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Active starch-gelatin films for shelf-life extension of marinated salmon

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Abstract

Biodegradable active films were obtained by casting, using glycerol plasticized oxidized corn starch (OS) and bovine gelatine (BG) blends (1:1 mass ratio), with and without ethyl lauroyl arginate (LAE) as antimicrobial agent (1.3 g LAE/100g polymer). Water vapour barrier capacity and colour of the films conditioned at 53 or 88 % relative humidity were determined. Both LAE incorporation and high RH promoted film browning, coherently with the progression of Maillard reactions between amino groups of gelatine or LAE and carbonyl groups of oxidized starch. These compounds imparted antimicrobial properties to the films with and without LAE, both exhibiting antilisterial activity in *in vitro* tests. Packaging of marinated salmon samples in these films greatly reduced the total viable counts, which remained below the legal limit after 45 storage days at 5ºC. Nevertheless, films were not effective at controlling weight loss of salmon samples during the cold storage.

Keywords: Biodegradable films, corn starch, bovine gelatine, LAE, marinated salmon

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1. Introduction

Active packaging protects food products against chemical contaminants, oxygen, moisture, mechanical damage or deterioration caused by spoilage microorganisms (Rhim et al., 2013; Sung et al., 2013). Although petroleum-derived synthetic plastics have been commonly used for food packaging, their serious environmental impact has driven current research towards biobased/biodegradable food packaging materials (Byun & Kim, 2014). Of the biodegradable polymers, starch is one of the most promising materials due to its ready availability, low cost and food compatibility. Nonetheless, starch films have some drawbacks, mainly associated with their highly hydrophilic nature (Du et al., 2008; Ortega-Toro et al., 2014).

Different strategies have been used to improve properties of starch films, such as blending with other polymers o chemical modification (Masina et al., 2016). Starch oxidation promotes hydrophobicity of starch chains (-OH number reduction) through the oxidative cleavage of the C-2 and C-3 bond of the anhydroglucose units, giving rise to di-aldehyde starch (DAS) (Yu et al., 2010, Du et al., 2008). DAS can act as a crosslinking agent when blended with proteins through the condensation reaction between carbonyl and amino groups (Azeredo and Waldron, 2016), thus improving the film functionality (Martucci and Ruseckaite, 2009; Rhim et al., 1998). Starch-gelatin blend films exhibited good mechanical performance (Acosta et al., 2015; Fackhoury et al., 2013). They are food contact materials and could be used as carriers of antimicrobials to obtain active films. Promotion of crosslinking in the matrix by starch oxidation could favour the film ability to control the antimicrobial release (De Oliveira Pizzoli et al., 2016), while enhancing the film water vapour barrier capacity.

LAE (N-α-lauroyl-l-arginine ethyl ester monohydrochloride) is a potent antimicrobial agent, derived from lauric acid, L-arginine, and ethanol, with a wide spectrum of antimicrobial activity (Muriel-Galet et al., 2014), reported to cause cell growth inhibition

47 or death by increasing the permeability of the cell membrane, as a consequence of the
48 membrane protein denaturation (Rodriguez et al., 2004). It is considered as GRAS
49 (Generally recognized as safe) by the FDA with a maximum dose of up to 200 ppm
50 (Kang et al., 2014), and accepted as food additive (E243, in Europe) for several food
51 products (Hawkins et al., 2009; Higueras et al., 2013).

52 Fish and seafood products are highly perishable mainly due to microbial spoilage,
53 (Aubourg et al., 2007) which make them good candidates for preservation by using
54 antimicrobial packaging. Moreover, they are highly susceptible to *Listeria spp.*
55 contaminations, which represent a huge problem for food safety, due to the high
56 resistance of the bacteria under refrigeration conditions (Cornu et al., 2006) and the
57 high mortality rate associated with listeriosis (Scallan et al., 2011). Therefore,
58 development of effective strategies to reduce the initial contamination levels and to
59 inhibit the growth of these bacteria in fish products is essential. Antimicrobial packaging
60 containing LAE may be a good means of facing up to this issue, since LAE has been
61 successfully proven to be effective at controlling the growth of *Listeria moncytogenes* in
62 different food matrices, such as roasted turkey (Jiang et al., 2011), frankfurters (Porto-
63 Fett et al., 2010), cheese (Soni et al., 2012) or cooked cured ham (Stopforth et al.,
64 2010). The application of LAE for the purposes of salmon preservation has been
65 reported to require a higher dose than the maximum allowed, 200 ppm, since its
66 partitioning into the lipid phase reduces the molecules available for direct contact with
67 bacteria (Kang et al., 2014). Its inclusion in a food compatible polymer matrix could
68 mitigate this problem, improving the effectiveness of the active compound through its
69 controlled release to the potentially contaminated product surface.

