1 **TITLE**

2 **Effect of oxygen concentration and temperature on the viability of small-**
3 **sized mussels in hermetic packages**

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5 Marta Bernárdez, Laura Pastoriza

6 *Instituto de Investigaciones Marinas (IIM-AECSIC). Eduardo Cabello, 6. 36208. Vigo. Spain*

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9 **ABSTRACT**

10 The objective of this study was to evaluate the behaviour of small-sized (~19 g/unit)
11 Mediterranean mussels (*Mytilus galloprovincialis*) packaged hermetically with different
12 proportions of oxygen in the interior of the package (20% and 83%) and storage
13 temperatures (2 °C and 7 °C). The most favourable conservation conditions (83% O₂
14 and 2 °C) were compared with larger mussels (~24 g/unit). On day 3 of storage with
15 low %O₂ at 2 °C, the concentration of volatile fatty acids in the intervalval fluid was
16 double that for the mussels packaged with 83% O₂ and this difference increased
17 during storage. Higher temperatures (7 °C) produced a larger and more rapid
18 accumulation of ammonium nitrogen with concentrations of 49 mg N/L on day 6. Lower
19 concentrations were observed at the end of storage at 2 °C (27 mg N/L on day 9).
20 From day 7, the increase of N-TVB measured in the intervalval fluid of the small
21 mussels at 2 °C was more pronounced than in the larger mussels. The organoleptic
22 evaluation, percentage mortality and microbial counts collectively indicated that a high
23 quality product was maintained on day 8-9 with mussels packaged with 83% O₂ and
24 stored at 2 °C. Inadequate temperatures have a stronger negative impact on shelf life
25 than low oxygen concentrations.

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27 **KEYWORDS:** mussel, refrigerated, modified atmosphere, ammonium, volatile fatty
28 acids

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1 **1. Introduction**

2 In 2006, 48.5% of seafood products destined for human consumption, such as
3 crustaceans and bivalve molluscs, were marketed in a live, fresh state. This strategy
4 provides high financial rewards and has increased in recent years due to technological
5 advances, improved logistics and an increase in demand. Furthermore, more intense
6 aquaculture has allowed the wholesale price of shellfish to become more affordable
7 and has led to an increase in consumption per capita in recent years (FAO, 2009).

8

9 Once withdrawn from their environment, shellfish have a limited life which depends on
10 the species and the conditioning during transport and sale. Stress-inducing conditions
11 such as oxygen deficiency, the presence of metabolites, temperature and humidity are
12 important factors to consider for the cultivation and transport of fish and live shellfish.
13 Venugopal (2006) indicates some suitable preparation systems, such as the packaging
14 of prawns in sealed oxygenated plastic bags or open tanks cooled to 8-10 °C to induce
15 hibernation. The packaging of lobsters in moist straw, seaweed or wood shavings is
16 also recommended. Low concentrations of dissolved oxygen have been shown to
17 reduce survival and depress the immune response of cultivated scallop (Chen et al.,
18 2007) and post-transport recovery decreases with emersion time and is strongly
19 influenced by temperature (Christophersen, Román, Gallagher & Magnesen, 2008). In
20 other mollusks different critical thermal limits have been observed. These limits depend
21 on the species or the farmed area and have an influence on an adequate oxygenation.
22 To this extent, Morley, Hirse, Pörtner and Peck (2009) have proved in limpets and
23 clams how temperatures above the ideal cause the establishment of anaerobic
24 metabolic pathways.

25 These factors should also be considered during commercialization. Temperature is a
26 key factor for the conservation of foodstuffs. Higher temperatures tend to accelerate
27 the processes of decomposition following animal death and are one of the causes of
28 ante-mortem stress leading to poor-quality meat (Haard, 1998). Temperature is also a
29 limiting factor for the shelf life of live shellfish. An adequate storage temperature for
30 clams reduces the appearance of altering compounds, and provides a higher quality

