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Modeling the Effect of Active Modified Atmosphere Packaging on the Microbial Stability and Shelf Life of Gutted Sea Bass

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Abstract: The aim of the study was the evaluation and mathematical modeling of the effect of active modified atmosphere packaging (MAP), by the incorporation of CO_2 emitters in the package, on the microbial stability and shelf life of gutted sea bass during refrigerated storage. Gutted sea bass samples were packaged in modified atmosphere (50% CO_2–40% N_2–10% O_2) with and without CO_2 emitters (ACT-MAP, MAP) (gas/product volume ratio 3:1) and stored at isothermal conditions: 0 °C, 5 °C, and 10 °C. The gas concentration in the package headspace (%CO_2, %O_2) and microbial growth (total viable count, TVC, *Pseudomonas* spp., *Enterobacteriaceae* spp., lactic acid bacteria) were monitored during storage. The microbial growth was modeled using the Baranyi growth model, and the kinetic parameters (microbial growth rate, lag phase) were estimated at the tested temperature and packaging conditions. The results showed that the ACT-MAP samples presented significantly lower microbial growth compared to the MAP samples. The growth rate of the total viable count at 0 °C was 0.175 and 0.138 d^{-1} for the MAP and ACT-MAP sea bass, respectively ($p < 0.05$). The shelf life of the MAP sea bass at 0–10 °C (based on a final TVC value: 7 log CFU g^{-1}) was extended 4–7 days with the addition of a CO_2 emitter in the package. The CO_2 concentration in the ACT-MAP samples was stabilized at approximately 60%, while the CO_2 in the MAP samples was approximately 40% at the end of the shelf life.

Keywords: modified atmosphere packaging; active packaging; CO_2 emitters; sea bass; shelf life modeling

1. Introduction

Modified atmosphere packaging (MAP) has been established as an efficient method to preserve refrigerated fish and extend its shelf life. The application of such hurdles reduces the rate of fish product deterioration and spoilage caused by microbial growth. The application of MAP may result in a better retention of quality attributes and shelf life prolongation of fish, minimally processed fish products (e.g., marinated), and ready-to-cook commodities [1]. The most commonly used gases in MAP used for fish are oxygen (O_2), carbon dioxide (CO_2), and nitrogen (N_2), as well as their concentrations in the package headspace, which depend on the specific fish product and the mechanism of spoilage that limits the shelf life of the final food product [2]. Microbial growth in food products is inhibited by CO_2, which is the most widely used gas for the MAP of fish [1]. The concentration of CO_2 has been

reported to play a major role in the inhibition of microbial growth and thus may delay the growth of respiratory organisms, such as *Pseudomonas* spp. and *Shewanella putrefaciens* [3].

The applicability of MAP on fish and its preservative effect have been reported in the literature, indicating a shelf life extension of several days, depending on species and storage temperature [4–8]. A significant diversification in the dominant spoilage bacteria in MAP fish is observed, which may be attributed to their geographical origination, as well as differences in water temperature and storage conditions [1,3,9–12].

Although the importance of MAP technology in the fish industry is well established and several studies evaluate the effect of MAP on fish quality and shelf life, a small number of mathematical models have been developed in order to express the combined effect of storage temperature and headspace composition on the growth of spoilage bacteria. The effect of CO_2 on the growth kinetics for *Photobacterium phosphoreum* and *Shewanella putrefaciens* was quantified and modeled for MAP cod [13]. The results showed that the microbial spoilage of packed cod stored with various concentrations of CO_2 was accurately predicted from the effect of CO_2 on *P. phosphoreum* growth in model substrates.

The temperature behavior of the natural microflora on red mullet was examined in another study by Koutsoumanis et al. [14]. The growth of the spoilage bacteria *Pseudomonas* spp., *S. putrefaciens*, *Brochothrix thermosphacta*, and lactic acid bacteria was modeled as a function of temperature and the concentration of CO_2 in MAP. Combined models were developed and comparatively assessed based on polynomial, Belehradek, and Arrhenius equations. The effect of storage temperature and CO_2 concentration on the growth of *Carnobacterium maltaromaticum*, *Serratia proteamaculans*, *Yersinia intermedia*, and *Shewanella baltica*, as well as on the growth of a mixed culture of the four species, has also been investigated [15]. These species were identified as the organisms responsible for spoilage in MAP mackerel fillets. A modified Arrhenius model has been reported by Tsironi et al. [16] that describes the lactic acid bacteria growth in MAP gilthead seabream fillets, and its applicability was validated at different storage conditions (0–15 °C and 20–80% CO_2) [17]. Such mathematical models may predict microbial growth and consequently the shelf life of the studied MAP fish product as a function of packaging and refrigerated storage conditions. Under this context, predictive models for MAP fish products may enable the selection of appropriate MAP conditions in order to achieve adequate shelf life extension and minimize food waste and costs. Additionally, the shelf life models combined with the use of time/temperature integrators (TTI) could be an effective tool for monitoring the quality of chilled fish during distribution and storage, allowing better management and optimization of the chill chain from production to the point of consumption [17].

Despite the advantages of MAP on the shelf life of packed fish and fish products, it may be space-demanding due to the need for a high gas volume/product volume ratio in order to show a significant preservative effect on fish products. At the same time, high concentrations of CO_2 in the package headspace and increased gas volume/product ratios may result in the significant dissolution of CO_2 in the fish tissue. Carbon dioxide emitters have been reported in the literature, and they are also known to utilize the O_2 in the package headspace so as to form CO_2 and enhance the concentration of a CO_2/N_2 headspace in the package without requiring an additional insertion of gas [18–23]. Another significant drawback of using MAP is the significantly shortened shelf life once MAP integrity is compromised. Novel smart packaging solutions have been developed for the controlling and/or monitoring of the package integrity and gas concentration in the headspace [1,24].

The aim of the study is the evaluation and mathematical modeling of the effect of active modified atmosphere packaging by the incorporation of CO_2 emitters into the package on the microbial stability and shelf life of gutted sea bass during refrigerated storage.

2. Materials and Methods

2.1. Raw Material

Marine cultured sea bass (*Dicentrarchus labrax*) (weight: 300 g/fish approximately, capture zone: Aegean Sea, Greece) from the same batch were provided by a leading Greek aquaculture company (Selonda S.A.). Fish were cultivated in net pens and harvested (age 14–20 months approximately). After being ice shocked and slaughtered, fish was put into ice (0 °C), size sorted, and transported to the processing line within 1 day after harvesting. Fish samples were gutted and rinsed with tap water in the industrial processing line. Samples were transported directly to the laboratory in polystyrene boxes with an appropriate quantity of flake ice (0 °C) within 2–4 h.