70 The aim of the present study was to characterize antimicrobial films based on oxidized
71 corn starch and bovine gelatin, by analysing the effect of LAE incorporation on the film
72 functional properties, as well as to study the effectiveness of the films at controlling

73 microbial growth (with special emphasis on the antilisterial activity) in marinated salmon
74 and at extending its shelf life.

75

76 ## 2. MATERIALS AND METHODS

77 ### 2.1. Materials

78 Corn starch (Roquette Laisa España, S.A.), bovine gelatin type A (BG) (Sancho de
79 Borja, S.L., Zaragoza, Spain), ethyl-lauroyl-arginate (LAE) at 10 % wt in ethanol
80 (Vedeqsa, Lamirsa, Terrassa, Spain) were used. Sodium periodate was supplied by
81 Sigma-Aldrich (Madrid, Spain). Glycerol, magnesium nitrate and potassium chloride
82 were supplied by Panreac Química S.A. (Castellar de Vallès, Barcelona, Spain).
83 Microbiological products were supplied by Scharlab (Barcelona, Spain). *Listeria*
84 *innocua* (CECT 910) was supplied by Colección Española de Cultivos Tipo (CECT,
85 Burjassot, Valencia, Spain).

86

87 ### 2.2. Film preparation

88 Starch was oxidized according to the procedure described by Wang et al. (2015), using
89 sodium periodate. Starch (10 % wt.) and oxidant were dispersed in distilled water at 1:1
90 molar ratio, with respect to the glucose units (solution pH 3.5). Reaction occurred at 35
91 ºC for 4 h, under magnetic stirring in the dark. Afterwards, dispersion was vacuum
92 filtered to stop the reaction. The filtrate was washed three times with distilled water at
93 8000 rpm (Ultraturrax T25, Janke and Kunkel, Germany) for 30 seconds and vacuum
94 filtered. Moisture content of the oxidized starch (OS) was determined gravimetrically.

95 Films were prepared with a 1:1 mass ratio of OS dry solids and BG, using glycerol
96 (0.25 g/g dry polymer blend) as plastizicer, with (OS:BG:LAE) and without LAE
97 (OS:BG). LAE was added at 0.013 g/g of dry polymer blend. The LAE concentration

was fitted in order not to exceed the legal limit (200 ppm, Kang et al., 2014) when released into the food. OS was dispersed in distilled water (2 % wt.) and then gelatinized under stirring at 99ºC for 1 h. BG was dispersed in distilled water (2 % wt.) under magnetic stirring at 40 ºC. OS and BG dispersions were mixed and glycerol was added. LAE was afterwards added to this dispersion. Formulations were finally vacuum degasified.

The films were obtained by casting the mass of film-forming dispersion containing 1.5 g of solids per casting plate (15 cm diameter). After drying for 48 h at 45 % RH and 25 ºC, the films were peeled off and conditioned for one week at either 53 or 88 % RH, using saturated solutions of magnesium nitrate or potassium chloride, respectively. These conditions were chosen because 53 % RH is a common storage condition for the films, whereas 88 % can be the equilibrium value in the films when applied to food products with intermediate a_w values.

2.3. Film characterization

2.3.1. Moisture content and Water vapour permeability (WVP)

Moisture content (g of water per 100 g of dry film) was gravimetrically determined (six replicates) by desiccation in a convection oven (60 ºC, 24 h) and subsequent equilibration in desiccator containing P_2O_5, until constant weight.

Water vapour permeability was determined using the standard method (ASTM E96-95), and considerations of McHugh et al. (1993) for hydrophilic films. Circular film samples were fitted to 3.5 cm diameter Payne permeability cups (Elcometer SPRL, Hermelle/s Argenteau, Belgium) containing distilled water and then put into desiccators with the controlled RH at 5 ºC. Six replicates were made per film formulation and RH gradient (53-100 % and 88-100 %). The cups were weighed every 1.5 h, for 24 h. WVP values

were determined as previously described (Atarés et al., 2010) from the slope of the weight loss *vs.* time relationship when the steady state was reached.

2.3.2. Optical properties: transparency, colour and gloss

The film CIE-L*a*b* colour coordinates (lightness, Lab*; chrome, Cab* and hue, hab*; D65 illuminant/10º observer) and internal transmittance (Ti) were obtained from the reflectance spectra measured on both black and white backgrounds, by using a spectrocolorimeter (CM-3600d, Minolta Co., Tokyo, Japan), as previously described by Atarés et al. (2010). The infinite reflectance (R_∞) of the films were obtained by applying the Kubelka–Munk theory for multiple scattering (Hutchings, 1999). Measurements were taken on the free film surface with six replicates per formulation and conditioning conditions.

