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Effect of the presence of ethyl lauroyl arginate on the technological properties of edible fish gelatin films

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Sumary

Physical, chemical and antimicrobial properties of fish gelatin films with different concentrations of ethyl lauroyl arginate (LAE) were studied. Optical properties of film-forming solution did not vary with increased LAE content. However, pH and surface tension increased. The incorporation of LAE into the formulation increased moisture and solubility of the films. In addition, the presence of LAE affected mechanical properties, making films stronger and more flexible; it had no effect on water vapor permeability. Finally, films with LAE significantly increased antimicrobial properties against *Listeria innocua*, *Shewanella putrefaciens and Pseudomonas fluorescens*, *but not against Aeromonas hydrophila.* These antimicrobial films could be a used as an alternative technology for extending shelf-life of fresh products.

Keywords: fish gelatin films; ethyl lauroyl arginate; physical, chemical and antimicrobial properties

Introduction

Reduction of bacterial growth which can cause fresh fish deterioration is of great importance to abate possible affects on consumer health. Thus the development of antimicrobial films as a tool to improve the microbiological quality of food is currently receiving great efforts as they act against bacterial growth. Proteins have been used as the basis of edible films and coatings (EFC)

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formulations from both plant-based proteins and animal-based proteins (Otero-Tuárez *et al.*, 2019).

Biopolymers could be used as carriers of a wide variety of additives (including antimicrobial agents), while possibly acting as barriers against moisture, water vapor, gases, and solutes. The properties of antimicrobial films are related to their capability to control the release of active compounds which slows food spoilage. Antimicrobial EFC are a means of controlling the diffusion of the antimicrobial compound to the food surface (Dehghani *et al.*, 2018). The scientific literature describes several categories of active compounds that can potentially be incorporated into EFC, such as essential oils, probiotic bacteria, bacteriocins, organic acids, polypeptides and fatty acid esters (Dehghani *et al.*, 2018). LAE is an ester recently approved in Europe (EU, 2014) it is developed from arginine and lauric acid. LAE is an amphiphilic antimicrobial that has been shown to have very high antimicrobial activity against foodborne bacteria such as *Staphylococcus aureus*, *L. innocua, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella enterica* (Moreno *et al.*, 2018). Several studies have been conducted to evaluate the antimicrobial efficacy of LAE, either directly, through a food package or through an EFC. Consequently, Moreno *et al.* (2017) developed active starch-based films and bovine-derived gelatin with LAE as an antimicrobial agent (1.3 g LAE/100 g polymer). These films inhibited the growth of *Listeria*. Subsequently, they were applied to marinated salmon samples, reducing viable total counts in them for up to 45 days in storage at 5 ºC.

Fish gelatin (FG) is an alternative to gelatin from bovine and porcine origin because it is not associated with the risk of bovine spongiform encephalopathy outbreaks. In addition, it has properties that characterize it such as gel resistance, viscosity, gelification and melting points (Chuaynukul *et al.*, 2018). Being that FG is a thermoreversible hydrocolloid with good barrier properties it is a suitable matrix to be used as an edible coating on foods. Since LAE dissolves easily in water at temperatures above 20 ºC (Nerin *et al.*, 2016) the FG in its aqueous phase can be easily mixed with LAE at temperatures higher than 40 ºC. Due to their strong gelling capacity, these antimicrobial films can be used as active packaging for fresh fish, thus improving the quality of fresh fish products.

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The objective of this research was to study compatibility and characterize films based on FG with different concentrations of LAE; including their antimicrobial properties. The specific objectives were: i) to evaluate the physical-chemical properties of the film-forming solution; ii) to determine the technological and mechanical properties of the resulting films; and iii) to evaluate the antimicrobial capacity of the active films against four bacterial strains associated with the deterioration and pathogenicity of fresh fish.

