Journal of Agricultural, Food Science & Biotechnology

JAFSB, 2(2), 110-119, 2024
https://doi.org/10.58985/jafsb.2024.v02i02.41
ISSN (Print): 2959-3417

Research Article

Synergistic effect of edible antimicrobial coatings, modified atmosphere, and sanitization on the shelf life of fresh hake medallions

Víctor Otero-Tuárez[1*], Víctor Bozal[2] and Juan I. Maté[2,3]

1. Faculty of Agricultural Sciences, Universidad Laica Eloy Alfaro de Manabí, 130214, Manta-Ecuador
2. Department of Agronomy, Biotechnology and Food, Universidad Pública de Navarra, 31006, Pamplona-Spain
3. IS-FOOD. Research Institute for Innovation & Sustainable Development in Food Chain. Universidad Pública de Navarra, 31006, Pamplona-Spain

Article Information
Received: 30 January 2024
Revised: 12 March 2024
Accepted: 13 March 2024
Published 24 March 2024

Academic Editor
Prof. Dr. Gian Carlo Tenore

Corresponding Author
Prof. Dr. Víctor Otero-Tuárez
E-mail: victor.otero@uleam.edu.ec
Tel: +593-52678299-52623740, Ext.145

Keywords
Edible antimicrobial coating; fish gelatin; ethyl lauroyl arginate; sanitization; modified atmosphere packaging.

Abstract

Fresh fish is a highly perishable product, due to its high nutrient content and high water activity and as a consequence, there is a demand for new preservation strategies for this product. In this study, fresh hake medallions were subjected to three preservation processes: (i) surface sanitization by sodium hypochlorite (NaOCl); (ii) surface treatment with lauroyl arginate ethyl (LAE) directly or incorporated into an edible fish gelatin-based coating; and (iii) modified atmosphere packaging (MAP). The effect of these treatments on the evolution of microbiological quality was studied individually and in combination. The samples which were only treated with NaOCl did not evolve distinctly from the control samples. However, the combination of an edible fish gelatin coating with LAE and MAP had a powerful synergistic effect on the growth of all bacterial groups analyzed in the research. The sanitized medallions coated with edible gel carrying LAE and packaged in MAP significantly delayed the growth of all bacterial groups for up to 12 days of refrigerated storage. Therefore, the synergistic effect of combining several preservation technologies is promising for ensuring the microbiological quality of fresh fish.

1. Introduction

Nowadays, there is a high demand for products that can be kept fresh for as long as possible under refrigerated conditions. Initial microbiota is an influential factor in fresh fish shelf life. That initial microbiota is comprised of native microbiota, which are natural bacterial populations found on the skin, gills and in the digestive tract [1]. Depending on the processing and storage conditions, this initial microbiota can grow rapidly and uncontrollably. Therefore, good manufacturing practices are critical to ensure the microbiological quality of fresh fish which is normally done by minimizing bacterial growth in the fish muscle [2]. However, other techniques are needed to help reduce the microbial load and extend the shelf life of fresh fish. As the microbiota present on the skin can contaminate the muscle during the process of decapitation, evisceration and filleting, an application of a surface treatment based on sodium hypochlorite (NaClO) has been reported to significantly reduce the initial

microbial load present on the fish skin [3]. As *Pseudomonas* spp. and *Shewanella putrefaciens* along with other microorganisms can develop under aerobic conditions at low temperature (4 °C), refrigeration should be combined with another modern conservation technique such as the use of modified atmosphere packaging (MAP) [4]. Some studies have shown that a high concentration of CO_2 and a low concentration of O_2 extend shelf life. Thus, Carrión-Granda et al. [5] demonstrated that the combination of MAP and refrigeration is an effective strategy for prolonging the shelf life of fresh fish. These authors established the ideal mixture of gases to extend the shelf life of hake medallions was 50% CO_2, 45% N_2 and 5% O_2.

Some microorganisms such as lactic acid bacteria (LAB) or *Photobacterium phosphoreum* grow under refrigeration and MAP conditions. In order to reduce the growth of these microorganisms, the addition of other preservation methods such as the use of antimicrobial agents is necessary [6]. Currently, food preservation requires a combination of distinct processes to achieve good microbiological quality without affecting the sensory quality of the finished product. For this reason, in recent years, consolidated techniques have been developed such as antimicrobial edible films and coatings. Edible films and coatings (EFC) based on fish gelatin (FG) are commonly used as a carrier for antimicrobial agents. These EFC serve as an oxygen and/or moisture barrier, increasing shelf life and improving the appearance of coated foods. In a study, Otero-Tuárez et al. [7] developed and characterized FG based edible films and coatings which incorporated LAE as an antimicrobial agent in order to improve the microbiological quality of fish products. LAE is known for its powerful antimicrobial effect because it is a cationic surfactant that causes bacterial death when it induces an alteration of the membrane potential and cell permeability [8]. This antimicrobial agent was categorized as GRAS (generally recognized as safe) by the Food and Drug Administration (FDA) in 2005, by the European Food Safety Authority (EFSA) in 2007 [9] and approved by the European Union as a food additive [10]. The aim of the present research was to study the individual and combined effects of sanitization with NaOCl, use of MAP packaging and the application of LAE by immersion or through incorporation into an edible coating on the development of the microbiological quality of fresh hake medallions under refrigeration.

2. Materials and methods

2.1 Materials

To produce the edible films with and without LAE, FG was procured by LAPI Gelatin (Empoli, Italy), the glycerol was supplied by Sigma (Barcelona, Spain) and the LAE (85% purity) marketed as Mirenat-P/100 was obtained by Vedeqsa, LAMIRSA group (Barcelona, Spain). The NaOCl (10% w/v) used in disinfection was procured by Panreac Química SA (Barcelona, Spain).

