

Investigation of soluble gas stabilization combined with modified atmosphere packaging on the shelf life of cooked blue mussels (*Mytilus edulis*)

Investigação da pré-solubilização de gás combinada com atmosfera modificada na vida útil de mexilhões cozidos azuis (*Mytilus edulis*)

Investigación de la presolubilización de gas combinada con envasado en atmósfera modificada sobre la vida útil de mejillones azules cocidos (*Mytilus edulis*)

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Abstract

For the use of carbon dioxide in modified packaging (MA) systems, is it necessary a high gas/product ratio to ensure the bacteriostatic effect of fishery food. The use of techniques such as CO₂ Soluble Gas Stabilization (SGS), can reduce the size of package necessary to maintain the effectiveness of MA. Thus, the effect of the MA packaging combined with soluble gas stabilization (SGS) as pre-treatment was studied to investigate the shelf life of cooked blue mussels. The samples were subjected at 100% food-grade CO₂ (96%) for 2 h (SGS), in large flexible pouch. After SGS treatment, 100 g of mussels were packaged under modified atmosphere and air. Initial CO₂ concentration under SGS packages was 70.0 % and remained constant (72.5 %) due the pre-treatment with CO₂. Physicochemical properties (pH, water content, firmness and water-holding capacity) were not influenced significantly SGS treatments. The SGS samples had reduced growth of mesophilic and psychrotrophic during storage. The application of SGS resulted in an increased microbiological shelf life (19 days) compared with the packaging with air (5 days) and can contribute to improved quality of cooked mussels.

Keywords: Blue mussels; Carbon dioxide; Modified atmosphere; Soluble gas stabilization.

Resumo

Para o uso do dióxido de carbono em sistemas com atmosfera modificada (AM), é necessária uma elevada relação gás/produto para assegurar o efeito bacteriostático em pescado e seus derivados. O uso de técnicas como a pré-solubilização do CO₂ (SGS) pode reduzir o tamanho da embalagem necessária para manter a efetividade da AM. Assim, o efeito do envase sob AM combinada com a pré-solubilização de gás (SGS) como pré-tratamento foi estudado para investigar a vida útil de mexilhões azuis cozidos. As amostras foram submetidas à 100 % de CO₂ de grau alimentício (96 %) por 2 h (SGS), em embalagem grande e flexível. Após o tratamento SGS, 100 g de mexilhões foram envasados sob atmosfera modificada e ar. A concentração inicial de CO₂ nas embalagens com SGS foi de 70 % e permaneceram constantes (72,5 %) devido ao pré-tratamento com CO₂. As propriedades físico-químicas (pH, teor de água, firmeza e capacidade de retenção de água) não foram influenciadas significativamente pelos tratamentos com SGS. As amostras com SGS tiveram crescimento reduzido de mesófilos e psicrotróficos durante o armazenamento. A aplicação do SGS resultou em um aumento da vida útil microbiológica (19 dias) comparada com o envase com ar (5 dias) e podem contribuir para melhorar a qualidade de mexilhões cozidos.

Palavras-chave: Mexilhões azuis; Dióxido de carbono; Atmosfera modificada; Pré-solubilização de gás.

Resumen

Para el uso de dióxido de carbono en sistemas de envasado modificado (EM), es necesaria una alta relación gas/producto para garantizar el efecto bacteriostático de los pescados y sus derivados. El uso de técnicas como la Presolubilización de Gas CO₂ (SGS) puede reducir el tamaño del paquete necesario para mantener la efectividad de EM. Por lo tanto, se estudió el efecto del empaque de EM combinado con la estabilización de gas soluble (SGS) como pretratamiento para investigar la vida útil de los mejillones azules cocidos. Las muestras se sometieron a CO₂ de grado alimenticio al 100% (96%) durante 2 h (SGS), en una bolsa grande y flexible. Después del tratamiento con SGS, se envasaron 100 g de mejillones en atmósfera modificada y aire. La concentración inicial de CO₂ en los envases SGS fue del 70,0% y se mantuvo constante (72,5%) debido al pretratamiento con CO₂. Las propiedades fisicoquímicas (pH, contenido de agua, firmeza y capacidad de retención de agua) no se vieron influenciadas significativamente en los tratamientos con SGS. Las muestras de SGS tenían un crecimiento reducido de mesófilos y psicrotóxicos durante el almacenamiento. La aplicación de SGS dio como resultado una mayor vida útil microbiológica (19 días) en comparación con el envasado con aire (5 días) y puede contribuir a mejorar la calidad de los mejillones cocidos.

Palabras clave: Mejillones; Dióxido de carbono; Atmósfera modificada; Presolubilización de gas.

1. Introduction

The blue mussel (*Mytilus edulis*) is a bivalve mollusk with great importance in the Norwegian and European seafood market, where Europe produces 34 % of the total EU production (EUMOFA, 2019). However, this product is very perishable and requires processes that can improve their shelf life. An alternative could be a fresh cooked ready-to-be-used product (Lima et al., 2013). For this, the preservation of mussels depends on techniques that can reduce the microbial count associated with the maintenance of its sensory characteristics. Carbon dioxide (CO₂) is a gas that has been extensively studied for use in foods, especially in modified atmosphere (MA) systems (Lambert et al., 1991; Hotchkiss & Langston, 1995). It is applied to food as a mixture of gases in the packaging step, without further control of the atmosphere during storage (López-Vásquez & Vanaclocha, 2004; Simpson et al., 2009).

The efficiency of packaging under modified atmosphere can be increased in non-respiring foods, especially seafood, adopting a gas/product (g/p) ratio adequate to ensure the availability of CO₂ and to avoid packaging collapse (or the “snug-down” effect) due to the high solubility of CO₂. For seafood, a g/p ratio of 2:1 or 3:1 is recommended (Sivertsvik & Birkeland, 2006), and alternative methods to decrease the headspace volume, could contribute to more transport and environmental efficient packages.