Materials and methods

Materials

For the elaboration of films with and without LAE, FG was provided by LAPI Gelatin (Empoli, Italy), and the glycerol was supplied by Sigma (Barcelona, Spain). The LAE (85% purity) marketed as Mirenat-P/100 was supplied by Vedeqsa Grupo LAMIRSA (Barcelona, Spain). The strains described in table 1 were used to evaluate antibacterial activity, which were provided by the Spanish Type Culture Collection (CECT) of the University of Valencia. Bacteria were cultured according to CECT recommendations.

Protocol for the preparation of films with and without LAE

100 mL of film-forming solution (FFS) were prepared for each treatment. For FFS without LAE (control) a 10% (w/w) mixture of FG was prepared and 3% (w/w) glycerol was added as a plasticizer. The mixture was then dissolved in distilled water with constant agitation for 30 minutes at 70 °C. For the preparation of FFS with LAE, the following amounts were added to individual mixtures of the control formulation: 0.5%, 1%, 5% and 10% (w/w). The indicated concentrations were calculated considering the purity of 85% of Mirenat-P/100 LAE (g of LAE/100 g of dry gelatin).

To make films with and without LAE the FFS were poured into 14 cm diameter glass plates. Twenty grams of FFS without LAE were poured into the glass plates. On the other hand, for the FFS with LAE lower weights were established in order to integrate 2 g of LAE in each film while obtaining films with homogeneous thicknesses with and without LAE. The films were dried

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in a climatic chamber (Weis Technick, Germany) at 27 °C and 75% RH for 15 hours. Finally, the films were conditioned in a desiccator with a saturated solution of sodium bromide (57% RH) for 24 hours at room temperature (22 °C ± 2 °C).

Conditioning of FFS

Due to the fact that gelatin is thermoreversible and solid at room temperature all the FFS were conditioned at 50 ºC for analysis.

pH

The pH was measured 5 times for each FFS with a high precision pH meter (GLP 22 Crison model, Barcelona, Spain).

Turbidity

The turbidity of the FFS was measured in triplicate for each treatment. The measurements were made with a nephelometer, model Turbiquant 300 IR - 3000 T (Merck KGaA, Darmstadt, Germany). The calibration of the equipment was carried out with a formazin standard calibration set (Turbiquant 1000 IR) with: 0.02, 10, 100 and 1000 nephelometric turbidity units (NTU).

Surface tension

The surface tension of all FFS was measured five times for each of the treatments, using the Du Nouy ring method with the aid of a digital tensiometer (K9 Krüss, Germany). The tensiometer is equipped with a platinum ring which was immersed in the FFS and then raised. The maximum force required to move the ring through the interface corresponded to the surface tension expressed in mN m^{-1}. Previously, distilled water was used to check the calibration of the equipment; which was approximately 70 mN m^{-1} at room temperature.

Thickness of films

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The thickness was determined with a digital micrometer (model ID-F125, Mitutoyo Corp., Tokyo, Japan) with an accuracy of 1 μm, at 10 different points on each film. The thickness was measured after balancing the films at 57% RH prior to the characterization of their technological properties.

Color

Color was measured in triplicate on each film using a Minolta Croma Meter CM-2500d colorimeter (Konica Minolta, Valencia, Spain). The colorimeter had been previously calibrated on the surface of a standard white plate. The values of the calibration plate were: L* = 99.34; a* = -0.07; and b* = -0.11. Samples of films with and without LAE were placed on a white surface for analysis. The color parameters L*, a* and b* were represented using the CIELAB color space, with the following scale: L* = 0 (dark) to L* = 100 (light); -a* = -100 (green) to +a* = +100 (red), -b* = -100 (blue) to +b* = +100 (yellow). The color difference (ΔE*) of films compared to films without LAE was calculated using the following formula:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where: $\Delta L^* = \Delta L^*_{\text{with LAE}} - \Delta L^*_{\text{without LAE}}$; $\Delta a^* = \Delta a^*_{\text{with LAE}} - \Delta a^*_{\text{without LAE}}$; $\Delta b^* = \Delta b^*_{\text{with LAE}} - \Delta b^*_{\text{without LAE}}$

Transparency

The transparency of films with and without LAE was determined by measuring absorbance at a wavelength of 600 nm, using a double beam UV-visible spectrophotometer (model UH5300, Hitachi, Tokyo, Japan). The transparency of the films was calculated using the equation:

$$T = Abs_{600}/x$$

Where: Abs_{600} is the absorbance value at 600 nm and x is the thickness of the films (150 ± 22 μm).