2.2 Experimental design

An experimental design was established, as described in Table 1.

Table 1. Treatments used in the experimental design.

Block 1	Block 2
C: control (untreated)	**C:** control
D: treated with NaClO	**DL:** treated with NaOCl and immersed in LAE
CM: not disinfected and packaged in MAP	**DLM:** treated with NaOCl, immersed in LAE and packaged in MAP
DM: treated with NaClO and packaged in MAP	**DLG:** treated with NaOCl, coated with FG with LAE
	DLGM: treated with NaOCl, coated with FG with LAE and packaged in MAP

2.3 Preparation of the fish medallions

The experimental trials required 12 kg of fish. Fresh hake was purchased from a local fish market in Pamplona, Spain and, was transported to the laboratory under refrigeration. If not sanitized (samples C and CM), the fish was decapitated, gutted, and filleted in a laminar flow chamber. Medallions with an average weight of 30±4 g were obtained. The sanitized hake (samples other than C and CM) was treated by immersion for 1 min in a solution of 250 mg L^{-1} of NaOCl in distilled water at 4±2 °C. After disinfection, the fresh hake was gutted, the head was removed and filleted in the same way as described above. Thus, sanitized 30±4 g medallions were obtained.

The preparation of the film-forming solutions (FFS)

https://doi.org/10.58985/jafsb.2024.v02i02.41

with and without LAE was based on the process adapted by Otero-Tuárez et al. [7]. To treat the fish medallions with LAE, the antimicrobial agent was dissolved in distilled water under constant stirring for 30 min at 70 °C. The solution was cooled to 25 °C after which the hake medallions were immersed in the solution for 1 min.

Three hundred milliliters of film-forming solution enriched with LAE were prepared. A 10% (w/w) aqueous solution of FG in distilled water was obtained, to which up to 3% (w/w) glycerol was added. Everything was dissolved in constant agitation for 30 min at 70 °C. During stirring the LAE was added at 5% (w/w) and the FFS was cooled to 30 °C. The hake medallions were immersed in the treatment solution for 1 min. The coated medallions were dried in a horizontal dry air cabinet to remove the excess coating (Sanplatec™ Dry-Keeper A-Type, Spain).

2.4 Packaging and storage of fish medallions

Polypropylene trays heat-sealed with polyethylene-polypropylene-ethylene-vinyl-alcohol-polypropylene (PE-PP-EVOH-PP) film was used for packaging. Three medallions per treatment were placed in each package. The trays were sealed with air or MAP (50% CO_2, 45% N_2 and 5% O_2) depending on the treatment. Storage was carried out for up to 12 days at 4±2 °C.

2.5 Microbiological analysis

For each treatment, hake medallions were analyzed in triplicate at 0, 4, 8 and 12 days of storage. The hake medallions were aseptically weighed, placed in sterile plastic bags (BagPage, Interscience-France) and homogenized with 225 mL of Buffered Peptone Water (pH=7±0.1 at 25 ºC) (Cultimed, Spain) using a Stomacher (Stomacher 400, London-UK) for 2 min. Decimal dilutions were prepared and then sown on the surface of the culture medium (agar) in a 9 cm diameter Petri dishe using a sterile spiral seeder (Eddy Jet 2 for spiral seeding IUL, USA). Table 2 describes the microorganisms that were evaluated for each sample and medium nutrient. The results were expressed in log CFU g^{-1}. The maximum growth ranges (6 log CFU g^{-1} mesophilic bacteria and 3 log CFU g^{-1} *Enterobacteriaceae*) are in accordance with European regulations [11]. In addition, values <1 log CFU g^{-1} were considered as undetectable growth of microorganisms.

2.6 Data analysis

All tests were performed in triplicate. The statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL., USA). Duncan's multiple range test was used to observe significant differences ($P \leq 0.05$) between the means of the variables.

Table 2. Microorganisms associated with fish spoilage and culture conditions.

Microorganisms	Culture medium	Incubation
Enterobacteriaceae	Violet Red Bile Glucose Agar (VRBG)	37 °C; 24 h
Mesophilic bacteria	Plate Counting Agar (PCA)	30 °C; 48 h
Psychrotrophic bacteria	Plate Counting Agar (PCA)	5 °C; 7 days
Pseudomonas spp.	*Pseudomonas* Agar (PS Agar)	30 °C; 48 h
Lactic acid bacteria	De Man, Rogosa and Sharpe Agar (MRS)	30 °C; 5 days
H_2S producing bacteria	Iron Agar (IA)	30 °C; 48 h

3. Results and discussion

3.1 Appearance

Throughout the 8 days of storage, the FG coated medallions appeared brighter than the other treatments. The medallion samples acquired a brown color in the control group with and without surface disinfection. The presence of exudates in the samples treated with MAP was very evident. In treatments with edible FG-based antimicrobial coatings, the appearance did not change during storage, no exudates were present, and the texture remained firm. The smell was more intense after 8 days of storage; and for the control group, relatively unpleasant after 12 days.