The film gloss was measured at a 60º incidence angle, following the ASTM standard D-523 (ASTM, 1999), using a flat surface gloss meter (Multi-Gloss 268, Minolta Co., Tokyo, Japan). Measurements were taken on the free film surface with 15 replicates per formulation.

2.3.3. In vitro antimicrobial activity of the films

The antimicrobial activity of the films against *Listeria innocua* (CECT 910) was analysed. The strain, which was initially kept frozen in TSB with 30 % glycerol, was regenerated by inoculating a loopful in 10 mL TSB. After incubation (24 h at 37 ºC), 10 μl were transferred into 10 mL TSB, which was incubated for 24 h at the same temperature to obtain the culture with exponential growth phase. Tubes with 10 mL of TSB were inoculated with 10^4 CFU/mL of *L.innocua*. Film samples (stored for 1 week and 5 months), 5.3 cm in diameter, were introduced into the inoculated tubes, using inoculated tubes without film as control. Tubes were incubated at 37 ºC for 0, 5 and 24

148 h and bacterial counts were performed. Palcam agar was used as the specific medium
149 for *Listeria*. All of the tests were run in duplicate.

150

151 **2.4. Preparation and characterization of salmon samples**

152 Fresh salmon, purchased in a local supermarket, was marinated covering the steaks
153 with a 1:1 wt. sucrose-sodium chloride blend, applying 2 kg weight to favour leaking, at
154 5 ºC for 48 h. Marinated steaks were peeled, cross-sliced diagonally in 4mm thickness
155 slices, from which sample disks (5.3 cm diameter, 10 ± 0.5 g) were obtained. All of the
156 samples were vacuum packaged and stored at -20 ºC until analyses.

157 Marinated salmon was characterized as to its moisture content, water activity (a_w), pH
158 and colour, in comparison to fresh salmon. The moisture content (g of water per 100g
159 of salmon) was gravimetrically quantified in six replicates (5-10 g each) by desiccation
160 in a vacuum oven at 60 ºC. Water activity and pH were determined in 6 replicates using
161 a Dew Point Water Activity Meter 4TE (Lérida, Spain) and a pH meter (Mettler Toledo
162 Seven Easy pH meter, Switzerland), respectively. The optical properties of marinated
163 salmon were measured in the same way as the films (section 2.3.2.)

164 Initial levels of coliforms and Total viable Counts (TVC) were determined in 10 g
165 random sampled marinated salmon. These were aseptically placed inside a stomacher
166 filter bag with 90 mL of buffered peptone water and homogenized for 90 seconds in
167 Stomacher (Bag Mixer 400, Interscience). The homogenate was ten-fold diluted and
168 plated out. TVC were determined in Plate Count Agar incubated at 30 ºC for 72 h, while
169 coliforms were determined in Violet Red Bile Agar incubated at 37 ºC for 48 h. All of the
170 tests were run in triplicate.

171

172

2.5. Antilisterial activity of the films in marinated salmon

Marinated salmon samples (10 g each) were placed in sterile Petri dishes (5.3 cm of diameter), inoculating *Listeria innocua* (10^2 CFU/cm^2) on the sample surface. Samples were coated with the films (OS:BG and OS:BG:LAE) conditioned at 88 % RH for 1 week. Uncoated, inoculated samples were used as control. Bacterial growth was monitored throughout the storage at 5 ºC for 45 days. Each sample was aseptically homogenized in Stomacher with 90 mL of peptone water for 90 seconds. Ten-fold dilutions were made and poured onto agar PALCAM, prior to incubation at 37 ºC for 48 h. Each film formulation was tested in duplicate.

2.6 Shelf-life of packaged marinated salmon

Salmon samples vacuum packaged in the obtained films (1 week conditioned at 88 % RH) were stored for 45 days at 5 ºC (in duplicate), in order to monitor the preservation ability of the films during storage time. Vacuum packaged samples in conventional polyamide/low density polyethylene (PA/LDPE) bags (La Pilarica S.A., Paterna, Valencia, Spain) were used as control. The growth of TVC and Coliforms was analysed after 25, 35 and 45 days of storage, as described in section 2.4. In parallel, sample weight loss and colour was also monitored. Colour measurements were carried out as described in section 2.3.2 for the films, at four points on the salmon surface covered with an optical glass. Four samples were considered per sampling day. The total color difference ΔE*, as compared to the initial, was calculated using equation 1:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \text{Equation 1}$$

2.6. Statistical analysis

197 The statistical analysis was performed through analysis of variance (ANOVA) using
198 Statgraphics Centurion XVI s for Windows 5.1 (Manugistics Corp., Rockville, Md.).
199 Fisher's least significant difference (LSD) at the 95 % confidence level was used to
200 compare treatments.

