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The effect of high oxygen modified atmospheres on the quality degradation of packed live blue mussels (*Mytilus edulis*)

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ABSTRACT

Oxygen (O_2) plays a vital role in the blue mussel (*Mytilus edulis*) survival, microbial growth, and volatile organic compounds (VOCs) formation during transport and storage. This study measures the impact of different atmospheres (% $CO_2/O_2/N_2$): 30/40/30; 40/60/0; and 0/60/40 on blue mussels' spoilage. Quality parameters were monitored at 4 °C for up to 16 days of storage, including headspace composition (% CO_2/O_2), pH, mortality, microbial analysis, and VOCs concentrations. Selected-ion flow-tube mass spectrometry (SIFT-MS) was used for real-time VOCs measurements. At the end of storage, odor activity values (OAVs) were calculated to assess VOCs that contributed to the overall odor profiles (OAV ≥1). Different atmospheres result in a different shelf life in which B60 condition (% $CO_2/O_2/N_2$: 40/60/0) prolonged the life span of blue mussels for nine days of storage. A total of 23 compounds were identified in packed blue mussels. The evolution of ethanol; 3-methyl-1-butanol; 2,3-butanediol; acetone; 2,3-butanedione; dimethylamine (DMA); dimethyl sulfide; dimethyl disulfide; and methyl mercaptan were associated with mortality and total plate count (TPC >7 log CFU g^{-1}). This study identifies dimethyl sulfide and methyl mercaptan as the primary contributors to malodor of blue mussels at the end of storage (OAVs = 182–66716).

1. Introduction

European shellfish culture is dominated by blue mussels (*Mytilus edulis*) and Mediterranean mussels (*M. galloprovincialis*). The total mussel production in 2018 was 533,000 tonnes, of which 82% were traded in live or fresh condition (Hough, 2022). Besides being an excellent source of nutrients, live bivalves are vulnerable to mortality and spoilage. Insufficiency of oxygen and an increase of temperature result in metabolic changes and stress, which are the primary causes of mortality (Li, Sun, Zhang, Wang, & Li, 2019; Pastoriza, Bernárdez, Sampedro, Cabo, & Herrera, 2004). Right after mussels perish, either autolysis, lipid oxidation, and microbial activity are significantly increased. The degradation of carbohydrates, amino acids, and fatty acids leads to metabolite production affecting further deterioration (Bongiorno et al., 2015; Muller et al., 2012).

Modified atmosphere packaging (MAP) is typically applied in food industry in which the air surrounding the product is replaced by an alternative gas mixture (mainly CO_2, O_2, N_2, or combined) (Vanderroost, Ragaert, Devlieghere, & De Meulenaer, 2014). Studies on different atmosphere compositions with a combination of O_2 and N_2 have been done on Mediterranean mussels, while none of those studies addressed blue mussels (Bernárdez & Pastoriza, 2011, 2013; Odeyemi, Burke, Bolch, & Stanley, 2018; Pastoriza et al., 2004). Moreover, how atmospheric conditions influence the mortality and metabolism in blue mussels is still insufficiently understood.

VOCs such as alcohols, aldehydes, ketones, acids, esters, sulfuric

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compounds, and amines are associated with the spoilage of marine products (Boziaris & Parlapani, 2017; Wierda, Fletcher, Xu, & Dufour, 2006). The unpleasant odors related to VOCs formation are often caused by microbial metabolism. Specific spoilage organisms (SSOs) such as *Shewanella* spp., *Photobacterium phosphoreum*, *Pseudomonas, Brochothrix thermosphacta,* and *Enterobacteria* showed metabolic activity that results in sensorial changes (Kuuliala et al., 2018; Leroi, 2010; Odeyemi et al., 2018; Parlapani, Mallouchos, Haroutounian, & Boziaris, 2017). Thus, packaging technologies should support product quality by enabling respiration and preserving the product's freshness (Odeyemi et al., 2018). For this reason, oxygen-rich atmospheres can be considered as an approach to maintain live mussel quality.

Selected-Ion Flow-Tube Mass Spectrometry (SIFT-MS) is a real-time, non-destructive, fast, and sensitive device to evaluate VOCs' evolution. SIFT-MS has been used for several purposes, e.g., examining the VOCs in packages of autoclaved shrimp (Broekaert, Noseda, Heyndrickx, Vlaemynck, & Devlieghere, 2013), raw Atlantic salmon (Kuuliala et al., 2018), peeled gray shrimp (Noseda, Goethals, et al., 2012), poultry (Ioannidis et al., 2018), and cheese (Castada, Wick, Harper, & Barringer, 2014), but not yet for live blue mussels packed under high O_2 atmospheres. This study aimed to evaluate the effect of oxygen-rich atmospheres on the spoilage of fresh blue mussels stored at a low temperature. The relationship between mortality, microbial growth, and VOCs production is investigated to clearly identify spoilage metabolism and malodor formation during refrigerated storage.

2. Materials and methods

2.1. Sample preparation

Live blue mussels (*Mytilus edulis*) with a bodyweight of ~15 ± 5 g (jumbo size) were brought from the Netherlands. The sample was packed in a jute bag and transported to the Research Unit Food Microbiology and Food Preservation (FMFP) the day after, with the temperature maintained at 2.0–7.0 °C during transportation. In this research, two different batches of mussels were employed at separate time points (August and October 2020) and labelled as Batches 1 and 2, to evaluate the effect of natural variation from biological replication.

2.2. Packaging and storage

After arrival, 10 to 12 individual live mussels were packed (150 ± 2 g) using a MECA 900 tray sealer (DecaTechnic, Herentals, Belgium). Multilayer trays (dimensions 187 × 137 × 25 mm, black PP/EVOH/PP, O_2 transmission rate 0.5–13 cm^3/m^2.24 h.0.1 MPa at 23 °C, and 0% RH; Decapac, Herentals, Belgium) and cover film (OPA/EVOH/OPA/PE/PP, O_2 transmission rate 6.57 0.5–13 cm^3/m^2.24 h.0.1 MPa at 23 °C, and 50% RH, Bemis, France) were used for packaging. The mussels were packed under three different gas conditions (% $CO_2/O_2/N_2$), i.e. A40 (30/40/30), B60 (40/60/0), and C60 (0/60/40) with a gas/product ratio of 2 : 1 (mL: g) before being stored at 4.0 ± 0.7 °C for up to 16 days. Regularly (days 0, 2, 5, 7, 9, 12, 14, and 16), three random packages were analyzed for each batch of the experiment to conduct technical replication.

2.3. Headspace composition (% CO_2/O_2), pH, and mortality

The headspace gases (% CO_2/O_2) were measured using a gas analyzer (CheckMate® 9900 CO_2/O_2, Dansensor A/S, Ringsted, Denmark). The pH of mussel's liquid was determined at 6.2 ± 0.7 °C using a pH electrode (Lab® 427, Mettler Toledo GmbH, Schwerzenbach, Switzerland) connected with a pH meter (SevenCompact, Mettler Toledo AG, Schwerzenbach, Switzerland). Before measurement, a saline peptone solution (PPS; 0.85% g/dL NaCl, 0.1% g/dL peptone) was used to homogenize a total of 20 g of mussel flesh at a ratio of 1:9 (g: mL). The mortality was evaluated by tapping on the mussel shells, at which the shells that remained open were considered dead. The mortality is expressed as the percentage of dead mussels per package. The proposed retail quality standard for packed live blue mussels entails ≤20% mortality per package (Bernárdez & Pastoriza, 2011).

2.4. Microbial analysis

Mussel flesh was aseptically weighed (20.0 ± 0.2 g) in a sterile stomacher bag, then ten-fold diluted in PPS. Stomacher Lab Blender (LED Techno, Heusden-Zolder, Belgium) was used for 1 min to homogenize the sample prior to serial dilutions. The spread plate method was used to determine the total psychotropic count (TPC) on Marine Agar (MA, Difco Le Pont de Claix, France); to determine *Pseudomonas* on *Pseudomonas* Agar (PA, Oxoid) with the addition of *Pseudomonas* CFC supplement (SR 103 E, Oxoid); and to determine *Brochothrix thermosphacta* on Streptomycin Sulfate Thallous Acetate Actidione Agar (STAA, Oxoid) supported with selective supplement (SR 151 E, Oxoid). The pour plate method was used to enumerate lactic acid bacteria (LAB) on Man Rogosa Sharpe Agar (MRS, Oxoid, Hampshire, UK); hydrogen sulfide (H_2S) producers on Iron Agar Lyngby (IAL, Oxoid) supplemented with L-cysteine hydrochloride (Fluka, Steinheim, Germany); and Enterobacteriaceae on Violet Red Bile Glucose Agar (VRBGA, Oxoid). Plates were incubated at 37 °C for one day (VRBGA), 22 °C for two (PA and STAA), three (MRS and IAL), and five (MA) days.

