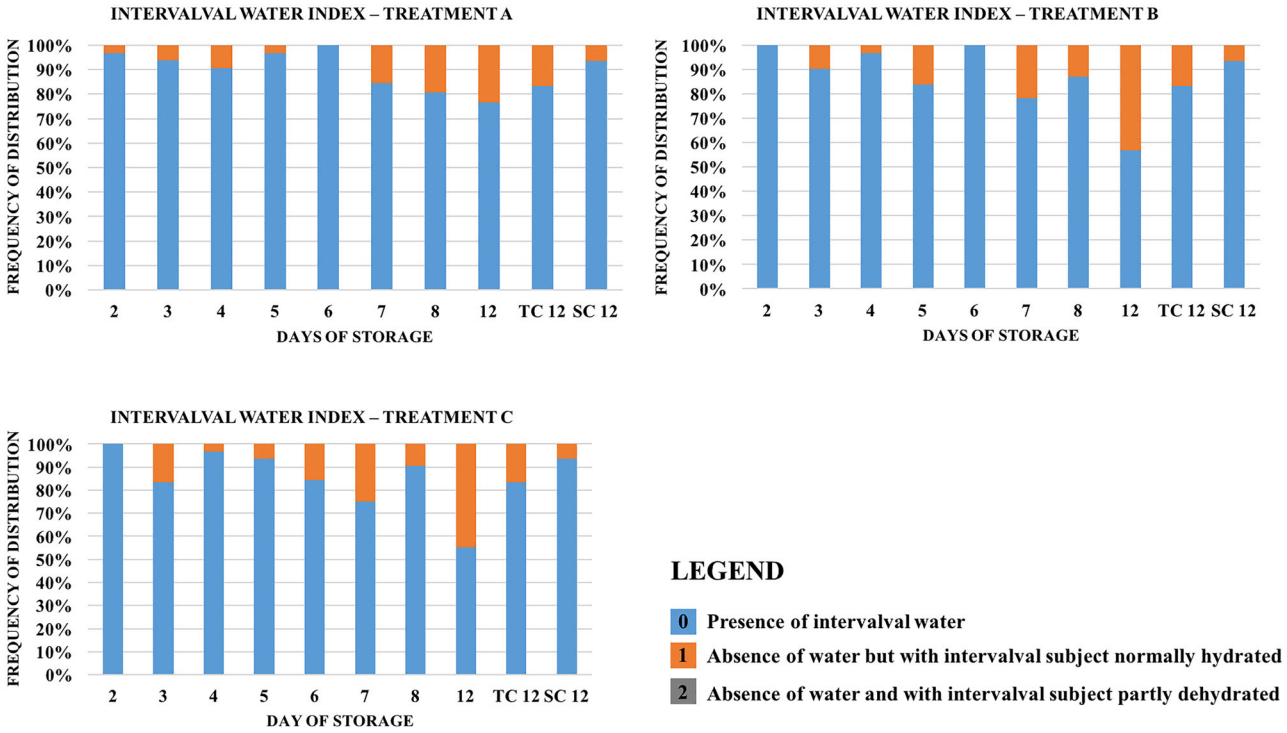


FIGURE 2 Variations in intervalval water index (IWI) of oysters according to the treatment undergone (A–B–C) and the cold storage time (*t*2–*t*12) compared to the controls (TC 12—tank control at 12 days; SC 12—seawater control at 12 days). Level 0 (blue)—presence of intervalval water; Level 1 (orange)—absence of water but with intervalval subject normally hydrated; Level 2 (gray)—absence of water but with intervalval subject partly hydrated.

(white/cream color to transparent), smell (sea/seaweed up to mud/mire), taste (sealike/salty up to bitter/ammonia), and texture (chewiness, hardness, and juiciness) using a scale ranging from 5 (maximum intensity) to 1 (minimum intensity) for each descriptor.

2.9 | Statistical analysis

Data concerning biomorphological parameters (TW, IWW, total SW, fresh FW, and CI), of nutritional traits (chemical composition, fatty acid profile, sterol, and vitamin E content), and of TAS measured at the different treatments and the storage time were submitted to analysis of variance, followed by Duncan's comparison test; whereas sensorial analysis was measured taking into account the different treatments. Generalized Linear Model procedure was used to determine the fixed effects of treatment, time, and their interaction. Significance was set at $p < 0.05$. Correlation between water weight and CI was assessed through Pearson's correlation coefficients, setting significance thresholds at the $p < 0.01$ levels (two-tailed). All statistical tests were conducted using the software package SPSS (IBM SPSS Statistics 23.0 for Windows, 2020; SPSS). Data concerning sensory traits were reported on a graph using Excel.

3 | RESULTS

3.1 | Biomorphometric parameters

The effects of different treatments (A, B, and C) and the cold storage time on oyster biometric parameters are shown in Table 1. At 12 days of storage, no significant differences were observed for the TW, SW, and FW compared to day 0 within each treatment. However, the TW showed a significant increase at day 6 for A and B treatments compared to all other times, except for day 4. During the same storage days, the higher TW values coincided with significantly higher SW and FW. At the end of the trial (day 12), there were no significant differences between in TW and SW for all treatments and both controls; for the FW, instead, the tank control (TC) showed a significantly higher value than B and C treatments, whereas no significant differences were recorded between the seawater control (SC) and the three treatments.

IWW was the only parameter that significantly decreased over time within each treatment; however, this loss did not affect TW as described above. At 12 days of storage, the two controls showed a significantly higher IWW than all of the treatments.