3.2 Microbial quality

The total counts of microorganisms without sanitized (control) and the sanitized with NaClO samples are shown in Fig. 1. No LAB growth was observed for any of the treatments during the 12-day storage period. At zero days the total counts of H_2S producing bacteria, mesophilic and psychrotrophic bacteria were significantly lower ($P<0.05$) in the treatment with disinfection (<4 log CFU g^{-1}) compared to the counts observed in the control group (4 log CFU g^{-1}). Bacterial counts of mesophilic bacteria and *Enterobacteriaceae*

https://doi.org/10.58985/jafsb.2024.v02i02.41

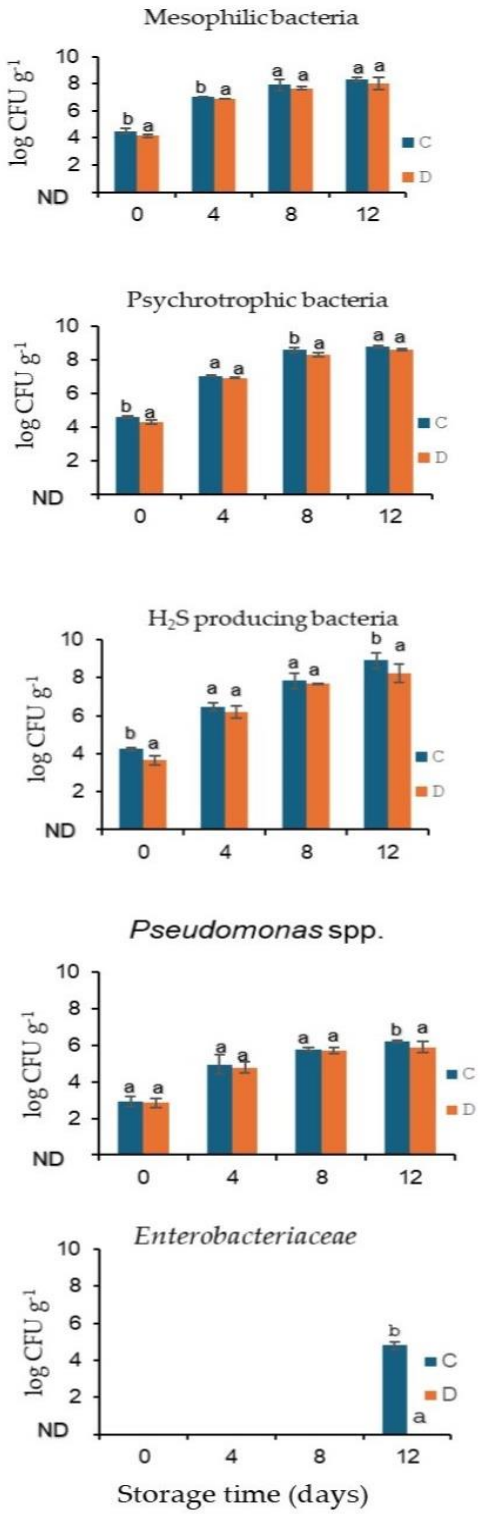

Figure 1. Total counts of microorganisms in fresh medallions stored for 12 days at 4 °C. Medallions without disinfection (C). Medallions with disinfection (D). Different letters (a, b,) indicate significant (P<0.05) differences between treatments. ND, non-detectable.

did not exceed the legal limit allowed for marketing (6 log CFU g^{-1} and 3 log CFU g^{-1}, respectively) [11]. After 4 days of cold storage, the total mesophilic bacteria count of the disinfected samples exceeded 6 log; which was slightly lower than the counts obtained for the control sample (7 log CFU g^{-1}). For the other microorganisms (psychrotrophic bacteria, *Pseudomonas* and H_2S producing bacteria), bacterial growth was slightly lower in the disinfected samples than in the controls. However, no *Enterobacteriaceae* growth was seen until 12 days of storage for the control group (4.8 log CFU g^{-1}), in contrast to the disinfected samples, where there was no bacterial growth during storage (<1 log CFU g^{-1}). NaOCl-based sanitation had no significant effect on bacterial growth compared to the control group, except for enterobacteria, whose growth in the disinfected samples was not detected at any point during the 12-day storage period.

The total counts of microorganisms in the fresh fish medallions were similar to those reported by Otero-Tuárez et al. [3] who used hake medallions filleted in the laboratory under similar conditions. These authors reported bacterial growths at zero days similar to those obtained in this research due to the effect of sanitation (mesophilic bacteria <6 log CFU g^{-1} and *Enterobacteriaceae* <1 log CFU g^{-1}). They also reported undetectable values of LAB (<1 log CFU g^{-1}). Therefore, the effect of sanitation on the initial microbial load of fresh fish was demonstrated, especially for the growth of *Enterobacteriaceae* and LAB. These results highlight the need for better handling practices to ensure the microbiological quality of fresh fish.

3.3 Effect of disinfection and the use of MAP on the microbiological quality of fresh hake medallions

The effect of packaging under modified atmosphere on the evolution of the microbiological quality of fresh, sanitized or unsanitized hake medallions was evaluated (Fig. 2). At time zero, no significant differences (P>0.05) were observed between the sanitized and unsanitized samples for all the microorganisms analyzed, except for the counts of mesophilic bacteria and H_2S producing bacteria. A very slight significant difference (P<0.05) was observed between the control groups (without and with sanitation without MAP) compared to the counts of these bacterial groups in the samples packaged with MAP (with and without sanitation).