2.2. Modified Atmosphere Packaging

Gutted fish samples were packed in high-density polyethylene (HDPE) pouches under modified atmospheres (50% CO_2–40% N_2–10% O_2) (Boss NT42N, Bad Homburg, Germany). One fish was placed in each package (samples coded as MAP). Gas headspace was evaluated using the CheckMate 9900 O_2/CO_2 device (PBI Dansensor, Ringsted, Denmark). The gas/product volume ratio (g/p) was 3:1. In order to evaluate the effect of active MAP on the microbial stability and shelf life of fish, selected CO_2 emitters were put in each package in half the number of samples (coded as ACT-MAP). CO_2 emitters (dimensions 300 mm × 130 mm × 40 mm) were provided by McAirlaid's Inc. (Steinfurt, Germany).

2.3. Shelf Life Study

All packages (MAP and ACT-MAP) were stored at controlled isothermal conditions (0 °C, 5 °C, and 10 °C) in high-precision (±0.2 °C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan). Temperature monitoring in the incubators was based on electronic, programmable miniature dataloggers (COX TRACER®, Belmont, NC, USA).

For microbiological enumeration, a representative sample of 10 g was transferred to a sterile stomacher bag with 90 mL of sterilized Ringer solution (Merck, Darmstadt, Germany) and was homogenized for 60 s with a Stomacher (BagMixer ® interscience, France) [12]. Samples (0.1 mL) of 10-fold serial dilutions of fish homogenates were transferred into the appropriate media on Petri dishes for the enumeration of total aerobic viable count (TVC) and *Pseudomonas* spp. TVC was enumerated on plate count agar (PCA, Merck, Darmstadt, Germany) after incubation at 25 °C for 72 h, whereas *Pseudomonas* spp. were enumerated on cetrimide agar (CFC, Merck, Darmstadt, Germany) after incubation at 25 °C for 48 h. For lactic acid bacteria (LAB) and *Enterobacteriaceae* spp. enumeration, the pour-plate method was used. Lactic acid bacteria (LAB) were enumerated on De Man Rogosa Sharpe agar (MRS, Merck, Darmstadt, Germany) followed by incubation at 25 °C for 96 h. For *Enterobacteriaceae* spp. enumeration violet red bile glucose agar (VRBG, Merck, Darmstadt, Germany) was used, which was incubated at 25 °C for 48 h. Two replicates of at least three appropriate dilutions were enumerated.

2.4. Data Analysis

The microbial growth was described using the Baranyi growth model [25]. For curve fitting, the program DMFit was used (available at http://www.combase.cc/index.php/en/). The kinetic parameters, microbial growth rate (k in d^{-1}), and lag phase (λ in d) were estimated at all tested temperature and packaging conditions.

The temperature-dependence of the microbial growth rates constants, k, was modeled by the Arrhenius Equation (1):

$$\ln k = \ln k_{ref} - (Ea/R)(1/T - 1/T_{ref}) \quad (1)$$

where k_{ref} is the microbial growth rate at a reference temperature, T_{ref} (e.g., 4 °C for chilled food), T is the temperature in K, E_a is the activation energy that indicates the temperature dependence of the

selected mode of degradation, and R is the universal gas constant. The activation energy (E_a) values were estimated from the slope of Arrhenius plots of ln(k) versus ($1/T_{ref} - 1/T$) by linear regression [26].

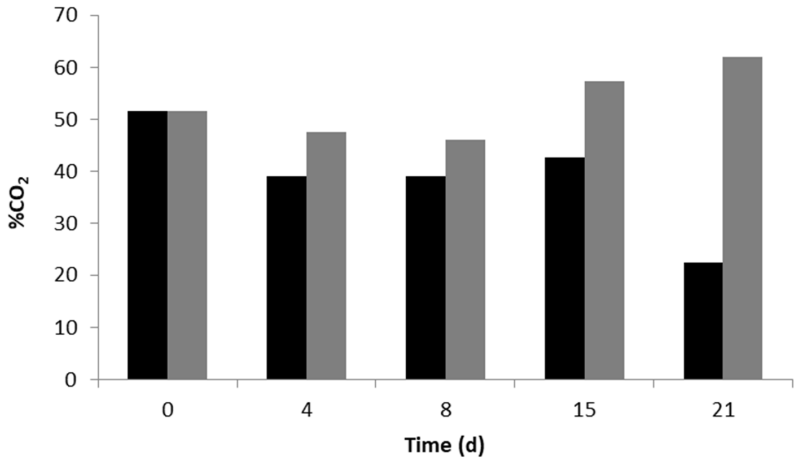
2.5. Statistical Analysis

Analysis of variance (ANOVA) at a significance level of 95% was used for the analysis of the gas concentration levels (%CO_2, %O_2), microbial growth kinetic parameters (k, λ), and shelf life (t_{SL}) of MAP and ACT-MAP gutted sea bass samples (STATISTICA® 7.0, StatSoft Inc., Tulsa, OK, USA). Significant differences were calculated according to Duncan's multiple range test ($\alpha = 0.05$).