201

202 **3. RESULTS AND DISCUSSION**

203 **3.1. Physical and antimicrobial properties of the films**

204 Table 1 shows the moisture content and WVP values of the films conditioned at either
205 53 or 88 % RH. Moisture content slightly increased at the highest RH, coherently with
206 the shape of the water sorption isotherms of hygroscopic polymers, since the two RH
207 levels correspond to different points of the isotherm plateau (Jimenez et al., 2013). LAE
208 incorporation did not cause significant differences in the moisture content of the films
209 ($p>0.05$). The WVP of the films was higher than that of oil-derived polymers, coherently
210 with the hydrophilic nature of starch and gelatin. The WVP increased significantly at 88-
211 100 % RH when compared to that at 53-100 % due to the higher mean moisture
212 content in the films in contact with higher RH levels. Water molecules interact with the
213 film matrix, which leads to a plasticizing effect of the polymer structure and enhances
214 water diffusion across the film, as previously reported by Jiménez et al. (2013) for
215 starch films. Nevertheless, the obtained WVP values were lower than those obtained
216 for starch-gelatin blend films with non-oxidized starch (Moreno et al., 2016). LAE
217 incorporation did not significantly affect the water barrier properties of the films
218 ($p>0.05$).

219 Table 2 shows the optical parameters of the films, namely the colour coordinates,
220 lightness (Lab*), chrome (Cab*) and hue (hab*), internal transmittance (Ti) at 430 nm
221 and gloss at 60º. Both the increase in RH and LAE incorporation had a slight, but
222 statistically significant ($p<0.05$) effect on the colour of the films, reducing lightness and

223 hue and increasing chrome. These changes suggest the progression of browning
224 reactions between the carbonyl groups of OS and the amino groups of gelatin and
225 LAE, thus yielding brown products, such as Maillard compounds. As water availability
226 increases (high RH), molecular mobility is promoted and, hence, carbonyl-amino
227 reaction take place to a larger extent. The small molecular size of LAE may enhance its
228 reactivity, which would boost the browning process. In agreement with the colour
229 changes, the internal transmittance of the films at low wavelength was reduced by LAE
230 incorporation, which was further enhanced at high RH. This is the result of the greater
231 light absorption of the brown compounds between 400 and 500 nm.

232 All of the films had gloss values of under 60, hence they could be described as only
233 slightly glossy. Both LAE addition and the highest RH produced some gloss changes,
234 but these could not be considered as relevant, considering the variability of the gloss
235 values.

236 After 5 months' conditioning at 53 % RH, the lightness of OS:BG films was significantly
237 reduced to 65.7 ± 1.6, at the same time as hue changed from 79.2 ± 0.3 to 76.3 ± 1.3
238 and colour saturation increased from 32.4 ± 0.7 to 37.9 ± 1.9 ($p<0.05$). These changes
239 point to the progression of the carbonyl-amino condensation reaction throughout
240 storage time and the progressive formation of Maillard compounds. Browning
241 progressed to greater extent in LAE-free films and no significant differences were
242 observed as regards the colour parameters of films with and without LAE after 5
243 storage months.

244 Table 3 shows the results obtained for the analysis of the antilisterial effectiveness of
245 the films stored for 1 week and 5 months at 53 % RH. Both films without LAE exhibited
246 antimicrobial ability at controlling the growth of *L.innocua*, after 5 and 24 h of incubation
247 at 37 ºC. This could attributed to the Maillard compounds resulting from the carbonyl-
248 amino reaction, which have previously been reported to show antimicrobial activity

(Hauser et al., 2014; Wu et al., 2014). Hauser et al. (2014) demonstrated that films with Maillard products showed strong antimicrobial activity through different mechanisms, such as the generation of hydrogen peroxide; while Einarsson et al. (1983) observed that these compounds exhibited a greater inhibitory effect against Gram positive bacteria than against Gram negative. The enhancement of the films' browning during storage time, as previously commented on, yielded a greater formation of active compounds and the antimicrobial effectiveness of the LAE-free films after 5 months of storage increased in comparison with that of films stored for 1 week. The inhibition of *Listeria* growth, in terms of log reduction in comparison to the control sample, were 1.69 and 2.38 CFU/mL, respectively for films stored for 1 week and 5 months. The incorporation of LAE into the films led to a total bactericidal effect at all incubation times. The minimally inhibitory concentration (MIC) of LAE reported against *Listeria monocytogenes* was 8 μg/mL (Higueras et al., 2013), and the concentration reached in the medium, assuming a total release from the films, would be 200 μg/mL. Therefore, the MIC value would be exceeded in the culture medium, acting against the microbial cells effectively.