After 12 days of storage, the mesophilic and

https://doi.org/10.58985/jafsb.2024.v02i02.41

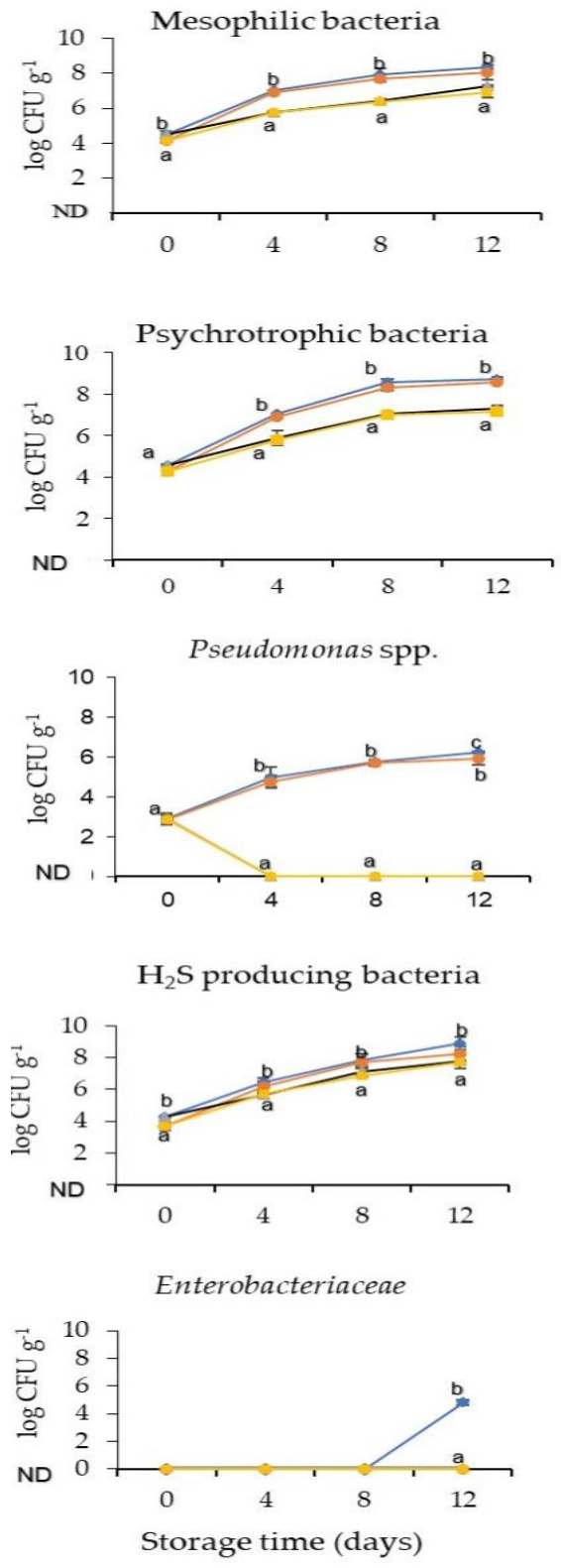

Figure 2. Total counts of microorganisms in fresh medallions stored for 12 days at 4 °C; Control medallions without disinfection (C-♦); Medallions with disinfection (D-●); Control with MAP (CM-▲); Medallions with disinfection and MAP (DM-■); Different letters (a, b, c) indicate significant (P<0.05) differences between treatments. ND, non-detectable.

psychrotrophic bacterial counts, in samples with MAP (7 log CFU g^{-1}) were significantly lower (P<0.05) than the bacterial counts of the packaged samples without MAP (>8 log CFU g^{-1}). The difference between the maximum values of bacterial growth in medallions packaged with MAP and those packaged without MAP was established at approximately 1.5 log CFU g^{-1}. Therefore, the application of MAP for fresh, previously sanitized medallions could extend shelf life.

Pseudomonas spp. growth in samples packaged with MAP (<1 log CFU g^{-1}) was significantly lower (P<0.05) than in samples packaged without MAP (>5 log CFU g^{-1}) throughout the 12 days of storage. This effect on *Pseudomonas* growth retardation was observed from the 4th day of storage for both sanitized and unsanitized samples packaged with MAP. Similarly, no growth of *Enterobacteriaceae* was detected during storage of medallions packaged with MAP (<1 log CFU g^{-1}), in contrast to medallions packaged without MAP (>5 log CFU g^{-1}) in the 12 days of storage.

The use of MAP did not have a critical effect on H_2S producing bacteria. As a rule, during storage microbial counts of MAP packaged treatments were significantly lower (P<0.05) in contrast to non-MAP treatments with a difference of 0.5 to 1 log CFU g^{-1}. The maximum values of bacterial growth in MAP treatments are 5.7 log CFU g^{-1}, 7 log CFU g^{-1} and 7.7 log CFU g^{-1} at 4, 8 and 12 days of storage, respectively. Following the use of MAP, significant (P<0.05) delays in microbial growth were observed throughout storage compared to samples that were packaged without MAP. In contrast, there were no observed significant differences (P<0.05) in the bacterial counts between the two MAP-treated samples (with and without sanitization). Therefore, with initial levels of bacterial population (> 4 log CFU g^{-1}) disinfection did not significantly affect the H_2S producing bacteria count when MAP was used. In contrast when MAP was not used, microbial growth was significantly higher (P<0.05) at 12 days of storage.

No proliferation of LAB was observed for any of the treatments during the 12-day storage period for both MAP and non-MAP packaged medallions. This was possibly due to the good handling and hygiene practices applied during the experimental trial.