3. Results and Discussion

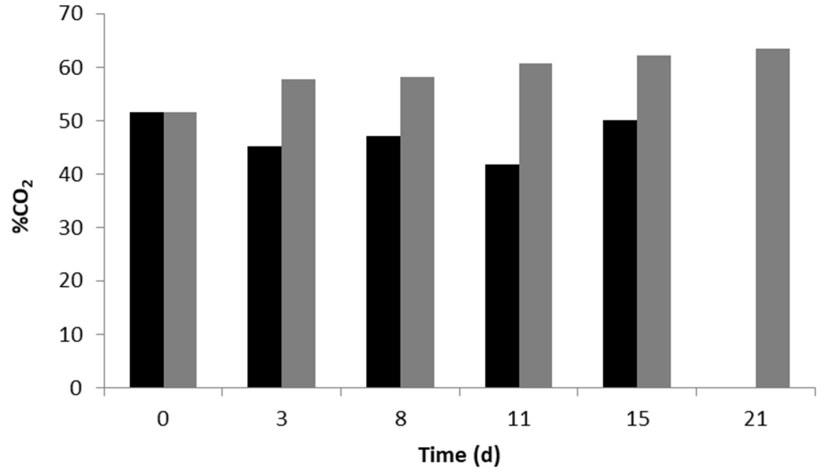
3.1. Gas Changes

Gas (CO_2, O_2) concentration (%) in MAP and ACT-MAP gutted sea bass package headspace during the isothermal storage (0, 5, 10 °C) are presented in Figures 1 and 2, respectively. The CO_2 concentration in the MAP gutted sea bass package headspace decreased (from the initial value of 50% to the value of 40% approximately) up to 15, 11, and 4 days of storage at temperatures of 0, 5, and 10 °C, respectively, and then increased (to the initial value of 50% approximately) due to the metabolic activity of the spoilage bacteria. The CO_2 concentration in the ACT-MAP gutted sea bass package headspace initially decreased (e.g., from the value of 50% to the value of 46% at a storage time of 8 days and storage temperature of 0 °C; Figure 1), which was attributed to the CO_2 dissolution in the fish flesh. Afterwards, the CO_2 concentration increased, reaching the maximum values, indicating that the CO_2 emitter started to produce CO_2 due to the contact of the emitter ingredients in the liquid absorber pad and the liquid loss. The highest CO_2 concentration values (60.5%–63.5%) were observed at the end of the storage period at all the tested temperatures. Bjerkeng et al. (1995) observed that the CO_2 concentration in the MAP + CO_2 emitter packaged cod fillets decreased to 40% approximately within a few days, and then increased to a maximum stable value of 50%. Hansen et al. [20] reported similar CO_2 gas behavior for an MA + CO_2 emitter (60% CO_2–40% N_2) packaged salmon fillets. For the MAP (g/p: 3/1) samples, the CO_2 concentration decreased to 40% (Day 4) and then increased. For the MAP + CO_2 emitter (g/p: 1/1), the CO_2 concentration initially decreased to 45% (Day 1) and then increased and reached 65–70% during storage. They also observed that CO_2 concentrations above 60% in the package headspace caused inflation or bulging [19–21]. Hansen et al. [22] demonstrated that the MAP + CO_2 emitter packaged cod loins presented 35–37% CO_2 concentration in the package headspace (g/p: 1.6/1.0) when the absorption of CO_2 was stabilized (Day 1). For the MAP cod samples (g/p: 1.6/1.0), the CO_2 concentration decreased to the stable value of 26–27%.



(**a**)

Figure 1. *Cont.*



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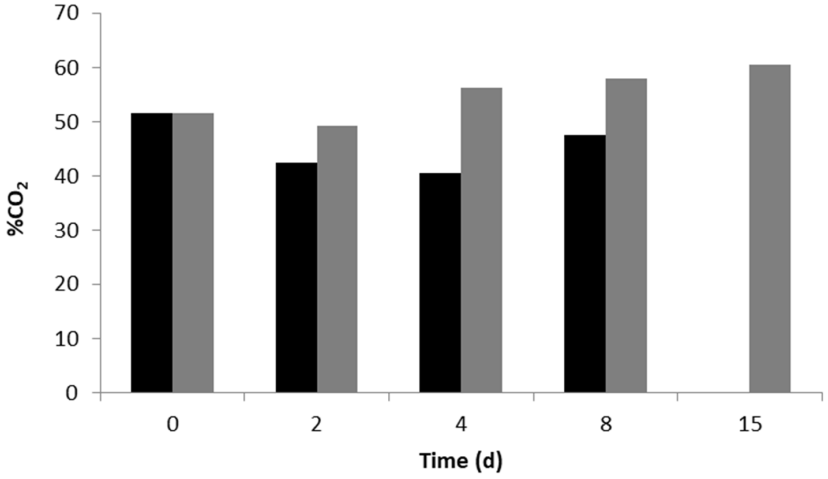
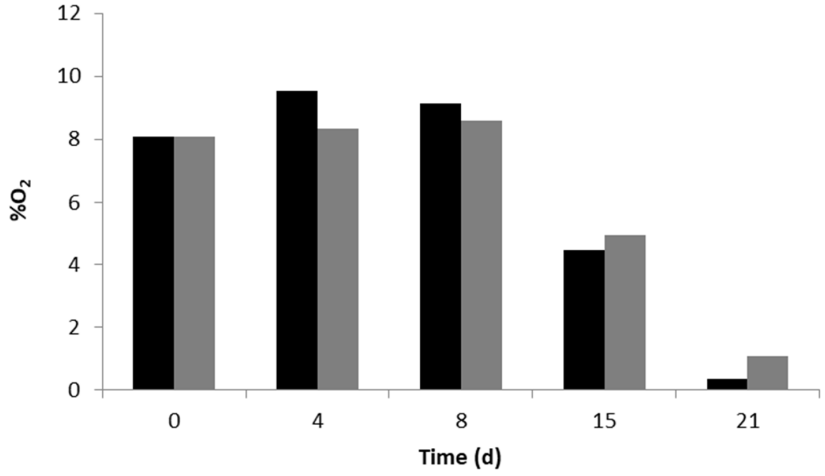


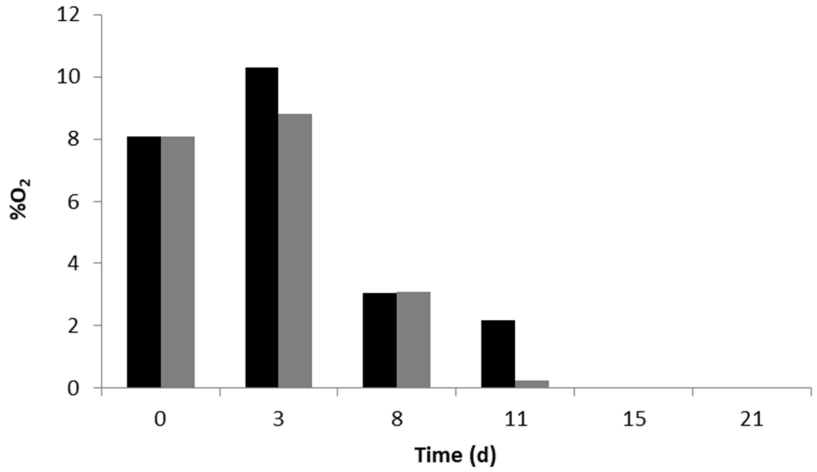
(**c**)

Figure 1. CO_2 concentration in the sea bass package headspace during the isothermal storage at (**a**) 0 °C, (**b**) 5 °C, and (**c**) 10 °C (grey bars ACT-MAP, back bars MAP). MAP: modified atmosphere packaging, ACT-MAP: active modified atmosphere packaging with CO_2 emitters.



(**a**)

Figure 2. *Cont*.