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Moisture content

Moisture content was determined by the weight loss of a film with and without LAE after drying according to the protocol previously published by Rubilar *et al.* (2013). The films were balanced for three days at 57% and 75% RH. Subsequently, the samples were dried in a convection oven at 105 ºC for 24 hours and were weighed again after cooling the samples in a chamber at 0% RH to avoid rehydration due to ambient humidity. Five repetitions were carried out. The moisture content was expressed as a percentage with respect to the initial weight, employing the following equation:

$$Moisture\ (\%) = \frac{W_o - W_f}{W_o} x100$$

Solubility in water

The solubility of films with and without LAE was determined according to the method performed by Rubilar *et al.* (2013) expressed as the solubilized dry matter content after 24 hours in water with agitation. Five repetitions were performed. First, the initial matter content was determined by drying the samples at 105 ºC to constant weight (M_i). The films were then weighed and immersed in 50 mL of distilled water. Finally, after agitation for 24 hours the undissolved sample was extracted and dried again at 105 ºC to constant weight (M_f) to determine the weight of the dry matter that did not solubilize in the water. The solubility of the films was calculated using the following equation:

$$Solubility\ in\ water\ (\%) = \frac{M_i - M_f}{M_i} x100$$

Mechanical properties

For the analysis of the mechanical properties, film strips 25.4 mm wide by 8 mm long were cut with the aid of a double shear. Ten strips were prepared for each formulation; all were balanced at 57% and 75% RH for 3 days prior to analysis. The mechanical properties of the strips were determined according to ASTM D882 (ASTM, 2000) by a tensile test using the texturometer, Texture Analyzer TA-XTZi (Surrey, Great Britain). The film strips were placed between two tweezers and the tensile test was carried out. The following parameters were employed: initial distance between tweezers 5 cm, and test speed 0.8 mm s^{-1}. The Texture Export program was

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used to analyze the results and illustrate them on a graph; Force vs Distance, tensile strength (N mm^2) and elongation at the breaking point (%).

Water vapor permeability (WVP)

For the calculation of WVP the corrected ASTM E96-92 (ASTM, 2000) method was used. This gravimetric procedure consists of sealing the film to be evaluated in a methacrylate cell containing 6 mL of distilled water. After sealing, the cells were placed in a desiccant booth previously balanced at 0% RH. Permeability was measured using a humidity gradient at 100% RH and room temperature (25 ºC). The test began by measuring the weight of the cell with water and film at time zero. The methacrylate cells were thereafter placed in the desiccant chamber (Sanplatec Corp. Japan) equipped with a fan (Elco, Milano, Italy), producing an air velocity of 2.5 m s^{-1}, thus ensuring a homogeneous RH throughout the chamber. A total of five cells were tested for each type of film processed. The WVP was calculated by multiplying the steady-state water vapor transmission rate by the sample thickness and dividing by the difference in water vapor partial pressure across the sample (McHugh ***et al.***, 1993).

$$WVP = \frac{WVTR * thickness}{[PA1 - PA2]}$$

Where: WVTR = water vapor transfer rate. PA1 = pressure on the film exerted by in a methacrylate cell. PA2 = pressure to which the film is subjected inside in a methacrylate cell.