3.2. Film application to extend shelf-life of marinated salmon.

Moisture content, water activity and pH of marinated salmon were significantly reduced ($p<0.05$) when compared to the values of fresh salmon, due to the osmotic dehydration (water loss and solid gain), reaching values of 52.4±0.8 g water/g salmon (70±4 g water/g fresh salmon), 0.925±0.017 and 5.98±0.03, respectively (0.988±0.002 and 6.21 ±0.13, respectively in fresh salmon). Linked to these changes, the concentration of pigments increased in the tissue, thus modifying the selective light absorption properties of the muscle. Whereas the hue of salmon (45±4) was not significantly affected by marinating (45 ± 2, in fresh salmon), lightness (38±2) and colour saturation

275 (chrome: 16±2) were significantly reduced (41±2 and 24±3, respectively in fresh
276 salmon), as previously reported by Lerfall et al. (2016).

277 Regarding the initial microbiological quality, marinating did not affect the microbial flora
278 of the fresh salmon. No Coliforms were detected either in fresh or marinated salmon
279 and TVC were 3.8 ±0.5 log CFU/g, thus indicating good hygienic fish handling practices
280 (legal limit: 10^6 cfu/g, EC, 2007).

281

282 *3.2.1. In vivo antilisterial activity of the films in marinated salmon*

283 Figure 1 shows the counts of *Listeria innocua* in inoculated marinated salmon samples
284 coated with OS:BG and OS:BG:LAE films, as well as those of uncoated samples
285 (control) throughout the storage time at 5 ºC. The application of both films entailed
286 growth reduction in comparison with the control sample. During the first 25 storage
287 days, the LAE presence in the films did not imply significant differences in the *Listeria*
288 counts. However, at the end storage time (45 days), the growth inhibition compared to
289 the control was significantly greater ($p<0.05$) for the samples coated with OS:BG films
290 (1.87 log CFU/g of reduction compared to the control sample) than for those coated
291 with the OS:BG:LAE film (reduction of 0.98 log CFU/g). Hence, the incorporation of
292 LAE into the films did not result in an improved antilisterial activity in salmon samples,
293 despite the stronger activity observed in the in vitro test for OS:OB:LAE films.
294 Assuming a total release of the active compound, its more limited antilisterial action in
295 salmon could be attributed to the predominant partitioning of the compound in the fat
296 phase, thus making it unable to act against the bacteria prevalent in the aqueous
297 phase, as previously reported (Kang et al., 2014). This result suggests that the
298 inclusion of LAE in the OS:BG films is not enough effective to limit its migration into the
299 fat phase, in order to enhance its antimicrobial action in salmon samples. However, the
300 active compounds generated in OS:BG films were effective at controlling the *Listeria*

301 growth in salmon in the same way as that observed for in vitro tests. This compounds
302 are water-soluble and can act against bacteria, inhibiting their growth during a long
303 period of time. In fact, the counts after 25 storage days were lower than those initial
304 values.

305 *3.2.3. Shelf-life of packaged marinated salmon*

306 The shelf life of marinated salmon samples, vacuum packaged in the OS:BG
307 biodegradable films was compared to that of samples packaged in commercial
308 synthetic plastic (control). Figure 2 shows the TVC counts over 45 storage days at 5 ºC
309 for all samples. Coliform counts were not detectable in any case. The application of
310 films with and without LAE resulted in a reduced cell population as compared to the
311 control sample. In the early stages of the storage, a similar bactericidal effect of both
312 OS:BG and OS:BG:LAE films was observed, which suggests the Maillard products
313 were prevalent in the antimicrobial action, since no LAE was present in OS:BG films.
314 After 25 days, TVC exceeded the established legal limit (10^6 CFU/g) for control
315 samples, whereas a marked count reduction (4.7 log CFU/g) when compared to the
316 control was observed for samples packaged in OS:BG films with and without LAE. At
317 the end of storage, OS:BG:LAE packaged samples had 10^4 CFU per gram of sample,
318 exhibiting a reduction of 2 log CFU/g when compared to the legal limit. OS:BG
319 packaged samples, showed a smaller reduction (1 log CFU/g). The antimicrobial
320 effectiveness of the films against the natural microflora of marinated salmon seems to
321 be enhanced by the presence of LAE, but this effect was only appreciated after a long
322 storage time.

323 However, despite the good antimicrobial properties of the films, they were not effective
324 at controlling the moisture loss of the samples due to their relatively high WVP values
325 (Table 1). In fact, whereas no mass loss occurred in the samples packaged in the
326 conventional plastic film, samples packaged in OS:BG films, with and without LAE

exhibited a linear mass loss in line with the storage time (0.063, 0.086 and 0.105 at 25, 35 and 45 storage days, respectively), with a slope of 0.0025 day^{-1} and a final mass loss of nearly 10 %. This mass loss implied a progressive reduction in both the product moisture content and a_w, mainly at surface level, which affected the sample colour and could also contribute to the antimicrobial activity of the films (combined hurdles).