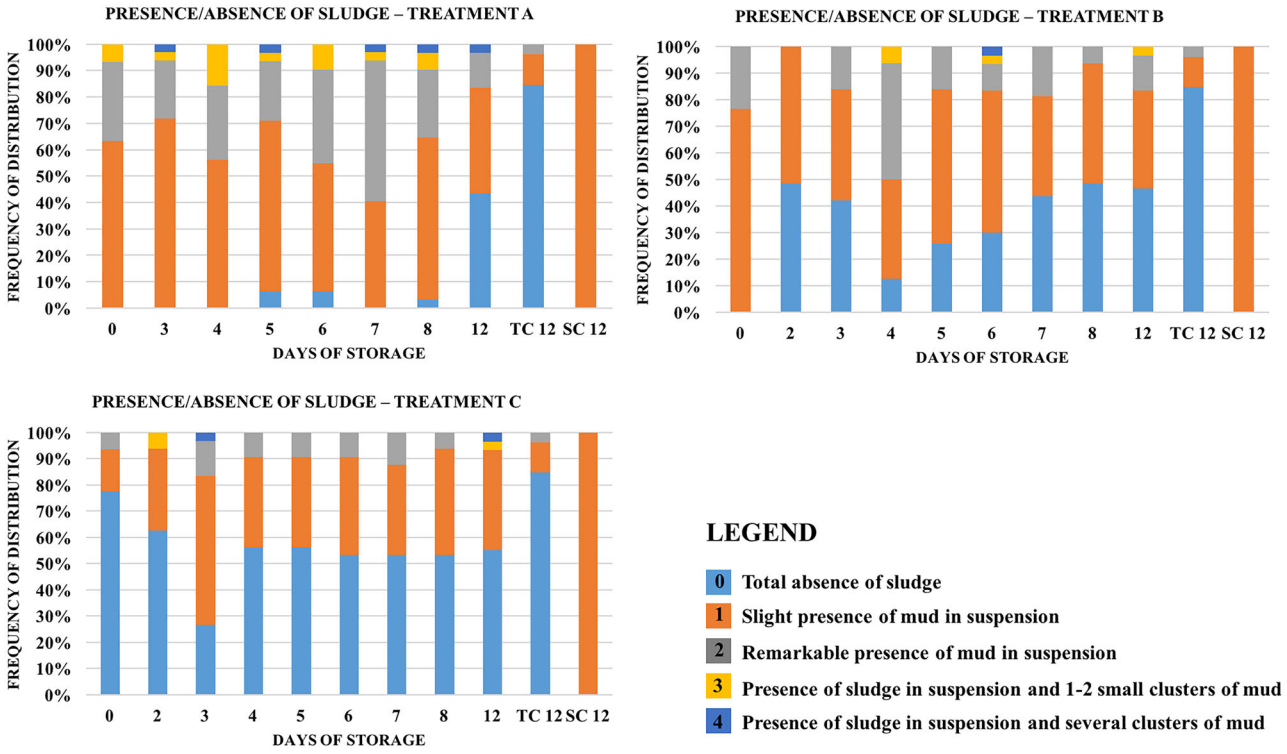


FIGURE 3 Variations in the "presence" or "absence" of sludge in the intervalval cavity of oysters according to the treatment undergone (A–B–C) and the cold storage time (*t*0–*t*12) compared to the controls (TC 12—tank control at 12 days; SC 12—seawater control at 12 days). Level 0 (blue)—total absence of sludge; Level 1 (orange)—slight presence of mud in suspension; Level 2 (gray)—remarkable presence of mud in suspension; Level 3 (yellow)—presence of sludge in suspension and 1–2 small clusters of mud; Level 4 (dark blue)—presence of sludge in suspension and several clusters of mud.

During the 12-day experiment, the CI calculated, on the bases of the FW, had significantly increased over time in all treatments. Interestingly, Pearson's correlation showed that IWW negatively correlated with the CI ($r = -0.95$, $p = 0.000$); therefore, as the IWW decreased, the CI increased. At 12 days, the TC did not show any significant difference compared to all treatments, whereas the SC had a significantly lower CI compared to B and C treatments.

Figure 1 shows the variations in the opening status of oysters according to the treatment undergone and the cold storage time. For all three treatments, there was an increase in the percentage of oysters with open valves that were able to reseal when energized (level 1) over the storage period. However, the C treatment (Figure 1c) showed a decline in quality starting from day 6 of cold storage, with 3.13%, 15.63%, 3.13%, and 13.79% of the samples exhibiting open oysters that were unable to respond to any stimulus (level 2) at 6, 7, 8, and 12 days, respectively. Although A and B treatments (Figure 1a,b), recorded 6.67% and 10% of the samples, respectively, with open oysters that were unable to respond to any stimulus, only at the end of the trial (day 12). At 12 days, the two controls (TC12;SC12) exhibited the highest percentages of oysters with fully closed valves (level 0), although between them TC12 also recorded 13.33% of oysters with open valves that were unable to respond to any stimulus.

The variations in IWI of oysters, according to the treatment they underwent and the cold storage time, are reported in Figure 2. During the 12-day experiment, IWI decreased over cold storage time for all three treatments, with the exception of day 2 for C treatment (Figure 2c) and day 6 for A and B treatments (Figure 2a,b). At the end of the experiment, treatments B and C (Figure 2b,c) recorded the highest percentage of subjects with absence of intervalval water but with normally hydrated flesh (level 1) (43.3% and 44.83%, respectively) compared to A treatment (23.33%). Both controls showed the highest percentage of oysters with the presence of intervalval water (level 0) compared to all treatments.

In regards to the visual evaluation of the "presence" or "absence" of sludge in the intervalval cavity (Figure 3), it was observed that the highest percentage of samples with a total absence of sludge (level 0) was found in treatment C (Figure 3c) for all storage times considered. This was followed by treatment B (Figure 3b), and finally treatment A (Figure 3a). At 12 days, the TC showed the highest percentage of samples with a total absence of sludge compared to all treatments. On the other hand, the SC recorded 100% of

TABLE 1 Biomorphometric parameters of oysters subjected to different treatments and refrigerated storage times compared to the controls at 12 days.