Generally, the microbial count values presented in

https://doi.org/10.58985/jafsb.2024.v02i02.41

this research is lower than those reported in the scientific literature, except for the H_2S producing bacteria that showed higher colony numbers. Gutting and filleting under aseptic conditions in the laboratory, together with the application of NaOCl and MAP produced a significant reduction of the microbial load. The results derived from the combination of these techniques can provide significantly better microbiological quality of fresh fish even after storage. This was reflected in the results obtained in the counts of *Pseudomonas*, LAB and *Enterobacteriaceae*. Due to the combined application of good handling practices, sanitization with NaOCl and packaging with MAP, no growth of the mentioned microorganisms was detected during the 12 days of refrigerated storage.

Kykkidou et al. [12] packaged fresh swordfish (*Xiphias gladius*) with the same MAP concentrations used in this research (50% CO_2, 45% N_2 and 5% O_2). These authors reported total viable microorganism counts similar to those shown in this research with the application of MAP (<7 log CFU g^{-1}) and without the application of MAP (>8 log CFU g^{-1}) after 12 days of storage. On the other hand, Carrión-Granda et al. [5] reported growth values of psychrotrophic bacteria similar to those reported in this research after 4 days of storage in fresh hake with the same MAP conditions. In addition, Silbande et al. [13] who evaluated the microbiological quality of tuna (*Thunnus albacares*) reported data similar to this research on the microbial development of mesophilic and psychrotrophic bacteria for both MAP and non-MAP treatments during the 12-day storage period. In addition, the *Pseudomonas* count was below the detection threshold until day 10 of storage. Similar mesophilic and psychrotrophic bacteria count results were found by Garrido et al. [14]. They evaluated the microbiological quality of sea bream (*Sparus aurata*) packaged with MAP (40% CO_2, 30% N_2, 30% O_2) and stored at 4 °C. The high amount of CO_2 is the common denominator in all the research with MAP. Its effect on bacterial growth is the probable cause of microbial count reduction.

3.4 Effect of a combination of disinfection, use of MAP and the presence of LAE on the microbiological quality of fresh hake medallions

The evolution of the bacterial counts after application by immersion in a LAE solution or incorporated into an antimicrobial edible coating on disinfected fresh hake medallions with and without MAP is shown in Fig. 3. The total mesophilic bacteria count on day zero for the control group (4.8 log CFU g^{-1}) were significantly higher ($P<0.05$) than the rest of the treatments (4.1 log CFU g^{-1}) due to the surface treatment (NaOCl) applied to the fresh fish. For day 4, medallions packaged with MAP and LAE (DLGM and DLM) showed a lower bacterial count (non-detectable levels, <1log CFU g^{-1}) and were significantly lower ($P<0.05$) than counts obtained in samples with LAE and without MAP (DL and DLG) (>4.2 log CFU g^{-1}). The counts of both trials were well below the control group (5.1 log CFU g^{-1}). On the other hand, after 8 days of storage, bacterial growth was significantly higher in all treatments. However, the growth of mesophilic bacteria in the samples with antimicrobial coating (DLGM) (4.6 log CFU g^{-1}) was significantly lower than the counts obtained in the uncoated samples (DLM) (5.2 log CFU g^{-1}). These counts were lower compared to all other treatments which were >6 log CFU g^{-1}. This delay in the bacterial growth in DLGM and DLM samples (>5 log CFU g^{-1}) was significant throughout the 12 days of storage compared to the control group (>8 log CFU g^{-1}).

The combined use of antimicrobial edible coatings and MAP resulted in a significant ($P<0.05$) reduction in mesophilic bacteria growth up to 4 days of storage. This reduction was not observed when only MAP was used. Thus, this effect was attributed to a synergy of the use of MAP and LAE alone or incorporated into an edible coating. Furthermore, FG was found to play an important role as a diffusivity controller of the antimicrobial agent, thus, helping to maintain optimal levels of total viable microorganisms (<6 log CFU g^{-1}) when used in combination with MAP. This research also shows that LAE alone or added to an FG-based edible coating was not able to cause a relevant and prolonged antimicrobial effect. Therefore, the FG edible coating together with the LAE and without MAP did not affect the bacterial growth. However, the combination of an edible coating of FG with LAE and MAP had a powerful effect on the growth of mesophilic bacteria. These results are consistent with those obtained by Moreno et al. [15] who studied the shelf life of marinated salmon coated with starch film

