ELEVATED CONCENTRATIONS OF OXYGEN ON THE STABILITY OF LIVE MUSSEL STORED REFRIGERATED

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ABSTRACT

The stability of bivalve molluscs was studied in live mussels (*Mytilus galloprovincialis*) packaged under modified atmospheres. Studies of physical, chemical, microbiological and sensory parameters have detailed these changes in the live packaged mussels. The highest survival was achieved at high oxygen concentrations, i.e. 75%, in the absence of carbon dioxide. The improved storage conditions promote shelf life in 80% of the packaged mussels, reaching a storage life of 6 days when held at 2-3ºC, whereas control molluscs packaged in air did not exceed 3-4 days when stored under the same conditions. Sensory analysis of cooked products from live mussels packaged under 75% oxygen:25% nitrogen were optimum throughout the period of storage.

Secondly, the results for the most effective treatment were compared with those for an approximate 75-80% oxygen mixture achieved by applying partial vacuum to the packaging machine. This option employed only oxygen and decreased packaging time, which reduced mussel packaging stress by avoiding elevated vacuum conditions. It provided operational and economic advantage to commercial mussel packaging.

Keywords: mussels, modified atmosphere, oxygen, stability, refrigerated, freshness, mortality

INTRODUCTION

MAP technology (modified atmosphere packaging) is used to improve the quality and extend the shelf life for a wide range of fresh and refrigerated foods, including raw and cooked meats, fish, fresh pasta, fruit and vegetables, and more recently, coffee, tea and brewery products. The technology is expanding to other refrigerated foods with a limited shelf life as MAP effectively suppresses the growth of the main microbial flora which may be randomly present in the foodstuff. The advantages and disadvantages of MAP have been periodically evaluated [1, 2, 3, 4, 5] and its use does not eliminate the need for appropriate control of the storage conditions, especially temperature, as well as an adequate protocol for food manipulation and sanitation at each preparation stage. There have been many studies involving all food classes, raw [6, 7], frozen [8, 9], cooked, pre-cooked and prepared dishes [10, 11]. These studies researched alternative methods to promote the stability and safety of refrigerated food. However, understanding the application of MAP technology to live mussels is still limited, and the challenge offer to develop a packaged product with a superior shelf life compared to present commercial methods.

Deterioration of the live mussel, as for any foodstuff, can occur at any stage between raw material harvest and product consumption [16]. These stages are diverse, and for the mussel include: habitat, purification, processing, packaging, distribution, retail display, transport, storage and consumer use. Being a living product, special care is needed at every stage since stress accelerates instability and thus hastens mortality. Generally, MAP-treated mussels suffer longer processing times than those packaged under traditional ways since more manipulation is required. The extended processing can easily lead to mortality, rapid deterioration and departure from a natural and fresh appearance during storage.

The purpose of the study was to develop a modified storage atmosphere, distinct from air, which would maintain mussel quality and longevity with a natural and fresh appearance during 6 days of refrigeration. To increase the range of marketing options for the fresh mussel market, modified atmospheres may reduce microbial growth and other reactions that may limit the storage life of refrigerated food. A study of modified atmosphere microbiological preservation procedures was not considered necessary, since the research centred on achieving the stability and longevity of a live product, while

maintaining the sensory qualities demanded by the consumer. The work to research is of enormous interest for the fishery sector of Galicia (NW Spain), since mussel is the most widely marketed Spanish bivalve. The mussels are highly valued, for their excellent meat quality, size and for the high production levels (approximately 262,000 tonnes/year representing 45% of global production). The mussel is an important commodity for national and international markets.

MATERIALS AND METHODS

Sample preparation

Live mussels (60 kg) from mussel farms in the Ría of Arosa (Galicia-Spain), were maintained in sea water for 48h in a purifier station before transfer to the processing plant. After mechanically removing the byssus, they were brushed and submerged in refrigerated water (6ºC) for 6 minutes to reduce stress. One kg portions of mussels were manually placed in high-density polyethylene (HDPE) barrier containers (G10, 264mm x 165mm x 51mm, Polimoon S.A., Spain), the mixture of gases was injected and the surface was sealed by a multilayer film (GPO1570, Polimoon S.A., Spain).. The mussels should be adjusted to achieve a tight fit inside the containers, preventing easy shell opening, increasing viability. Care must be taken to avoid possible tearing of the containers by the sharp edges of the bivalve shells. The packaging machine used in the study was an 900VG ELTON PACK-MEGA, S.A. (Barcelona, Spain).