2.5. Quantification of VOCs by SIFT-MS

A selected-ion flow-tube mass spectrometer (Voice 200, Syft Technologies™, Christchurch, New Zealand) was used to quantify VOCs in the package headspace (suction rates SIFT-MS: 17.9–33.2 mL/min). The target compounds (Table 1) were selected based on literature (Kuuliala et al., 2019; Noseda, Goethals, et al., 2012). The MAP package was connected to a nitrogen bag with Teflon tubing to avoid the internal package from collapsing during the SIFT-MS measurement. For each package, the headspace VOCs concentrations were measured for 300 s. In addition, a Nalophan bag (Nalophan™, Scentroid, and Whitchurch-Stouffville, ON, Canada) containing 100% nitrogen was used as a background.

All measured concentration were adjusted in accordance to suction rates. The average, standard deviation, and relative standard deviation (SD%) of the target compound concentrations were calculated for each package. In case multiple product ions were measured for a target VOC, one product ion was selected for further quantification based on the following criteria: 1) compound concentration had SD% < 25% for each atmosphere condition, 2) large branching ratio, 3) avoid conflicts of m/z, 4) the product ion resulting in the lowest VOC concentration was chosen (Kuuliala et al., 2019).

The limit of quantification (LOQ) is the smallest concentration that can be quantitatively examined at its true value, and was calculated by using IUPAC equation (Noseda, Ragaert, et al., 2010):

$$LOQ = X_{background} + 6^{*}SD_{background} \quad (1)$$

where $X_{background}$ is the average and $SD_{background}$ is the standard deviation of concentrations measured in the background (100% N_2 bag). LOQ values were calculated for each VOC at each day of analysis. The screening of spoilage indicators was determined by comparing compound concentrations to the LOQ. VOCs with a concentration > LOQ in at least 33% from all atmospheric packages were considered for further statistical analysis (Kuuliala et al., 2019). The LOQ values are presented in Supplementary Tables 1 and 2 Accordingly, 13 out of 23 compounds listed in Table 1 were relevant for all atmospheric conditions.

2.6. Odor activity value

Odor activity values (OAVs) were determined by calculating the ratio

Table 1
Precursor ion, mass to charge ratios (m/z), branching ratios (b), reaction rate coefficients (k), and product ions for volatile organic compounds (VOCs) quantification by using selected-ion flow-tube mass spectrometry (SIFT-MS).

VOC	Precursor ion	m/z	b (%)	k (molecule cm^{-3} s^{-1})	Product ion
Alcohols					
2,3-Butanediol	NO^+	89	100	2.3 E−09	$C_4H_9O_2^+$[a]
	NO^+	107		2.3 E−09	$C_4H_9O_2^+.H_2O$
Ethanol	NO^+	45	100	1.2 E−09	$C_2H_5O^+$[a]
	NO^+	63			$C_2H_5O^+.H_2O$
	NO^+	81			$C_2H_5O_+.2(H_2O)$
3-Methyl-1-butanol	H_3O^+	71	100	2.8 E−09	$C_5H_{11}^+$[a]
	NO^+	87	85	2.3 E−09	$C_5H_{11}O^+$
Isobutyl alcohol	NO^+	73	95	2.4 E−09	$C_4H_9O^+$
	O_2^+	33	50	2.5 E−09	CH_5O^+
Aldehydes					
3-Methylbutanal	NO^+	85	100	2.4 E−09	$C_5H_9O^+$[a]
Nonanal	NO_2^+	69	100	3.20E−09	$C_9H_{17}^+$[a]
Decanal	NO^+	155	100	3.30E−09	$C_{10}H_{19}O$
Acids					
Acetic acid	NO^+	90	100	9.0 E−10	$NO^+.CH_3COOH$
	NO^+	108		9.0 E−10	$NO^+.CH_3COOH.H_2O$
3-Methylbutanoic acid	NO^+	132	70	2.5 E−09	$C_5H_{10}O_2{\cdot}NO^+$
Ketones					
Acetone	NO^+	88	100	1.2 E−09	$NO^+.C_3H_6O$[a]
Acetoin/Ethyl actate	NO^+	118	100	2.5 E−09	$C_4H_8O_2{\cdot}NO^+$[a]
2,3-Butanedione	NO^+	86	65	1.3 E−09	$C_4H_6O_2^+$[a]
Butanone	NO^+	102	100	2.8 E−09	$NO^+.C_4H_8O$
Esters					
Ethyl acetate/Acetoin	H_3O^+	89	100	2.9 E−09	$CH_3COOC_2H_5{\cdot}H^+$
	H_3O^+	107		2.9 E−09	$CH_3COOC_2H_5{\cdot}H^+.H_2O$
	O_2^+	31	20	2.4 E−09	CH_3O^+[a]
	O_2^+	61	40	2.4 E−09	$C_2H_5O^{2+}$[a]
Amines					
Ammonia	H_3O^+	18	100	2.6 E−09	NH_4^+
	H_3O^+	36			$NH_4^+.H_2O$
	O_2^+	17	100	2.4 E−09	NH_3^+
Dimethylamine (DMA)	H_3O^+	46	100	2.1 E−09	$(CH_3)_2NH.H^+$[a]
Trimethylamine (TMA)	NO^+	59	100	1.6 E−09	$(CH_3)_3N^+$
Sulfur compounds					
Carbon disulfide	O_2^+	76	100	7.0 E−10	CS_2^+
Dimethyl sulfide	H_3O^+	63	100	2.2 E−09	$(CH_3)_2S{\cdot}H^+$
	NO^+	62	100	2.2 E−09	$(CH_3)_2S^+$[a]
	O_2^+	47	25	2.2 E−09	CH_3S^+
	O_2^+	62	60	2.2 E−09	$(CH_3)_2S^+$
Dimethyl disulfide	H_3O^+	95	100	2.6 E−09	$(CH_3)_2S_2{\cdot}H^+$
	NO^+	94	100	2.4 E−09	$(CH_3)_2S_2^+$[a]
Dimethyl trisulfide	H_3O^+	127	100	2.8 E−09	$C_2H_6S_3H^+$ [a]
	H_3O^+	145		2.8 E−09	$C_2H_6S_3H^+.H_2O$
	NO^+	126	100	1.9 E−09	$C_2H_6S_3^+$
Hydrogen sulfide	H_3O^+	35	100	1.6 E−09	H_3S^+
	H_3O^+	53		1.6 E−09	$H_3S^+.H_2O$
Methyl mercaptan	H_3O^+	49	100	1.8 E−09	$CH_4S{\cdot}H^+$[a]
	H_3O^+	67		1.8 E−09	$CH_4S{\cdot}H^+.H_2O$

[a] Product ions selected for real-time VOCs quantification.

of the compound concentration with the olfactory threshold (OT). The OAVs with scores >1 indicate that the VOCs contribute to the overall odor (Zhou et al., 2021). OAVs were calculated from the end-of-storage data to investigate the contribution of a single compound to the overall spoilage odor.

2.7. Statistical analysis

Data were analyzed by using IBM SPSS statistics 27.0. Parametric factorial pattern ANOVA was conducted with CO_2/O_2, mortality, pH, and microbial growth data as dependent variables, while headspace conditions and storage time were treated as independent variables. Normality was measured by Q-Q plots, and the Levene test was used to assess homogeneity. For the post hoc analysis, the Tukey-b test was applied. Estimated marginal means were employed to assess the difference batches of experiment (Batch 1 and Batch 2).

3. Results

3.1. Headspace composition (% CO_2/O_2)

Headspace compositions were measured to assess the atmosphere change inside the A40, B60, and C60 packages and compared between the two batches of experiment (Table 2). Similar trends were obtained in both experiments, in which oxygen decreased as carbon dioxide increased throughout the storage period. By the end of storage, O_2 levels on mussels packed under the B60 atmosphere were maintained at 2.4 (Batch 1) and 24.4% (Batch 2). Conversely, the production of CO_2 increased to 63.8–84.3% over storage time. Mussel packages with 60% oxygen combined with 40% nitrogen (C60) exhibited anaerobic conditions after days 9 (Batch 1) and 12 (Batch 2), as O_2 levels ran out and CO_2 concentrations increased during storage. Anaerobic atmospheres were also reached for A40 on days 12 and 16 in Batch 1 and Batch 2, respectively. The experiments demonstrate that a dynamic change in the headspace occurred differently for each atmospheres.