https://doi.org/10.58985/jafsb.2024.v02i02.41

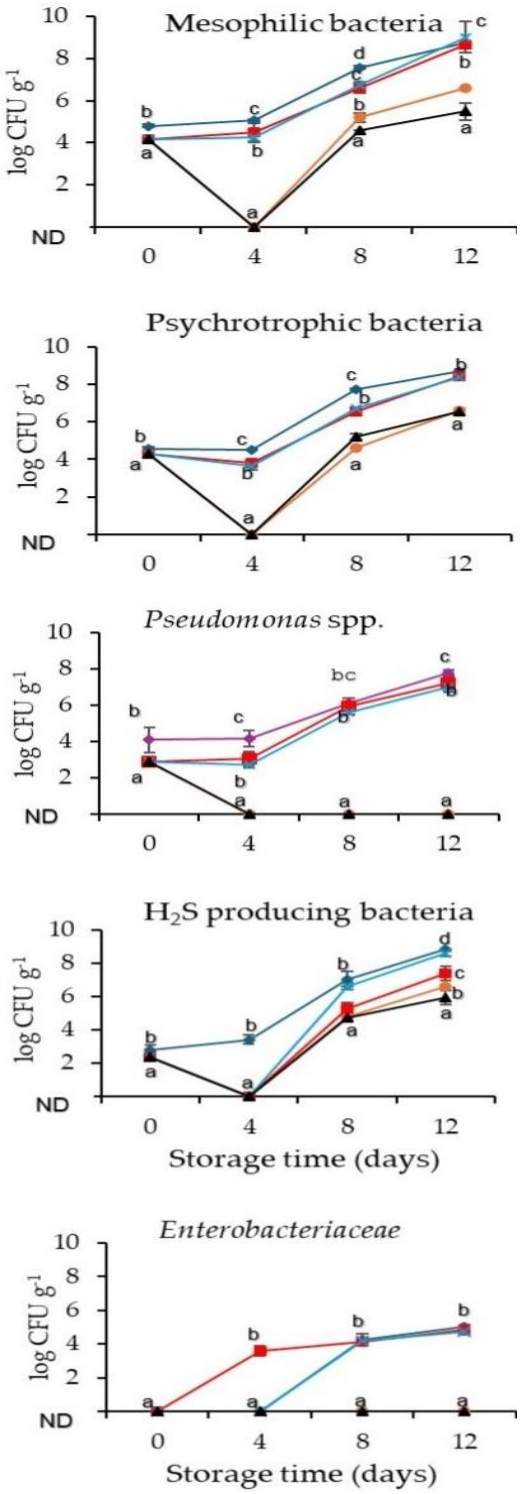

Figure 3. Total counts of microorganisms in fresh medallions stored for 12 days at 4 °C; Control medallions without disinfection (C-♦); Medallions disinfected plus LAE (DL-■); Medallions disinfected and covered with fish gelatin with LAE (DLG-x); Disinfected medallions plus LAE with MAP (DLM-●); Medallions disinfected and coated with fish gelatin and MAP (DLGM-▲) Different letters (a, b, c) indicate significant (P<0.05) differences between treatments.

and bovine gelatin with and without LAE. The authors reported that the growth of mesophilic bacteria did not exceed 6 log CFU g^{-1} during storage after using edible coatings together with LAE which was a similar effect to that obtained in this research.

The psychotropic bacteria on day zero for the control group was 4.5 log CFU g^{-1}, significantly higher (P<0.05) than the rest of the treatment (4.3 log CFU g^{-1}) due to the applied disinfection. After 4 days of storage, a significant delay (P<0.05) in growth was observed (<1 log CFU g^{-1}) in the samples treated with MAP (DLGM and DLM) in relation to the rest of the treatments (>3 log CFU g^{-1}). However, a considerable increase was observed after 8 days of storage. Significant differences were observed between the values (P<0.05): 4 log CFU g^{-1} for DLM treated samples and 5 log CFU g^{-1} for the DLGM treated ones. The other treatments presented significantly high values (>6 log CFU g^{-1}). This delay in the growth of psychrotrophic bacteria for the DL and DLG treatments (>6 log CFU g^{-1}) was maintained through the 12 days of storage. It was significantly different than the rest of the groups (C, DL, DLG) that had a count of approximately >8 log CFU g^{-1}.

The effectiveness of LAE's presence on the evolution of the psychrotrophic bacteria also seems to be linked to the presence of MAP, as in the case of the mesophile bacterial group. If compared with previous results (Fig. 1), MAP does not have such an effect on these microorganisms. Therefore, the result of the presence of LAE is what illustrates the synergy between LAE and MAP. The effect of LAE with MAP was evident at 4 days storage with the level of bacterial proliferation below 1 log CFU g^{-1}. The effect of the antimicrobial coatings alone was not sufficient since the results were not significantly (P>0.05) different from the control. The action of LAE alone and LAE integrated into FG produced an effect against bacterial growth until 8 days of storage. However, by 12 days the count levels were close to those of the control. Higueras et al. [16] who applied an edible coating based on chitosan with 5% LAE on chicken obtained similar results.

In the disinfected samples, *Pseudomonas* growth at time zero was 2.9 log CFU g^{-1}, which was significantly lower (P<0.05) than the control (4.1 log CFU g^{-1}) due to the disinfection. After 4 days and until the end of storage, the samples treated with MAP (DLGM and

https://doi.org/10.58985/jafsb.2024.v02i02.41

DLM) had non-detectable levels (<1 log CFU g^{-1}) of microbial counts. On the other hand, a significantly lower level of microbial counts was observed for DLG and DL (<3 log CFU g^{-1}) in contrast to the control (>4 log CFU g^{-1}). This could be attributed to the LAE treatment alone and the antimicrobial coating. However, after 8 days of storage, the values were >5 log CFU g^{-1}. This delay was also significant in relation to the control group until 12 days of storage.

Therefore, the results indicate the presence of MAP contributed to the growth retardation of *Pseudomonas* and that the effect of LAE alone and the FG with LAE was limited. Becerril et al. [17] evaluated the in vitro antimicrobial activity of LAE by microdilution in broth. They demonstrated the limited effect of LAE on *Pseudomonas*; since the cells of this bacterium (Gram-negative) have a cell wall based on lipopolysaccharides that makes them resistant to antimicrobial agents such as LAE.

The growth of H_2S producing bacteria at day zero for the control group (>2.7 log CFU g^{-1}) had significantly higher counts ($P<0.05$) compared to the rest of the samples (2.4 log CFU g^{-1}) due to the disinfection. After 4 days of storage, the control samples showed bacterial counts of 3.4 log CFU g^{-1} which were significantly higher ($P<0.05$) than the rest of the samples (<1 log CFU g^{-1}). After 8 days of storage, the bacterial counts in the DL, DLGM and DLM treatments were significantly lower (<6 log CFU g^{-1}) than the control and DLG treatments (>6 log CFU g^{-1}). This significantly lower level of H_2S producing bacterial growth was exhibited even after 12 days of storage.