Antimicrobial properties of films

The culture conditions of the selected bacteria are shown in Table 1. The gelatin discs melt, lose their disk shape and spread over the whole surface of the agar at temperatures higher than 25 ºC, thus all bacterial strains were incubated at 22 ± 2 ºC for 48 hours. All tests were performed in triplicate. The disc diffusion method was used to evaluate the antibacterial activity of FG-based films with and without LAE for four strains of bacteria: *L. innocua, A. hydrophila, P. fluorescens* and *S. putrefaciens*. Discs 17 mm in diameter were cut from each film formulation using a hallow hole corer and placed on agar plates inoculated with a bacterial concentration of 10^7 to 10^8 CFU mL^{-1} of each strain. The diameters of the inhibition zone were measured using a caliber and expressed as inhibition areas in mm^2 excluding the disc diameter.

Statistical analysis

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Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL., USA). Significant differences between different LAE concentrations were determined using ANOVA and Duncan's multiple range post hoc tests (95% confidence level).

Results and discussion

Physical Characteristics of FFS

The addition of LAE (pH 3) into the FFS led to acidification. Thus, the pH was reduced from 5.4 in FFS without LAE to 5.17 in FFS with 10% LAE. These values were within the range indicated by Nerin *et al.* (2016) who stated that LAE causes acidification of the aqueous solution. It is important to note that LAE is stable between pH 3 and 7 maintaining its antimicrobial activity within this range (Higueras *et al.*, 2013).

In addition, all the FFS had a very transparent appearance and obtained a value of zero NTU. There was a very slight tenuous yellow coloration that increased with the antimicrobial agent concentration. The presence of LAE significantly decreased ($p < 0.05$) the surface tension of FFS from 48.7 ± 1 mN m^{-1} (control) to 26.6 ± 1 mN m^{-1} (10% LAE) improving wettability. No significant differences ($p > 0.05$) were observed between FFS with distinct LAE concentrations. The gelatin molecules were probably saturated with LAE affecting the surface tension of FFS. This is consistent with Bai et al. (2018) who reported the surface tension of the aqueous solution to gradually decrease with the LAE concentration (0.001% to 0.6%, w/w) due to its cationic surfactant character.

Physical characteristics of films

The transparency levels of films with and without LAE were quantified by absorbance at 600 nm. For all cases the absorbance readings were less than 1 unit. These values illustrate how all the films, with and without LAE, were very transparent. These results were similar to those reported by Hosseini *et al.* (2013) who evaluated the functional properties of FG films with chitosan and concluded that these were very transparent. In the present research, the application of the LAE did not affect the optical properties of the FG based films. These transparent films, in addition to providing good mechanical and barrier properties, allow the consumer to observe important food quality characteristics such as color and appearance.

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The thickness of the films with and without LAE was homogeneous for all treatments (150 ± 2 μm). The values (ΔE^*) were < 3 units, thus the difference in color of the films with LAE in relation to films without LAE was not visible to the human eye (Mokrzycki & Tatol, 2011). On the other hand, high L* values were observed in all films. The highest values were found in films with 5 and 10% LAE (94.2 ± 0.2 and 94.35 ± 0.2, respectively) which were significantly different (p < 0.05) relative to films without LAE. L* values close to 100 indicate very clear and glossy films. No trend was observed for either a* or b* values. Films with 1% LAE expressed the highest b* value (4.7 ± 0.3) which was significantly different (p < 0.05) from the other films. These b* values expressed a slight yellow tonality in all films. In contrast, results presented by Moreno et al. (2017a), who studied starch films mixed with bovine gelatin and LAE, highlight how the incorporation of LAE had a slight effect on the color of the films, reducing luminosity (L* = 73.4) and tone. These authors concluded that these changes were due to the progression of browning reactions between the carbonyl groups and the amino groups of gelatins and LAE, thus giving brownish products, probably due to Maillard's reaction.