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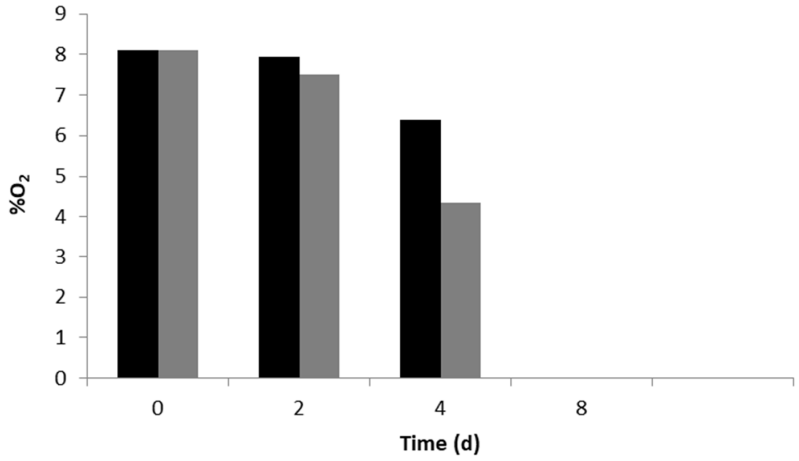

(**c**)

Figure 2. O_2 concentration in the sea bass package headspace during the isothermal storage at (**a**) 0 °C, (**b**) 5 °C, and (**c**) 10 °C (grey bars ACT-MAP, black bars MAP).

With regard to the O_2 concentration, it showed a descending trend (Figure 2), while at the end of the storage period, the O_2 concentration exhibited zero levels, which was related to the increased microbial population of the total viable counts, for both MAP and ACT-MAP gutted sea bass samples ($p > 0.05$; no statistically significant differences between ACT-MAP and MAP samples for most cases).

3.2. Microbial Growth during Refrigerated Storage

The microbial load of TVC, *Pseudomonas* spp., *Enterobacteriaceae* spp., and LAB in MAP and ACT-MAP gutted sea bass stored at temperatures 0, 5, and 10 °C is depicted in Figures 3–6, respectively. The experimental data were fitted to the Baranyi growth model, and the respective kinetic parameters at each condition were determined, as presented in Table 1.

The initial total viable count for the fresh gutted sea bass was approximately 4 log CFU g^{-1} (Figure 3) [27]. The MAP and ACT-MAP gutted sea bass samples presented similar TVC growth curves. The ACT-MAP gutted sea bass samples reached the value of 7 log CFU g^{-1} on days 22, 17, and 10 at 0, 5, and 10 °C, respectively. The MAP gutted sea bass samples reached the same log CFU g^{-1} value quicker on days 18, 10, and 6 at 0, 5, and 10 °C, respectively. The significant inhibitory effect of CO_2 emitters was observed for the total viable microbial growth due to a lag phase increase in all the ACT-MAP samples ($p < 0.05$) (e.g., 6.7 d for ACT-MAP samples compared to 1.8 d for the respective MAP samples stored at 5 °C; Table 1). The rates of TVC growth were also lower for the ACT-MAP samples compared

to the MAP samples ($p < 0.05$; apart from the ACT-MAP samples stored at the lowest temperature of 0 °C). Hansen et al. [19–21] measured the TVC of the MAP and MAP + CO_2 emitter packaged salmon fillets to be from 5 to 6 log CFU g^{-1} after 15 days compared to the packaged under vacuum salmon samples that reached the same value after 7 to 10 days. It has been reported that MAP packaging with CO_2 enrichment is the most effective packaging technique for the microbial growth inhibition and shelf life extension of sea bass [28,29]. More specifically, increasing the CO_2 and reducing the O_2, the aerobic bacteria and Gram-negative bacteria growth (e.g., *Pseudomonas* spp., *Shewanella* spp.) is delayed.

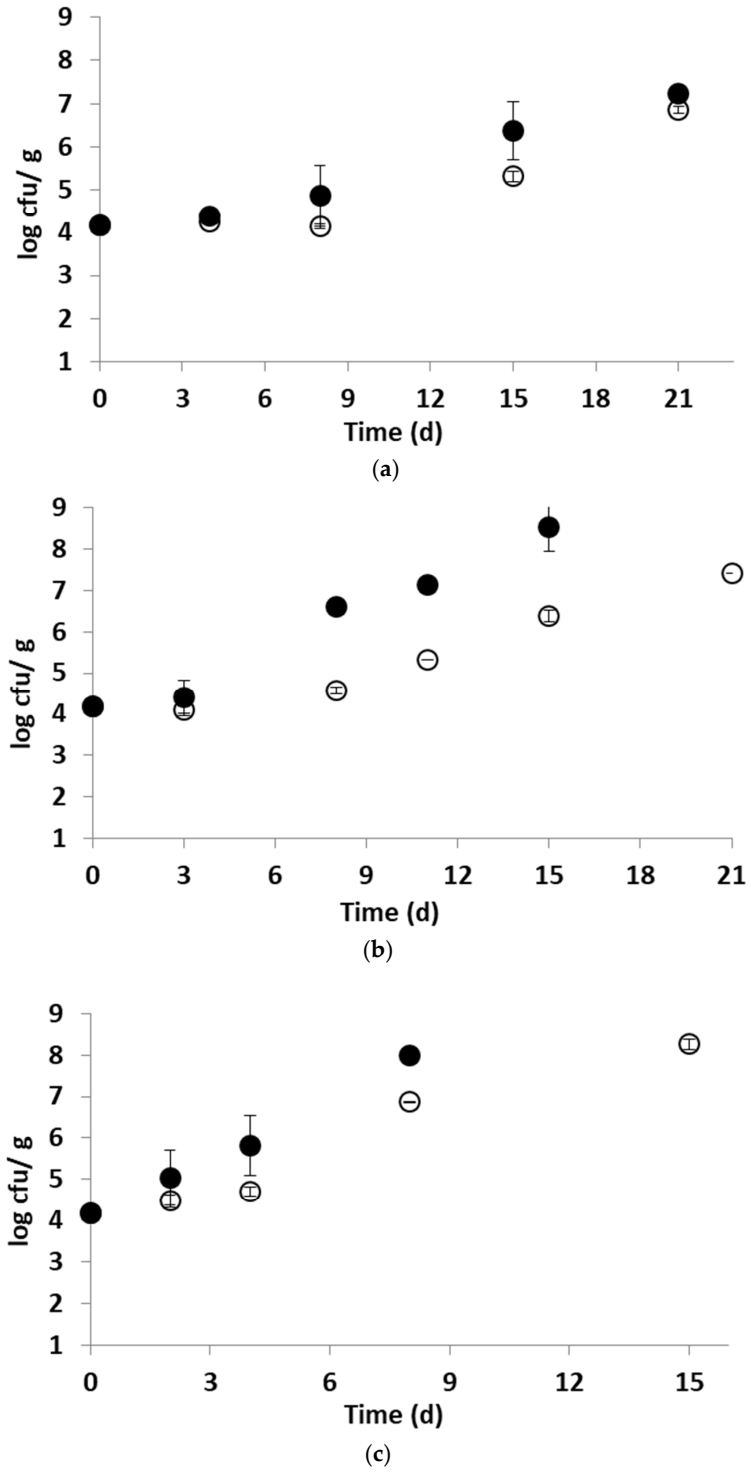

Figure 3. Total viable count in MAP and ACT-MAP sea bass during the isothermal storage at (**a**) 0 °C, (**b**) 5 °C, and (**c**) 10 °C (○ ACT-MAP, ● MAP).