1 product with a prolonged marketable period (Sadok, Uglow & El-Abed, 2003).
2 Additionally, the behaviour of live shellfish can vary with species, the locality of
3 cultivation or withdrawal and the time of the year. Robson, Kelly and Latchford (2007)
4 showed that the onset of deterioration for distinct species of live crustaceans stored at
5 4 °C depended on the habitat of origin, with the intertidal specimens being the most
6 resistant. The storage temperature may also alter the shelf life. For example, the shelf
7 life of *Necora puber* is ~13 days at 4 °C and ~ 2 days at 20 °C.
8 Conservation of live bivalve molluscs in hermetic packages allows the product to reach
9 the consumer in an ideal state. Modified atmosphere packaging (MAP) is an effective
10 alternative for prolonging the commercial life of these molluscs. Combined with oxygen-
11 rich atmospheres and with respect to unpackaged molluscs, this system can provide
12 the adequate gas mixture and humidity for maintaining live mussels (Pastoriza,
13 Bernárdez, Sampedro, Cabo, & Herrera, 2004) in addition to the characteristic
14 organoleptic qualities of clams (Gonçalves, Pedro, Duarte & Nunes, 2009).
15 Furthermore, it has been shown that the size (large or medium) of Mediterranean
16 mussels and the oxygen concentration inside the package can influence quality and
17 shelf life (Bernárdez & Pastoriza, 2011).

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19 The objective of the present study is to determine the shelf life of commercial small
20 sized-mussels packaged under oxygen-rich atmospheres, their resistance compared to
21 medium-sized mussels and the production of basic metabolites. The impact on shelf
22 life through the appearance of acidic metabolites during storage at different
23 temperatures will also be investigated.

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25 **2. Materials and methods**

26 *2.1. Materials and equipment*

27 The gas, containers and packaging machine were described in Bernárdez and
28 Pastoriza (2011). The proportion of CO₂ and O₂ in the package headspace was
29 determined using a PAK 12P headspace analyzer (Abiss, Barcelona, Spain).

1 Spectrophotometric measurements were performed on a Cecil 3041
2 Spectrophotometer (Cecil, Cambridge, England).

3 *2.2. Sample collection and preparation for analyses*

4 Live mediterranean mussels (*Mytilus galloprovincialis*) from mussel farms in the Ría
5 de Arosa (Galicia, Spain) were maintained in seawater for 12 h at a purification station
6 before transfer to the processing plant. Analytics were performed to verify that the
7 mussels met the microbiological criteria specified in commission regulation (EC)
8 2073/2005. Following mechanical removal of the byssus, the mussels were tightly
9 packed by hand in 1 kg containers (Tecnopack Plastics, Barcelona, Spain) and then
10 filled with the corresponding gas and sealed.

11 These mussels were collected in March 2006 and were classified by size, whereby 1
12 kg of small and medium mussels was equivalent to 52 ± 4 and 41 ± 3 units,
13 respectively. The percentage oxygen in the packaged atmospheres was $20\% \pm 1$ (air)
14 or $83\% \pm 2$. The packages were stored refrigerated at 2 ± 1 °C or 7 ± 1 °C. The
15 conditions of each batch are defined in Table 1.

16 For microbiological and chemical analyses, live mussels (22-25) were removed from
17 each batch and opened. The shell liquor and meat were collected separately for
18 analysis.

19 *2.3. Quantification of mortality*

20 Three containers from each batch were removed daily over the 10 days of storage.
21 Mortality was evaluated by tapping on gaping bivalve shells and those mussels whose
22 shells remained open were considered to be dead. The result is expressed in
23 percentage with regards to the number of total units in the 3 packages.

24 *2.4. Chemical analysis*

25 The analysis of ammonium content in the samples was determined following the
26 method of Solorzano and described by Parsons (1984). Total volatile bases (TVB-N),
27 expressed as mg TVB-N per L of liquor or mg/100g of meat, were determined as
28 described by Lücke and Geidel (1935) and modified by Antonacopoulos (1960). Total
29 volatile fatty acids (VFA) were quantified by titration with 0.01mol equi/L KOH using *m*-
30 cresol purple (Merck, Darmstadt, Germany) indicator (Bernárdez & Pastoriza, 2011).

2.5. Microbiological analysis

A 25 g sample (20g meat and 5 g shell liquor) was mixed with 225 mL of 0.1 g/100 mL peptone water for 1 minute in a Lab-Blender-400 Stomacher. Serial decimal dilutions of the mixture were made in 9 mL of 0.1 g/100mL peptone water. The diluted samples were spread on Plate Count Agar (Oxoid, Basingstoke, England) supplemented with 1 g/100 mL NaCl and incubated at 20 °C for 72 h under aerobic conditions. The results are expressed as log CFU/g of sample. Using the most probable number method (MPN, 5 tubes/dilution), the quantity of fecal coliforms and *Escherichia coli* was determined and verified using the indole test and seeding on Levine agar (Oxoid, Basingstoke, England). Results are expressed as MPN/100g of sample.