Treatment	Storage time (day)	Total weight (g)	Intervalval water weight (g)	Shell weight (g)	Fresh flesh weight (g)	Condition index
A	0	$72.68 \pm 2.96^{c–g}$	7.29 ± 0.51^{a}	$52.85 \pm 2.30^{b–f}$	$12.53 \pm 0.51^{d–g}$	63.90 ± 1.45^{o}
	2	$74.81 \pm 3.67^{b–e}$	6.97 ± 0.69^{ab}	$54.63 \pm 2.71^{b–e}$	$13.20 \pm 0.64^{b–f}$	67.06 ± 0.85^{lmn}
	3	62.55 ± 2.5^{gh}	$5.52 \pm 0.43^{b–g}$	44.89 ± 1.91^{fg}	$12.13 \pm 0.50^{d–g}$	69.61 ± 1.70^{ilm}
	4	80.95 ± 4.21^{abc}	$6.32 \pm 0.73^{a–d}$	59.83 ± 3.13^{ab}	14.79 ± 0.64^{ab}	$73.03 \pm 1.94^{f–i}$
	5	$74.52 \pm 4.35^{c–f}$	$6.02 \pm 0.59^{a–e}$	55.05 ± 3.42^{bcd}	$13.44 \pm 0.65^{b–e}$	70.63 ± 1.92^{hil}
	6	86.40 ± 2.75^{a}	6.63 ± 0.54^{abc}	64.30 ± 2.22^{a}	15.46 ± 0.54^{a}	70.91 ± 1.80^{hil}
	7	$71.01 \pm 3.36^{c–g}$	$4.31 \pm 0.50^{f–m}$	$53.89 \pm 2.78^{b–e}$	$12.85 \pm 0.46^{c–f}$	$76.66 \pm 1.74^{b–g}$
	8	56.96 ± 2.64^{h}	$3.21 \pm 0.37^{i–n}$	42.73 ± 2.14^{g}	11.01 ± 0.52^{g}	$78.30 \pm 1.75^{a–f}$
	12	68.21 ± 3.15^{efg}	$4.32 \pm 0.52^{f–m}$	$51.71 \pm 2.52^{c–f}$	$12.17 \pm 0.47^{d–g}$	$75.48 \pm 2.08^{d–h}$
B	0	$65.97 \pm 3.13^{e–h}$	7.03 ± 0.52^{ab}	46.33 ± 2.35^{efg}	$12.59 \pm 0.45^{c–g}$	65.10 ± 1.39^{mn}
	2	63.70 ± 2.95^{gh}	$4.93 \pm 0.10^{d–h}$	46.26 ± 2.39^{efg}	$12.49 \pm 0.46^{d–g}$	$72.29 \pm 1.58^{g–l}$
	3	64.09 ± 1.98^{fgh}	$4.36 \pm 0.46^{f–l}$	$46.86 \pm 1.47^{d–g}$	$12.85 \pm 0.47^{c–f}$	$75.84 \pm 1.85^{d–h}$
	4	$79.90 \pm 3.36^{a–d}$	$6.40 \pm 0.26^{a–d}$	58.67 ± 2.61^{abc}	14.82 ± 0.56^{ab}	70.96 ± 1.79^{hil}
	5	$70.05 \pm 3.63^{d–g}$	$4.13 \pm 0.48^{g–m}$	$52.67 \pm 2.89^{b–f}$	$13.24 \pm 0.50^{b–f}$	$77.98 \pm 1.78^{b–f}$
	6	84.11 ± 3.5^{ab}	$5.00 \pm 0.46^{d–h}$	63.80 ± 2.97^{a}	15.30 ± 0.47^{a}	$76.18 \pm 1.58^{c–h}$
	7	63.30 ± 2.58^{gh}	$3.12 \pm 0.40^{i–n}$	$48.49 \pm 2.08^{d–g}$	11.67 ± 0.42^{efg}	$80.68 \pm 1.95^{a–d}$
	8	63.43 ± 2.40^{gh}	$3.16 \pm 0.31^{i–n}$	$47.38 \pm 1.96^{d–g}$	$12.88 \pm 0.46^{c–f}$	$81.10 \pm 1.28^{a–d}$
	12	62.75 ± 3.14^{gh}	$3.30 \pm 0.40^{i–n}$	$47.91 \pm 2.45^{d–g}$	11.54 ± 0.56^{fg}	$79.22 \pm 1.69^{a–e}$
C	0	67.79 ± 3.25^{efg}	6.69 ± 0.37^{abc}	$48.91 \pm 2.64^{d–g}$	$12.17 \pm 0.48^{d–g}$	64.73 ± 1.35^{mn}
	2	$72.53 \pm 4.14^{c–g}$	$5.73 \pm 0.42^{b–f}$	$52.44 \pm 3.36^{b–f}$	14.35 ± 0.64^{abc}	$71.87 \pm 1.46^{g–l}$
	3	$69.92 \pm 2.46^{d–g}$	$3.50 \pm 0.40^{h–n}$	$53.27 \pm 2.06^{b–f}$	$13.15 \pm 0.52^{b–f}$	$79.60 \pm 2.06^{a–d}$
	4	67.76 ± 3.32^{efg}	$3.65 \pm 0.40^{h–n}$	$51.32 \pm 2.57^{c–f}$	$12.78 \pm 0.57^{c–g}$	$79.19 \pm 1.40^{a–e}$
	5	$65.18 \pm 2.62^{e–h}$	$4.66 \pm 0.46^{e–i}$	$48.29 \pm 1.98^{d–g}$	$12.21 \pm 0.53^{d–g}$	$73.70 \pm 1.98^{e–i}$
	6	$64.27 \pm 2.76^{e–h}$	3.06 ± 0.36^{lmn}	$48.34 \pm 2.06^{d–g}$	$12.87 \pm 0.52^{c–f}$	82.21 ± 1.27^{ab}
	7	63.28 ± 1.87^{gh}	2.50 ± 0.36^{n}	$48.54 \pm 1.52^{d–g}$	$12.23 \pm 0.45^{d–g}$	83.89 ± 1.97^{a}
	8	$70.38 \pm 2.99^{d–g}$	$3.74 \pm 0.36^{h–n}$	$52.85 \pm 2.33^{b–f}$	$13.77 \pm 0.64^{a–d}$	$79.11 \pm 1.29^{a–e}$
	12	$64.65 \pm 2.07^{e–h}$	2.77 ± 0.29^{mn}	$50.03 \pm 1.71^{d–g}$	11.84 ± 0.40^{efg}	81.71 ± 1.58^{abc}
Tank control	12	$70.07 \pm 3.35^{d–g}$	$4.69 \pm 0.51^{e–i}$	51.49 ± 2.53^{cf}	$13.80 \pm 0.65^{a–d}$	$76.05 \pm 2.07^{c–h}$
Seawater control	12	68.11 ± 2.44^{efg}	$5.37 \pm 0.32^{c–g}$	$49.53 \pm 1.86^{d–g}$	$13.20 \pm 0.47^{b–f}$	$71.35 \pm 1.19^{g–l}$
Treatment effect		$p = 0.011$	$p = 0.000$	$p = 0.108$	$p = 0.009$	$p = 0.000$
Time effect		$p = 0.000$	$p = 0.000$	$p = 0.000$	$p = 0.000$	$p = 0.000$
Treatment × time effect		$p = 0.000$	$p = 0.001$	$p = 0.000$	$p = 0.000$	$p = 0.005$