The undetectable level of H_2S producing bacteria was possibly due to the antimicrobial effect of LAE whose cationic surfactant character can affect the viability of the bacteria. As the days of storage passed the antimicrobial effect decreased in the medallions with LAE alone. However, bacterial growth was more intensely inhibited when fish samples were coated with LAE incorporated FG and packaged with MAP. This H_2S producing bacteria growth retardation was possibly due to the controlled migration of the antimicrobial agent through the coating combined with the effect of MAP. Higueras et al. [16] used edible chitosan coatings with 5% LAE on fresh chicken and reported H_2S counts below 4 log CFU g^{-1} H_2S until day 6 of storage, coincident with the results obtained in this research.

The growth of *Enterobacteriaceae* at zero storage time did not exceed the detection threshold for any treatments. However, after 4 days of storage, the DL treatment (3 log CFU g^{-1}) was significantly higher ($P<0.05$) than the rest of the treatments (<1 log CFU g^{-1}). However, after 8 days of storage, the bacterial population of the control treatment (>5 log CFU g^{-1}) and DLG (>4 log CFU g^{-1}) had increased, while the rest of the treatments that were packaged with modified atmosphere did not exceed the detection threshold (<1 log CFU g^{-1}). Treatments with MAP presented significantly lower results ($P<0.05$) than treatments without MAP. This pattern was observed after 12 days of storage.

An inhibition synergy between LAE and MAP was evident by the lack of growth of *Enterobacteriaceae* microorganisms (<1 log CFU g^{-1}). The LAE by itself or when incorporated into FG seems to have had a limited effect on the studied microorganisms since these treatments reached values of microbial counts similar to the control. Pattanayaiying et al. [18] used nisin and LAE in pullulan coatings on fresh chicken meat. These authors reported a delay in the growth of *Enterobacteriaceae* due to the effect of LAE. After 12 days of storage, they reported that the counts of *Enterobacteriaceae* did not exceed 3 log CFU g^{-1}, as in this research. The initial microbial load of the chicken was higher than that of the hake due to the fact that they did not use NaOCl as an initial surface treatment. Multiple authors have reported that LAE has a powerful bactericidal effect on the microorganisms evaluated. In another study, Haghighi et al. [19] reported growth inhibition of *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium* and *Campylobacter jejuni* by chitosan and gelatin-based edible films with LAE in antimicrobial trials.

Finally, no growth of LAB was detected for any of the treatments during the 12-day storage period (<1 log CFU g-1). These results are consistent with those obtained by Otero-Tuárez et al. [3]; wherein LAB did not grow during storage due to the sanitization with NaOCl and the application of good handling and hygiene practices.

https://doi.org/10.58985/jafsb.2024.v02i02.41

4. Conclusions

Sanitization significantly reduced the initial microbial load in fresh fish but not enough to extend its shelf life. The use of MAP significantly reduced the bacterial counts, reaching undetectable levels for *Pseudomonas* and *Enterobacteriaceae* during the storage time. It did; however, increase the exudates which negatively affected the appearance of the fresh fish inside the packages. On the other hand, the effect of LAE alone delayed bacterial growth during the first days of storage. However, at the end of storage microbial growth was considerably high. Notwithstanding, the addition of LAE to an edible FG-based coating significantly slowed the growth of H_2S producing bacteria and mesophilic bacteria; thus, improving the microbiological quality of fresh fish. The combined application of several preservation technologies such as sanitization, the incorporation of LAE into an edible FG-based coating and packaging with MAP reduced the microbial load of hake medallions significantly due to the observed synergistic effect between the technologies mentioned. Good handling practices and conservation at 4 °C are critical factors in maintaining the microbial quality of fresh fish and are the baseline for conservation.

Authors' contributions

Performed the experiments, V.B.; Wrote and revised the manuscript, V.O.T. and J.M.; Responsible for the statistical treatment, writing and review of the manuscript, V.B. and V.O.T.; Conceived the research project, guided the students in carrying it out and coordinated the writing of the article, V.O.T. and J.M.

Acknowledgements

The authors would like to thank the Food Technology, Control and Safety Research Group (ALITEC) and the Research Institute for Innovation and Sustainable Development in the Food Chain (IS-FOOD) of the Public University of Navarra.

Funding

This work was carried out thanks to funding provided by SENESCYT, the Secretariat of Higher Education, Science, Technology and Innovation of Ecuador; the Food Technology, Control and Safety Research Group; and the Research Institute for Innovation and Sustainable Development in the Food Chain of the Public University of Navarre, Spain.

Availability of data and materials

All data will be made available on request according to the journal policy

Conflicts of interest

The authors have no conflicts of interest or competing interests to declare that are relevant to the content of this article.