Table 2
Oxygen (O_2), carbon dioxide (CO_2) concentrations (%), and pH value under different atmospheres (% $CO_2/O_2/N_2$): A40 (30/40/30), B60 (40/60/0), and C60 (0/60/40).

Analysis	Headspace	Time of storage (d)							
		0	2	5	7	9	12	14	16
Experiment 1									
O_2 (%)	A40^{z}	42.7 ± 1.5^{a}	38.7 ± 0.8^{a}	27.0 ± 5.2^{b}	21.0 ± 5.3^{b}	4.8 ± 4.2^{c}	0^{c}	0^{c}	0^{c}
	B60^{x}	63.2 ± 0.8^{a}	52.8 ± 5.2^{b}	46.3 ± 2.2^{bc}	38.3 ± 2.4^{cd}	26.3 ± 7.6^{ef}	12.4 ± 6.2^{fg}	17.8 ± 2.6^{gh}	2.4 ± 3.3^{i}
	C60^{y}	59.1 ± 2.7^{a}	51.6 ± 5.2^{b}	28.0 ± 2.4^{c}	13.3 ± 5.2^{d}	0^{e}	0^{e}	0^{e}	0^{e}
CO_2 (%)	A40^{y}	24.8 ± 1.9^{a}	27.5 ± 0.8^{a}	39.3 ± 3.2^{b}	45.8 ± 4.3^{c}	60.6 ± 2.4^{d}	67.3 ± 1.0^{e}	68.8 ± 0.2^{e}	71.2 ± 2.3^{e}
	B60^{x}	35.3 ± 1.1^{a}	22 ± 8.8^{a}	37.1 ± 1.4^{b}	44.1 ± 0.9^{b}	55.9 ± 5.7^{c}	71.7 ± 3.7^{d}	71.0 ± 1.9^{d}	84.3 ± 3.5^{e}
	C60^{z}	2.4 ± 10.0^{a}	16.6 ± 10.0^{b}	28.8 ± 2.1^{c}	44.1 ± 1.4^{d}	58.0 ± 0.3^{e}	58.7 ± 0.2^{e}	63.4 ± 1.7^{e}	63.8 ± 4.0^{e}
pH	A40	6.3 ± 0.1^{a}	6.2 ± 0.0^{a}	6.2 ± 0.1^{a}	6.2 ± 0.0^{a}	6.1 ± 0.1^{ab}	6.1 ± 0.2^{ab}	5.9 ± 0.1^{b}	
	B60	6.3 ± 0.1^{a}	6.3 ± 0.1^{a}	6.3 ± 0.0^{a}	6.2 ± 0.0^{ab}	6.1 ± 0.1^{ab}	6.1 ± 0.1^{ab}	5.9 ± 0.1^{c}	6.0 ± 0.1^{bc}
	C60	6.3 ± 0.1^{a}	6.1 ± 0.1^{a}	6.2 ± 0.1^{a}	6.2 ± 0.2^{a}	6.0 ± 0.0^{a}	6.1 ± 0.2^{a}	5.7 ± 0.1^{b}	
Experiment 2									
O_2 (%)	A40^{z}	40.6 ± 0.9^{a}	39.4 ± 1.8^{a}	31.4 ± 0.3^{b}	28.0 ± 2.4^{b}	22.2 ± 1.3^{c}	11.3 ± 0.8^{d}	4.9 ± 4.2^{e}	0.1 ± 0.5^{e}
	B60^{x}	59.1 ± 0.8^{a}	58.8 ± 1.6^{a}	51.4 ± 1.30^{b}	47.9 ± 3.2^{bc}	45.0 ± 2.0^{cd}	34.3 ± 2.6^{e}	29.6 ± 1.1^{f}	24.4 ± 1.8^{g}
	C60^{y}	61.6 ± 1.3^{a}	50.8 ± 2.5^{b}	37.6 ± 2.5^{c}	28.7 ± 2.7^{d}	18.7 ± 5.7^{e}	0^{d}	0^{d}	0^{d}
CO_2 (%)	A40^{y}	27.7 ± 4.7^{a}	22.9 ± 0.7^{a}	28.4 ± 0.9^{a}	35.8 ± 1.5^{b}	39.3 ± 1.5^{b}	51.6 ± 1.0^{c}	60.3 ± 4.7^{d}	67.1 ± 0.5^{e}
	B60^{x}	36.3 ± 5.6^{ac}	27.6 ± 1.7^{b}	32.3 ± 0.5^{ab}	39.8 ± 0.4^{c}	41.2 ± 0.6^{c}	53.6 ± 0.4^{d}	56.2 ± 1.1^{d}	63.8 ± 2.7^{e}
	C60^{z}	0.3 ± 0.5^{a}	5.6 ± 0.4^{a}	16.9 ± 0.8^{b}	28.0 ± 2.7^{c}	36.2 ± 6.2^{d}	59.1 ± 1.8^{e}	58.9 ± 1.8^{e}	63.5 ± 0.8^{e}
pH	A40	6.6 ± 0.1^{a}	6.6 ± 0.0^{a}	6.4 ± 0.1^{ab}	6.4 ± 0.0^{ab}	6.3 ± 0.1^{ab}	6.3 ± 0.0^{ab}	6.1 ± 0.0^{b}	6.3 ± 0.4^{ab}
	B60	6.6 ± 0.1^{a}	6.5 ± 0.0^{abc}	6.6 ± 0.1^{ab}	6.3 ± 0.0^{cde}	6.4 ± 0.1^{bcd}	6.2 ± 0.0^{de}	6.2 ± 0.1^{de}	6.1 ± 0.1^{e}
	C60	6.6 ± 0.1^{ab}	6.8 ± 0.1^{a}	6.6 ± 0.1^{ab}	6.3 ± 0.2^{bc}	6.2 ± 0.2^{cd}	6.1 ± 0.2^{cd}	6.0 ± 0.1^{cd}	6.0 ± 0.0^{d}

Values represent mean ± standard deviation (n = 3).
[a-i] Indicates the significant difference ($p \leq 0.05$) of pH, O_2, or CO_2 values during storage when analyzed by Tukey B's HSD test.
[x-z] Indicates the significant difference ($p \leq 0.05$) among headspace compositions when analyzed by Tukey B's HSD test.

3.2. Mortality

Mortality increased over storage time (Fig. 1A and B), with different atmospheres resulting in a significant distinction in mortality rate ($p \leq 0.05$). In the first batch, mortality reached 20% within seven days in the A40 and C60 packages, while under atmosphere B60, 20% mortality was seen after nine days of storage. Packages with B60 atmosphere promoted 100% viability in the first five days (Batch 1) and nine days (Batch 2). In Batch 1, the A40 and C60 packages retained 19–63% of live mussels prior to a 100% death rate on days 12 and 9, respectively. The test showed that with oxygen decline, mortality occurred differently for each condition (Fig. 1C and D). For B60, generally mortality occurred at higher O_2 levels in comparison with the other two atmospheres. The latter showed similar trends in the relation between mortality and O_2

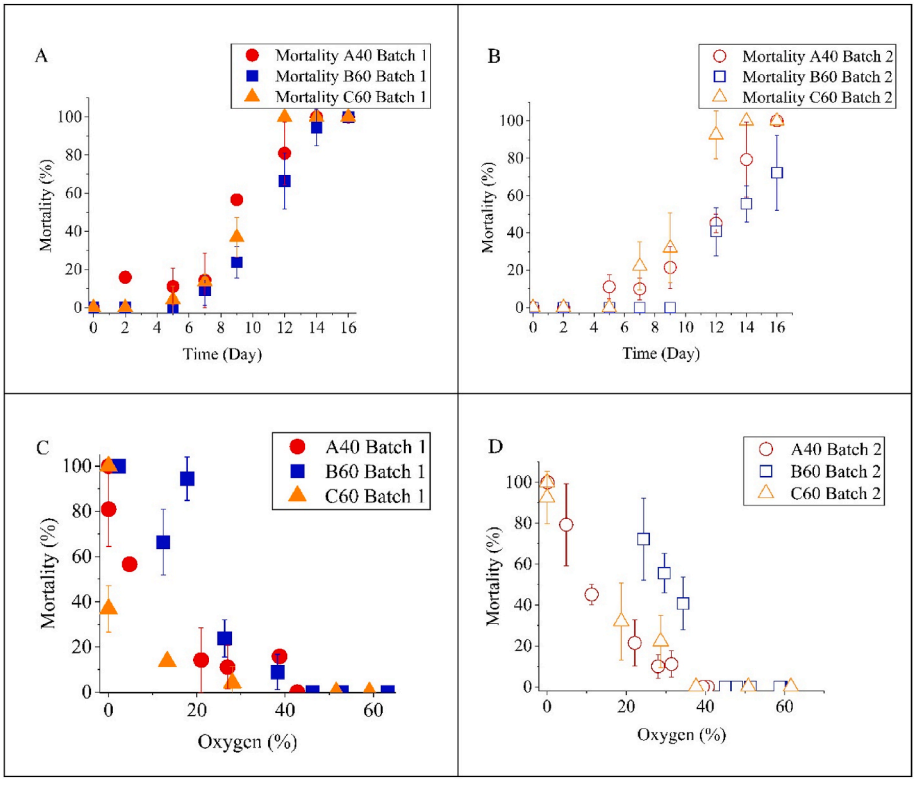

Fig. 1. Mortality as a function of time (A–B) and oxygen level (C–D) under different atmosphere conditions (% $CO_2/O_2/N_2$): A40 (30/40/30), B60 (40/60/0), and C60 (0/60/40) in Batches 1 and 2 of the experiment. The values represent the mean ± standard deviation (n = 3).

levels.