Note: Different superscript letters (a–o) within the same column indicate a significant difference ($p < 0.05$).

the samples with a slight presence of mud in suspension (level 1).

3.2 | Biochemical composition

The chemical composition of the oysters was influenced by both the treatment and the cold storage times, with the exception of the ash content (Figure 4). For the protein percentage, on day 12, treatment A showed a significantly higher value both in respect to the day in which the oysters were harvested from the sea (postharvest) and in respect to the other treatments and the two controls. Treatment B did not show any significant difference compared to postharvest and treatment C. The two controls recorded the lowest protein percentage ($p < 0.05$) in respect to all of the treatments.

The initial lipid contents underwent a significant decrease during the cold storage time for all of the treatments. The lowest percentage ($p < 0.05$) was reached for the oysters of the B treatment, followed by A and C treatments, between which no significant difference was found.

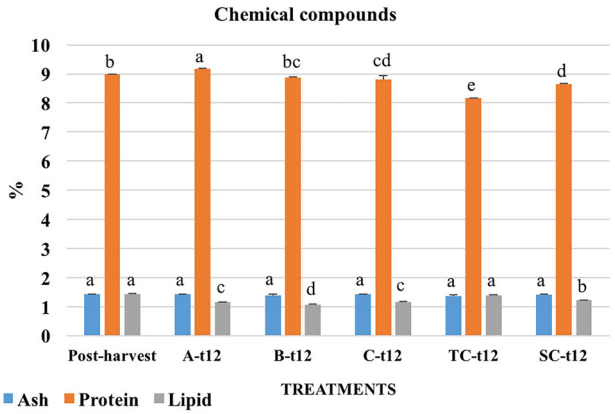


FIGURE 4 Effect of different short-storage treatments (A-*t*12—treatment A at 12 days; B-*t*12—treatment B at 12 days; C-*t*12—treatment C at 12 days) on percentage of chemical compounds compared to the postharvest oysters and controls (TC-*t*12—tank control at 12 days; SC-*t*12—seawater control at 12 days). Blue bars—ash percentage; orange bars—protein percentage; gray bars—lipid percentage. Different letters on the bars of the same color indicate a significant difference ($p < 0.05$).

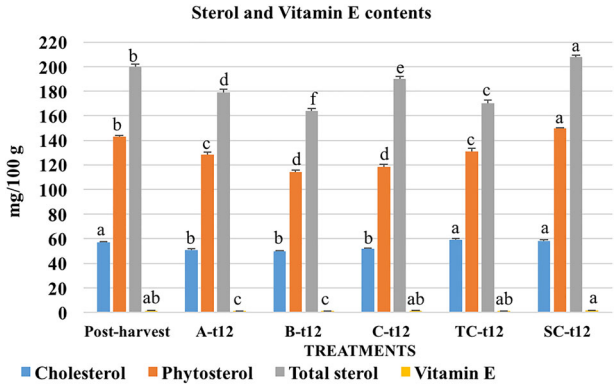


FIGURE 5 Effect of different short-storage treatments (A-*t*12—treatment A at 12 days; B-*t*12—treatment B at 12 days; C-*t*12—treatment C at 12 days) on the sterols and vitamin E content compared to the postharvest oysters and controls (TC-*t*12—tank control at 12 days; SC-*t*12—seawater control at 12 days). Blue bars—cholesterol; orange bars—phytosterol; gray bars—total sterol; yellow bars—vitamin E. Different letters on the bars of the same color indicate a significant difference ($p < 0.05$).

Both controls showed a higher lipid percentage ($p < 0.05$) compared to all of the other treatments.

Regardless to the type of the treatment and the cold storage time, results recorded for the chemical composition are in agreement with those reported by other authors for 100 g of raw oyster (Sáenz, 2007).

3.3 | Fatty acid profiles

The fatty acid profiles of oysters that underwent different treatments and cold storage times are shown in Table 2.

Total saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) were most abundant, followed by monounsaturated fatty acids (MUFAs). Among the fatty acids detected in the present study, C16:0 (palmitic acid) was the major fatty acid, followed by C20:5*n*3 (EPA), C22:6*n*3 (DHA), and C18:1*n*9 (oleic acid) in all of the treatments, these results were comparable with those observed by van Houcke et al. (2016). From immediately postharvest to the end of each experiment (A-*t*12, B-*t*12, and C-*t*12), total SFA and MUFA significantly decreased; on the contrary, total PUFAs, including total $n - 3$ PUFAs and $n - 6$ PUFA, showed a significant increase. Consistent with these latter results, the ratio *n*3/*n6* was higher at the end of the experiments (*t*12) compared to postharvest for all treatments.