The initial *Pseudomonas* spp. has been reported to be between 2.5 and 3.9 log CFU g^{-1} for sea bass [30–32]. In this study, at initial storage times, *Pseudomonas* counts were also low (ranging from the initial value of 3.0 to the value of 4.0 log CFU g^{-1}), reaching the value of 6.0 log CFU g^{-1} (Figure 4). The *Pseudomonas* spp. growth rates were significantly lower for ACT-MAP samples compared to MAP samples stored under the same time–temperature conditions ($p < 0.05$). At a storage temperature of 0 °C, the decrease of the *Pseudomonas* spp. growth rate (0.165 compared to 0.108 d^{-1}) was accompanied by a lag phase increase (from 2 to 8 d), showing that the use of the CO_2 emitter led to a significant inhibition of *Pseudomonas* spp. growth.

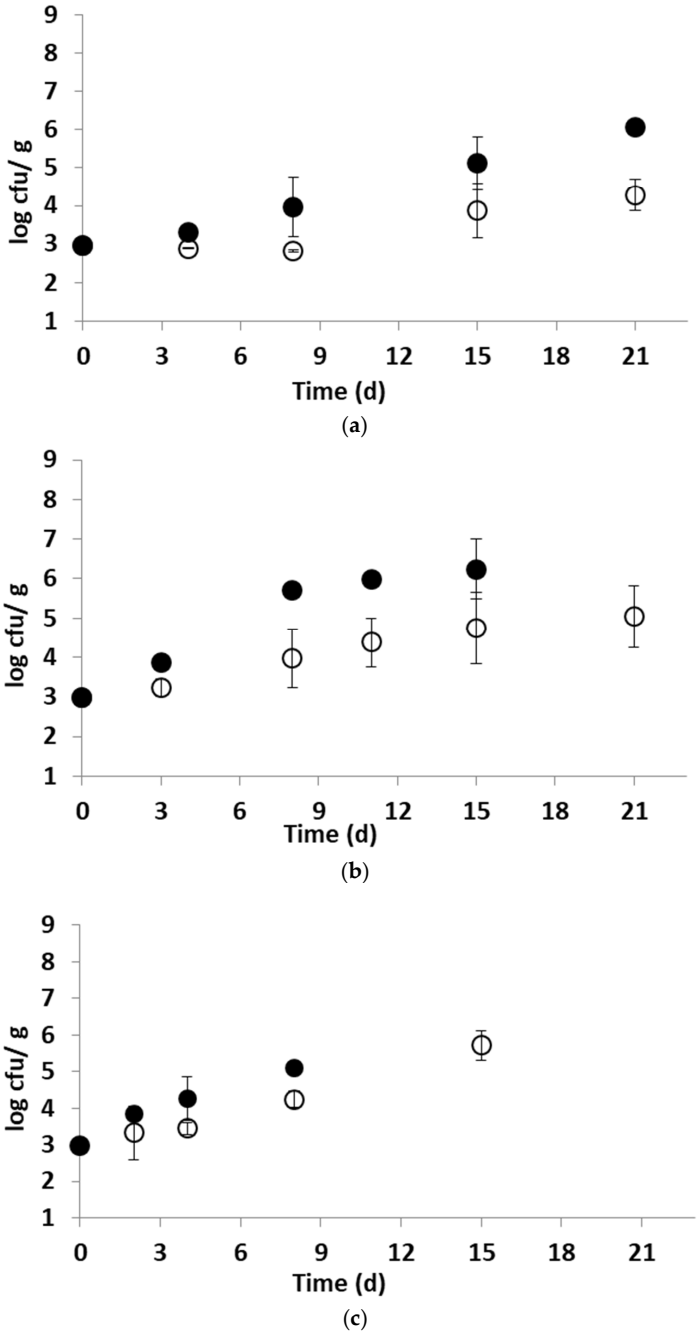

Figure 4. *Pseudomonas* spp. count in MAP and ACT-MAP sea bass during the isothermal storage at (**a**) 0 °C, (**b**) 5 °C, and (**c**) 10 °C (○ ACT-MAP, ● MAP).

Enterobacteriaceae spp. and LAB have also been reported in sea bass fillets (smaller extent) [29]. In this study, the initial population of *Enterobacteriaceae* spp. and LAB was low (Figure 5). *Enterobacteriaceae*

spp. presented increased growth rates under MAP with increasing storage temperatures from 0 °C to 10 °C for both MAP and ACT-MAP samples. ACT-MAP samples presented an *Enterobacteriaceae* spp. count of approximately 6.0 log CFU g^{-1} after 21 and 15 days at 5 °C and 10 °C, respectively, and MAP samples after 15 and 8 days of storage at 5 °C and 10 °C, respectively. CO_2 emitter use decreased significantly the growth of *Enterobacteriaceae* spp. at temperatures of 0–10 °C ($p < 0.05$).