Figure 3 shows the development of the colour attributes for the different salmon samples packaged in the conventional and developed films throughout storage time. Whereas no significant changes in colour coordinates were observed for the control samples, taken the variability in the values into account, the samples packaged in OS:BG films, with and without LAE, exhibited similar significant reduction in lightness, which can be mainly attributed to the water loss. For samples packaged in OS:BG films with LAE, no significant changes in either hue or colour saturation (chrome) occurred during storage; however, in the samples in contact with films without LAE, a significant increase in hue and chrome values occurred after 25 days of storage, which points to a certain discoloration effect. However, subsequent changes lead these colour parameters closer to the initial values. The potential oxidation of the salmon pigments, such as carotenoids, could explain this initial development, and this effect on the colour could be subsequently mitigated by the progressive water loss, which enhanced the concentration of the natural pigments. After 45 days of storage, the differences in the sample lightness are the main factor that contributes to the total colour difference as compared to the initial sample. These values were 6±3 for plastic packaged samples, close to the natural variability in the samples, and 11±5 and 16±6, respectively, for samples in contact with OS:BG and OS:BG:LAE films. Therefore, despite the good antimicrobial properties of the OS:BG films, their ability to preserve the quality of salmon during storage are compromised by their poor water vapour barrier properties, which in turn, affect the product lightness imparting a non-acceptable colour difference (Hutchings, 1999) when compared to the initial product.

354

4. CONCLUSIONS

Oxidized starch-gelatin blend films, with and without LAE, were highly effective at controlling microbial growth, exhibiting antilisterial activity in marinated salmon, greatly extending the product shelf life in terms of microbial spoilage. However, the films were not effective at controlling weight loss due to their insufficient water vapour barrier capacity. Films with LAE showed *in vitro* bactericidal effect against *Listeria innocua*, but they were less effective in marinated salmon where similar antilisterial activity was observed for films with and without LAE. TVC in salmon samples remained below the legal limit after 45 storage days, which represents a shelf-life extension. However, salmon samples underwent darkening in line with desiccation. Therefore, a multilayer film, including a high water vapour barrier layer, would be necessary to guarantee the quality of the marinated salmon throughout such a lengthy storage period.

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500

Table

Table 1 Moisture content (X_w, g water per 100g dry film) and water vapor permeability (WVP) of the film samples conditioned at 53 or 88% RH. RH gradients used for WVP tests are indicated.

	RH (%)	OS:BG	OS:BG:LAE
X_w (g water/100 g dry film)	53	$12.9 \pm 1.5^{a,A}$	$12.0 \pm 1.8^{a,A}$
	88	$16.4 \pm 1.8^{a,A}$	$15.9 \pm 1.9^{a,A}$
WVP x 10^7 ($g \cdot m^{-1} s^{-1} Pa^{-1}$)	53-100	$3.1 \pm 0.3^{a,A}$	$3.2 \pm 0.5^{a,A}$
	88-100	$6.6 \pm 0.9^{a,B}$	$5.8 \pm 0.3^{a,B}$

Different superscript lower case letters in the same row indicate significant differences due to LAE incorporation ($p<0.05$).

Different superscript upper case letters (A, B) in the same column indicate significant differences due to RH ($p<0.05$).

Table 2 Optical parameters (lightness (L*), chrome (C_{ab}*), hue (h_{ab}*)), internal transmittance (T_i) at 430nm and gloss at 60º of films conditioned at 53 and 88% RH.

	RH (%)	OS:BG	OS:BG:LAE
L*	53	$75.5 \pm 0.4^{b,B}$	$73.4 \pm 0.8^{a,B}$
	88	$74.5 \pm 0.2^{b,A}$	$69.8 \pm 0.6^{a,A}$
C_{ab}*	53	$32.4 \pm 0.7^{b,A}$	$34.4 \pm 0.3^{a,A}$
	88	$35.2 \pm 0.9^{b,B}$	$36.3 \pm 0.3^{a,B}$
h_{ab}*	53	$79 \pm 0.03^{b,B}$	$77.2 \pm 0.2^{a,B}$
	88	$78.5 \pm 0.5^{b,A}$	$75.9 \pm 0.3^{a,A}$
T_i (430nm)	53	$0.695 \pm 0.009^{b,A}$	$0.577 \pm 0.007^{a,A}$
	88	$0.606 \pm 0.017^{b,B}$	$0.525 \pm 0.012^{a,B}$
Gloss (60 º)	53	$57 \pm 9^{b,A}$	$21 \pm 10^{a,A}$
	88	$25 \pm 5^{b,B}$	$33 \pm 8^{a,B}$

Different superscript lower case letters (a, b) in the same row indicate significant differences due to LAE incorporation ($p<0.05$).