Among treatments, the highest total SFAs values ($p < 0.05$) were recorded for treatments A and B, which were also higher than the TC, whereas they showed no significant difference in SC. The total MUFAs were not affected by the treatments. Total PUFAs, including $n - 3$ PUFAs and $n - 6$ PUFAs, were significantly higher in the C treatment compared to all other treatments and both controls. However, the A treatment had the best *n*3/*n*6 ratio due to the lower value of total $n - 6$ PUFA recorded compared to B and C treatments.

3.4 | Sterol and vitamin E content

For vitamin E, a small but significant decrease was observed over time, with the exception of the C treatment at 12 days. Vitamin E contents were significantly higher in the C treatment and both controls than A and B treatments. No significant differences were found between the C treatment and both controls (Figure 5).

Total sterol content (cholesterol and phytosterols) underwent a significant decrease over time for all treatments. Moreover, the two controls showed significantly higher values compared to all of the treatments for all components analyzed, except for the content of phytosterols between A treatment and TC. Among treatments no differences ($p < 0.05$) in the cholesterol content were found, whereas the highest values of phytosterol and total sterols ($p < 0.05$) were founded for the A treatment (Figure 5).

3.5 | Total antioxidant status

A significant decrease in the antioxidant status of oysters over time was observed in all treatments (Table 3). Specifically, treatment A showed a significant decrease at as early

TABLE 2 Fatty acid profile of oysters subjected to different treatments, from postharvest to at the end of the refrigerated storage time (*t*12) compared to the controls.

Fatty acid	Postharvest	A-*t*12	B-*t*13	C-*t*12	TC-*t*12	SC-*t*12
Saturated (SFA)						
C14:0	9.35 ± 0.13^{a}	5.94 ± 0.19^{c}	6.26 ± 0.13^{bc}	4.95 ± 0.23^{d}	6.57 ± 0.22^{b}	6.48 ± 0.21^{b}
C15:0	0.81 ± 0.17^{ab}	0.52 ± 0.02^{b}	1.05 ± 0.02^{a}	0.95 ± 0.02^{ab}	0.68 ± 0.08^{ab}	0.65 ± 0.06^{ab}
C16:0	34.87 ± 0.93^{a}	28.30 ± 0.59^{b}	26.26 ± 0.25^{bc}	21.14 ± 0.45^{d}	24.94 ± 0.47^{c}	28.15 ± 0.39^{b}
C17:0	2.01 ± 0.17^{a}	1.62 ± 0.08^{ab}	1.65 ± 0.05^{ab}	1.64 ± 0.21^{ab}	1.43 ± 0.13^{b}	1.32 ± 0.07^{b}
C18:0	9.64 ± 0.17^{a}	5.95 ± 0.61 cd	7.21 ± 0.11^{b}	6.83 ± 0.36^{bc}	5.39 ± 0.28^{d}	6.32 ± 0.23^{c}
C20:0	0.23 ± 0.04^{ab}	0.12 ± 0.008^{c}	0.34 ± 0.03^{a}	0.30 ± 0.05^{ab}	0.20 ± 0.02^{bc}	0.17 ± 0.01^{bc}
Total SFA	56.93 ± 0.67^{a}	42.47 ± 0.19^{b}	42.79 ± 0.23^{b}	35.82 ± 0.20^{d}	39.22 ± 0.12^{c}	43.10 ± 0.94^{b}
Monounsaturated (MUFA)						
C14:1	0.11 ± 0.01^{b}	0.11 ± 0.01^{b}	0.10 ± 0.00^{b}	0.00 ± 0.00^{c}	0.10 ± 0.00^{b}	0.20 ± 0.05^{a}
C16:1 $n-7$	8.24 ± 0.14^{a}	5.91 ± 0.25^{c}	5.30 ± 0.20^{d}	6.92 ± 0.25^{b}	5.91 ± 0.08^{c}	5.91 ± 0.16^{c}
C18:1 $n-9$	16.66 ± 0.23^{a}	9.16 ± 0.12^{c}	10.58 ± 0.35^{b}	9.33 ± 0.18^{c}	9.74 ± 0.20^{c}	9.42 ± 0.27^{c}
C20:1 $n-9$	1.68 ± 0.24^{a}	1.60 ± 0.24^{a}	1.28 ± 0.19^{a}	1.64 ± 0.21^{a}	1.52 ± 0.04^{a}	1.39 ± 0.16^{a}
Total MUFA	26.70 ± 0.46^{a}	16.79 ± 0.06^{b}	17.27 ± 0.06^{b}	17.89 ± 0.22^{b}	17.28 ± 0.16^{b}	16.84 ± 0.53^{b}
Polyunsaturated (PUFA $n-3$)						
C18:3 $n-3$	2.47 ± 0.11^{a}	2.60 ± 0.01^{a}	1.46 ± 0.07^{c}	2.26 ± 0.07^{ab}	2.08 ± 0.08^{b}	2.34 ± 0.12^{ab}
C18:4 $n-3$	0.59 ± 0.05^{d}	1.79 ± 0.09^{a}	1.12 ± 0.08^{c}	1.92 ± 0.05^{a}	1.29 ± 0.02^{bc}	1.48 ± 0.12^{b}
C20:3 $n-3$	0.61 ± 0.01^{f}	3.26 ± 0.08^{a}	2.06 ± 0.08^{d}	2.90 ± 0.04^{b}	2.34 ± 0.08^{c}	1.35 ± 0.05^{e}
C20:5 $n-3$ (EPA)	4.60 ± 0.10^{d}	16.19 ± 0.09^{c}	17.34 ± 0.24^{b}	18.31 ± 0.32^{a}	17.81 ± 0.04^{ab}	16.05 ± 0.48^{c}
C22:5 $n-3$	0.00 ± 0.00^{e}	0.06 ± 0.02^{d}	0.12 ± 0.00^{c}	0.16 ± 0.01^{b}	0.20 ± 0.00^{a}	0.12 ± 0.02^{bc}
C22:6 $n-3$ (DHA)	2.98 ± 0.02^{f}	12.24 ± 0.16^{e}	12.59 ± 0.05^{d}	15.64 ± 0.02^{a}	14.61 ± 0.11^{b}	13.47 ± 0.29^{c}
Total PUFA $n-3$	8.28 ± 0.15^{d}	23.91 ± 0.12^{b}	22.12 ± 0.40^{c}	25.56 ± 0.30^{a}	23.73 ± 0.18^{b}	21.36 ± 0.79^{c}
Polyunsaturated (PUFA $n-6$)						
C18:2 $n-6$	2.10 ± 0.07^{a}	1.81 ± 0.06^{ab}	2.03 ± 0.14^{a}	1.57 ± 0.20^{b}	1.96 ± 0.13^{a}	1.91 ± 0.06^{a}
C20:4 $n-6$	2.98 ± 0.08^{ab}	2.77 ± 0.05^{b}	3.15 ± 0.09^{ab}	3.39 ± 0.27^{a}	3.17 ± 0.11^{ab}	3.23 ± 0.15^{a}
Total PUFA $n-6$	8.07 ± 0.10^{f}	16.83 ± 0.03^{e}	17.78 ± 0.22^{d}	20.61 ± 0.06^{a}	19.75 ± 0.09^{b}	18.62 ± 0.51^{c}
Total PUFA	16.35 ± 0.21^{d}	40.74 ± 0.13^{c}	39.90 ± 0.18^{c}	46.17 ± 0.25^{a}	43.48 ± 0.28^{b}	39.99 ± 1.30^{c}
$n-3/n-6$	1.02 ± 0.02^{d}	1.42 ± 0.01^{a}	1.24 ± 0.03^{b}	1.23 ± 0.01^{b}	1.20 ± 0.01^{bc}	1.14 ± 0.01^{c}