Moisture content

The moisture levels of films conditioned at 75% RH were significantly higher (p < 0.05) than those values observed in films conditioned at 57% RH. At this RH moisture content of the films without LAE was 17.8%. This percentage was not significantly different (p > 0.05) from that of films containing 0.5%, 1% and 5% LAE. However, the moisture content of films with 10% LAE (20.9%) was significantly different (p < 0.05) from that of the other films with lower concentrations. A similar pattern was reported in films conditioned to 75 % RH. Although there were no significant differences (p > 0.05) between the moisture content levels of the films, an increasing trend was observed as the LAE concentration increased. Ma *et al.* (2016) reported the moisture content of chitosan films to increase significantly with increasing LAE concentration. In addition, Jiang *et al.* (2010) found the moisture content of fish gelatin-based films to be provided by water molecules in the biopolymer structure. In this sense, the increase in the moisture content of the FG films with LAE could be explained by the interaction of the gelatin molecules that are of hydrophilic character with those of LAE.

Solubility in water

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The solubility levels of the films with 0%, 0.5% and 1% LAE were within the range of 28% to 30%; there were no significant differences ($p > 0.05$) among them. However, films with 5% or 10% LAE were found to have significantly higher solubility values compared to the other films with lower LAE concentrations. This was possibly caused by the hydrophilic character of the LAE and its interaction with the FG molecules as stated before.

Rubilar *et al.* (2016) reported LAE to have high solubility in water due to its polar properties and low water-oil equilibrium partition coefficient (Kow < 0.1); hence it tends to concentrate in the aqueous phase of foods. The high solubility of LAE in water could be the main factor explaining the increase in solubility of antimicrobial films with concentrations of 5% and 10% LAE. Furthermore, the homogeneous dispersion of the LAE in the coating matrix, together with its high solubility would allow an efficient migration of the antimicrobial agent on the coated food surface.

Water vapor Permeability (WVP)

The WVP values range from 4.7 to 5.4 g μm kPa^{-1} h^{-1} m^{-2}. There were no significant differences ($p > 0.05$) among films. However, a trend towards an increase in WVP was observed as the concentration of LAE increased. According to Etxabide *et al.* (2015) WVP in a film involves a two-step process that includes initial absorption and subsequent diffusion of the film. The increase in WVP values could be explained by changes in protein structure that would favor the diffusion stage. It is likely that the non-polar part of the LAE would provide the gelatin with improved water vapor barrier properties.

In addition, Etxabide *et al*. (2016) reported WVP values increase with increasing pH, probably due to a greater degree of denaturation of proteins and, therefore, to a greater deployment that would facilitate the permeation of water vapor molecules. This is due to the fact that with an acid pH the WVP is lower. The results of this research are consistent with the literature as the LAE significantly affected the pH of the films, which could have slightly influenced the WVP rate.

Mechanical properties of films

The tensile strength (TS) of the films conditioned at 57% RH with 1% LAE (27.7 N mm^{-2}), with 5% LAE (30.6 N mm^{-2}) and with 10% LAE (31.8 N mm^{-2}) had significantly higher values ($p < 0.05$)

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than films containing 0.5% or 0% LAE. A similar pattern was observed in the TS of films conditioned at 75% RH (Fig. 1). The increase was progressive as the concentration of LAE increased, with the higher TS observed in films with LAE at 1%, 5% 10% and significantly different ($p < 0.05$) than films without LAE. The TS of all films conditioned at 57% RH were significantly higher ($p < 0.05$) than of those films conditioned at 75% RH. According to these results, LAE increased film TS. The possible cause was the interaction between the FG molecules and the LAE. As discussed above, LAE is a cationic surfactant that can interact with anionic biopolymers through electrostatic interactions; producing an antiplasticising effect that would lead to increased TS.

FG is a negatively charged anionic biopolymer (Hosseini *et al*., 2013) with an isoelectric point between pH 6 and 9 (Gómez-Estaca *et al*., 2011). Therefore, it may experience associative interactions to form a soluble polyelectrolyte complex. As Staroszczyk *et al.* (2014) indicated in the formation of antimicrobial films, it is important to characterize the compatibility of their components and the intermolecular interactions that may occur between the matrix and the antimicrobial agent, as they ultimately affect the structure and determine the properties of the film. Rubilar *et a*l. (2016) demonstrated a significant increase in TS for chitosan films with LAE (at high and low concentrations), however no studies were found to explain this phenomenon. They indicated how LAE could produce some antiplasticising effect in the film which could be the reason for the increase in TS. Despite the increase in humidity that would produce a plasticizing effect in the EFC, the addition of the LAE produced the opposite effect, an increase in the TS which was consistent with the results herein presented.