Different superscript upper case letters (A, B) in the same column indicate significant differences due to RH ($p<0.05$).

Table 3 Effect of the films, conditioned for 1 week and 5 months at 53 % RH, on the growth and survival of *Listeria innocua* (CECT 910) at 37 ºC. Bacterial counts obtained at initial time (0 h), 5 and 24 h of incubation. Average values and standard deviation.

Formulation	Initial time	5h	24h
	Log (CFU/mL)	Log (CFU/mL)	Log (CFU/mL)
Control Listeria	$4.33 \pm 0.09^{A,a}$	$6.59 \pm 0.06^{B,b}$	$8.78 \pm 0.05^{B,c}$
OS:BG 1 week	$4.37 \pm 0.09^{A,a}$	$4.6 \pm 0.4^{A,a}$	$7.099 \pm 0.007^{A,b}$
OS:BG 5 months	$4.307 \pm 0.106^{A,a}$	$4.1 \pm 0.6^{A,a}$	$6.4 \pm 1.0^{A,b}$
OS:BG:LAE 1 week	NDG*	NDG*	NDG*
OS:BG:LAE 5 months	NDG*	NDG*	NDG*

Different superscript lower case letters (a, b) in the same row indicate significant differences among the different formulations ($p<0.05$).

Different superscript upper case letters (A,B,C) in the same column indicate significant differences among the different times of incubation for the same formulation ($p<0.05$).

* No Detected Growth.

Figure

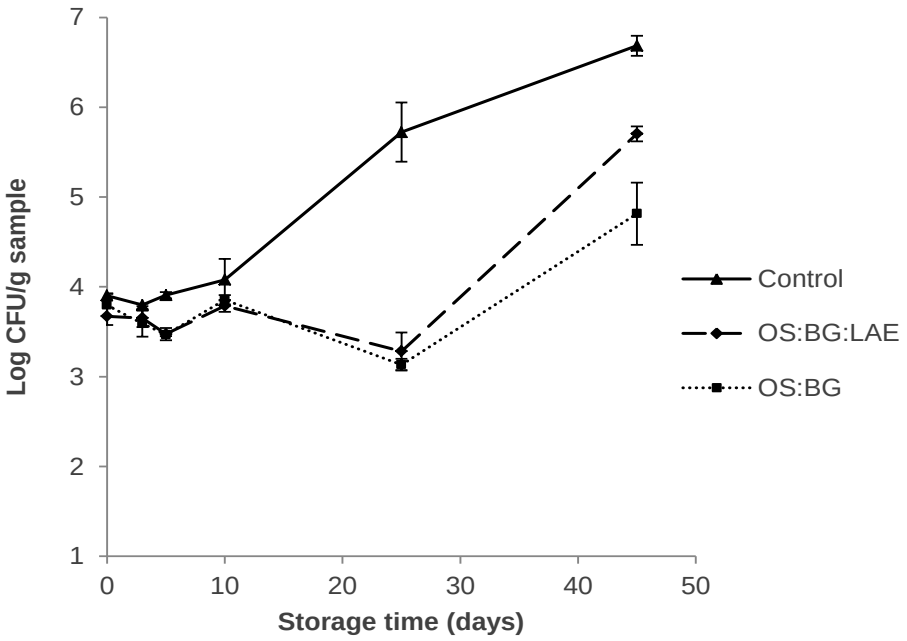

Figure 1 Microbial counts obtained at initial time, 3, 5, 10, 25 and 45 days of storage of marinated salmon kept at 5 ºC inoculated with *Listeria innocua,* either without film coating (Control) or completely coated with both film formulations (OS:BG and OS:BG:LAE) conditioned for 1 week at 88 % RH. Mean values and standard deviation.