The gas mixtures were supplied by Carburos Metálicos, S.A. (Barcelona, Spain). Selected mixtures were tertiary, containing oxygen, carbon dioxide, and nitrogen (5%O_2, 20%CO_2, 75%N_2 and 20% O_2, 50%CO_2, 30% N_2), binary, without carbon dioxide (50% O_2, 50% N_2 and 75% O_2, 25% N_2) and oxygen only (100% O_2, with a partial vacuum in the mixing zone, producing a final atmosphere of 75-80% O_2). Experimental results were compared with control air, which is the usual method of commercial packaging in 3 and 5kg mesh bags. Mussels were placed in a refrigerated chamber at 2-3ºC for storage.

Two studies, based on the selected gas mixture, were carried out as follows:

The first study investigated tertiary and binary gas mixtures, by determining the greatest mollusc survival in each mixture. Mortality was evaluated by tapping on gaping bivalve shelves and observing mussel closure. Any shells gaping after taping were considered dead.

The second study compared the results for the most effective treatment in the first study (75% O_2 : 25% N_2) with an approximate 75-80% oxygen mixture achieved by applying a partial vacuum to the packaging machine. This option provided an operational and economic advantage to commercial mussel packaging. The system employed only oxygen and indirectly decreased packaging time which reduced mussel packaging stress by avoiding elevated vacuum conditions.

In the first study, 5 packages of each gas mixture were taken daily for sampling, whereas in the second study 10 packages were taken. The approach permitted the comparison of mixtures, an assessment of the deterioration process, and the selection of the optimal conditions by studying the parameters outlined below. The proportion of CO2 and O2 in the package headspace was determined using a PAK 12P headspace analyser (Abiss, Control and Supplies, Barcelona, Spain).

Chemical analyses

Mussel samples were homogenized with water at a ratio of 1:2 for pH determinations. Total volatile bases (TVB) were determined according to Lücke and Geidel [12] with modifications by Antonacopoulos [13]. TVB is expressed as mg TVB-N/100g tissue.

Microbiological analyses

A series of standard microbiological determinations were carried out to ascertain the effectiveness of the experimental treatments against the growth of microbial flora normally acquired by the product during inadvertent contamination in industrial processing plants. Samples for microbiological analyses were prepared by blending 10 g of product and 90 mL of sterile peptone (Cultimed S.A., Madrid, Spain) in a Lab-Blender-400 Stomacher, followed by serial (10-fold) dilution in sterile peptone water. Triplicate microbiological analyses were performed in all cases.

Total viable counts (TVC) were measured by spreading 0.1 ml aliquots of serially diluted samples on Plate Count Agar (Difco, Madrid, Spain) supplemented with 0.5% NaCl. Three plates/dilutions were incubated for 5 days at 20°C [14]. Results are expressed as the logarithm of colony forming units (log CFU/g) per sample.

Using the Most Probable Number (MPN, 5 tubes/dilution) method, total coliforms were determined in 2% Brilliant Green Bile Broth (Cultimed S.A.) after incubation at 37±1°C for 24-48 hours. Results are expressed as MPN/100g of sample. From the total positive coliforms, the presence or absence of faecal coliforms was verified by incubation at 44.5ºC for 48h. *Escherichia coli* was verified by the indole test and seeding on Levine agar. Results are expressed as MPN/100g of sample [15].

The presence of S*almonella* was confirmed by a validated immunoassay test (Bioser, Barcelona, Spain), which permitted the detection of mobile and non-mobile salmonella organisms in food. Results are expressed as presence or absence/25g of sample.

Sensory analyses

Mussels were steam-cooked at 100ºC for 3 min and served into one valve to esperienced panelists at aproximately 35ºC. Odor, flavor and texture were scored up to 6 days of storage by a five-member panel following a 10 point-scale according to the characteristics shown in Table 1.

Statistical analysis

Statistical analyses were applied to compare mortality values of fresh mussels packaged under a commercial gas mixture consisting of 75%O_2, 25%N_2 or a gas mixture of similar composition prepared by applying partial vacuum at the factory (see second study). Significant differences between the samples were calculated by StatView software using one-way analysis of variance (ANOVA) ($p < 0.05$) by Fischer's test.

RESULTS AND DISCUSSION

Considering the evolution of the different gas mixtures, independent of the tertiary or binary condition, a decrease in oxygen was usually accompanied by increases in carbon dioxide throughout the storage period (Table 2). The ability to maintain relatively constant oxygen uptake under conditions of low oxygen availability is important for organisms that may frequently encounter low dissolved oxygen (hypoxia) in their environment [17]. Accordingly, in the gas mixtures with low oxygen concentrations, for example sample A (5%), the oxygen was almost completely consumed by the molluscs. Consequently, reduced O_2 increased mortality, which reached 93% after 6 days. As the oxygen composition of the initial mixture increased, samples C and E with 50% and 75% oxygen, respectively, the residual oxygen in the containers after 6 days storage dropped to 32% and 56% respectively. Both levels were adequate to keep the mussels alive.