References

1. Boziaris, I.S.; Parlapani, F.F. Specific Spoilage Organisms (SSOs) in Fish; Elsevier Ltd, 2017; ISBN 9780081005026.
2. Parlapani, F.F.; Mallouchos, A.; Haroutounian, S.A.; Boziaris, I.S. Microbiological spoilage and investigation of volatile profile during storage of sea bream fillets under various conditions. Int. J. Food Microbiol. 2014, 189, 153–163. https://doi.org/10.1016/j.ijfoodmicro.2014.08.006.
3. Otero-Tuárez, V.; Carrión-Granda, X.; Fernández-Pan, I.; Fernández, T.; Maté, J.I. Combined effect of antimicrobial edible coatings with reduction of initial microbial load on the shelf-life of fresh Hake (*Merluccius merluccius*) Medallions. Int J Food Sci Technol 2019, 54, 835–843, https://doi.org/10.1111/ijfs.14003.
4. Parlapani, F.F.; Kormas, K.A.; Boziaris, I.S. Microbiological changes, shelf life and identification of initial and spoilage microbiota of sea bream fillets stored under various conditions using 16S RRNA gene analysis. J. Sci. Food Agric. 2015, 95, 2386–2394. https://doi.org/10.1002/jsfa.6957.
5. Carrión-Granda, X.; Fernández-Pan, I.; Rovira, J.; Maté, J.I. Effect of Antimicrobial Edible Coatings and Modified Atmosphere Packaging on the Microbiological Quality of Cold Stored Hake (Merluccius Merluccius) Fillets. J Food Qual. 2018, 1–12. https://doi.org/10.1155/2018/6194906.
6. Boziaris, I.S.; Parlapani, F.F. Chapter 3 - Specific Spoilage Organisms (SSOs) in Fish. In The Microbiological Quality of Food; Bevilacqua, A., Corbo, M.R., Sinigaglia, M., Eds.; Woodhead Publishing Series in Food Science, Technology and Nutrition; Woodhead Publishing, 2017, pp 61–98 ISBN 978-0-08-100502-6.
7. Otero-Tuárez, V.; Fernández-Pan, I.; Ignacio Maté, J. Effect of the Presence of Ethyl Lauroyl Arginate on the Technological Properties of Edible Fish Gelatin Films.

https://doi.org/10.58985/jafsb.2024.v02i02.41

Int. J. Food Sci. Technol. 2020, 55, 2113–2121. https://doi.org/10.1111/ijfs.14454.

8. Rodríguez, E.; Seguer, J.; Rocabayera, X.; Manresa, A. Cellular effects of monohydrochloride of L-arginine, Nα- Lauroyl Ethylester (LAE) on exposure to *Salmonella typhimurium* and *Staphylococcus aureus.* J. Appl. Microbiol. 2004, 96, 903–912. https://doi.org/10.1111/ j.1365-2672.2004.02 207.x.
9. Nerin, C.; Becerril, R.; Manso, S.; Silva, F. Ethyl Lauroyl Arginate *(LAE):* Antimicrobial Activity and Applications in Food Systems; Elsevier Inc., 2016; ISBN 9780128007235.
10. EU Commission Regulation (EU) No 506/2014 of 15 May 2014; amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards Ethyl lauroyl arginate as a preservative in certain heat-treated meat products.
11. EC Commmission Regulation No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs. J. Eur. Union 2005, L322, 1–19, https://doi.org/10.1016/B978-0-12-385007-2.00012-7.
12. Kykkidou, S.; Giatrakou, V.; Papavergou, A.; Kontominas, M.G.; Savvaidis, I.N. Effect of thyme essential oil and packaging treatments on fresh mediterranean swordfish fillets during storage at 4 °C. Food Chem. 2009, 115, 169–175. https://doi.org/10.1016/j.foodchem.2008.11.083.
13. Silbande, A.; Adenet, S.; Smith-Ravin, J.; Joffraud, J.J.; Rochefort, K.; Leroi, F. Quality assessment of ice-stored tropical yellowfin tuna (*Thunnus albacares*) and influence of vacuum and modified atmosphere packaging. *Food Microbiol.* 2016, 60, 62–72. https://doi.org/10.1016/j.fm.2016.06.016.
14. Garrido, M.D.; Hernández, M.D.; Espinosa, M.C.; López, M.B. Enhanced quality characteristics of refrigerated seabream (*Sparus aurata*) fillets packed under different systems (Modified atmosphere vs. vacuum). J. Aquat. Food Prod. Technol. 2016, 25, 156–168, https://doi.org/10.1080/10498850.2013.838814.
15. Moreno, O.; Cárdenas, J.; Atarés, L.; Chiralt, A. Influence of starch oxidation on the functionality of starch-gelatin based active films. Carbohydr Polym 2017, 178, 147–158, https://doi.org/10.1016/J.CARBPOL. 2017.08.128.
16. Higueras, L.; López-Carballo, G.; Hernández-Muñoz, P.; Gavara, R.; Rollini, M. Development of a Novel Antimicrobial Film Based on Chitosan with LAE (Ethyl-Nα-Dodecanoyl-l-Arginate) and Its Application to Fresh Chicken. Int. J. Food Microbiol. 2013, 165, 339–345, https://doi.org/10.1016/j.ijfoodmicro.2013.06.003.
17. Becerril, R.; Manso, S.; Nerin, C.; Gómez-Lus, R. Antimicrobial activity of lauroyl arginate ethyl (LAE), against selected food-borne bacteria. Food Control 2013, 32, 404–408. https://doi.org/10.1016/j.foodcont.2013. 01.003.
18. Pattanayaiying, R.; H-Kittikun, A.; Cutter, C.N. Optimization of Formulations for Pullulan Films Containing Lauric Arginate and Nisin Z. LWT - Food Science and Technology 2015, 63. 1110–1120, https://doi.org/10.1016/J.LWT.2015.04.016.
19. Haghighi, H.; De Leo, R.; Bedin, E.; Pfeifer, F.; Siesler, H.W.; Pulvirenti, A. Comparative analysis of blend and bilayer films based on chitosan and gelatin enriched with LAE (lauroyl arginate ethyl) with antimicrobial activity for food packaging applications. Food Pac. Shelf Life. 2019, 19, 31–39. https://doi.org/10.1016/ j.fpsl.2018.11.015.

https://doi.org/10.58985/jafsb.2024.v02i02.41