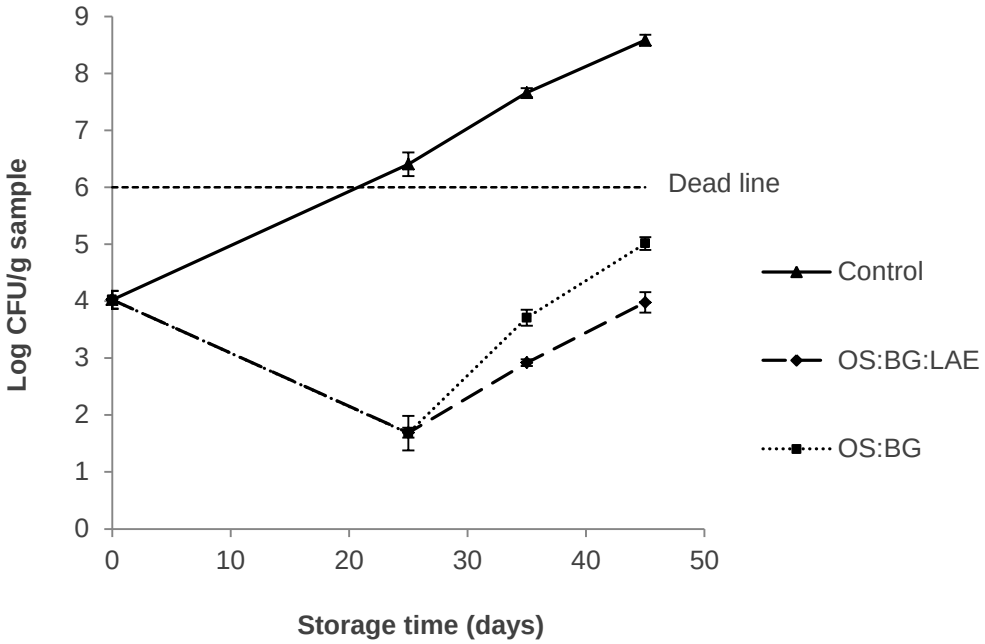

Figure 2 TVC counts obtained at initial time, 3, 25, 35 and 45 days of storage of marinated salmon at 5 ºC*,* vacuum packaged in commercial synthetic plastic (Control), or with both film formulations (OS:BG and OS:BG:LAE) conditioned for 1 week at 88 % RH. Mean values and standard deviation.

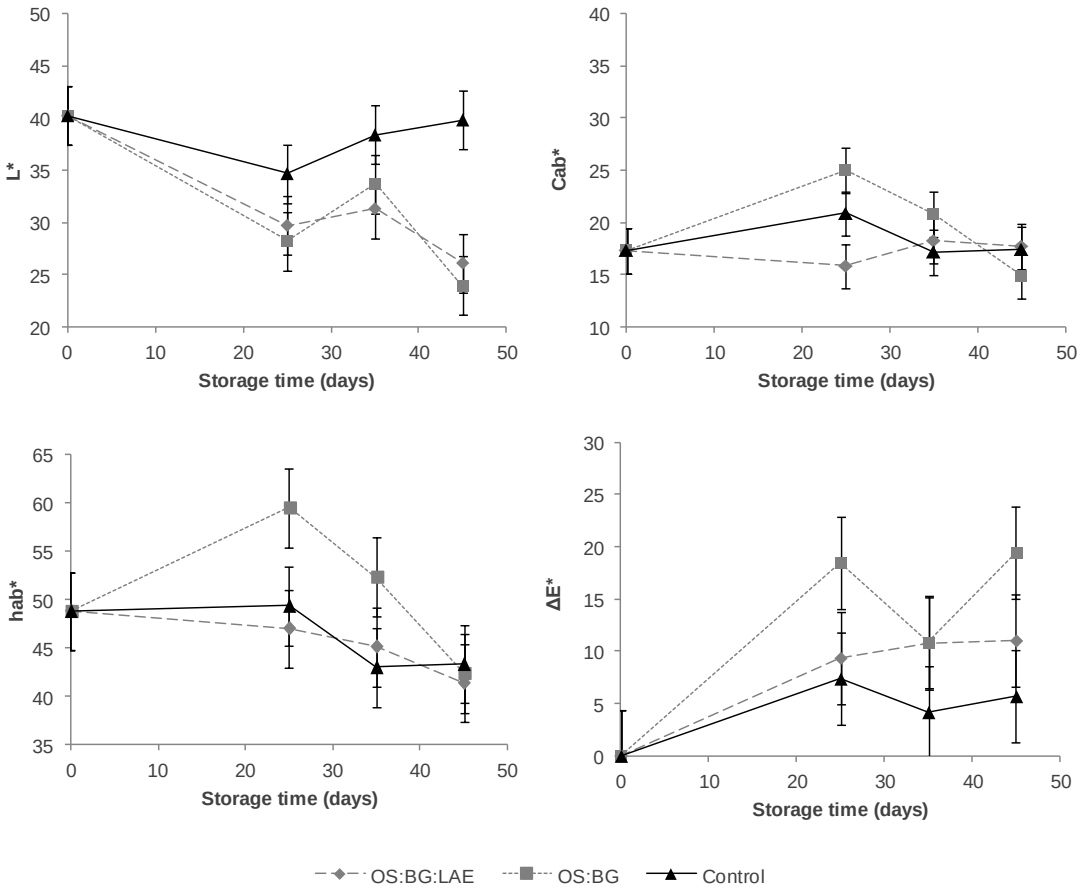

Figure 3 Evolution of colour (lightness (L*), chrome (C_{ab}*), hue (h_{ab}*) and ΔE* with respect to the initial) of the marinated salmon samples packaged with synthetic plastic (Control) and both film formulations (OS:BG and OS:BG:LAE) throughout 45 days of storage at 5 ºC.