Diluted samples of liquor were spread on Iron Agar Lyngby (nutrients and agar by Cultimed, Barcelona, Spain; media supplements by Sigma, Steinheim, Germany) supplemented with 0.5 g/100 mL NaCl and incubated at 17 °C for 5 days under aerobic conditions to determine total viable counts (TVC) and H₂S-producing bacteria. The results are expressed as log colony forming units (log CFU/mL) per sample.

2.6. Sensorial analyses

A panel of 8 persons was trained to evaluate the sensory properties of odour and taste of cooked mussels (5 women and 3 men; age 38 ± 13 y.o.). The mussels were steam-cooked at 100 °C for 4-6 min and one valve was served at approximately 35 °C to each experienced panellist and scored on a 10-point scale following Pastoriza et al. (2004) method.

2.9. Statistical analysis

Statistical analyses were used to compare mortality and chemical values of four batches of the fresh mussels packed under modified atmospheres. Significant differences between the samples were calculated with Statistica 6.0 package (Statsoft, Oklahoma, USA) using a Student's test with a significance level of 95%.

3. Results and discussion

The analysis of mussels packaged under modified atmospheres indicates a dependence on the initial concentration of oxygen, mussel size and storage

temperature. This dependence is observed in the analysis of ammonium, TVB-N and VFA in the intervalval fluid of the live mussels. Considering the percentage mortality and the organoleptic analysis, the shelf life of good quality Mediterranean mussel can be determined for the different batches (Table 1).

3.1. Effect of the initial oxygen concentration

The effect of the initial oxygen concentration is illustrated by comparing batch 1 (20% O₂) and 3 (83% O₂) in figures 1, 2 and 3. The accumulation of ammonium in the intervalval fluid was lower in batch 1 mussels throughout storage, with values of 18.42 ± 0.13 and 28.21 ± 1.48 mg N/L on day 8, respectively. The presence of ammonium is due to the metabolism of proteinic compounds (Hawkins & Bayne, 1992). Despite the observed increase during storage in both batches, the difference between the batch packaged with low and high O₂ content is indicative of a more active aerobic metabolism in the latter. For the same reason, higher concentrations of volatile nitrogenous products were associated with batch 3 mussels after 8 days of storage, with values of 22.3 ± 0.2 mg N/L, which compares to batch 1 mussels with values of 12.0 ± 0.6 mg N/L. Sadok, Uglow and Haswell (1999) observed a reduction of O₂ in the mantle cavity fluid during emersion of *Mitylus edulis*. Yet, the haemolymph oxygen tension did not become zero, and the authors postulated that oxygen uptake and low levels of aerobic metabolism may take place in emerged mussels. Total volatile fatty acids (VFA) are a group of short-chain fatty acids mainly produced in invertebrates by the anaerobic metabolism of carbohydrates, although in certain molluscs this production can occur when oxygen is available (Widdows, Bayne, Livingstone, Newell & Donkin, 1979). The VFA results in Fig. 3 show a greater and more rapid accumulation of these compounds in the batch packaged with a low concentration of O₂ (batch 1) from day 2 of storage, reaching values of 165.9 ± 8.5 mg VFA/L on day 8. In contrast, VFA concentration in batch 3 mussels increased slowly to 67.9 ± 6.4 mg VFA/L (day 8). The differences in ammonium, TVB-N and VFA concentrations between these two batches during storage were statistically significant (Table 2). Oxygen deficiency can be a major control of the energy available to mussels. For example,

1 mussels close their shells at low tide and decrease their metabolic rate (Famme,
2 Knudsen & Hansen, 1981) whereas in other cases, such as in waters with low
3 dissolved O₂ concentration, there is a tendency toward anaerobic metabolism (de
4 Zwaan, 1983).

5

6 *3.2. Results according to mussel size packaged in oxygen-rich atmospheres*

7 The effect of mussel size is illustrated by comparing batch 3 (small) and 4 (medium) in
8 figures 1, 2 and 3. The sequence of results in the analysis of ammonium and volatile
9 bases is similar for these batches, although the values were greater (and significantly
10 different) for the smaller mussels throughout storage. The measured concentrations in
11 batch 3 mussels were 35.1 ± 0.9 mg N/L of ammonium and 24.31 ± 0.4 mg of TVB-N/L
12 on day 9, compared to 26.2 ± 1.5 mg and 13.9 ± 0.6 mg (respectively) for the larger
13 mussels.