Note: Different superscript letters (a–f) within the same row indicate a significant difference ($p < 0.05$).
Abbreviation: MFA, monounsaturated fatty acid; PFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

as 3 days of storage, reaching a significantly lower value at *t*12 compared to treatments B and C and the two controls. Treatments B and C exhibited a significant decrease starting from the fifth to sixth day of storage, respectively. Compared to the controls, these two treatments reached significantly higher values at the end of the experiment only in respect to the TC. At 12 days, the SC recorded the best antioxidant status compared to all others ($p < 0.05$).

3.6 | Microbiological analysis

Data reported in Table 4, showed that after 2 (treatment B), 3 (treatment C), or 8 days (TC) of depuration, independently by the treatment, the concentrations of *E. coli* in 100 g flesh and intervalval water decreased from 9 to 0 MPN/100 g, maintaining an absence of fecal contamination up until the end of the trials. A slight decrease of the *E. coli* value was observed when oysters underwent treatment A (without depuration), which remained in any case well below the acceptability limit of 230 MPN/100 g both at the beginning and at the end of the cold storage period (from 8 to 7 MPN/100 g), whereas the *E. coli* value in SC maintained the same value after 12 days (9 MPN/100 g).

The low values of *E. coli* found in the oysters immediately postharvest are due to the fact that the samples came from waters classified as A zone. *Salmonella* spp. was always completely absent.

TABLE 3 Total antioxidant status of oysters subjected to different treatments and refrigerated storage times compared to the controls at 12 days.

Treatment	Storage time (day)	Total antioxidant status (mmol/L equivalent Trolox)
A	Postharvest	8.14 ± 0.01^{b}
	2	8.12 ± 0.01^{b}
	3	7.89 ± 0.02^{c}
	4	7.51 ± 0.10^{d}
	5	6.70 ± 0.12^{f}
	6	6.06 ± 0.03^{g}
	7	5.30 ± 0.07^{h}
	12	2.50 ± 0.15^{m}
B	Postharvest	8.14 ± 0.01^{b}
	2	8.14 ± 0.01^{b}
	3	8.10 ± 0.01^{b}
	4	8.10 ± 0.02^{b}
	5	7.70 ± 0.14^{c}
	6	7.30 ± 0.05^{e}
	7	6.55 ± 0.05^{f}
	12	4.62 ± 0.09^{i}
C	Postharvest	8.14 ± 0.01^{b}
	2	8.15 ± 0.00^{b}
	3	8.14 ± 0.01^{b}
	4	8.14 ± 0.01^{b}
	5	8.10 ± 0.02^{b}
	6	7.84 ± 0.05^{c}
	7	7.22 ± 0.03^{e}
	12	4.62 ± 0.10^{i}
Tank control	12	4.02 ± 0.09^{l}
Seawater control	12	9.16 ± 0.01^{a}
Treatment effect		$p = 0.000$
Time effect		$p = 0.000$
Treatment × time effect		$p = 0.000$

Note: Different superscript letters (a–l) within the same column indicate a significant difference ($p < 0.05$).

3.7 | Sensorial assessment

At the end of the experiment, appearance, smell taste, and texture scores of oysters that were only packaged and refrigerated (A treatment) were significantly higher than depurated oysters (B and C treatments). No significant differences were found between B and C treatments for smell and texture, while for appearance of edible parts and taste, significantly lower values were recorded in treatment C. These results indicated that the panelists detected a decrease of all qualitative attribute of the flesh of oysters that were subjected to the depuration process (Figure 6), although the oysters were still acceptable for raw consumption.

4 | DISCUSSION

In the present study, the biomorphometric parameters, the nutritional and sensorial traits, and the microbiological status were used as valid indicators to investigate the effect of an innovative depuration device on the quality

TABLE 4 Microbiological analysis of oysters subjected to different treatments.