Soluble Gas Stabilization (SGS) is a method able to reduce the size of packing, where CO₂ is solubilized in the product before packaging, with the potential to prevent package collapse, even with low g/p ratios in relation to the traditional MA packaging (Sivertsvik & Birkeland, 2006; Rotabakk et al., 2008). The SGS treatment has been shown good results in several foods, including shrimp, sardine, cheese, chicken breast, chicken drumsticks, fish soup, oysters, and others (Sivertsvik & Birkeland, 2006; Mendes et al., 2008; Jakobsen & Risbo, 2009; Rotabakk et al., 2010; Al-Nehlawi et al., 2013; Rode et al., 2015; Soares et al., 2015).

Lima et al., (2016) combined the integrated cooking and vacuum-cooling system and modified atmosphere packaging (50 % CO₂/50% N₂) in cooked *Perna perna* mussels and the combination of processes was efficient in the product stored over 25 days. However, for this, it was necessary a high g/p ratio (4.75) to ensure the effectiveness of MA system. MA combined with SGS as pre-treatment can contribute to improve quality of cooked mussels and to reduce the g/p ratio and the packaging size applied, giving a convenient product that is a good alternative for consumers. Thus, the aim of this study was to investigate the effect of soluble gas stabilization combined with modified atmosphere packaging on the packaging system, microbiological and physicochemical behavior of blue mussels.

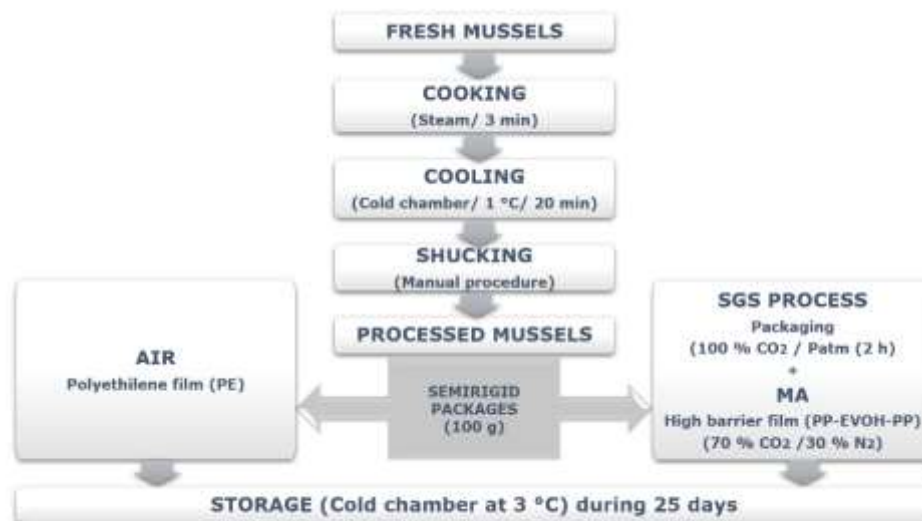
2. Methodology

2.1 Raw material, processing and sampling

Live blue mussels (*Mytilus edulis*) farmed in the Mid-Norway region were delivered overnight to the laboratory and stored in a cold room at 1 ± 1 °C until processing the following day.

The mussels were processed according to flowchart in Figure 1. First, the mussels were sorted where dead or damaged shells were removed, followed by cleaning and removal of the byssus before cooking at 100 °C for 3 minutes using a steam convection oven (Zanussi FCV/E10L, Pordenone, Italy). The samples were cooled in a cold chamber (Metos BC161, Metos Norway, Oslo, Norway) at a temperature of 1 °C for 20 minutes with high air velocity before the meat was manually removed from the shells. The peeled muscles were randomly divided into two different treatments.

Figure 1. Flowchart of the procedure adopted for processing and packaging of mussels.



Source: Authors (2021).

2.2 Temperature profile during the heat treatment

The temperature profile during heat treatment was logged every 10 second using thermocouples type K (PR. Eletronics Inc., San Diego, USA) connected to a temperature acquisition system (Ellab, Denmark). The thermocouples were positioned inside the shell and into the mussel meat. Another thermocouple was placed inside the oven to measure the temperature in the steam/air mixture.

Lethality (F-value) and inactivation during processing were calculated according to Mafart et al. (2010) where F-value for *Clostridium botulinum* was chosen according Lima et al., (2013).

2.3 Packaging treatments

Cooked mussel meat (100 ± 2 g) were placed in PP-EVOH-PP trays (Tray E1540, 380 mL, EDV packaging solutions, Barcelona, Spain) according to each treatment:

a) **Treatment 1** - mussels packaged under air atmosphere (AP) using over-wrapped PE-film with a high oxygen permeation.

b) **Treatment 2** - SGS process before modified atmosphere (MA) mussels were carried out for 2 hours at 1 ± 1 °C in trays placed inside multilayer plastic bag (20- μ m thickness polyamid (PA)/70- μ m thickness polyethylene (PE) 700 × 500 mm,

Star-pack produktie B.V. Waalwijk, Netherlands). SGS was performed with 100% food-grade CO₂ (96.8 ± 0.7 % CO₂) inside the bags (95% vacuum, CVP Fresh Vac Model A-600, Downers Grove, IL, USA) with a total pressure of 1 atm. Bag's oxygen transmission rate 30 cm³.m⁻².d⁻¹.atm⁻¹. The SGS-bag's were large enough to ensure excess availability of CO₂ (filling degree approx. 7.0% [mL product/mL package volume]). Immediately after SGS treatment, the mussels were MA packaged. Mussels were subsequently flushed with food grade CO₂ and N₂ (AGA, Linde Gas, Stavanger, Norway) mixed on a gas mixer (Witt KM 100-2m, Witt Gasetechnik, Witten, Germany). The initial gas was 70/30 % CO₂ and N₂. Trays were covered with barrier top web film (90 µm Polyamide/polypropylene, NOP101, Cryovac) using a semi-automatic tray sealer (Mondini, Italy). O₂ and CO₂ transmission rate of film were 40 cm³.m⁻².d⁻¹.atm⁻¹ and 150 cm³.m⁻².d⁻¹ atm⁻¹ at 23 °C, 0 % RH, respectively. O₂ transmission rate of tray was 0.004 cm³.d⁻¹.atm⁻¹.

All samples were stored at 3 °C (±1°C) until assessments of physicochemical (after 3, 10, 15, 19 and 22 days of storage); and microbiological analysis (after 4, 11, 16, 19 and 22 days of storage).