14 The results shown graphically in Fig. 3 (VFA) indicate little and non-significant
15 differences between both batches (Table 2) with values of 81.5 ± 4.3 and 75.6 ± 0.3
16 after 9 days of storage. The differences in the basic compounds generated according
17 to mussel size may be caused by a greater metabolic rate in larger individuals despite
18 a lower specific metabolic rate (metabolic rate per unit of body mass) (Lucas, 1993).
19 Lower concentrations of ammonium have been observed for large mussels in the
20 intervalval fluid compared to medium-sized mussels when packaged with different
21 modified atmospheres (Bernárdez & Pastoriza, 2011).

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23 *3.3. Effect of storage temperature for mussels packaged in oxygen-rich atmospheres*

24 The effect of storage temperature is illustrated by comparing batch 2 (7 °C) and 3 (2
25 °C) in Figs. 1, 2 and 3. The evolution of the content of ammonium and basic volatile
26 substances was more pronounced from day 3 for the mussels stored at 7 °C, at which
27 point the differences between both batches became significant (Table 2). On day 6, the
28 values of ammonium nitrogen and TVB-N in the intervalval fluid of the mussels stored
29 at the higher temperature were approximately 2 and 1.6 times higher than those stored
30 at 2 °C, respectively.

1 A strong change in VFA concentration was observed between day 6 and 7 (from 101.1
2 mg/L to 230 mg/L) for the mussels stored at the higher temperature. In contrast, the
3 increase was slow and gradual until day 9 for the mussels stored at 2 °C.
4 Temperature is an important factor for maintaining the quality of live and dead fishery
5 products. For example, sensory evaluation results of scallop adductor muscle indicated
6 that decomposition progressed faster at 5 °C than at 0 °C with a higher accumulation of
7 D-lactic acid (Kawashima & Yamanaka, 1992). Similarly, after incubating cultivated
8 clams for 1 week at different temperatures, Morley et al. (2009) observed a significantly
9 higher concentration of the end products of anaerobic metabolism at 7.5 °C than at
10 lower temperatures. Studies of oxygen consumption by shrimp maintained in cultivation
11 tanks showed that specific metabolic rates increased by a factor of between 1.6 and 2
12 when temperature increased from 2 to 8 °C (Daoud, Chabot, Audet & Lambert, 2007).

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14 *3.4. Changes in mortality, sensorial assessment and microbial abundance of packed* 15 *mussels during storage*

16 The mortality rate in the distinct batches indicates that the higher temperature mussels
17 (batch 2) reached the highest mortality levels within the shortest time (Table 3). The
18 recorded mortality on day 6 was 28% compared to only <10% for the other batches. An
19 important increase of non-viable mussels was produced in the batch packaged with
20 20% O₂ (batch 1) in the following hours, and the batch was considered unsuitable on
21 day 7 of storage. Batches 3 and 4 packaged with oxygen-rich atmospheres and
22 maintained at 2 °C presented the lowest mortality throughout storage, with the larger
23 mussels (batch 4) being more resistant.

24 Conditioning temperatures for molluscs which are very different from their optimum
25 temperature can cause high rates of mortality. Christophersen et al. (2008) indicated
26 that the resistance to physiological collapse depends on time, temperature, season and
27 ontogenetic stage. According to these authors, adult scallops were least affected by
28 simulated transport at temperatures that deviated from ambient, and mortality
29 commenced earlier for spat than for juveniles. For example, the percentage survival in
30 larvae was 8-16% versus 40-43% in juveniles when the temperature was 2-5 °C above

1 or 11-15 °C below the ambient temperature. For live clams, it has been shown that the
2 most notable conservation temperature is 10 °C (Sadok et al., 2003), whereas
3 conservation with ice provides a good quality product with a long shelf life for mussels
4 cultivated in Canada (Harding, Couturier, Parsons & Ross, 2004). In contrast, placing
5 crustaceans directly on ice will cause death within a few hours, probably by thermal
6 shock (Robson et al., 2007).