Under our experimental conditions, the period of mussel survival without water depends on factors such as surrounding air temperature and humidity and the possibility of intermittent gaping. Mussel viability was variable, depending on the oxygen and carbon dioxide concentrations in the containers (Figure 1). Contact with high concentrations of CO_2 negatively influenced samples A and B, which contained 20% and 50% CO_2, producing mussel mortality of 93% and 95%, respectively, after 6 days refrigeration (Figure 1). Conversely, higher oxygen concentrations were usually accompanied by increased bivalve longevity. In samples C and E, with oxygen concentrations of 50% and 75%, respectively, the mortality was 17% and 5% after 4 days of refrigeration and 33% and 10% following 6 days (Figure 1). For air, (sample D), the gas usually used in commercial packaging for this mollusc, mortality reached 33% and 77% after 4 and 6 days of refrigeration (Figure 1), respectively, under the same storage conditions.

A further important consideration was the influence of the container fill level on refrigerated mollusc mortality. Figure 2 shows the percent bivalve mortality in gas mixtures with higher oxygen concentrations (atmospheres C and E with 50% and 75%, respectively) in totally-filled and semi-filled containers. With 50% oxygen, the percentage of dead mussels was 26% and 49% for totally-filled and semi-filled containers, respectively. The best results were obtained with samples at the highest oxygen concentration (75%). Mortality only reached 10% and 29% for totally-filled and semi-filled containers, respectively, after 6 days storage (Figure 2). Filling the containers in a favorable atmosphere delays shell gaping, with a possible alteration of the

aerobic/anaerobic metabolism producing greater survival. It is well known that when exposed to air, *Mytilus edulis* can maintain both aerobic and anaerobic metabolism via shell gaping [18, 19, 20].

The pH of fresh mussel flesh was 6.47, and 7.01 for the inter-valve liquid. In the edible meat, the pH increased slightly but remained within acceptable quality values for fresh mussel (Table 3). The pH of the muscle reached 6.74 on day 6 in atmosphere C (data not shown). In the inter-valve liquid, pH tended to decrease slightly. Slight decreases in the pH of the inter-valve liquid may be produced by anaerobic metabolism caused by removing the bivalve from its natural habitat [21].

The total volatile base values were determined for both flesh and inter-valve liquid (Table 3). The total volatile bases were low and little discernable variation was observed after 6 days storage. Values oscillated between 8.47 mg and 10.42 mg TVB-N/100g for day 1 and 6, respectively. These values are similar to those obtained by Lee et al [22] in fresh mussel muscle and by Thomson [23] in ice-stored clam. When the conditions were favorable, TVB increased rapidly, as indicated in the data from Cantoni and others [24]. The TVB increase in mussels refrigerated at 2ºC over 6 days was 48.57%. These values are similar to those obtained in atmosphere C of this study, with TVB increasing to 52.76% (data not shown) over the same period.

Ammonia excreted by the mussel from aerobic metabolism is included in the TVB. When the concentration increases, the oxygen to nitrogen (O:N) ratios are modified, producing a lower ratio under anaerobic conditions. The O:N, derived from respiration and NH_4-N excretion, indicated an unfavorable metabolic balance, during stressed refrigerated storage [25, 26].

The presence of *Vibrio* and other potential bacterial pathogens such as *Escherichia coli*, *Salmonella* and *Shigella* have been detected in cultivated mussels at retail markets [27, 28, 29]. Some of these bacteria form part of the natural marine microflora and may be accumulated by shellfish during feeding [30]. S*almonella* spp. is among the most important groups of global food pathogens. These species are often the agents of gastroenteritis associated commonly with consumption of contaminated shellfish, particularly oysters and live mussels [31, 32]. Contaminating bacterial load is occasional,

and almost always a consequence of sewage outfalls close to the cultivation zone. However, improper processing or poor sanitation practices on the boats and in processing plants, should not be ruled out as other possible sources of bacterial contamination.

Bacterial indicators of bivalve molluscs quality and safety in this study followed the established limits defined by the Spanish authorities: of <300 fecal coliforms/100g +<230 *E. coli*/100g, and the absence of *Salmonella*/25g [33]. All samples met the regulations and were below established limits, which certifies the quality and safety of the molluscs cultivated in NW Spain and packaged in modified atmospheres for 6 days (Table 4). No *Salmonella* was observed in 25g of sample. No fecal coliforms or *Escherichia coli* were recovered following 6 days treatment and the number of aerobes was 4.32 log CFU/g, which is lower than the established limit for live bivalve molluscs of 5 log CFU/g [14]. These findings agree with previous results for bivalves from NW Spain [34].