3.3. pH

The pH exhibited a similar trend under all tested conditions ($p \leq 0.05$). The initial pH was 6.3 and 6.6 for Batches 1 and 2, respectively (Table 2). There was no difference in pH during five days of storage before the pH gradually decreased until day 16. Throughout the storage time, pH was reduced to 5.9 ± 0.1, 5.9 ± 0.1, 5.7 ± 0.2 (Batch 1) and 6.3 ± 0.4, 6.1 ± 0.1, 6.0 ± 0.0 (Batch 2) for the A40, B60, and C60 packages, respectively. Thus, the significant pH reduction was associated with storage time.

3.4. Microbial growth

Pseudomonas, B. thermosphacta, and Enterobacteriaceae were counted in minimal numbers, amounting to <5, <3, and <1 log CFU g^{-1}, respectively. Overall, Batch 1 showed higher values of TPC and LAB (Fig. 2) in comparison with Batch 2, the first already starting from a higher initial contamination.

There was no difference of TPC between A40 and B60 packages, meanwhile a noticeable microbial growth was shown in Atmosphere C60 ($p \leq 0.05$). Initially, the TPC value stood at 7.2 and 6.0 log CFU g^{-1} for Batches 1 and 2, respectively. There was a >1 log difference between the two independent batches. Within nine days of storage, an increase of TPC value was presented in conditions C60 (7.8 and 7.8 log CFU g^{-1}), then followed by conditions A40 (7.9 and 7.1 log CFU g^{-1}) and B60 (7.8 and 7.2 log CFU g^{-1}).

LAB counts presented a low value, initially amounting to 3.3 and 1.9 log CFU g^{-1}. By the end of storage, LAB in Batch 1 prevailed in all atmospheres, reaching more than 8 log CFU g^{-1}. In Batch 2, LAB predominated only in spoiled mussels exposed to the B60 atmosphere (7.2 log CFU g^{-1}). A significant difference in LAB count was observed only in the C60 packages, presenting the lowest value ($p \leq 0.05$, mean value = 4.9). This result indicates the domination of other bacteria in conditions C60. Conversely, during storage, H_2S producers showed a slight growth presenting low values (<5.5 log CFU g^{-1}) in all atmospheric conditions. These results highlighted that blue mussels' atmosphere conditions and mortality impacted microbial growth variations. At the same time, a natural variation induced the difference in the bacteria domination

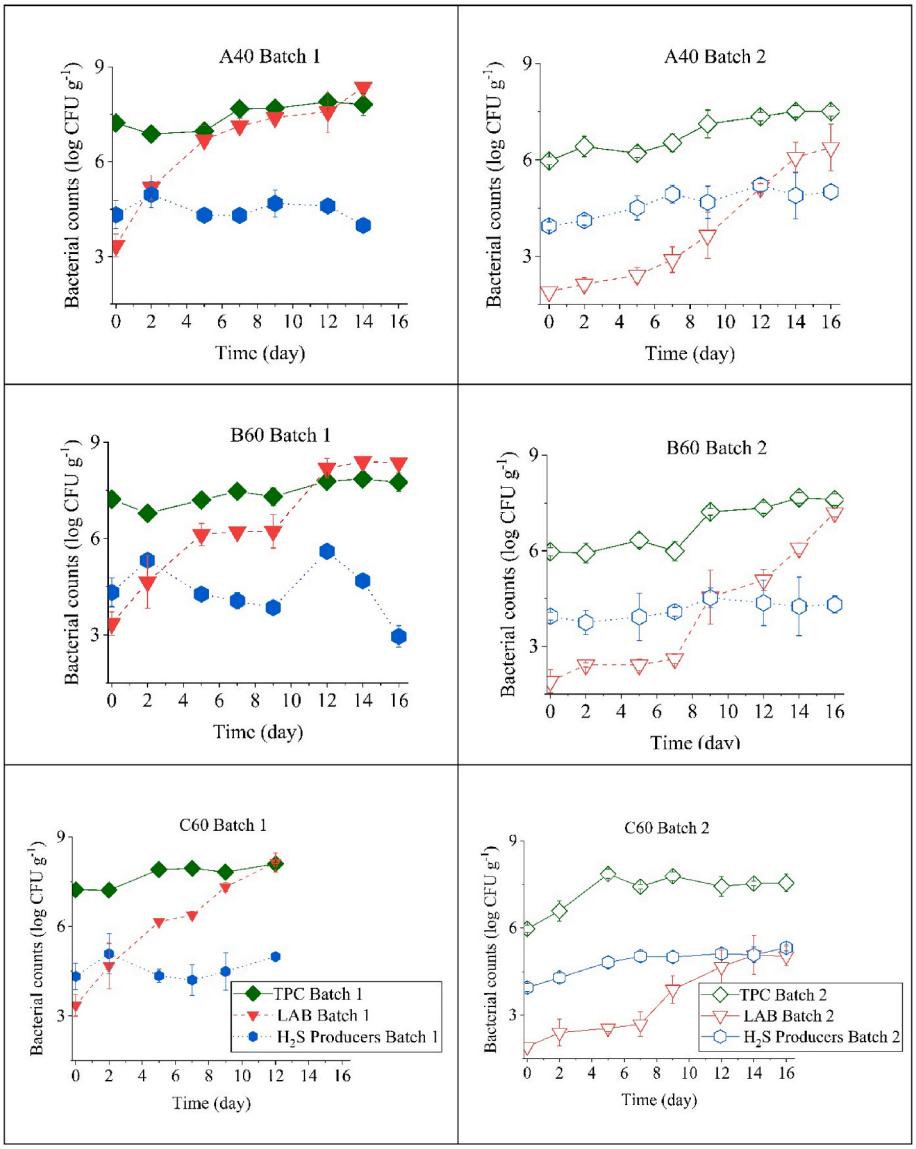

Fig. 2. Counts of total viable psychrotrophic bacteria (TPC), lactic acid bacteria (LAB), and H_2S producers in blue mussels stored under different atmosphere conditions (% $CO_2/O_2/N_2$): A40 (30/40/30), B60 (40/60/0), and C60 (0/60/40) at 4 °C in Batches 1 and 2 of the experiment. The values represent the mean ± standard deviation (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

between Batches 1 and 2.

3.5. Volatile organic compounds (VOCs)

The evolution of alcohols, aldehydes, acids, ketones, esters, amines, and sulfur compounds was monitored in blue mussels under refrigerated storage. The study implies that different MAP atmospheres result in distinct VOC profiles.

3.5.1. Alcohols

The concentration of 3-methyl-1-butanol and 2,3-butanediol increased as mortality reached 60% along with oxygen depletion (Batch 1). When mortality reached 80–100%, and TPC >7.9 log CFU g^{-1}, only conditions A40 and C60 (Batch 1) showed concentrations of 3-methyl-1-butanol and 2,3-butanediol above 1000 μg/m^3. Earlier in Batch 2 (20–40% mortality rate), average concentrations of 3-methyl-1-butanol increased by 312–463 μg/m^3, whereas 2,3-butanediol accumulated at less than 400 μg/m^3 (Fig. 3). By the end of storage, C60 accumulated the highest quantities of ethanol, reaching 97036 (Batch 1) and 132347 μg/m^3 (Batch 2). The study depicted that a gradual increase in alcohol levels was pronounced in A40 and B60 packages (Batch 2).