2.4 Headspace gas volume and composition

Gas in packages headspace were determined by submerging the package under water and measure the resultant force by a texture analyzer (Stable Micro System Ltd., TAXT plus Godalming, UK), according described in detail by Rotabakk et al., (2007). Buoyancy force measurements were made at 26 s, 28 s and 30 s. An average of the three measurements was used for statistical analysis of the data.

The headspace gas composition was performed on each tray using an oxygen and carbon dioxide analyzer (Checkmate 9900 analyzer, PBI-Dansensor, Ringsted, Denmark). From packages headspace an aliquot (20 mL) was collected with a syringe through of a rubber foam septum on the package out surface (Nordic Supply, Skodje, Norway) added to avoid introduction of false atmosphere into the gas analyzer.

2.5 Physicochemical analysis

The water loss during cooking was measured gravimetrically, in triplicate, and was divided by the initial mass of the product (g) and reported as a percentage (%).

The pH was measured in triplicate, directly in the mussels with a pH meter (Orion 420 A-plus Benchtop, Thermo Electron Cooperation, Cambridgeshire, U.K.) using a spear tip pH electrode (Thermo, Thermo Electron Cooperation).

Water content (%) was measured in duplicate by drying in an oven at 105 °C for 18 h according to Skipnes et al., (2007).

Water holding capacity (WHC) was calculated in duplicate. Samples were weighted before and after centrifugation at 1800 min⁻¹ for 15 minutes at 4 °C as described in detail by Skipnes et al., (2007).

The instrumental texture (firmness) was carried out once on each package, measuring the maximum shear strength using a Kramer shear cell (KSC) coupled to texturometer (Stable Micro System Ltd., TAXT plus Godalming, UK), using a 500 N load cell. Approximately 50 g of mussels from each package were subjected to the test at a rate of 3 mm s⁻¹ and the results of firmness expressed as N g⁻¹.

2.6 Microbiological counts

The microbiological count was performed by total viable counts (APC) and psychrotrophic counts (PC), in the raw and cooked mussels. Proximate 10 g of raw or cooked mussels were homogenized 1:10 with peptone water (1% NaCl (w/v) and 0.1% peptone (w/v) for 120 s in a Stomacher 400 Laboratory Blender (AJ Steward Company LTD, London, England). For the raw material the APC and PC counts were performed in aliquots, from suitable dilutions were added to melted and temperate (45 °C) iron agar with an overlay (Iron Agar Lyngby, Oxoid CM 964, Basingstoke, England). The agar was supplemented with

0.04% L-cysteine, and incubated at 20 ± 1 °C for 3 days, for APC counts and for PC counts, were incubated at 7 ± 1 °C for 10 days. Black colonies were counted as H₂S-producing bacteria and APC were counted as the total of black and white colonies.

Microbiological analyses of cooked mussels, during storage (0, 4, 11, 16, 19 and 22 days), APC and PC bacteria count was determined by a spread plate count method with plate count agar (PCA, Merck, Darmstadt, Germany) added 1% NaCl, in order to support growth of the salt requiring bacteria. Plates were incubated at 20 ± 1 °C for 3 days to APC, at 7 ± 1 °C for 10 days to PC. Duplicates were prepared for each package analyzed (4 for each experimental point).

2.7 Statistical analysis

The results were analyzed to check significant differences for physicochemical parameters and head space gas volume and composition, between the processes tested and during storage time, using one-way analysis of variance (ANOVA). In case of significant effects ($P < 0.05$), the mean values were compared using Tukey's test. The data analyzed were obtained from triplicate experiments and analyses.

3. Results and Discussion

3.1 Characterization of raw and processed mussels

pH values obtained in this work (Table 1) showed the same range of values as raw mussels with a high microbial load. This reflects the microbiological characteristics of their habitat, and mainly the water quality (Lima et al., 2013). The heat process is important to separate the meat of the shells and to reduce the microbial load in the cooked product.

Table 1. Raw and cooked mussels characterization.

Properties	Raw mussels	Cooked mussels
pH	6.8 ± 0.12	6.59 ± 0.02
Water content	82.8 ± 1.3 %	72.03 ± 1.0 %
Microbiological counts (H ₂ S)	4.07 ± 0.56 log (CFU/ g)	Not detected
Microbiological counts (APC)	5.81 ± 0.78 log (CFU/ g)	1.32 ± 0.5 log CFU/g
Microbiological counts (PC)	5.01 ± 0.41 log (CFU/ g)	1.08 ± 0.45 log CFU/g

Source: Authors (2021).

According Table 1, the microbial count of cooked mussels was reduced close to 4 log for APC and PC, while H₂S bacteria was not detected. Mendes et al., (2011) detected H₂S bacteria after 3 days in octopus in air and negligible quantity in SGS samples after 13 days, and attributed the low count of H₂S to be a result of inhibition by *Pseudomonas* spp. In this study, H₂S bacteria was not detected after heat treatment and neither during the storage, probably due the same phenomenon (discussed subsequently).

The short time thermal process reached 4 log reductions, 2 log more than obtained by Cavalheiro et al., (2012) who observed 2 log reductions for mussels processed with vapor cooking-vacuum cooling.

3.2 Water loss after processing

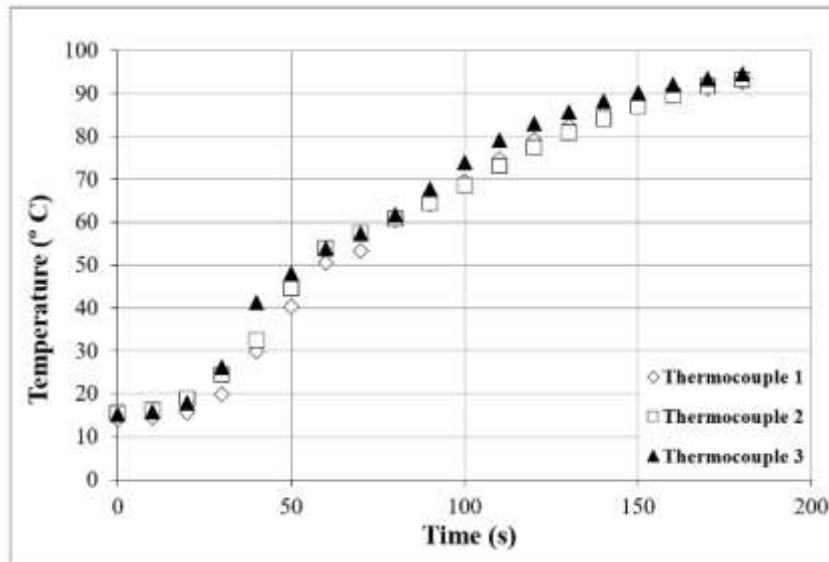
The average water loss during processing (cooking and cooling) was 30.53 ± 0.72 % (Table 1) resulting in a cooked product with water content around 76.2 %. Cavalheiro et al., (2012) evaluated the cooking weight loss in mussels under vapor cooking-water immersion cooling, vapor cooking-vacuum cooling and immersion cooking-immersion vacuum cooling, average time of 7.5 min obtained average water losses of 39.1 %. Lower water loss during the process is important to maintain the texture