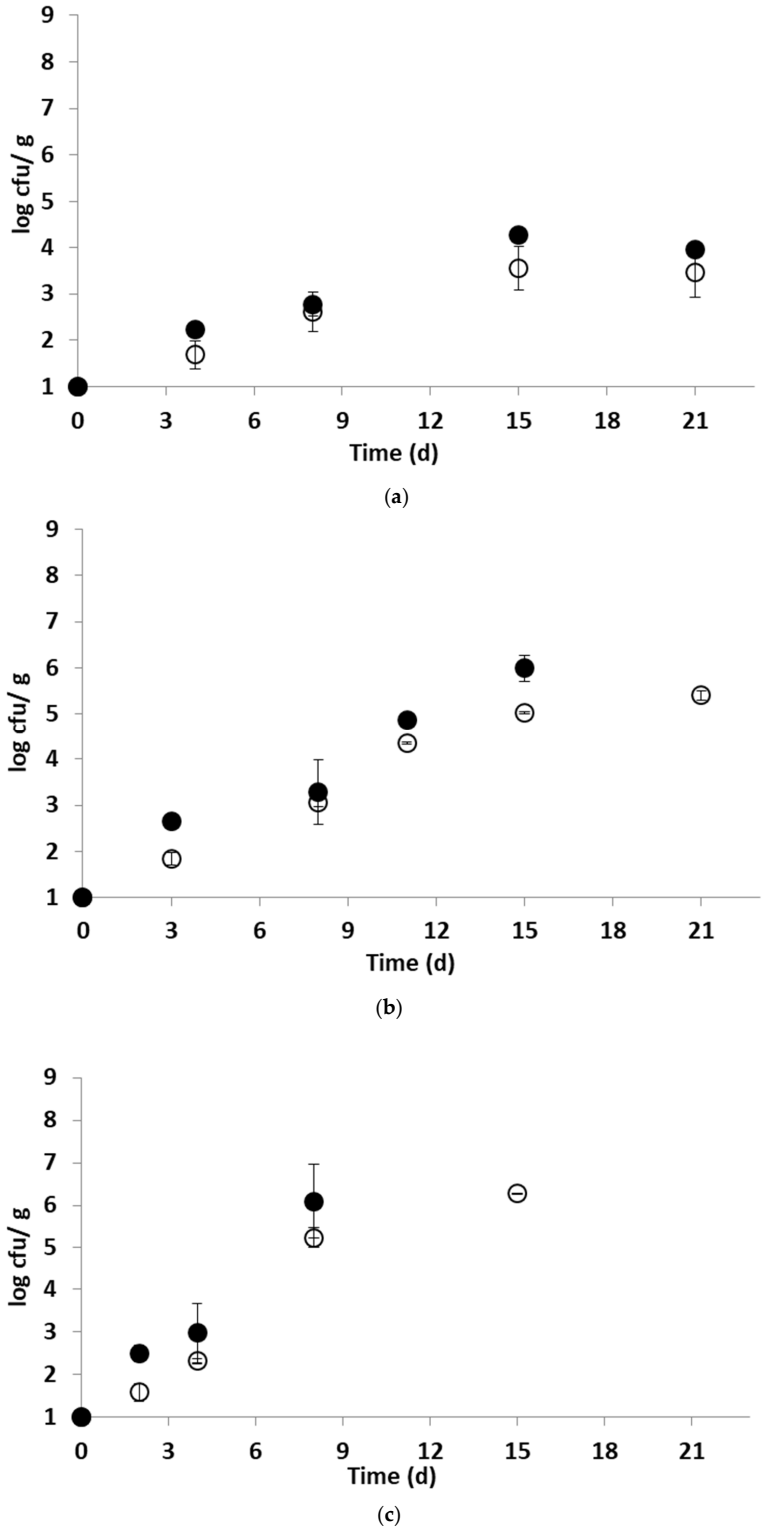

Figure 5. *Enterobacteriaceae* spp. count in MAP and ACT-MAP sea bass during the isothermal storage at (**a**) 0 °C, (**b**) 5 °C, and (**c**) 10 °C (○ ACT-MAP, ● MAP).

Lactic acid bacteria (LAB) counts remained well below the spoilage level of 6.0 log CFU g^{-1} [17], increasing with the storage time–temperature increase. Both ACT-MAP and MAP samples presented similar LAB growth behavior (Figure 6). No mathematical modeling of LAB growth was performed. It was concluded that the CO_2 concentration in the package headspace did not present a statistically significant effect on LAB in MAP and ACT-MAP samples ($p > 0.05$) [20,33]. Turan and Kocatepe [29] reported that the initial LAB count was 2.03 log CFU g^{-1}, and no more than 4.91 log CFU g^{-1} was measured using different packages.

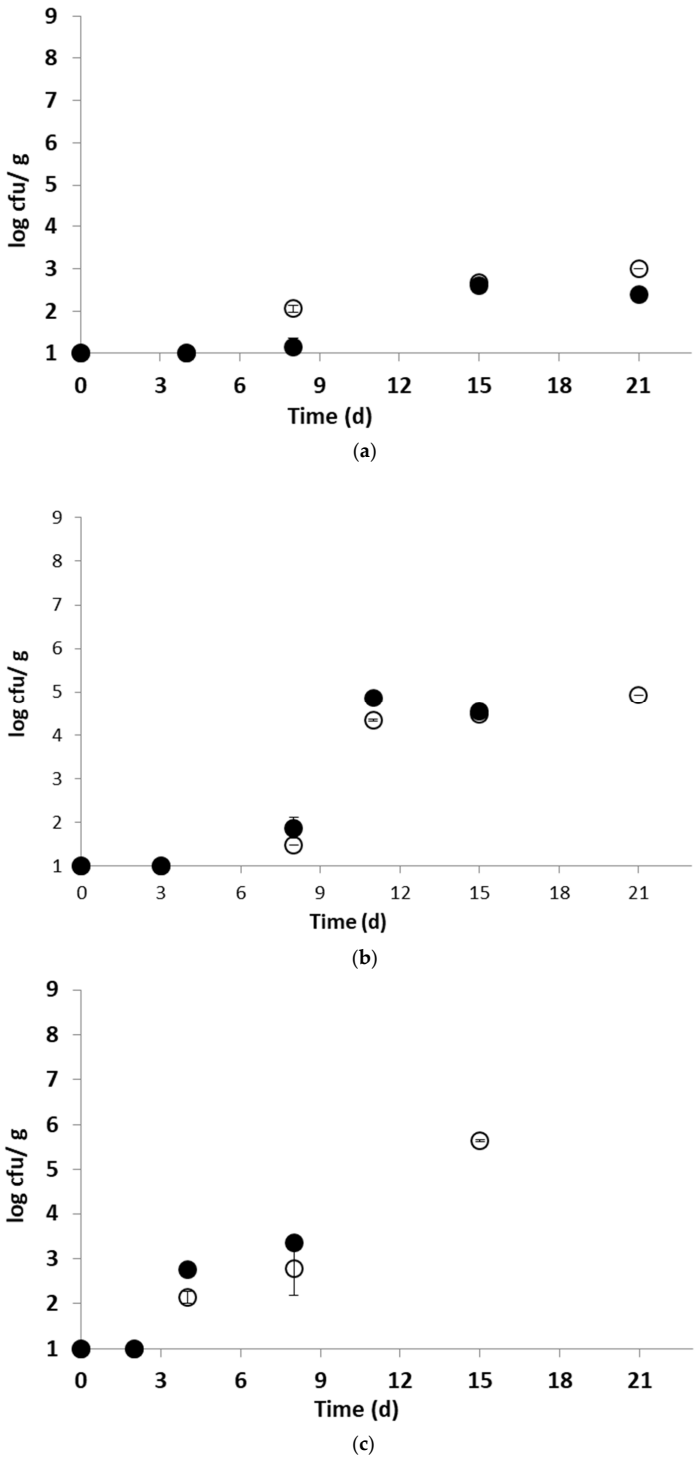

Figure 6. Lactic acid bacteria count in MAP and ACT-MAP sea bass during the isothermal storage at (**a**) 0 °C, (**b**) 5 °C, and (**c**) 10 °C (○ ACT-MAP, ● MAP).