The addition of the antimicrobial compounds also influenced the gelatin films´ elongation at the breaking point (EB) (Fig. 2). In films conditioned at 57% RH, a gradual increase in EB was observed as the LAE concentration increased, with significantly higher values ($p < 0.05$) with 1% LAE (61.1%), 5% LAE (62.5%) and 10% LAE (60.8%) in contrast to films without LAE (53.5%). In the films conditioned at 75% RH, a similar pattern was observed, yet no significant differences were found ($p > 0.05$). However, a tendency to increase the percentage of EB was observed. No significant differences ($p > 0.05$) were established between the films conditioned at 57% and 75% for any of the concentrations.

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According to these results, the plasticising effect of LAE was limited because there were no important differences between the EB of gelatin films with distinct LAE concentrations at both RH levels. It is very likely that saturation would have been reached in the plasticising potential. Thus it is plausible that the maximum EB of gelatin films is around 60%.

The scientific literature reports variable ranges of EB in FG films. This variability is dependent on the extraction conditions to which the collagen has been subjected for the gelatin extraction. Le *et al.* (2015) investigated the optimal conditions for extracting gelatin and preparing FG films. They evaluated temperatures (70 ºC, 80 ºC and 90 ºC) and extraction times (15 minutes to 3 hours). At a temperature of 70 ºC they obtained an EB in the gelatin films of 46.1 ± 7.4%. On the other hand, Jiang *et al.* (2010a) reported EB values of 68% in FG films obtained by thermal extraction (55 ºC for 180 minutes). Huang *et al.*, (2019) indicated the FG extraction process to influence the length of polypeptide chains and the functional properties of gelatin. These final properties depend on processing parameters such as temperature, time and pH.

The results illustrate how the mechanical properties of FG-based films depend on the molecular interactions of LAE with the film matrix. As has been discussed recently, with LAE concentration increases saturation of the plasticizer is likely to occur, affecting the flexibility, brittleness, and stretchability of films.

Antimicrobial capacity of films

The antimicrobial activity of films with and without LAE against the bacteria tested is shown in Fig. 3. FG films without LAE served as a control to determine the possible intrinsic antibacterial effect. As expected, no area of inhibition was observed against the four bacteria tested for gelatin films without LAE. Therefore, the differences in the area of inhibition observed for films containing LAE could only be attributed to the effect that this antimicrobial agent has on each strain when diffused into inoculated media. Antimicrobial effect of gelatin films with different concentrations of LAE was observed against *L. innocua, P. fluorescens* and *S. putrefaciens* but not against *A. hydrophila*. The antibacterial activity of gelatin films containing 10% LAE was significantly higher ($p < 0.05$) than that observed by films with lower concentrations of LAE. However, the antibacterial activity of films was effective even at a concentration of 0.5% LAE.

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No antibacterial activity against *A. hydrophila* was detected at any concentration of LAE. No positive *in vitro* test against *A. hydrophila* has been reported. However, there are studies that show these bacteria to be very resistant to antibacterial agents. Albertos *et al.* (2015) reported *A. hydrophila* to be the most resistant to the antimicrobial effect of films based on chitosan with essential oils. In addition, Rodríguez *et al.* (2004) reported the effect of LAE to depend on the structure of bacterial cells. They concluded, that although LAE can cause alterations in the potential of the membrane, they observed viable cells at a microscopic level. It is likely that LAE did not affect the cellular viability of *A. hydrophila*, and thus, did not hinder its growth. Another possibility is that there was some alteration of the lipopolysaccharides of the external membrane that prevented their destabilization and later lysis against the cationic surfactant compounds. Another alternative hypotheses is that *A. hydrophila* was able to block the antibacterial compound by means of a specific inhibitor; even though the latter has not been determined (Hancock, 2002; Armas *et al.*, 2019).