7 The sensorial analysis of the cooked mussel (Table 4) indicates that from day 6 the
8 mussels in batches 1 and 2 were significantly different from those in batches 3 and 4,
9 with higher values in the latter. All scores decreased progressively, although with
10 greater intensity for those stored at 7 °C. The small- and medium-sized mussels stored
11 at 2 °C obtained good scores until day 9 of storage. Some tasters remarked that the
12 texture in the small mussels had a lower consistency on day 9, coincident with
13 spawning observed in some of the packages. It is possible that the higher rate of
14 consumption of energetic reserves during the pre-spawning period was reflected in the
15 organoleptic analysis. The storage temperature of live oysters has been shown to
16 influence the percentage acceptability of panellists based on smell (Lorca, Pierson,
17 Flick & Hackney, 2001). These workers reported that molluscs stored at 7 °C had a
18 75% acceptability level on day 3. This figure decreased to 45% at 21 °C, even though
19 small differences were noted in pH (for evaluating wholesomeness) and in the total
20 counts of halophilic microflora. The concentration of acidic or basic compounds in the
21 intervalval fluid may influence the sensorial assessment of the cooked product,
22 although their presence is diminished due to their volatile character. The accumulation
23 of acidic products in the intervalval fluid appears to be a more determining factor since
24 the batches with highest accumulation of acids (batch 1 and 2) obtained worse
25 organoleptic scores. Furthermore, it has been shown that the increase of VFA in shell
26 liquor is also detected in the tissues of Mediterranean mussels, although this depends
27 on the packing conditions (Bernárdez & Pastoriza, 2011). The legally-permitted
28 concentration of TVB-N is limited to range of 25-35 mg/100g (Directive 95/149/ECC) for
29 certain fish products. Moderate increases were observed in mussel meat for the 4
30 batches. The concentration of TVB-N on day 8 of storage, as mg TVB-N per 100g of

1 mussel tissue or meat, was 18.1 ± 0.1 for the batch low in O₂ (batch 1) and 21.0 ± 0.5
2 for the mussels stored at 7 °C (batch 2). The concentration in the small- and medium-
3 sized mussels reached 16.9 ± 0.9 and 15.3 ± 0.4 mg, respectively, from an initial value
4 of 12.6 ± 0.8 . These values are not elevated in any of the cases and maintained the
5 same order as the organoleptic analysis, that is, the mussels stored at the higher
6 temperature had a lower organoleptic scores and a higher concentration of TVB-N.

7 The levels of fecal coliforms and *E. coli* in the mussel batches did not exceed the 230
8 MPN/ 100g limit established by the European Community (EC 2073/2005). The total
9 count of aerobic microorganisms on the same sample on day 2 and 7 of storage
10 showed a mean value of 3.9 ± 0.23 log UFC/g in all batches. The stability of the
11 bacterial count in live mussels is justifiable provided that the immune system is capable
12 of combating their presence. Robson et al. (2007) also showed that crabs have the
13 potential to be transported alive at 4 °C without spoilage for 5 to 21 days depending on
14 the species. On the other hand, the total bacterial count in the intervalval fluid of live
15 mussels showed a substantial increase from day 6 of storage (Table 5). Also, the
16 presence of H₂S-producing bacteria was more evident in the last days of storage. Both
17 counts indicate a higher bacterial growth in mussels stored at 7 °C and a lower
18 concentration of bacteria in the larger mussels. This seems to indicate that microbial
19 development is favoured by higher temperatures and the presence of waste products
20 or decomposition products of dead mussels into each container (see percentage
21 mortality, Table 3) since these can be used substrates by bacteria. For instance, it is
22 known that proteolytic seawater bacteria have the capacity to metabolize dissolved free
23 amino acids in addition to some labile dissolved combined amino acids (Amano, Hara
24 & Taga, 1982). Other workers obtained microbial counts on marine agar of 6.3 and 4.0
25 log CFU/g in mussels stored at 9 °C and 2 °C after 3 days of storage, respectively
26 (Khan, Parrish & Shahidi, 2005). In addition, the increasing volume of intervalval fluid
27 with shell size is one of the probable causes of the lower bacterial concentration in the
28 medium-sized mussels.

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4. Conclusion

Small-sized Mediterranean mussels packaged in oxygen-rich atmospheres and stored for 8 days at 2 °C maintained the organoleptic characteristics for 5-6 days longer than a batch packaged in air only and displayed lower percentage mortality and lower rate of production of acidic compounds. Elevated storage temperature (7 °C) impairs the viability of the mollusc when packaged under modified atmospheres, and contributes to a higher percentage mortality in a shorter time and a lower organoleptic evaluation. This factor reduces the shelf life by 5-6 days compared to storage at 2 °C. When the batches of different sized mussels are compared a greater resistance was observed in the larger mussels which, along with the microbial analysis and the organoleptic evaluation, indicate an extended commercial life by at least one day with respect to the smaller mussels.