	Time	*Escherichia coli* (MPN/100 g)	*Salm.* presence/absence in 25 g	Storage time (days)	*Escherichia coli* (MPN/100 g)	*Salm.* presence/absence in 25 g	Storage time (days)	*Escherichia coli* (MPN/100 g)	*Salm.* presence/absence in 25 g
Sea	Postharvest	9	Absent	/	/	/	*t*12	9	/
A				*t*0	8	Absent	*t*12	7	Absent
B				*t*0	0	Absent	*t*12	0	Absent
C				*t*0	0	Absent	*t*12	0	Absent
Tank control				/	/	/	*t*12	0	/

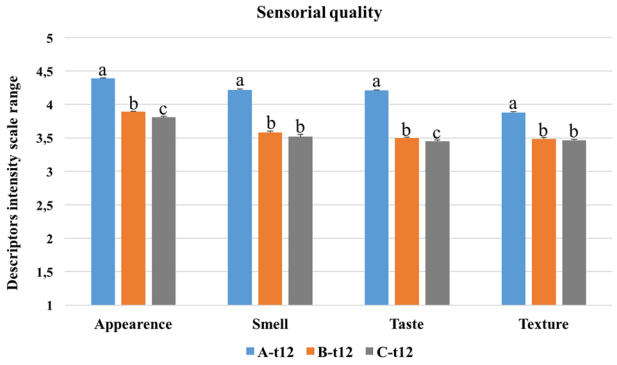


FIGURE 6 Effect of the treatments (A-*t*12—treatment A at 12 days; B-*t*12—treatment B at 12 days; C-*t*12—treatment C at 12 days) on the sensorial quality of the oysters. Different letters on the bars of the same color indicate a significant difference ($p < 0.05$).

of flat oyster (*O. edulis* L.). This system could be adopted as a postharvest management process before oysters' commercialization.

The biomorphometric parameters are simple measures to asses shellfish quality (Buzin et al., 2011). Nevertheless, they have been hardly used for similar studies so far. Regarding these measurements, in our study, was recorded a consistent loss of IWW over time for all treatments (A, B, and C) than in TC and SC, suggesting that the storage time in the refrigerated cell has a negative influence on this parameter. However, an interesting finding that emerged from our data is that this drastic decrease in IWW did not correspond to a simultaneous loss in TW (Table 1). At the same time, according to the negative correlation found between IWW and the CI, it was observed that as the IWW decreased and the CI increased. Overall, these results could suggest that intervalval water was transferred to the flesh to reintegrate water lost from the flesh during cold storage. Consistent with this assumption, the results recorded for the IWI showed that, despite a certain percentage of oysters with absence of intervalval water that was reported over time, the flesh always remained hydrated in all treatments up to 12 days (Figure 2). This condition could explain why water weight loss did not lead to a simultaneous decrease in the TW, as well as an increase in the weight of fresh meat. Unlike our study, Buzin et al. (2011) observed a significant decrease of the FW and TW after 8 and 15 days of cold storage, respectively, arranging the samples in bulk. These authors explained that the water from the flesh was transferred into the intervalval water, which in turn was lost after 15 days, with a concomitant significant decrease in the TW and dehydrated flesh. In this regard, we believe that the packaging system used in our study, where was paid attention to placing oysters horizontally with the lower valve located at the bottom, helped keep the valve closure and maintain homogeneous conditions

of temperature and humidity of the product, regardless to the treatment. It is important to remember that in order to keep all of the animals in this position and to avoid that they could open; the closure of each pack was assured by straps and lanyards. This kind of packaging is commonly applied by French shellfish farmers to commercialize oysters, whereas it is not common in Italy where the producers are more used to managing mussels that only require a labeled net to be placed on the market.

Concerning the open state of the oysters, treatments A and B showed a low percentage of open oysters that were unable to respond to any stimulus, only at the end of the trial (day 12). Moreover, this percentage was lower than that observed in the TC. Conversely, C treatment showed a decline in quality starting from day 6 of cold storage, reaching a percentage of open oysters that were unable to respond to any stimulus close to or higher than that of TC. These latter findings suggest that longer depuration times that precede the time in refrigeration cell could induce an alteration in the adductor muscle strength needed to keep the two oyster valves firmly closed. However, no treatments or controls showed gaping or dead oysters, probably due to the low storage temperature, where the metabolic rates of the oysters were kept at a maintained level as reported by Chinnadurai et al. (2013).

On the other hand, in agreement with Buestel et al. (2009), our results showed that a storage phase in filtered seawater (TC, C, and B treatments) results in cleaner oysters. Moreover, consistent with other similar studies, our system improved the product safety through complete elimination of *E. coli* (Bernard, 1989; Campbell et al., 2022). Conversely, the treatments in which the oysters did not undergo the depuration phase in filtered seawater (treatment A and SC) had the highest percentage of samples with a slight presence of mud in suspension and the *E. coli* concentration remained almost the same. Concerning the SC, although it was not in direct contact with the seabed, the consistent presence of mud in the intervalval cavity could have been due to the particularly rough sea during the winter period in which the experiment was performed. Overall, these finding revealed that the device essayed is able to assure high-quality water conditions during short-term storage, which may explain the significant reduction of sand and mud inside the oyster's pallial cavity and the complete elimination of *E. coli*. This result was achieved also without a biological filtration; it was essayed the possibility to employ a water recirculation system devoid of a biological filter, considering that this component should have occupied more space and could be removed for short-time storage of shellfish. This device could also be useful against mud worm (*Polydora* spp.) which use oyster shells as substrate to construct burrows, therefore causing damages to the organism and compromising marketability (Cilenti et al., 2017; Martinelli et al., 2020).

Another interesting result obtained in our study concerns the TAS. This parameter showed a significant decrease over time of storage in the cell for all treatments, although it was more pronounced in treatment A (without depuration). In this regard, Freitas et al. (2012) demonstrated that depuration reduces the oxidative stress of two clam species (*Ruditapes decussatus* and *Ruditapes philippinarum*), which is probably the reason for the highest TAS founded in the samples subjected to depuration processes in our experiment. A recent study investigated, in vitro, the effect of various essential oils against murine norovirus in order to assess their potential use as a depuration treatment in the shellfish industry for the reduction of the risks of exposure to this virus in oysters (Cozzi et al., 2023). However, the antioxidant properties of essential oils able to prevent autoxidation and prolong food shelf-life are widely known (Amorati et al., 2013). In this regard, the possibility to implement a depuration treatment of shellfish based on the use of these natural compounds could represent a valid future perspective to increase the effectiveness of this postharvest process on the oyster's antioxidant stability.

