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

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ABSTRACT

The objective of this study was to evaluate the behaviour of small-sized (~19 g/unit) Mediterranean mussels packaged hermetically with different proportions of oxygen in the interior of the package and stored at 2 °C and 7 °C. The most favourable conservation conditions (83% O_2 and 2 °C) were compared with larger mussels (~24 g/unit). On day 3 of storage with 20 %O_2 at 2 °C, the concentration of volatile fatty acids in the intervalval fluid was double that for the mussels packaged with 83% O_2 and this difference increased during storage. Higher temperatures produced a larger and more rapid accumulation of ammonium nitrogen with concentrations of 49 mg N/L on day 6. Lower concentrations were observed at the end of storage at 2 °C (27 mg N/L on day 9). From day 7, the increase of N-TVB measured in the intervalval fluid of the small mussels at 2 °C was more pronounced than in the larger mussels. The organoleptic evaluation, percentage mortality and microbial counts collectively indicated that a high quality product was maintained on day 8–9 at 2 °C with mussels packaged with 83% O_2. Inadequate temperatures have a stronger negative impact on shelf life than low oxygen concentrations.

1. Introduction

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

Once withdrawn from their environment, shellfish have a limited life which depends on the species and the conditioning during transport and sale.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

These factors should also be considered during commercialization. Temperature is a key factor for the conservation of foodstuffs. Higher temperatures tend to accelerate the processes of decomposition following animal death and are one of the causes of antemortem stress leading to poor-quality meat (Haard, 1998). Temperature is also a limiting factor for the shelf life of live shellfish. An adequate storage temperature for clams reduces the appearance of altering compounds, and provides a higher quality product with a prolonged marketable period (Sadok, Uglow, & El-Abed, 2003). Additionally, the behaviour of live shellfish can vary with species, the locality of cultivation or withdrawal and the time of the year. Robson, Kelly, and Latchford (2007) showed that the onset of deterioration for distinct species of live crustaceans stored at 4 °C depended on the habitat of origin, with the intertidal specimens being the most resistant. The storage temperature may also alter the shelf life. For example, the shelf life of *Necora puber* is ~13 days at 4 °C and ~2 days at 20 °C.

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Conservation of live bivalve molluscs in hermetic packages allows the product to reach the consumer in an ideal state. Modified atmosphere packaging (MAP) is an effective alternative for prolonging the commercial life of these molluscs. Combined with oxygen-rich atmospheres and with respect to unpackaged molluscs, this system can provide the adequate gas mixture and humidity for maintaining live mussels (Pastoriza, Bernárdez, Sampedro, Cabo, & Herrera, 2004) in addition to the characteristic organoleptic qualities of clams (Gonçalves, Pedro, Duarte, & Nunes, 2009). Furthermore, it has been shown that the size (large or medium) of Mediterranean mussels and the oxygen concentration inside the package can influence quality and shelf life (Bernárdez & Pastoriza, 2011).

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

2. Materials and methods

2.1. Materials and equipment

The gas, containers and packaging machine were described in Bernárdez and Pastoriza (2011). The proportion of CO_2 and O_2 in the package headspace was determined using a PAK 12P headspace analyser (Abiss, Barcelona, Spain). Spectrophotometric measurements were performed on a Cecil 3041 Spectrophotometer (Cecil, Cambridge, England).

2.2. Sample collection and preparation for analyses

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

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

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

2.3. Quantification of mortality

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

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

2.4. Chemical analysis

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

2.5. Microbiological analysis

A 25 g sample (20 g meat and 5 g shell liquor) was mixed with 225 mL of 0.1 g/100 mL peptone water for 1 min in a Lab-Blender-400 Stomacher. Serial decimal dilutions of the mixture were made in 9 mL of 0.1 g/100 mL 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/100 g 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 H_2S-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.

2.7. 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).