3.5.2. Ketones and amines

When mortality rates above 60%, blue mussels exhibited an increase in acetoin/ethyl acetate (178–997 μg/m^3) (Fig. 4). The C60 packages inhibited ketones and maintained 2,3 butanedione concentrations below 700 μg/m^3. In contrast, acetone concentration in B60 condition increased gradually, reaching 1609 and 1541 μg/m^3 in Batches 1 and 2, respectively. Similar to ketones, DMA concentrations in B60 packages exceeded 100 μg/m^3 when 60–100% of mussels perished and TPC levels >7 log CFU g^{-1}. The study revealed that mussels packed in the B60 atmosphere accumulated the most ketones during storage, whereas those packed in the C60 atmosphere accumulated the lowest rates of those compounds.

3.5.3. Sulfur compounds

The development of sulfur compounds was on higher level in the first batch than in the second one. Dimethyl sulfide concentrations increased

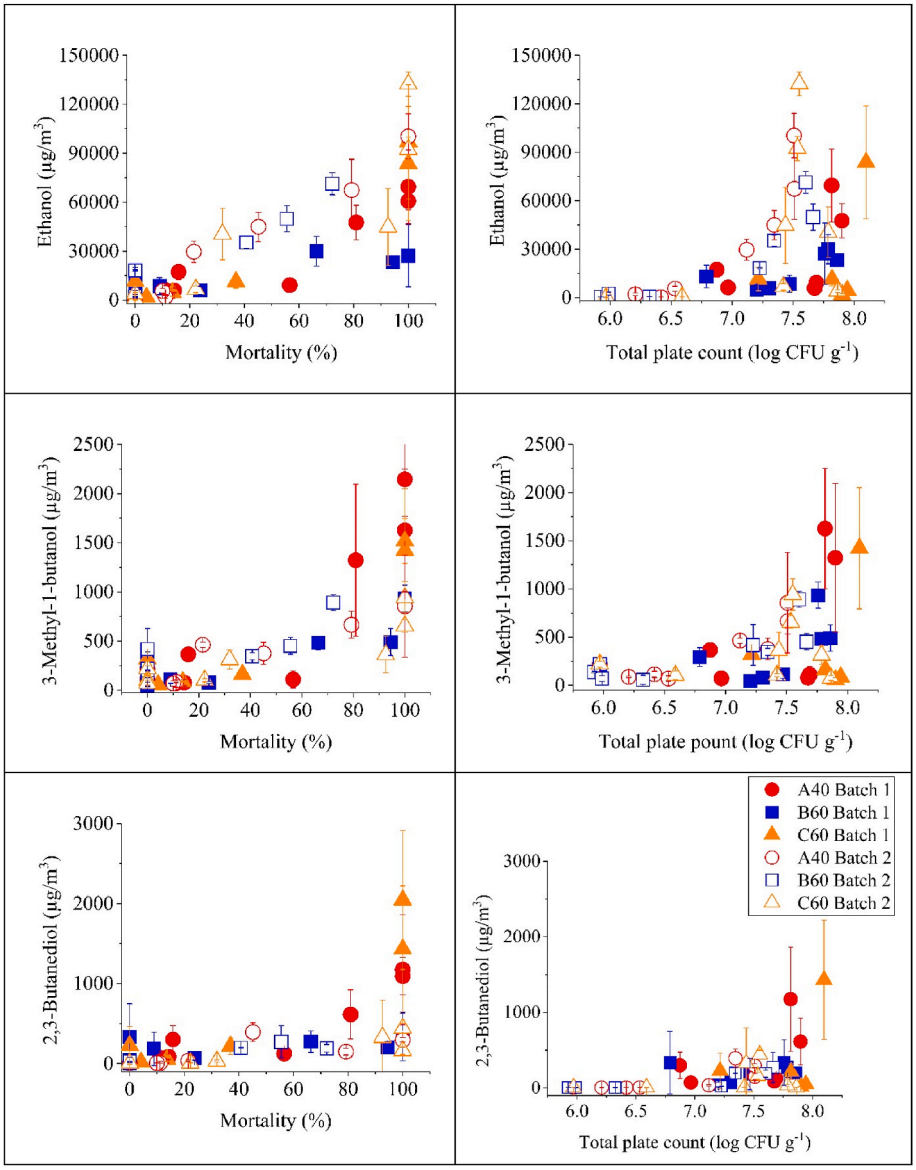

Fig. 3. Accumulation of alcohols as a function of mortality and total viable psychrotrophic count in blue mussels stored under different atmosphere conditions (% $CO_2/O_2/N_2$): A40 (30/40/30), B60 (40/60/0), and C60 (0/60/40) at 4 °C in Batches 1 and 2 of the experiment. The values represent the mean ± standard deviation (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

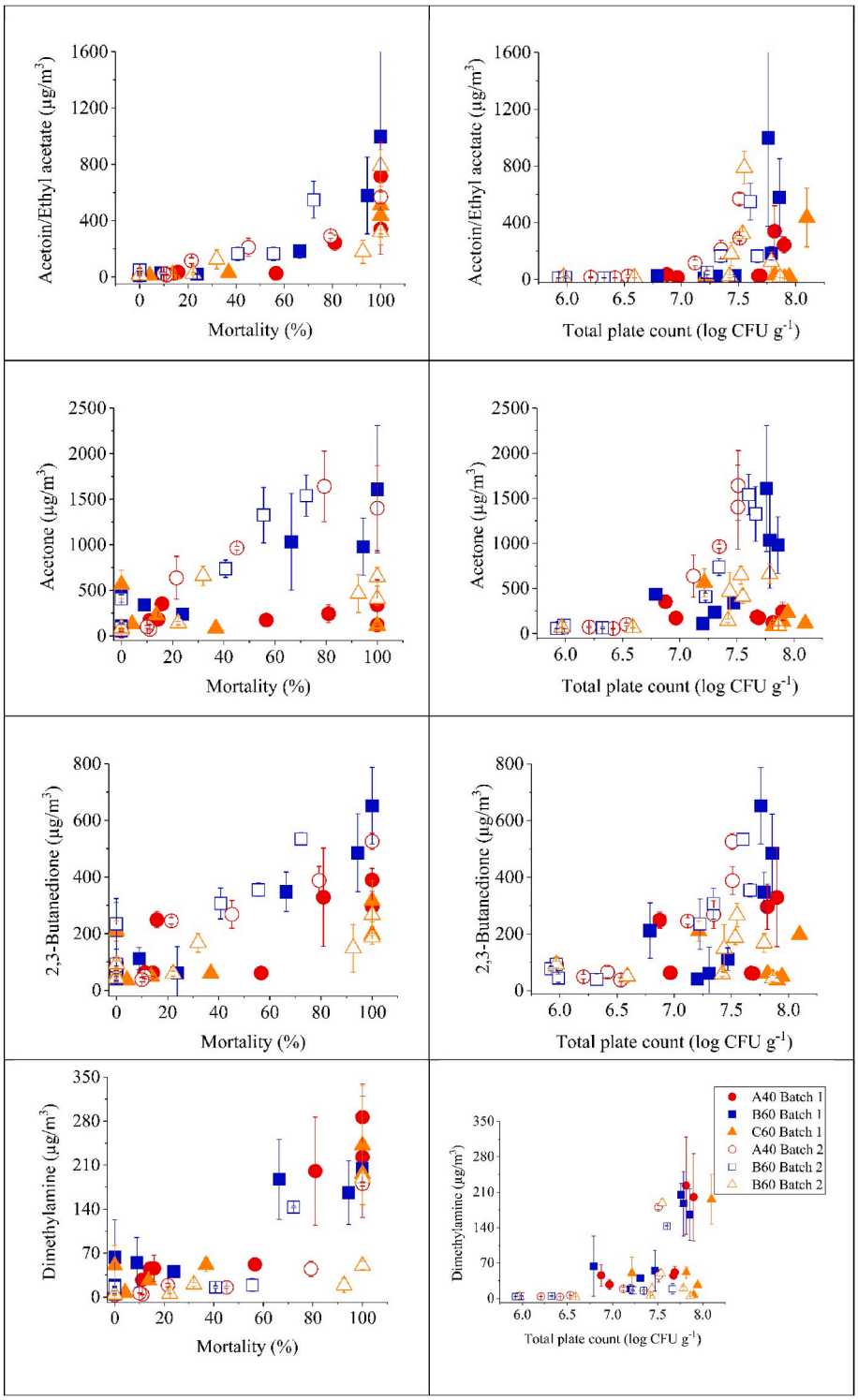

Fig. 4. Accumulation of ketones and dimethylamine (DMA) as a function of mortality and total viable psychrotrophic count in blue mussels stored under different atmospheres (% $CO_2/O_2/N_2$): A40 (30/40/30), B60 (40/60/0), and C60 (0/60/40) at 4 °C in in Batches 1 and 2 of the experiment. The values represent the mean ± standard deviation (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

as mortality occurred and subsequently escalated when mortality >60% (Fig. 5). Even though there was no clear trend of dimethyl sulfide accumulation between Batches 1 and 2, B60 packages pointed out the lowest accumulation over storage (10969 and 215243 μg/m^3). In the meanwhile, only for C60 conditions methyl mercaptan was significantly accumulated (86340 and 3467 μg/m^3) when all mussels died and TPC >7 log CFU g^{-1}. It is noteworthy to notice that a high degree of variability among samples primarily occurred at the end of storage. It appears that the accumulation of dimethyl sulfide and methyl mercaptan in decayed blue mussels escalate only by the end of storage.