As shown, the antimicrobial activity was more effective against *L. innocua* than against *S. putrefaciens* and *P. fluorescens*, gradually increasing from at 0.5% to 10% LAE. According to these results, Gram-positive bacteria is more sensitive than Gram-negative bacteria. This could be due to differences in cell membrane structure. Gram-positive bacteria are known to contain an outer layer of peptidoglycan, while Gram-negative bacteria contain an external phospholipid membrane. Therefore, the distinct cellular envelope should experience distinct types of interactions with antimicrobial agents (Bhawana *et al*., 2011). This theory was later corroborated by Deng *et al*. (2018) who evaluated the antibacterial activity of nanofiber films composed of chitosan/poly ethylene oxide and LAE against two pathogenic strains: one Gram-positive (*S. aureus*) and another Gram-negative (*E. coli*). They concluded lipopolysaccharides from the cell wall of Gram-negative bacteria probably have a barrier effect against antimicrobial compounds.

Conclusions

The incorporation of LAE into fish gelatin-based films affected their technological properties. FFS had a very transparent and homogeneous appearance, as did the films made with it. The

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antimicrobial agent did not affect WVP, but it did affect the mechanical properties; the TS and EB of the films are greater, resulting in stronger and more flexible films. Finally, the presence of LAE imparts antimicrobial properties to films with a more pronounced effect against Gram-positive bacteria than against Gram-negative bacteria. Thus, the addition of LAE into FG film formation constitutes the development of a new antimicrobial coating. The new coating is potentially very useful for the fishing industry wherein it can improve the microbiological quality of fresh fishery products and extend their shelf-life without affecting their sensory characteristics. The application of antimicrobial coating on fresh fish products will require a extended sensory analysis to guarantee their acceptance among consumers.

Conflicts of interest

The authors hereby declare there is no conflict of interests.

Data Availability Statement

Research data are not shared.

Ethical Guidelines

Ethics approval was not required for this research.

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Table

Table 1. Culture conditions for selected microorganisms

Microorganisms	CECT Nº	Charac.	Culture medium	Incubation
Listeria innocua	910	Gram +	Brain Heart Infusion	22 ± 2 ºC; 48 h
Aeromonas hydrophila	839	Gram -	Tripticase Soy Broth	22 ± 2 ºC; 48 h
Pseudomonas fluorescens	378	Gram -	Nutrient Broth II	22 ± 2 ºC; 48 h
Shewanella putrefaciens	5346	Gram -	Tripticase Soy Broth	22 ± 2 ºC; 48 h

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Figure

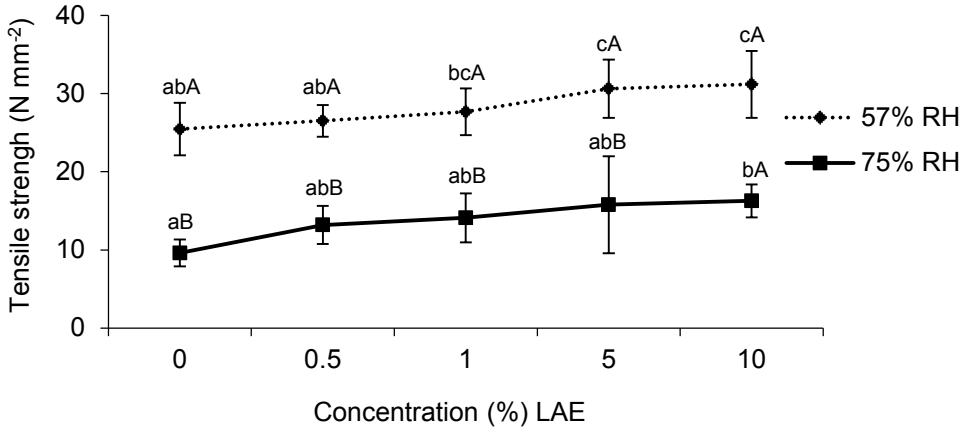

Figure 1. Tensile strength of fish gelatin-based films with and without ethyl lauroyl arginate (LAE). Different lower-case letters indicate significant differences among LAE concentrations; different upper-case letters indicate significant differences among RH conditions.