of the mussels, yield and quality.

3.3 Thermal processing parameters

Temperature profile inside mussels during the cooking is shown in Figure 2. Three minutes processing time was determined during previous trials (data not shown) after different processing times.

Figure 2. Temperature profiles of mussels during cooking process.



Source: Authors (2021).

Table 2 shows the results found for thermal lethality of microorganism calculated during the cooking process. All microorganisms, except non-proteolytic *Clostridium botulinum* Type E, can be inactivated according temperature history, where the F process showed high values comparing with F required for each target microorganism. To *C. botulinum*, the temperature was lower than required and decimal reduction only 0.59. This result is expected, since a 6-log reduction of this bacterium requires thermal process conditions corresponding to 90 °C for 10 min (European Commission, 1997).

Table 2. Evaluation of heat treatment for cooked mussels: literature data, lethality and number of decimal reductions for the target microorganisms.

Microorganism	Literature Information				F (lethality of process - min)		Evaluation**	Number of decimal reductions (γ)***
	D (min)	Z (°C)	Ref. Temp. (°C)	Source	F process	F required*		
Non-proteolytic <i>Clostridium botulinum</i> type E	1.5	10	90	ECFF (1996)	1.1	9.00	$F_P < F_R$	0.59
<i>Listeria monocytogenes</i>	0.3	7.0	70	ECFF (1996)	931.64	1.80	$F_P > F_R$	2217.76
<i>Vibrio parahaemolyticus</i>	3.2	7.5	60	Tucker & Featherstone (2010)	3949.26	19.20	$F_P > F_R$	603.88
<i>Pseudomonas fluorescens</i>	0.98	9.1	60	Adams (1995)	12410.75	5.88	$F_P > F_R$	2842.98
<i>Salmonella</i> spp.	0.1	10.6	65	Adams (1995)	2251.38	0.60	$F_P > F_R$	1792.53
<i>Staphylococcus aureus</i>	5.0	8.5	60	Adams (1995)	2952.26	30.00	$F_P > F_R$	152.07
<i>Escherichia coli</i>	15	8.8	60	Adams (1995)	248.85	90.00	$F_P > F_R$	2020.58

* Calculated with basis in the actual reference temperatures, and Z for each microorganism. For all microorganisms, 6 decimal reductions were considered.

** For pasteurization processes, a decimal reduction = 6 is enough; the least values found for F process in the calculations were used.

When $F_P > F_R$ we have high lethality for the applied process

When $F_P < F_R$ we have insufficient lethality comparing with the heat treatment in the experiments.

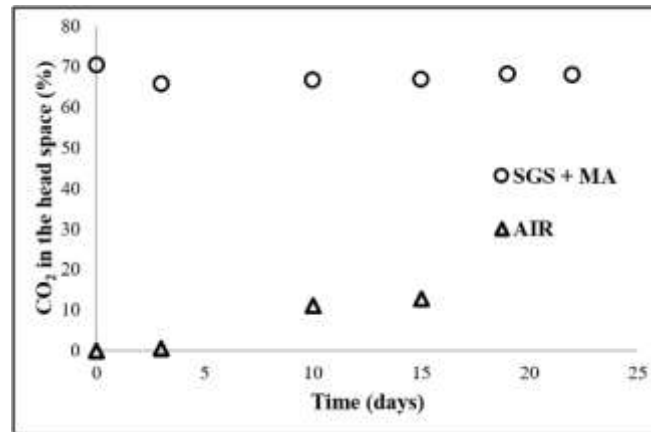
Source: Authors (2021).

In this study a short cooking process was used, with the objective just to open the shells and avoid losses in the quality of the product, due protein denaturation, influencing in the reduction of water holding capacity, shrinks muscle fibers, and causes connective tissue degradation (Ovissipour et al., 2013).

3.4 Storage trial of packaged mussels

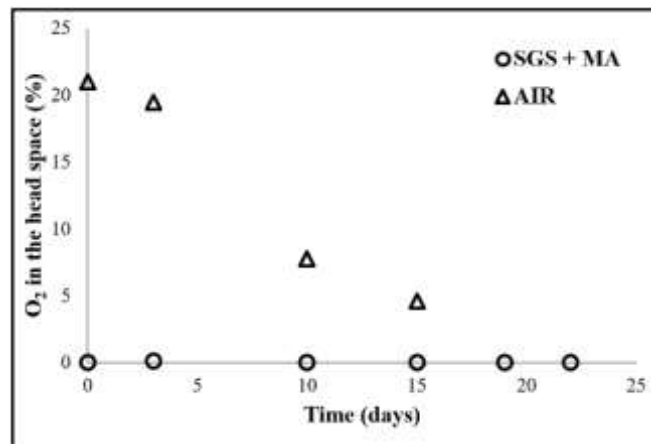
The gas composition in the packages' headspace (with SGS + MA) immediately after MA packaging was 70.3 ± 0.1 % CO_2 and 0.02 ± 0.01 % O_2 . The CO_2 and O_2 (%) concentration during storage are shown in Figure 3 and Figure 4, respectively. For sample packaged under SGS+MA there was not observed any significant difference ($P > 0.001$) on the CO_2 composition in the package headspace during the storage period. This is a good result, because usually is necessary to use a high g/p ratio to avoid collapse of the packages. Lima et al., (2016) applied conventional MA (50 % CO_2 /50 % N_2) on the cooked mussels and used a high g/p ratio (4.75) on the package to avoid the snug-down effect. However, it was observed a ranged from 50.70% (zero time) to 45.16% (at 24 h) of CO_2 solubilization in 24 h, which resulted in a reduction of g/p ratio during the storage. Our study shows that SGS process time of 2 hours was sufficient to dissolve CO_2 into the mussels to avoid counteract snug-down, with significantly increased CO_2 in the packaging.