Table 1. Growth kinetic parameters (growth rate, k in d^{-1}; lag phase, λ in d), for the total viable count, *Pseudomonas* spp. and *Enterobacteriaceae* spp. in gutted sea bass during the isothermal storage at 0–10 °C.

Storage Temperature	0 °C	5 °C	10 °C
	Total viable count		
MAP			
Growth rate, k (in d^{-1})	0.175 ± 0.010 [a]	0.337 ± 0.028 [a]	0.496 ± 0.027 [a]
Lag phase, λ (in d)	3.14 ± 0.63	1.83 ± 0.72	0.41 ± 0.27
R^2 fit	0.991	0.982	0.995
ACT-MAP			
Growth rate, k (in d^{-1})	0.138 ± 0.017 [b]	0.277 ± 0.016 [b]	0.308 ± 0.041 [b]
Lag phase, λ (in d)	10.6 ± 0.58	6.73 ± 0.41	1.04 ± 1.05
R^2 fit	0.996	0.999	0.949
Pseudomonas spp.			
MAP			
Growth rate, k (in d^{-1})	0.165 ± 0.005 [a]	0.347 ± 0.029 [a]	0.345 ± 0.142 [a]
Lag phase, λ (in d)	1.98 ± 0.56	-	-
R^2 fit	0.999	0.990	0.950
ACT-MAP			
Growth rate, k (in d^{-1})	0.108 ± 0.027 [b]	0.129 ± 0.006 [b]	0.191 ± 0.012 [b]
Lag phase, λ (in d)	8.05 ± 2.49	-	-
R^2 fit	0.949	0.994	0.987
Enterobacteriaceae spp.			
MAP			
Growth rate, k (in d^{-1})	0.245 ± 0.093 [a]	0.339 ± 0.103 [a]	0.625 ± 0.069 [a]
Lag phase, λ (in d)	-	-	-
R^2 fit	0.952	0.922	0.963
ACT-MAP			
Growth rate, k (in d^{-1})	0.204 ± 0.017 [a]	0.292 ± 0.019 [a]	0.378 ± 0.063 [b]
Lag phase, λ (in d)	-	-	-
R^2 fit	0.993	0.990	0.899

Mean values ± standard error based on the statistical variation in the kinetic parameters of the Baranyi growth model: regression analysis). [a,b] Different superscript in the same column for the estimated growth rates (k) of the same microorganism indicate statistically significant differences.

The temperature effect on the microbial growth rates (growth rate, k in d^{-1}) for TVC, *Pseudomonas* spp., and *Enterobacteriaceae* spp. and the lag phase (lag phase, λ in d) for the total viable count was described by the activation energy (E_a) estimated by the fitting the obtained data on the Arrhenius Equation (1). The estimated kinetic parameters for the tested microbial growth are presented in Table 2.

Table 2. Kinetic parameters of the Arrhenius model for the microbial growth rate and lag phase for the total viable count, *Pseudomonas* spp. and *Enterobacteriaceae* spp. in gutted sea bass during the isothermal storage at 0–10 °C.

	Arrhenius Model Parameters for the Microbial Growth Rate	Arrhenius Model Parameters for the Lag Phase
	Total viable count	
MAP		
$E_{a,k}$ (kJ mol^{-1})	67.2	213.4
k_{ref} (d^{-1})	0.279	1.05
R^2	0.981	0.990
ACT-MAP		
$E_{a,k}$ (kJ mol^{-1})	51.8	148.9
k_{ref} (d^{-1})	0.211	1.66
R^2	0.855	0.885

Table 2. *Cont.*

	Arrhenius Model Parameters for the Microbial Growth Rate	Arrhenius Model Parameters for the Lag Phase
	Pseudomonas spp.	
MAP		
$E_{a,k}$ (kJ mol^{-1})	47.8	-
k_{ref} (d^{-1})	0.252	
R^2	0.754	
ACT-MAP		
$E_{a,k}$ (kJ mol^{-1})	36.7	-
k_{ref} (d^{-1})	0.131	
R^2	0.951	
	Enterobacteriaceae spp.	
MAP		
$E_{a,k}$ (kJ mol^{-1})	60.0	-
k_{ref} (d^{-1})	0.342	
R^2	0.966	
ACT-MAP		
$E_{a,k}$ (kJ mol^{-1})	39.7	-
k_{ref} (d^{-1})	0.266	
R^2	0.993	

The activation energy (E_a) regarding the storage temperature effect on the TVC, *Pseudomonas* spp., and *Enterobacteriaceae* spp. growth rate were in the range of 37–52 kJ mol^{-1} and 48–67 kJ mol^{-1} for ACT-MAP and MAP samples, respectively, not showing statistically significant differences between the two group of samples ($p > 0.05$). However, the E_a value that describes the storage temperature effect on the lag phase was significantly higher for MAP samples compared to ACT-MAP samples (213 compared to 149 kJ mol^{-1}, respectively) (Table 2).

3.3. Shelf Life Estimation

The shelf life estimation of MAP fish was performed using the acceptability limit of 10^7 CFU g^{-1} for the total viable count [34]. Spoilage rejection for MAP + CO_2 emitter packaged fish has been reported at log 6.5–7.0 CFU g^{-1} (total viable count) [4,35]. Based on the TVC limit, indicating the shelf life acceptability of the product, and the temperature dependence of the microbial growth rates, based on Arrhenius kinetics, simple equations for MAP and ACT-MAP sea bass shelf life can be calculated using Equation (2):

$$t_{SL} = (\log N_l - \log N_o)/(k_{ref} \exp((-E_a/R)(1/T - 1/T_{ref}))) + \lambda_{ref} \exp((-E_a/R)(1/T - 1/T_{ref})) \quad (2)$$

where t_{SL} is the shelf life (d) of gutted sea bass, $\log N_l$ is the limit TVC load (7.0 log CFU g^{-1}), $\log N_o$ is the TVC at storage time zero (0), k_{ref} is the growth rate at the reference temperature T_{ref} (for this study 4 °C), E_a is the activation energy of TVC growth rate, R is the universal gas constant, and λ is the lag phase. The estimated shelf life of gutted sea bass at all the tested storage and packaging conditions, which were calculated using the developed mathematical model, is presented in Table 3.