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Figure

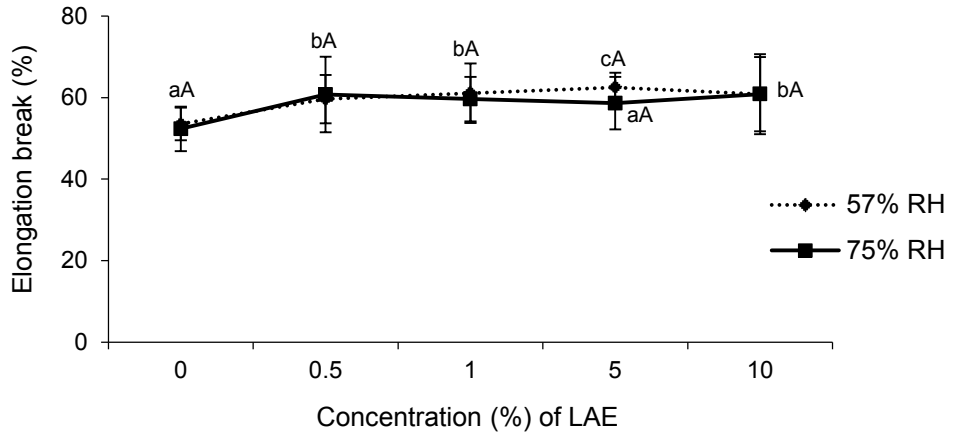

Figure 2. Elongation at the breaking point of fish gelatin-based films with and without ethyl lauroyl arginate (LAE). Different lowercase letters indicate significant differences among LAE concentrations. Different capital letters indicate significant differences among RH conditions.

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Figure

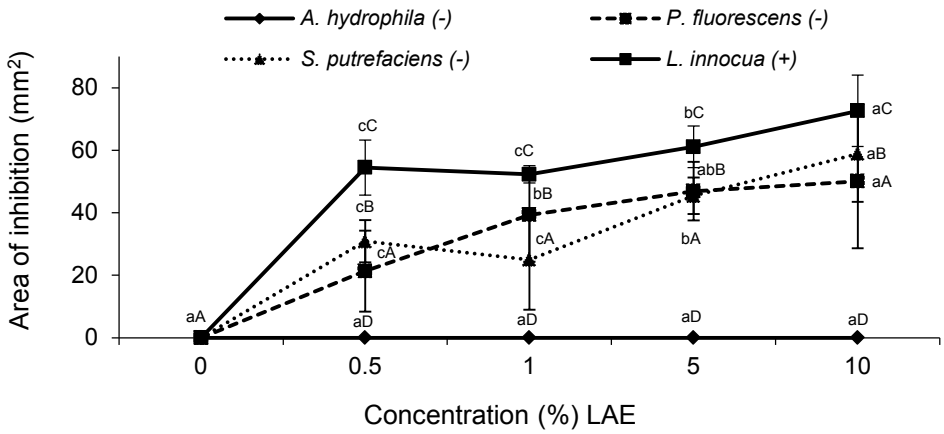

Figure 3. Antibacterial activity of fish gelatin-based films with ethyl lauroyl arginate (LAE) against *Aeromonas hydrophila, Pseudomonas fluorescens, Shewanella putrefaciens* and *Listeria innocua*. Different lower-case letters indicate significant differences among LAE concentrations. Different capital letters indicate significant differences among microorganisms.

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