Figure 3. Mean gas compositions for CO₂ content in the headspace of the packages during the storage time (Standard deviation less than 1 %).



Source: Authors (2021).

Figure 4 Gas compositions for O₂ content in the headspace of the packages during the storage time. (Standard deviation less than 1 %).



Source: Authors (2021).

An increased CO₂ content ($P < 0.001$), together with a significant decrease in O₂ level was observed in the mussels stored in air during the storage, was due to earlier deterioration mainly by psychotropic microorganisms producing CO₂. No changes were observed in the O₂ composition during the storage period, for the samples in SGS + MA. Other studies with chicken and Atlantic halibut found significant amounts of dissolved CO₂ (Rotabakk et al., 2010; Rotabakk et al., 2008), and demonstrates the possibility SGS gives to increase the amount of CO₂ in a package compared to traditional MA.

Total headspace gas volume (mL) in the packages changed significantly ($P < 0.001$) during the storage and among the treatments due production of CO₂ from the microorganisms in the air packaging. In the beginning of the storage time, SGS counteracted top web deflation in the packages (227.75 ± 4.30 mL), giving an almost flat top web. An increase in volume was observed the last two days for SGS samples (361.39 ± 10.49 and 374.62 ± 46.49 at day 19 and 22, respectively) also probably by microbiological growth.

3.5 Physicochemical properties

Table 3 presents pH, Firmness ($N \cdot g^{-1}$), water content (%) and Water Holding Capacity (WHC - %) results. pH was

significantly ($P=0.005$) different between the treatments only on third day, where mussels package under air had the highest pH, followed by SGS. Lower pH probably due absorbed CO_2 (Zhao et al., 1995). However, microbiological growth and absorbed CO_2 in the treatments did not affect significantly the pH during storage ($P>0.05$), reaching values of 6.51 for AP and 6.42 for SGS + MA packages. Masniyom et al., (2011) and Caglak et al., (2008), found a decreasing pH in mussels during storage in raw mussel stored under modified atmosphere. However, the influence of CO_2 on pH depends of the concentration of this gas applied inside the packages and of the microstructure of food. Lima et al., (2015) characterized the microstructure of male and female cooked mussels and observed high porosity in the cooked meat, and because this, the solubilization of CO_2 and, thus, their retention in cooked mussels depends on the network of capillaries is filled with water.

Table 3. Effect of storage time (days) and packaging treatments on physicochemical properties (pH, Firmness (N.g^{-1}), Water content (%) and Water Holding Capacity (WHC - %)).

Time (days)	pH		Texture (Firmness – N.g^{-1})	
	Air	SGS + MA	Air	SGS + MA
00	6.69 ^{aAB} ±0.04	6.46 ^{aAB} ±0.10	3.09±1.04 ^{aA}	3.10±1.34 ^{aA}
03	6.86 ^{aB} ±0.05	6.25 ^{bC} ±0.09	3.41±0.77 ^{aA}	2.74±0.69 ^{aA}
10	6.49 ^{aA} ±0.13	6.54 ^{aB} ±0.09	3.03±1.27 ^{aA}	3.94±0.34 ^{aA}
15	6.51 ^{aA} ±0.33	6.39 ^{aA} ±0.03	3.06±0.81 ^{aA}	3.95±1.07 ^{aA}
19	*	6.42 ^B ±0.04	*	3.87±0.79 ^A

Time (days)	Water content (%)		Water Holding Capacity WHC (%)	
	Air	SGS + MA	Air	SGS + MA
00	76.27 ^{aA} ±0.76	76.46 ^{aA} ±0.76	73.82±4.17 ^{aA}	73.80±3.16 ^{aA}
03	76.54 ^{aA} ±1.29	75.36 ^{aA} ±1.56	77.42±2.79 ^{aA}	77.93±6.07 ^{aA}
10	77.06 ^{aA} ±1.63	75.12 ^{bA} ±0.92	76.87±2.63 ^{aA}	78.61±7.85 ^{aA}
15	76.31 ^{aA} ±0.81	75.39 ^{aA} ±1.10	75.66±6.90 ^{aA}	80.49±2.56 ^{aA}
19	*	76.13 ^A ±0.72	*	77.77±2.62 ^A

* Not measured, samples discarded at 15th because deterioration. Lowercase letters between columns differ on the type of process. Uppercase letters between lines differ on the storage period. $P<0.05$.

Source: Authors (2021).