3.6. OAVs

The contribution of a single compound to the overall odor depends

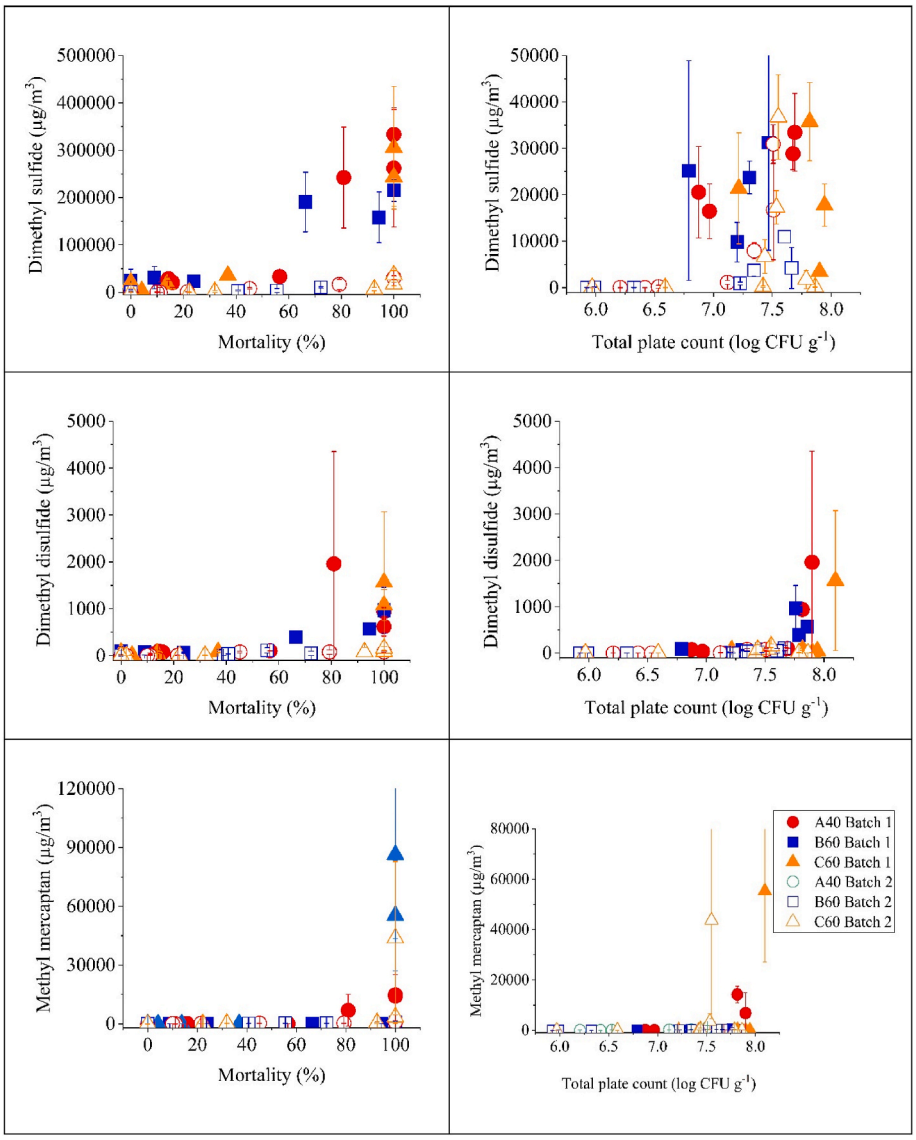

Fig. 5. Accumulation of sulfuric compounds as a function of mortality and total viable psychrotrophic count in blue mussels stored under different atmosphere conditions (% $CO_2/O_2/N_2$): A40 (30/40/30), B60 (40/60/0), and C60 (0/60/40) at 4 °C in Batches 1 and 2 of the experiment. The values represent the mean ± standard deviation (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

on the concentration and the odor activity values (OAVs). Each of the 23 VOCs might be associated with the predominant odor of spoiled blue mussels. A total of VOCs with OAVs ≥1 were detected in spoiled blue mussels at the end of storage (Table 3). Dimethyl sulfide with the highest OAV (2194–66716) contributed to the intensity of spoilage odor. Along with dimethyl sulfide, methyl mercaptan was also perceived as malodor (OAV = 182–43170). The contribution of ethanol to the olfactory mixture was modest (OAV = 1–2). Thereby, atmosphere conditions created a specific VOC profile that constituted a decaying odor. In the atmosphere B60, for instance, the intensity of pungent odor caused by dimethyl sulfide was lower (OAV = 2194–43048) compared to that in atmospheres A40 (OAV = 6187–66716) and C60 (OAV = 7352–61131). The OAVs study points to dimethyl sulfide and methyl mercaptan as the primary odorants in spoiled blue mussels after 16 days of storage.

4. Discussion

The study's objective is to investigate the impact of oxygen-rich atmospheres on blue mussels' quality which was determined based on the quantification of VOCs as well as the occurrence of mortality and microbial growth. The main finding was that different atmospheres result in blue mussels' quality differences. Blue mussels packed under atmosphere B60, combining high O_2 and CO_2 levels (% $CO_2/O_2/N_2$: 40/60/0), presented the lowest mortality rates with LAB dominating the total psychrotrophic count at the end of storage. Oxygen is essential for mussels' production, growth, and respiration (Srisunont, Srisunont, Intarachart, & Babel, 2022). For preservation purposes, live mussels are stored under high-oxygen atmospheres to decrease their metabolism, extend their lifespan and preserve their fresh appearance. However, the application of less than 80% of oxygen is beneficial in avoiding possible explosion hazards during the packaging process (Pastoriza & Bernárdez, 2012). Although the B60 atm presented the lowest mortality rate, mortality increased gradually despite the high remains of oxygen. Therefore, O_2 levels were not the only factor determining mussels' survival. Valve closure and gaping behavior in mussels are influenced by oxygen, temperature, and food availability (Kamermans, Saurel, Boudry, & Kamermans, 2022; Tang & Riisgård, 2016; Zamora et al., 2019). During post-harvest, bivalves absorb oxygen from the air by opening their shells and allowing air to enter the intra-valvular liquid (Jozić et al., 2017). In the absence of water and food, mussels tend to open the

Table 3
The odor activity values (OAVs) of the different VOCs measured in packages of spoiled blue mussels under different atmospheres (% $CO_2/O_2/N_2$): A40 (30/40/30), B60 (40/60/0), and C60 (0/60/40) in Batches 1 and 2 of experiment.