Table 3. Shelf life of MAP and ACT-MAP gutted sea bass during the isothermal storage at 0 °C, 5 °C, and 10 °C by using the developed mathematical model (limit for shelf life estimation = 10^7 CFU g^{-1} for the total viable count).

	Shelf Life (d)		
Storage temperature	0 °C	5 °C	10 °C
MAP	18	10	6
ACT-MAP	22	17	10

According to the results in Table 3, the CO_2 gas in the headspace of the ACT-MAP samples led to a shelf life extension of MAP gutted sea bass: 7 days at storage temperature 5 °C and 4 days at 0 °C and 10 °C. The use of CO_2 emitter in MAP significantly increased the shelf life of gutted sea bass at all the storage temperatures studied ($p < 0.05$). The beneficial effect of the use of CO_2 emitters in MAP and/or vacuum packages on the total quality and shelf life of fish products was demonstrated [19–22,36]. Bjerkeng et al. [36] reported the extension of the microbial (3 d extension, for the psychrophilic bacteria to reach 6 log CFU g^{-1}) and sensorial shelf life (4 d extension, based on the sensory criteria ammonia-like odor) of cod fillets. The vacuum + CO_2-emitter packaged cod fillets were found to have 11 days of shelf life, which was more than the 7 days for the MAP + liquid absorber packaged samples based on sensory, chemical (trimethylamine), and microbial analysis (TVC and H_2S-producing bacteria). Hansen et al. [19,20] reported a shelf life maintenance of cod and salmon fillets (based on microbial, sensory, and texture analysis) at lower g/p ratios (e.g., 1.3–1.0/1.0 with the CO_2-emitter compared to 3.9–3.0/1.0). Packaging in MAP (60% CO_2–40% O_2) and CO_2 emitter at a lower g/p ratio (1.3/1.0) or MAP at a higher g/p ratio (3.9/1.0) led to shelf life extension (14 to 21 d) of cod fillets both in terms of sensory acceptance and microbial growth compared to vacuum (7–14 d). More recently, Hansen et al. [21] showed that vacuum + –CO_2 emitter packaged cod fillets displayed 2 days of shelf life extension compared to vacuum-packed samples. The maximum shelf life (13 d) was reported for the MAP (60% CO_2–40% N_2) and CO_2 emitters.

In the present study, the incorporation of CO_2 emitters in the MAP of gutted sea bass has been investigated as a potential tool to maintain fish quality and further extend shelf life. The tested CO_2 emitters, prepared by $NaHCO_3$ and citric acid, produce CO_2 gas in contact with water that is obtained from liquid leaking from the fish flesh and thereby compensate for the reduced gas volume to product volume ratio. Thus, such CO_2 emitters may also simultaneously act as liquid absorbers [1]. In any case, CO_2 generators are used in the active packaging system for the purpose of increasing the lag phase and inhibiting the growth of bacteria in certain food products. Similar CO_2 pads have also been reported to sufficiently reduce the transport volume of MAP-farmed cod and Atlantic salmon [19,20]. Different types of CO_2 emitters have also been developed, which utilize the O_2 from the package atmosphere to form CO_2 and develop a CO_2/N_2 headspace into the package without implicating any gas insertion. Alternative techniques to generate CO_2 into the food package after sealing include the utilization of dry ice or carbonate in some cases in combination with weak acids [22,37]. It has been reported that the effect of CO_2 varies upon microorganisms. For example, moderate to high CO_2 concentrations (10–20%) can slow down the growth of aerobic bacteria, while the growth of LAB is stimulated by CO_2 [38]. Commercially available CO_2 emitters in many cases include ferrous carbonate and a metal halide catalyst to absorb O_2 and produce equal volumes of CO_2 [39,40].

The present article demonstrates the investigation of the effect of a CO_2 emitter-assisted MAP type as an effective active packaging system to prolong the shelf life of gutted sea bass in the cold chain. This study has been implemented at isothermal storage conditions in the range of 0–10 °C. Effective mathematical models must be validated at variable conditions, simulated time–temperature scenarios of the actual cold chain, and on independent fish batches. Additionally, since high concentrations of CO_2 are enabled, particular care should be taken in determining the safety of packed fish, especially when processing significantly extends the shelf life, such as for example high pressure [41–43]. For this

reason, the U.S. Food and Drug Administration (FDA) Seafood Hazard Analysis Critical Control. Points (HACCP) guidelines (Chapter 13) suggest a minimum oxygen transmission rate in the final package of fish of at least 10,000 cc/m^2/24 h at 24 °C to provide sufficient oxygen that allows growth of aerobic spoilage organisms before *Clostridium botulinum* toxin formation under moderate abuse temperatures, thus limiting the innovation and application of new packaging methods in the fish industry sector [44]. Future research should be focused on initiating hurdle technologies for optimum active packaging systems by incorporating multiple active components or active and smart functionalities in one system [38,45,46]. For example, the combined use of validated shelf life models for active MAP fish with an indicator with a function of CO_2 detection in the package headspace and a TTI would provide useful information on the probability of the quality deterioration of packed fish, allowing the better management and optimization of the cold chain from manufacture to consumption.

4. Conclusions

It is apparent that the selection of the optimal MAP parameters (i.e., gas/product volume ratio and initial headspace gas composition) is a complex issue and is a very important step in the design of MAP packages. Selection depends on the effect on microbial growth, desired food quality, and shelf life, as well as the appearance of a package. CO_2 emitters, which produce CO_2 in contact with water that is obtained from liquid leaking from the fish flesh, may enable reduced gas/product volume ratios in MAP fish and further increase shelf life. Increased shelf life can contribute to food waste reduction. In this study, the use of CO_2 emitters in MAP-packaged gutted sea bass (MAP: 50% CO_2–40% N_2–10% O_2) + CO_2 emitter, gas/product volume ratio 3:1) was recognized as effective, as it significantly decreased the microbial growth (TVC, *Pseudomonas* spp., *Enterobacteriaceae* spp.) and increased the shelf life of the fish product. The shelf life of MAP and ACT-MAP gutted sea bass based on microbial growth (limit of acceptability = 7.0 log CFU g^{-1} for TVC) was 18, 10, 6 and 22, 17, and 10 days at 0 °C, 5 °C, and 10 °C, respectively.

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