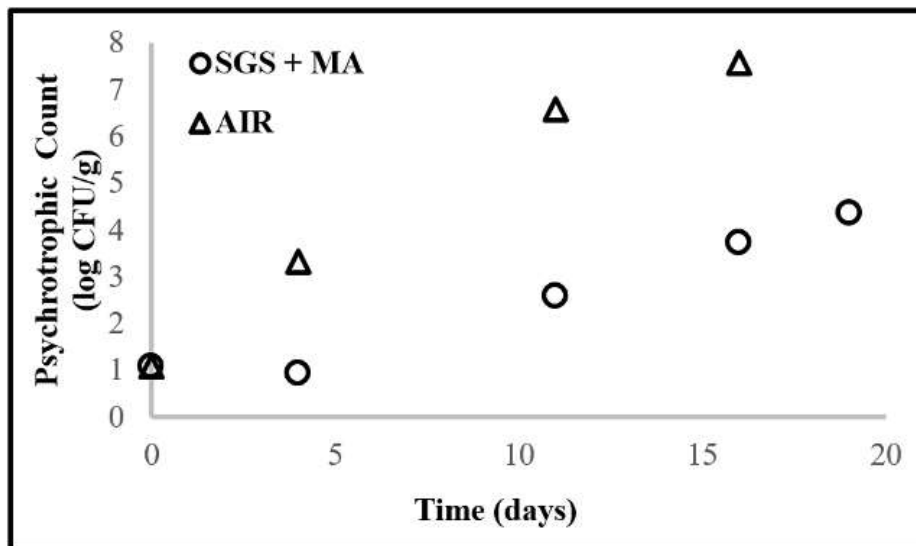
Mussels studied in this work presented high water content values (around 76 %). Usually, foods with high water content are very perishable because of high water activity and typically a high pH (6.7 – 7.1) (Goulas et al., 2005). Lima et al., (2013) found water content of 76.9 % in ready-to-eat mussels; on other hand, higher values (81%) using immersion cooling has been shown by Cordeiro et al., (2007). The water content remained stable during storage ($P = 0.631$), and packaging treatments not influenced significantly ($P= 0.0653$) on the water content. AP packed mussels showed little higher water content ($\approx 77\%$) when compared to SGS + MA ($\approx 76\%$). CO_2 absorbed by food stuff can encounter increased drip loss according to Sivertsvik & Birkeland, (2006), but there was not observed any effect of treatment ($P = 0.108$) or storage time ($P = 0.087$) on the water holding capacity in the current experiment. Masniyom et al., (2011) observed increased exudate, decreased pH and decreased WHC for raw mussel when using higher CO_2 concentrations. In our study, there was not observed any change on the WHC. No significant ($P= 0.213$) changes in texture (N.g^{-1}) was observed during storage and between treatments ($P=0.364$). Opposite behavior was observed in raw fishery products, where Sivertsvik et al., (2004) reported that high concentrations of CO_2 could promote

softening in the tissue, without negative effects in the meat.

3.6 Microbiological evaluation

Figure 5 shows the results for aerobic total count (APC) and Figure 6 for psychrotrophic (PC) count in cooked mussels. An effect of treatments and storage for both APC and PC counts was observed. The initial counts were low (1.32 log CFU/g for APC and 1.09 log CFU/g for PC). On day four, AP showed microbial growth for both APC and PC when compare with SGS + MA. Higher CO₂ concentration in SGS + MA treatments delayed the growth of the APC and PC when compare with other treatment (Figures 5 and 6). No previous studies using SGS treatment in cooked blue mussels has been found, only MAP with different CO₂ concentration to extend the shelf life of raw mussels (Masniyom et al., 2011; Goulas et al., 2005). SGS (100 % CO₂/2h) applied in shellfish decreased APC counts in ready-to-eat shrimp from 4.3 to 3.3 log CFU/g and PC from 5.2 to 4.9 log CFU/g and in Atlantic halibut was observed a decrease of 0.4 log CFU/g compared to MAP (Sivertsvik & Birkeland, 2006; Rotabakk et al., 2007).

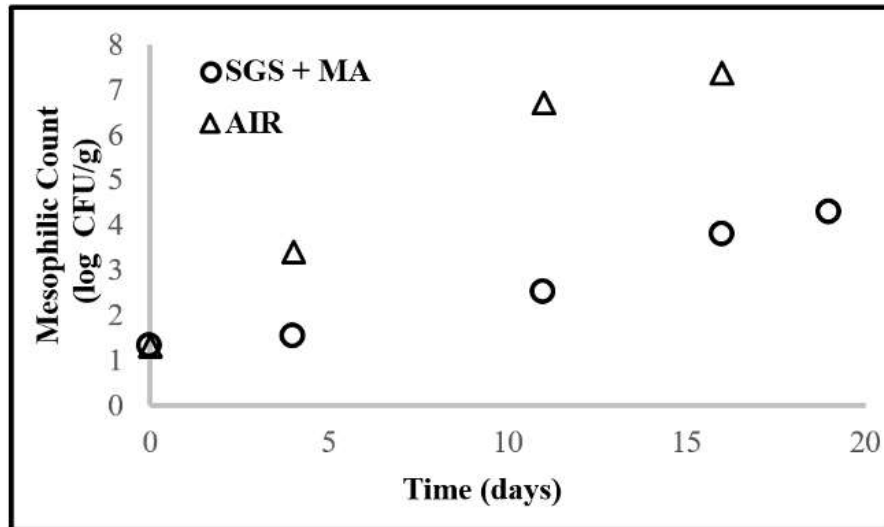
Figure 5. Microbiological counts for mesophilic. (Standard deviation less than 1 %).



Source: Authors (2021).

After 11 days of storage, the count of AP samples was 6.72 log CFU/g for APC and 6.58 log CFU/g for PC counts. For fish products, the total viable count for sensory rejection is typical around 7–8 log CFU/g and the acceptability limit are 6 log CFU/g for mesophilic aerobic bacteria. However, only psychrophilic bacteria count should be used as indicative of quality of fish (Mol et al., 2007) together with chemical, biochemical and sensory analyses (Khan et al., 2005). For the cooked mussel stored under air, in this study, it was possible to see clearly the colonies and physical changes (soft flesh and darker). In the 16th day of storage, AP reached 7.57 log CFU/g evidencing a spoiled product. Masniyom et al., (2011) also found fast increase (4 to 7 log CFU/g) at 15 days for raw mussel stored under air, higher than mussels under enriched atmospheres of CO₂.

Figure 6. Microbiological counts for psychrotrophic. (Standard deviation less than 1 %).



Source: Authors (2021).

For each analysis's day, mussels packaged under air (AP samples) were opened to check sensorial visual aspects, and it was concluded that AP samples presented good characteristics of odor to consumption until maximum of 5 days, due visible microbial colonies on the meat was observed after 7 days of storage.

Microbial levels at the end of shelf life for the SGS + MA APC: 4.30 log CFU/g and PC: 3.06 log CFU/g, lower than AP samples. However, off odor from the mussels upon opening of package indicated spoiled products at last storage day (22 days). High microbial activity could be observed by the O₂ consumption (Figure 4) indicating presence of respiratory spoilage bacteria, like *Shewanella* and *Pseudomonas* group present in seafood products, according Gram and Dalgaard, (2002). To SGS + MA treatment, there was absence of O₂ (0.02 % - 0.00 %) probably due their consumed by metabolism of bacteria and oxidative biochemical processes, as reported also in sardine stored under air in study testing SGS, vacuum and MA by Mendes et al., (2008).