VOC	OT (μg/m^3)	OAVs A40		OAVs B60		OAVs C60	
		Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
Alcohols							
2,3-Butanediol	149998[c]	<1	<1	<1	<1	<1	<1
Ethanol	54955[a]	1	2	<1	1	2	2
3-Methyl-1-butanol	1067[b]	2	<1	<1	<1	1	<1
Isobutyl alcohol	21221[a]	<1*	<1*	<1*	<1*	<1*	<1*
Aldehydes							
3-Methylbutanal	7[d]	15	30	18	31	18	23
Nonanal	6[e]	41	91	51	91	57	81
Decanal	13[e]	2*	6*	3*	6*	3*	6*
Acids							
Acetic acid	364[a]	<1*	<1*	<1*	<1*	<1*	<1*
3-Methylbutanoic acid	205[f]	<1*	<1*	<1*	<1*	1*	1*
Ketones							
Acetone	58610[a]	<1	<1	<1	<1	<1	<1
Acetoin/Ethyl acetate	2883[b]	<1	<1	<1*	<1*	<1	<1
2,3-Butanedione	18[a]	22	29	36	30	18*	15*
Butanone	147465[g]	<1*	<1*	<1*	<1*	<1*	<1*
Esters							
Ethyl acetate/Acetoin	9772[a]	<1	<1	<1	<1	<1	<1
Amines							
Ammonia	1889[h]	<1*	<1*	<1*	<1*	<1*	<1*
Dimethylamine (DMA)	2[g]	143	90	102	72	121	95
Trimethylamine (TMA)	2[g]	390	998	255*	565*	539*	557*
Sulfur compounds							
Hydrogen sulfide	27[a]	586*	4*	4*	<1*	1301*	1374*
Dimethyl sulfide	5[a]	66716	6187	43048	2194	61131	7352
Dimethyl disulfide	46[a]	13	2	21	1	23	3
Dimethyl trisulfide	5[b]	63*	96*	58*	94*	95*	102*
Carbon disulfide	654[g]	<1*	<1*	<1	<1	<1*	<1*
Methyl mercaptan	2[g]	7401	511	296	182	43170	21845

Values represent mean ± standard deviation (n = 3).
* Compounds did not meet the quantitative criteria (at least 33% concentrations of all independent packages over limit of quantification (LOQ) and 33% relative standard deviation below 25%).
[a] Devos et al. (1990).
[b] Buttery, Teranishi, Ling, and Turnbaugh (1990).
[c] Wu, Zhu, Tu, Duan, and Pan (2011).
[d] Noseda (2012).
[e] Buttery, Turnbaugh & Ling (1988).
[f] Zhao, Fan et al. (2021).
[g] Fazzalari (1978).
[h] Smeets et al. (2007).

valves to access O_2. Hence, frequent gaping activity is also susceptible to flesh desiccation and damage (Zamora et al., 2019).

Oxygen concentrations are closely linked to blue mussels' metabolism and microbial growth. Typically, the increase of bivalve longevity follows the oxygen levels. Still, the investigated oxygen-rich atmospheres, i.e. B60 (% $CO_2/O_2/N_2$: 40/60/0) and C60 (% $CO_2/O_2/N_2$: 0/60/40), pointed out distinctions in both oxygen change and mortality. The B60 condition slowed down the oxygen level reduction and extended viability for nine days of storage. Consistent with A40, the C60 packages preserved the mussel viability for seven days. The latter corresponds with a previous study (Pastoriza et al., 2004) in which a high oxygen level (% $CO_2/O_2/N_2$: 0/75/25) resulted in six days of shelf life at 2–3 °C. In both A40 and C60 packages, mussels survived under anaerobic conditions from day 9 onward. These conditions possibly induced anaerobic respiration in the remaining mussels before they perished. These findings comply with literature works on respiration. Blue mussels respond to limited oxygen by gradually closing the valves, reducing the respiration rate significantly (0.05–0.13 mg O_2/L), and no longer maintaining normal aerobic metabolism (Bernárdez & Pastoriza, 2013; Tang & Riisgård, 2016). Nonetheless, bivalves defend under limited oxygen concentration (anoxia) by consuming more energy from glycolysis, protein, and fat breakdown (Haider, Falfushynska, Timm, & Sokolova, 2020; Nguyen & Alfaro, 2020). Even so, the mussels suffer from environmental stress leading to cellular damage, which involves mitochondria as the chief function of aerobic respiration and makes the mussels prone to mortality (Li et al., 2019; Muller et al., 2012; Nguyen & Alfaro, 2020; Tang & Riisgård, 2016). The results obtained in this work indicate that mussels in all MAPs met the proposed commercial standard (mortality <20%) (Bernárdez & Pastoriza, 2011) for as long as seven (the A40 and C60 atmospheres) and nine days (the B60 atm) of storage.

In addition to mortality, atmosphere change impacts microbial proliferation. The initial TPC of 6.0 and 7.2 log CFU g^{-1} increased considerably within nine days. Several studies recommended the application of high O_2 and low CO_2 atmospheres to avoid acidification and potential toxicity on mussels (Bernárdez & Pastoriza, 2013; Odeyemi, Burke, Bolch, & Stanley, 2019; Pastoriza & Bernárdez, 2012; Pastoriza et al., 2004). However, our findings confirm that the absence of CO_2 in condition C60 led to the most rapid growth of bacteria. On that account, the microbial expansion might alter the weaker muscles and dissolve myofilament (Bernárdez & Pastoriza, 2011). CO_2 limits microbial growth by suppressing the gram negatives and favoring the gram positives (Noseda, Goethals, et al., 2012; Parlapani, Haroutounian, Nychas, & Boziaris, 2015). Atmospheres with a certain proportion of CO_2 in the packages protect blue mussels from microbial outgrowth. Under oxygen-nitrogen atmosphere (% O_2/N_2: 75/25), purified mussels retarded microbial growth resulting in TPC ≤5 log CFU g^{-1} within nine days of storage (Bernárdez & Pastoriza, 2013; Pastoriza et al., 2004), which was much lower than in the present study. Eight hours of

depuration in an aerated static tank at 4 °C was suggested to diminish microbial contamination in bivalves and remove spoilage bacteria such as *Shewanella* (Odeyemi et al., 2019).

LAB grow across a wide range of O_2 concentrations, despite the fact that they are likely favored in low oxygen environments and proliferate competitively under high CO_2 levels (Kuuliala et al., 2018; Pothakos, Devlieghere, Villani, Björkroth, & Ercolini, 2015). Under high oxygen, LAB initially presented minimal growth and then experienced a rapid increase when CO_2 elevated to more than 20%. By the end of storage, they grew dominantly under the A40 and B60 conditions. In both atmospheres, the LAB domination was more greater in blue mussels Batch 1 (8.3 and 8.4 log CFU g^{-1}) than in Batch 2 (6.4 and 7.2 log CFU g^{-1}). Under C60 packaging, the other groups of bacteria might predominate in spoiled blue mussels (Batch 2). In this case, natural variation may have affected the mussels' status, including temperature, tidal cycles, dissolved organic materials, nutritional states, feeding habits, reproductive conditions, and seasonality (Livingstone & Gallacher, 2003).

LAB metabolism was associated with acetic acid and lactic acid production, resulting in a reduced pH value (Leroi, 2010; Noseda, Goethals, et al., 2012; Pothakos et al., 2015). Considering that acetic acid levels (8–477 μg/m^3) in all packages were below the LOQ, the acidic environment was also induced by the accumulation of CO_2 as a byproduct of mussels and aerobic bacteria respiration. In wet products such as live blue mussels, the presence of CO_2 resulted in the formation of carbonic acid, depending on the solubility of CO_2, the initial pH, the pH buffering capacity, and the storage temperature (Noseda, Dewulf, et al., 2010). In all atmospheric conditions, there was no substantial growth of H_2S-producing organisms (<5,6 log CFU g^{-1}). In agreement with cod investigation, the growth of H_2S-producers remained low (≤6 log CFU g^{-1}) when the samples were stored under high O_2 concentration (Kuuliala et al., 2018). The presence of CO_2, especially under high O_2 levels, hindered the growth of H_2S producers (Kuuliala et al., 2018; Noseda, Goethals, et al., 2012).

This study highlights that most VOCs concentrations increased gradually after blue mussels largely died out or after 12 days of storage, while sulfur compounds, such as dimethyl sulfide and methyl mercaptan escalated significantly at the end of storage. Additionally, most VOCs rose exponentially with TPC >7 log CFU g^{-1} (Figs. 3–5). This finding corresponds to the other marine products, in which VOCs were detected when TPC surpassed 6.5 to 9 log CFU g^{-1} (Kuuliala et al., 2018, 2019; Mikš-Krajnik, Yoon, Ukuku, & Yuk, 2016). It is most likely that different microbial groups could have been responsible for VOC production. An increase in alcohol concentrations was notable in the present study. Previous examinations have revealed ethanol, 3-methyl-1-butanol, and 2,3-butanediol in bivalves (Cruz-Romero, Kerry, & Kelly, 2008; Odeyemi et al., 2018; Zhang, Li, Luo, & Chen, 2010). Ethanol concentrations in this study escalated to mg/m^3 ranges (11–132 mg/m^3). The increased trend of ethanol as a function of storage time has also been observed in other seafood, such as cod, salmon, and pangasius (Hindle et al., 2018; Kuuliala et al., 2018; Noseda, Islam, et al., 2012). With the lowering of oxygen and at a 100% mussel death rate, ethanol levels increased herewith exceeding the olfactory threshold (OT) of 54955 μg/kg (Devos, Patte, Rouault, Laffort, & Van Gemert, 1990). *Pseudomonas* is the primary producer of ethanol. However, other bacteria, including *Pseudoaltermonas, Carnobacterium, Psychrobacter,* and *Leuconostoc carnosum*, are also associated with alcohol production (Boziaris & Parlapani, 2017; Parlapani et al., 2020; Zagdoun, Coeuret, N'Dione, Champomier-Vergès, & Chaillou, 2020). *Carnobacteria*, for instance, catabolize amino acids into alcohols and aldehydes generating malty odors and bitter taste (Leroi, 2010). Glucose is widely used by heterofermentative or facultative heterofermentative LAB producing lactic acid, acetic acid, CO_2, and ethanol (Pothakos et al., 2015). The glycolysis metabolic pathway also contributes to alcohol generation in blue mussels. Under a hypoxic environment (low O_2 access), marine organisms consume more energy from glycogen, resulting in the upregulation of carbohydrate metabolism, producing alcohols and carbon dioxide as the end products (Haider et al., 2020; Li et al., 2019; Muller et al., 2012; Nguyen & Alfaro, 2020).