Regarding the biochemical composition, it is known that high quality of protein, low lipid content, and especially high proportion of PUFAs characterize the mollusc flesh, contributing to their nutritional value and organoleptic characteristics (Orban et al., 2002). In our study, the depuration step seems to have been the main cause of the protein loss in oysters. In fact, at the end of the experiment, the protein percentage was higher in samples submitted to treatment A compared to the TC, which recorded the lowest value, followed by SC, and the C and B treatments. Several studies reported that the ozone used as a disinfectant to increase the efficiency of depuration could have a negative effect on the protein level (Pardío-Sedas, 2015), leading to various modifications in their structure, composition, and functionality (Cataldo, 2003; Uzun et al., 2012). In particular, Pardío-Sedas (2015) demonstrated that, as a function of amount of ozone, the denaturation of muscle proteins has effects on free amino acids, shear force, and texture, leading to an undesirable degradation phenomenon in the oyster. Similarly, the results obtained by our sensorial analysis showed a significant decrease of all qualitative attributes for the samples subjected to the depuration system (B and C treatments) compared to those only packaged and refrigerated (A treatment), although the oysters were still acceptable for raw consumption. The ability to maintain high sensorial quality of oysters during the cold storage (refrigerated or super chilled) was also reported by other authors (Buzin et al., 2011; Dong et al., 2023). As showed by our results, it is possible that the oysters that were not purified were able to maintain a greater

percentage of oysters with the valves completely closed or open, but closes when stimulated and consequently able to retain the intervalval water and therefore freshness for longer. Thus, further investigations are needed in order to determine the changes in structural and functional properties of oyster proteins related to the amount of ozone used during the depuration process.

On the other hand, ozone seems not to be the factor responsible for the decrease of the lipid content in our study, consistent with what reported by Chen et al. (2014). These authors reported that the extent of PUFA oxidation is related to lipid content in food and exposure to O_3. In our study, the oysters subjected to the different treatments showed a significantly decrease in the percentage of lipids over time, mainly affecting the sterol, SFA, and MUFA content. Conversely, at the end of trials an increase in the amounts of total PUFA was observed, especially for the C treatment and TC, which underwent the most prolonged depuration process. In this regard, it is possible that in our study the depuration process made a difference. Freitas et al. (2012) revealed that depuration reduces the oxidative stress. Moreover, Çağlak et al. (2017) showed that the Mediterranean Mussel (*Mytilus galloprovincialis* Lamarck, 1819) contained higher PUFA levels after 72 h in a closed-circuit purification system than the initial samples (0th hour). In particular, the increment of $n-3$ PUFAs in our study may have been of endogenous origin, namely, C16:0 and C18:0 acids were converted C18:2 $n-6$ and C18:3 $n-3$ in a first instance and later to C20:4 $n-6$, C20:5 $n-3$ (EPA), and C22:6 $n-3$ (DHA) via fatty acid desaturation and elongation enzymatic steps. The ability of bivalves to metabolize essential PUFA precursors *ex novo* was demonstrated by Liu et al. (2013).

Moreover, was also observed that the variation of vitamin E content, although significant, was very small among postharvest and treatments A and B. This finding holds significant important as vitamin E represents the main lipophilic antioxidant in the muscle tissue of many fish and shellfish species, including oyster (Passi et al., 2002). In this regard, it is possible that it protected a large variety of oxidatio $n-$ sensitive biomolecules, including PUFAs during treatments and the cold storage period undergone to the oysters. It might be likely that all together these processes led to a predominance of total $n-3$ PUFAs compared to $n-6$ PUFAs obtained during the cold storage which led to an $n-3/n-6$ ratio superior to 1, which is the ratio recommended to have beneficial effects on human health (Chow, 2008). These results can be useful to producers as a powerful marketing tool, in the promotion of food of high economic and nutritional values, because the consumption of these fatty acids is increasingly recommended by health authorities (Grienke et al., 2014; Sargent & Tacon, 1999; Torstensen et al., 2004).

5 | CONCLUSIONS

From this study, group A treatment exhibited excellent results in nutritional quality ($n3/n6$ PUFA ratio and protein percentage) good hydration and flesh yield (CI), as well as a major sensorial quality for flat oysters. The oyster packaging system, combined with cold room storage, proved crucial for short-term preservation, also effectively preventing microbial growth at 4°C storage.

Our innovative device has had a positive influence on the significant reduction of the incident of sand and mud inside the oyster's pallial cavity, making the product cleaner, on the increase of microbiological safety, completely eliminating fecal contaminants, and on the flesh yield. Furthermore, extended depuration periods (C treatment and TC) positively impacted the total PUFAs content, as well as $n-3$ PUFAs and $n-6$ PUFAs.

Overall, these finding revealed that the essayed treatment device, associated with the refrigerated storage, could be recommended as a potential system for postharvest management of the Adriatic Sea flat oysters, although the potential effect of different amount of ozone on the proteins structural and sensorial changes will be investigated in more depth in future studies. The possibility to storage the products in land-based facilities rather than in the sea could in fact allow shellfish farmers to have more control over their product, enabling better selection, packaging, and traceability, ultimately maximizing market economic value for direct commercialization.

AUTHOR CONTRIBUTIONS

Giusy Rusco and Michele Di Iorio: Data curation; formal analysis; writing—original draft. **Alberto Felici**: Conceptualization; investigation; methodology; writing—original draft. **Livio Galosi**: Writing—original draft; data curation. **Nicolaia Iaffaldano**: Methodology; data curation; writing—original draft; writing—review and editing. **Alessandra Roncarati**: Conceptualization; investigation; methodology; writing—original draft; writing—review and editing; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

The trial was performed according to the European Directive 2010/63/UE and the Italian Legislative Decree 26/2014.

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