The storage time of the mussels were considered through of microbial results with undesirable characteristics for consumption, like off-flavors and visual changes as consequence from liberation of volatile compounds and enzymatic reactions. Gram and Dalgaard, (2002) reported that formation of amines, sulfides, alcohols, aldehydes, ketones, and organic acids with unpleasant and unacceptable off-flavors is a consequence of spoilage in foods due the microbial growth and metabolism.

The mussel's storage under SGS + MA showed good sensorial characteristics until 19th day. After that, the packages were inflation due microbial growth. The gas inflation probably was caused by production of undesirable primary metabolites such as trimethylamine from trimethylamine oxide, other amines and ammonia together with CO₂. Lu (2009) related the importance to observe that even in the environment with CO₂ concentrations there is the possibility for proliferation of psychrotrophic, facultatively anaerobic or strictly anaerobic pathogens, such as nonproteolytic *Clostridium botulinum* and *Listeria monocytogenes* in seafoods. New studies with the preservation of cooked mussels avoiding these pathogenic microorganisms should be performed.

4. Conclusion

The application in cooked blue mussel meat of soluble gas stabilization using 100% of CO₂ for 2 hours prior to modified atmosphere packaging solved the collapse of the packages thought dissolved CO₂ priori package and reduced the ratio gas volume/product for MAP. Beyond there was not changes in the CO₂ during storage period studied. In addition, SGS + MA

inhibited the mesophilic and psychrotrophic growth showing appositive bacteriostatic effect when comparing packaging under air atmosphere. The shelf life increased from 5 to 19th days showing that the SGS is a promising method to preserve mussels.

Future perspectives in this area are to evaluate the sensorial perception from the consumer about cooked mussels treated with SGS process and also to determine conditions for this method based on absorption and desorption mechanism for industrial application.

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References

- Al-Nehlawi, A., Saldo, J., Vega, L. F. & Guri, S. (2013). Effect of high carbon dioxide atmosphere packaging and soluble gas stabilization pre-treatment on the shelf life and quality of chicken drumsticks. *Meat Science*, 94: 1–8. <https://doi.org/10.1016/j.meatsci.2012.12.008>
- Caglak, E., Cakli, S. & Kilinc, B. (2008). Microbiological, chemical and sensory assessment of mussels (*Mytilus galloprovincialis*) stored under modified atmosphere packaging. *European Food Research and Technology*, 226:1293-1299. <https://doi.org/10.1007/s00217-007-0657-1>
- Cavalheiro, D., Schmidt, F.C., Rodrigues, L.G.G., Siga, C., Leitempergher, F. & Laurindo, J. B. (2012). Processing of *Perna perna* mussels using integrated process of cooking and vacuum cooling. *Journal of Food Process Engineering*, 36:2. <https://doi.org/10.1111/j.1745-4530.2012.00672.x>
- Cordeiro, D., Lopes, T. G. G., Oetterer, M., Porto, E. & Galvão, J. A. (2007). Qualidade do Mexilhão *Perna perna* Submetido ao Processo Combinado de Cocção, Congelamento e Armazenamento. *Boletim CEPPA*, 25(1):165-179.
- EUMOFA. European Market Observatory for Fishery and Aquaculture Products (2019). The EU Fish Market – 2019 Edition. <https://www.eumofa.eu/documents/20178/314856/EN_The+EU+fish+market_2019.>pdf
- European Commission. *Harmonization of safety criteria for minimally processed foods*. (1997). Report: FAIR Concerted action CT96-1020:36–37.
- Goulas, A. E., Chouliara, I., Nessi, E., Kontominas, M. G. & Sawaidis, I. N (2005). Microbiological, biochemical and sensory assessment of mussels (*Mytilus galloprovincialis*) stored under modified atmosphere packaging. *Journal of Applied Microbiology*, 98:752-760. <https://doi.org/10.1111/j.1365-2672.2004.02512.x>
- Gram, L. & Dalgaard, P. (2002). Fish spoilage bacteria – problems and solutions. *Current Opinion in Biotechnology*, 13:262-266. [https://doi.org/10.1016/S0958-1669\(02\)00309-9](https://doi.org/10.1016/S0958-1669(02)00309-9)
- Hotchkiss, J. H. & Langston, S. W. (1995). MAP of cooked meat and poultry products. In: Farber, J.M., Dodds, K.L., editors. Principles of modified-atmosphere and sous vide product packaging. Lancaster, Pa.: Technomic Publishing Co. 137-152.
- Jakobsen, M. & Risbo, J. (2009). Carbon dioxide equilibrium between product and gas phase of modified atmosphere packaging systems: Exemplified by semihard cheese. *Journal of Food Engineering*, 92:285-290. <https://doi.org/10.1016/j.jfoodeng.2008.11.007>
- Khan, M. A., Parrish, C. C. & Shahidi, F. (2005). Enumeration of total heterotrophic and psychrotrophic bacteria using different types of agar to evaluate the microbial quality of blue mussels (*Mytilus edulis*) and sea scallops (*Placopecten magellanicus*). *Food Research International*, 38:751-758. <https://doi.org/10.1016/j.foodres.2005.03.001>
- Lambert, A. D., Smith, J. P. & Dodds, K. L. (1991). Shelf-life extensions and microbiological safety of fresh meat – a review. *Food Microbiology*, 8(4):267-297. [https://doi.org/10.1016/S0740-0020\(05\)80002-4](https://doi.org/10.1016/S0740-0020(05)80002-4)
- Lima, M., Siga, C., Leitempergher, F., Lerin, L. A., Soares, L. S., Tosati, J. V., Rodrigues, N. B. & Monteiro, A. R. (2017). Mussel (*Perna perna*) processing by an alternative method and packaging under modified atmosphere to improve physicochemical and microbiological characteristics. *Journal of Food Processing and Preservation*, 41(3). <https://doi.org/10.1111/jfpp.12923>
- Lima, M., Soares, L. S., Tosati, J. V., Lerin, L. A., Vladimir de Oliveira, J. & Monteiro, A. R. (2015). Application of CO₂ in *Perna perna* Mussel: Evaluation of Absorption Mechanism During Soluble Gas Stabilization (SGS) Process. *Food Engineering Reviews*, 7, 250–257(2015). <https://doi.org/10.1007/s12393-014-9103-x>
- López-Vásquez, R. & A. Vanaclocha. 2004. *Tecnología de Mataderos*. Ediciones Mundi-Prensa.
- Lima, M., Melo, M. C. L. & Monteiro, A. R. (2013). Evaluation of the processing of *Perna perna* mussels: the influence of water quality involved in the cooling operations in the physico-chemical and microbiological characteristics of the product. *Journal of the Science of Food and Agriculture*, 93(13): 3322-3329. <https://doi.org/10.1002/jsfa.6178>
- Lu, S. (2009). Effects of bactericides and modified atmosphere packaging on shelf-life of Chinese shrimp (*Fenneropenaeus chinensis*). *LWT - Food Science and Technology*, 42:286–291. <https://doi.org/10.1016/j.lwt.2008.03.004>