With respect to aldehydes, a fluctuation of 3-methylbutanal, nonanal, and decanal concentrations (<600 μg/m^3) was observed. Thermal processes might cause the increase of 3-methylbutanal and nonanal in the early storage phase. Thermal processing as well as packaging procedures at temperatures of at least 22–50 °C can affect the rise of aldehydes (Lomelí-martín, Martínez, Welti-chanes, & Escobedo-avellaneda, 2021; Zhao, Hu, et al., 2021). Polypropylene (PP) trays were used as packaging materials. In agreement with the previous analysis, the migration of octanal, decanal, and nonanal from PP packaging material to products containing chicken breasts and vegetable oil has been reported. However, the concentrations were below the specific migration limits established by European Regulation (Aznar, Domeño, Osorio, & Nerin, 2020; Vera, Canellas, & Nerín, 2020).

Our study revealed the lowest accumulation of ketones in atmosphere C60 (% $CO_2/O_2/N_2$: 0/60/40). Different atmosphere compositions affected the evolution of ketones bringing acetoin/ethyl acetate and acetone levels below OT (Devos et al., 1990). The presence of these compounds are often triggered by *Pseudomonas, Pseudoalteromonas*, and *Psychrobacter* spp. (Boziaris & Parlapani, 2017). Apart from the evolution of ketones, acetoin and ethyl acetate cannot be distinguished by SIFT-MS since their product ions overlap (Van Kerrebroeck, Harth, Comasio, & De Vuyst, 2018). By known reaction rate constant (*k*), SIFT-MS relies on the chemical ionization of VOCs by selected precursor ions, such as NO^+, O_2^+, and H_3O^+ (Ioannidis et al., 2018). Nevertheless, the product ions of acetoin and ethyl acetate can not be confidently assigned in this study.

Some of amine productions have been associated with the spoilage of marine fish. In this study, DMA concentration increased, with the final concentration ranging from 143 to 286 μg/m^3. DMA is a secondary amine as a result of TMA degradation (Lou, Zhai, & Yang, 2021). However, it is important to highlight that DMA is not the consequence of microbial metabolism, but rather the action of autolytic enzymes (Boziaris & Parlapani, 2017). TMA prominently increased in the A40 packages reaching 1996 μg/m^3 and exceeding the very low OT of 2 μg/m^3 (Devos et al., 1990). Nonetheless, during the COVID-19 pandemic, using a hand sanitizer containing TMA before SIFT-MS assessment might result in an overestimation of TMA. High values and variability in LOQ indicated the presence of TMA pollution (Supplementary Tables 1 and 2). Amine groups such as trimethylamine, triethylamine, tripropylamine, tributylamine were utilized as a surfactant in the cleaning agents (Cho, Shin, & Jeong, 2019; Herrera-Márquez, Fernández-Serrano, Pilamala, Jácome, & Luzón, 2019; Ramprasad & Philip, 2018). Within 278 μg/m^3 detected in the headspace, TMA is designated as an old fishy odor of rotten oyster (Cheng et al., 2017). This compound is found in marine fish living at high salinity and low temperature, in the presence of *S. putrefaciens, P. phosphoreum, and* Vibrionaceae (Dalgaard, Gram, & Huss, 1993; Odeyemi et al., 2018; Serio, Fusella, Chaves López, Sacchetti, & Paparella, 2014). These bacteria utilize TMAO as the terminal electron acceptor in anaerobic respiration, resulting in off-odors and flavors due to TMA accumulation (Gram & Huss, 1996). High O_2 levels are often applied to inhibit these bacterial (Debevere & Boskou, 1996; Gram & Dalgaard, 2002; Noseda, Goethals, et al., 2012).

When all mussels died, or anaerobic conditions prevailed, sulfur compounds surged considerably. Prior research indicated that dimethyl disulfide was the most significant compound in spoiled Mediterranean mussels during storage (Odeyemi et al., 2018). Here we observed that two other sulfur compounds, i.e. dimethyl sulfide and methyl mercaptan, accumulated more significantly in decayed blue mussels. As deaths occurred, the dimethyl sulfide concentration escalated to mg/m^3 levels. In the second batches of experiment, mg/m^3 levels were only detected at the storage end. Similarly, methyl mercaptan raised up to mg/m^3 concentrations in the C60 packages when all mussels died and were considered microbiologically spoiled (TPC >7 log CFU g^{-1}). Under

low temperature and high salinity conditions, the proteolytic activity of *S. putrefaciens* accumulates biogenic amines, H_2S, and TMA (Odeyemi et al., 2018; Serio et al., 2014). The production of sulfur compounds can originate from dimethylsulfoniopropionate (DMSP) and L-methionine. DMSP is converted into dimethyl sulfide, while L-methionine is degraded into ammonia and methyl mercaptan. The formation of methyl mercaptan has been observed following the multiplication of *Pseudoalteromonas*, *Pseudomonas,* and *Shewanella* (Boziaris & Parlapani, 2017; Broekaert, Heyndrickx, Herman, Devlieghere, & Vlaemynck, 2013). Additionally, methyl mercaptan is a precursor to the rising of dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide by further chemical or enzymatic degradation (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015).

The accumulation of ethanol, dimethyl sulfide, and methyl mercaptan reached mg/m^3 levels during the final days of storage and may therefore be considered important spoilage indicators. When a VOC's concentration levels are lower below the application's quantitative thresholds, it is of limited significance in the development of packaging technology (Kuuliala et al., 2018). Although most VOCs were detected in low quantities, multiple compounds were significant to the overall odor when exceeding OTs and accumulating throughout storage. By the end of storage, dimethyl sulfide and methyl mercaptan were responsible for the primary odor attributes in all atmospheric conditions. These two compounds showed low OTs (5 and 2 μg/m^3) and the highest OAVs, indicating that they are easily recognized by the human nose and emit a pungent odor at higher concentrations (Devos et al., 1990; Noseda, Goethals, et al., 2012). Proteolytic activity of sulfur-containing amino acids (cysteine and methionine) produce sulfur compounds and generate an offensive odor (Boziaris & Parlapani, 2017; Haider et al., 2020; Ioannidis et al., 2018). Although the OAV results can be utilized to evaluate the compound's contribution to the overall odor of rotten blue mussels, it should be emphasized that odor contributions from single compounds may increase or decrease because of synergistic or antagonistic effects within VOCs interactions (Wu, Kan, Narale, Liu, & Sun, 2022).

5. Conclusions

Atmosphere composition affects the quality of live blue mussels under refrigerated storage. Compared to the other atmospheric conditions, a high level of oxygen (60%) combined with carbon dioxide (40%) extends the survival of blue mussels and suppresses the accumulation of VOCs. VOCs evolution closely represents the quality degradation of blue mussels during storage. Mussel's metabolism and microbial activities were in line with the measured VOCs profiles. Dimethyl sulfide and methyl mercaptan are identified as responsible for the primary odor in decayed blue mussels. However, considering its high concentrations reaching to the mg/kg range, also ethanol was potentially highlighted as a spoilage marker in all atmospheric conditions. Further research is required to determine the advanced statistical method and to develop the applicability of these VOCs as spoilage indicators of blue mussels. Also the microbial diversity responsible for spoilage needs to be further explored to develop a suitable atmosphere packaging for live blue mussels.

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CRediT authorship contribution statement

Susana Endah Ratnawati: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. **Lotta Kuuliala:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Christophe Walgraeve:** Supervision, Resources, Writing – review & editing. **Kristof Demeestere:** Resources, Supervision, Writing – review & editing. **Peter Ragaert:** Supervision, Writing – review & editing. **Frank Devlieghere:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal ties that could be perceived as having influenced the work described in this study.

Data availability

The data that has been used is confidential.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.114537.

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