The sensory characteristics (odor, flavor and texture) of the steam-cooked product were optimal after 6 days storage. Scores fluctuated between 10 and 8 after 1 day and 6 days of refrigerated storage (Table 5). Odor and flavor were those characteristics of fresh mussel (sea-like, seaweeds-like) whereas the texture of the cooked product maintained its firmness throughout the period of storage

Secondly, the binary commercial mixture of 75% oxygen was compared with an approximate 75-80% oxygen mixture prepared from a 100% oxygen mixture by applying a partial vacuum to the packaging machine (Table 6). These latter values oscillated between 71% and 79% oxygen. No significant differences were observed between the two preparations throughout 6 days of storage, with mortality values of 4.3% and 6.7% after 4 days, and 9.7% and 9.4% after 6 days of refrigeration (Table 6). The experimental results obtained are very similar, and therefore we recommend packaging of live mussel under modified atmospheres. The sensory studies revealed similar findings. The mixture prepared in the pilot plant of the factory presents an economical advantage by requiring only oxygen and reducing packaging time.

Modified atmospheres rich in CO2 are normally used to extend the shelf life of foods by inhibiting gram-negative aerobic flora when stored refrigerated [35, 36, 37]. However, in this study, the modified atmosphere was rich in oxygen with carbon dioxide

absent, since the latter causes mortality of bivalve molluscs. Meticulous sanitary conditions of the raw material at the origin and the processing plant are recommended to commercialize live bivalve molluscs in order to minimise flora alteration and pathogenic microorganisms at the distributional and sales points. Accordingly, bacterial alteration below recommended commercialisation level has shown to be minimised over the 6 days refrigeration period.

Mandatory purification conditions are required to certify suitable primary material for commercialisation of live mussels through exhaustive microbiological analysis. Furthermore, the storage temperature should match the recommended temperature for the refrigeration of fresh food, around 2-3ºC.

CONCLUSIONS

Modifying the storage atmosphere for live mussels, after removal from the cultivation site, has extended the commercial shelf life of this bivalve mollusc by 48 to 72 hours compared to current commercial storage practices. These improved storage conditions extend the natural and fresh appearance of the mussel, which have practical consequences for product distribution. The advances provide scientific knowledge and distribution economics, considering that the mussel is a highly valued product on national and international markets.

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Figure 1: Percentage mortality of live mussel packaged under a modified atmosphere for 6 days at 2-3ºC: A (5% O_2, 20% CO_2, 75% N_2); B (20% O_2, 50% CO_2, 30% N_2); C (50% O_2, 50% N_2); D (air) and E (75% O_2, 25% N_2).

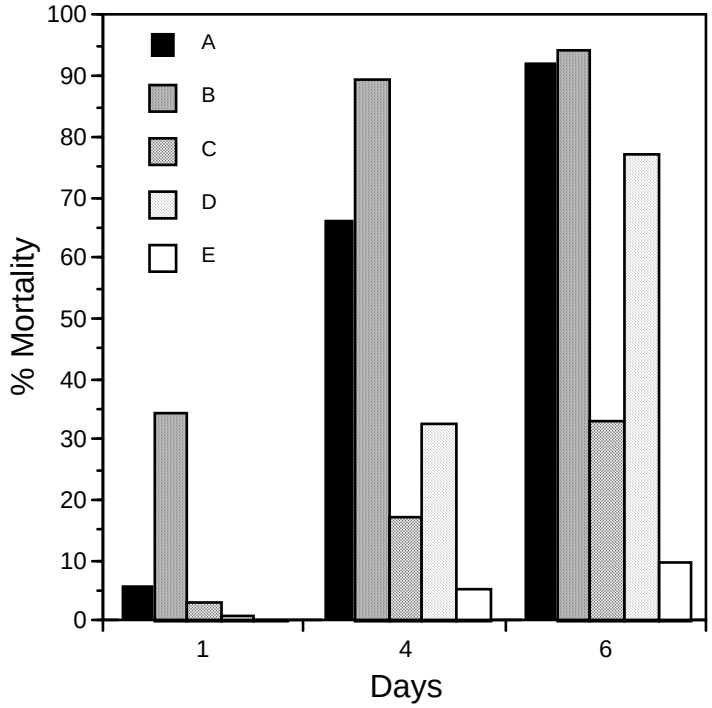

Figure 2: Percentage mortality of live mussel packaged under a modified atmosphere (50% O_2, 50% N_2 and 75% O_2, 25% N_2) in full or half-full packages for 6 days at 2-3ºC.