- Mafart, P., Leguérinel, I., Couvert, O. & Coroller, L. (2010). Quantification of spore resistance for assessment and quantification of heating processes: A never-ending story. *Food Microbiology*, 27:568 – 572. <https://doi.org/10.1016/j.fm.2010.03.002>
- Mendes, R., Silva, H. A., Anacleto, P. & Cardoso, C. (2011). Effect of CO₂ dissolution on the shelf –life of ready-to-eat *Octopus vulgaris*. *Innovative Food Science & Emerging Technologies*, 12:551-561. <https://doi.org/10.1016/j.ifset.2011.07.003>
- Mendes, R., Pestana, C. & Gonçalves, A. (2008). The effects of soluble gas stabilisation on the quality of packed sardine fillets (*Sardina pilchardus*) stored in air, VP and MAP. *International Journal of Food Science and Technology*, 43:2000-2009. <https://doi.org/10.1111/j.1365-2621.2008.01809.x>
- Masniyom, P., Benjama, O. & Maneesri, J. (2011). Extending the shelf-life of refrigerated green mussel (*Perna viridis*) under modified atmosphere packaging. *Songklanakarin Journal of Science and Technology*, 33(2):171-179.
- Mol, S., Erkan, N., Üçok, D. & Tosun, Y. (2007). Effect of psychrophilic bacteria to estimate fish quality. *Journal of Muscle Foods*, 18:120-128. <https://doi.org/10.1111/j.1745-4573.2007.00071.x>
- Ovissipour, M., Rasco, B., Tang, J. & Sablani, S. S. (2013). Kinetics of quality changes in whole blue mussel (*Mytilus edulis*) during pasteurization. *Food Research International*, 53:141–148. <https://doi.org/10.1016/j.foodres.2013.04.029>
- Rode, T. M., Hovda, M. B. & Rotabakk, B. T. (2015). Favourable effects of soluble gas stabilisation and modified atmosphere for suppressing regrowth of high pressure reated *Listeria innocua*. *Food Control*, 51:108-113. <https://doi.org/10.1016/j.foodcont.2014.11.006>
- Rosnes, J. T., Skåra, T. & Skipnes, D. (2011). Recent Advances in Minimal Heat Processing of Fish: Effects on Microbiological Activity and Safety. *Food Bioprocess Technology*, 4:833–848. <https://doi.org/10.1007/s11947-011-0517-7>
- Rotabakk, B. T., Lekang, O. I. & Sivertsvik, M. (2010). Solubility, absorption and desorption of carbon dioxide in chicken breast fillets. *Food Science and Technology*, 43:442-446. <https://doi.org/10.1016/j.lwt.2009.09.009>
- Rotabakk, B. T., Birkeland, S., Lekang, O. I. & Sivertsvik, M. (2008). Enhancement of modified atmosphere packaged farmed Atlantic Halibut (*Hippoglossus Hippoglossus*) fillet quality by soluble gas stabilization. *Food Science and Technology International*, 14(2):179-186. <https://doi.org/10.1177/1082013208092051>
- Rotabakk, B. T., Lekang, O. I. & Sivertsvik, M. (2007). Volumetric method to determine carbon dioxide solubility and absorption rate in foods packaged in flexible or semi rigid package. *Journal of Food Engineering*, 82:43-50. <https://doi.org/10.1016/j.jfoodeng.2007.01.013>
- Skipnes, D., Østby, M. L. & Hendrickx, M. E. (2007). A method for characterizing cook loss and water holding capacity in heat treated cod (*Gadus morhua*) muscle. *Journal of Food Engineering*, 80:1078–1085. <https://doi.org/10.1016/j.jfoodeng.2006.08.015>
- Simpson, R., Acevedo, C. & Almonacid, S. (2009). Mass transfer of CO₂ in MAP systems: Advances for non-respiring foods. *Journal of Food Engineering*, 92:233–239. <https://doi.org/10.1016/j.jfoodeng.2008.10.035>
- Sivertsvik, M. & Birkeland, S. (2006). Effects of Soluble Gas Stabilisation, Modified Atmosphere, Gas to Product Volume Ratio and Storage on the Microbiological and Sensory Characteristics of Ready-to-Eat Shrimp (*Pandalus borealis*). *Food Science and Technology International*, 12(5):445-454. <https://doi.org/10.1177/1082013206070171>
- Sivertsvik, M., Jeksrud, W. K., Vagane, A. & Rosnes, J. T. (2004). Solubility and absorption rate of carbon dioxide into non-respiring foods. Part 1: Development and validation of experimental apparatus using a manometric method. *Journal of Food Engineering*, 61(3):449-458. [https://doi.org/10.1016/S0260-8774\(03\)00167-5](https://doi.org/10.1016/S0260-8774(03)00167-5)
- Soares, L. S., Tosati, J. V., Lima, M., Lerin, L.A. & Monteiro, A. R. (2015). Evaluation of CO₂ absorption and desorption rate in oysters (*Cassostrea gigas*) using the soluble gas stabilisation method. *International Journal of Food Science and Technology*, 50:1089–1094. <https://doi.org/10.1111/ijfs.12743>
- Zhao, Y. Y., Wells, J. H. & Mcmillin, K. W. (1995). Dynamic changes of headspace gases in CO₂ and N₂ packaged fresh beef. *Journal of Food Science*, 60:571–575. <https://doi.org/10.1111/j.1365-2621.1995.tb09829.x>