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1 **Fig. 1.** Accumulation of ammonium (A), TVB-N (B) and VFA (C) in the intervalval fluid of live
2 Mediterranean mussels under MAP: bach 1 (■), bach 2 (□), bach 3(⊕) and bach 4(⊖). Vertical
3 bars represent the standard error (n=2).
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8 **(B)**

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Table 1
Conditions of packaging and storage in the study batches

Batch	[O ₂]	Mussel size	Storage temperature
1	20%	Small	2 °C
2	83%	Small	7 °C
3	83%	Small	2 °C
4	83%	Medium	2 °C

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Table 2
Significant differences in the analysis of the intervalval fluid of the 4 batches of mussels packed under modified atmospheres

Analysis	Day	Batches			
		1	2	3	4
Ammonium	2	a	b	c	c
	3	a	b	b	c
	6	a	b	c	d
	7	a	b	c	d
	8	a	b	c	d
	9	*	*	a	b
TVB	1	a	b	b	a
	2	a	b	c	d
	3	a	b	c	d
	6	a	b	c	d
	7	a	b	c	d
	8	a	b	c	a
	9	*	*	a	b
VFA	1	a	*	*	a
	2	a	a	b	*
	3	a	a	b	b
	6	a	b	c	c
	7	a	b	c	*
	8	a	b	c	*
	9	*	*	a	a

a-c are significantly different (p<0.05) between batches (n=2); * not sampled

TVB: total volatile bases; VFA: total volatile fatty acids

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1 **Table 3**
2 Percentage mortality of Mediterranean mussels packaged with distinct concentrations of oxygen
3 and stored at 2 or 7 °C.
4

Day	Batch 1			Batch 2			Batch 3			Batch 4		
	Mean	D st		Mean	D st		Mean	D st		Mean	D st	
1	0,6	1,0	a	1,0	1,0	a	1,0	1,0	a	0,0	0,0	a
2	3,0	1,6	a	2,5	1,2	a	2,6	3,1	a	0,0	0,0	b
3	3,5	2,4	a	1,6	2,7	a	2,4	1,2	a	1,6	1,3	a
6	9,6	7,4	a	28,5	4,5	b	5,4	3,2	a	9,0	1,9	a
7	21,6	4,9	a	24,4	8,5	a	7,5	7,6	b	6,4	2,6	b
8	32,1	10,0	a	23,4	5,4	a	15,2	6,2	b	8,1	3,0	b
9	49,9	12,3	a	*	*		17,1	4,8	b	10,5	1,7	b
10	*	*		*	*		*	*		12,6	5,4	

Mean and standard deviation (D st) of 3 packages (n=3)

a-c are significantly different (p<0.05) between batches

5

6 **Table 4**
7 Organoleptic evaluation of cooked mussel during storage
8

Day	Batch 1			Batch 2			Batch 3			Batch 4		
	Mean	D st		Mean	D st		Mean	D st		Mean	D st	
2	7,7	0,6	a	7,3	0,6	a	7,8	0,7	a	7,2	0,7	a
6	6,2	0,5	a	6,8	0,7	a	8,2	0,4	b	8,0	0,6	b
8	5,5	0,5	a	4,3	1,3	a	7,8	0,3	b	7,3	0,8	b
9	3,3	1,9	a	unfit			7,3	0,6	b	7,3	0,5	b

Mean and standard deviation (D st) of 8 panelist (n=3)

Scores were recorded in a 10-point scale with values > 7= ideal quality

a-c are significantly different (p<0.05) between batches

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1 **Table 5**
2 Microbiological counts in the intervalval liquid of packaged mussels (log CFU/mL)
3

Day	Batch 1		Batch 2		Batch 3		Batch 4	
	TVC	H2 S	TVC	H2 S	TVC	H2 S	TVC	H2 S
1	< 2,30	< 2,30	< 2,30	< 2,30	< 2,30	< 2,30	< 2,30	< 2,30
3	< 2,30	< 2,30	< 2,30	< 2,30	< 2,30	< 2,30	< 2,30	< 2,30
6	2,80 a	< 2,30	2,69 a	< 2,30	2,51 a	< 2,30	2,24 a	< 2,30
7	4,51 a	2,99 r	5,09 b	3,10 r	4,49 a	2,96 r	3,47 c	< 2,30
9	4,68 a	3,01 r	5,24 b	3,54 s	5,01 c	3,12 s	3,85 d	2,56 t

CFU: Colony Forming Unit; TVC: Total Viable Counts; H2 S: H2S-producing bacteria

a-c are significantly different ($p < 0.05$) between batches in TVC

r-t are significantly different ($p < 0.05$) between batches in H2 S

4