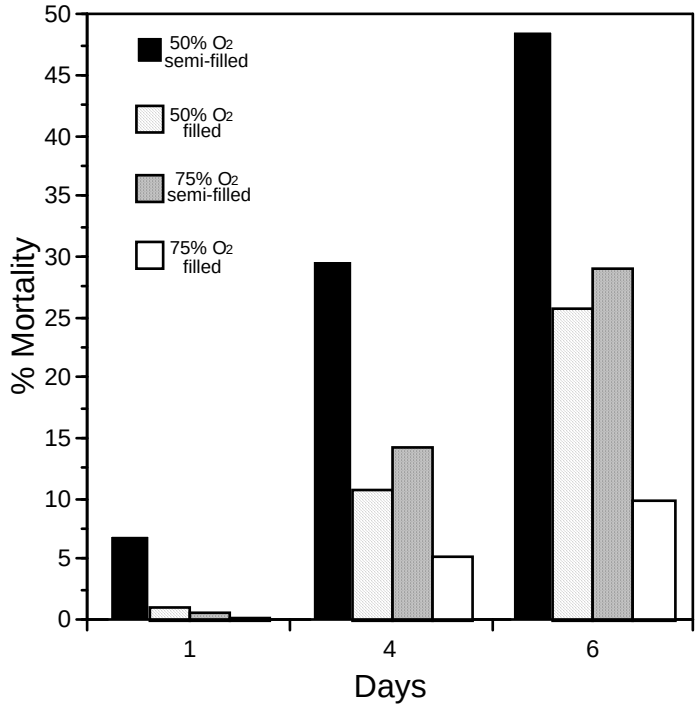

Table 1. Scale for sensory evaluation of steam-cooked mussel

SCORE	10	9-8	7-6	5	4-3	2-1
ODOR	characteristic sweet fresh	non-specific slightly sweet	neutral	slightly to ammonia	ammonia	very unpleasant
FLAVOR	characteristic mild	characteristic slightly mild	strong to mussel	slightly sour	sour	sickening
TEXTURE	very firm	firm	slightly firm	slightly soft	soft	very soft

Table 3. Values of total volatile bases (TVB) and pH of the live mussel packaged in gas mixture E at 2-3ºC.

Days	TVB	pH*	pH**
1	8.47 (0.20)	7.01 (0.01)	6.47 (0.04)
2	8.54 (0.10)	7.03 (0.02)	6.65 (0.03)
4	8.96 (0.08)	6.86 (0.01)	6.57 (0.01)
6	10.42 (0.19)	6.91 (0.01)	6.53 (0.01)

pH*: inter-valvar liquid. pH**: flesh. E: 75%O_2, 25%N2.
TVB: mg TVB-N/100g.
Values in parenthesis correspond to standard deviations.

Table 4. Total viable count (TVC), fecal coliform count (FCC) and *Escherichia coli* count of the live mussel packaged in gas mixture E at 2-3ºC.

Days	TVC	FCC	*E. coli*
1	3.88 (0.01)	2	2
4	3.92 (0.01)	5	2
6	4.27 (0.04)	0	0

E: 75%O_2, 25%N2. TVC (CFU/g), FCC (NMP/100g), *E. coli* (NMP/100g).
Values in parenthesis correspond to standard deviations

Table 5. Sensory characteristics of the live mussel packaged in gas mixture E at 2-3ºC.

Days	Odor	Flavor	Texture
1	9.75 (0.58)	9.83 (0.58)	9.80 (0.58)
2	9.69 (0.29)	9.77 (0.29)	9.70 (0.29)
4	8.33 (0.58)	8.60 (0.50)	8.50 (0.58)
6	8.17 (0.76)	8.27 (0.76)	8.33 (1.32)

E: 75%O_2, 25%N2. Values in parenthesis correspond to standard deviations

Table 6. Comparison of the mortality of live mussel in a commercial gas mixture (E) and in a mixture prepared in the plant of factory (P)

Days	E (%)	P (%)
1	0.31 (0.75)[a]	3.98 (1.69)[b]
4	4.32 (2.77)[a]	6.74 (3.00)[a]
6	9.72 (2.47)[a]	9.41 (4.42)[a]

E: Commercial mix: 75%O_2, 25%N_2. P: Mixture prepared in the plant of factory with 100% O_2 under partial vacuum to a concentration of approximately 75%O_2
Values in parenthesis correspond to standard deviations.
Values in the same row with same subscript are statistically equivalent