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STARCH-GELATIN ANTIMICROBIAL PACKAGING MATERIALS TO EXTEND THE SHELF LIFE OF CHICKEN BREAST FILLETS

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Abstract

Antimicrobial starch:gelatin (1:1) films containing N-α-lauroyl-l-arginine ethyl ester monohydrochloride (LAE) (10 % wt.) were used as food contact active layers in chicken breast fillets vacuum-packaged in polyamide/polyethylene pouches. Active layers were thermoprocessed (TP) or cast (OC) on the plastic film. Oxidized starch was used in OC coatings. Packaged chicken breast samples were stored at 4 ºC and their physicochemical properties (pH, colour and lipid oxidation) and microbial quality were analysed throughout storage. Both TP and OC films significantly ($p<0.05$) extended the shelf life of chicken breast fillets compared to control samples. The starch oxidation reaction in OC films promoted the formation of Maillard reaction compounds in the starch-gelatine blends, which enhanced the antimicrobial effectiveness of the OC films, but also promoted oxidative processes. This greatly affected the pH and colour parameters in OC packaged samples. Therefore, TP blend films containing LAE are

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recommended since they effectively extended the shelf life of chicken breast fillets without affecting the meat oxidation.

Keywords: LAE, starch, gelatin, antimicrobial, chicken breast

1. Introduction

Starch and gelatin has been extensively studied for the purposes of developing packaging materials because they are abundant, cheap and biodegradable materials, which are also edible and, thus, adequate for food contact purposes (Cazón, Velazquez, Ramírez & Vázquez, 2017). Starch-gelatin (S-G) based films obtained by both casting or thermoprocessing methods exhibited good mechanical resistance and extensibility and low oxygen permeability (Acosta, Jiménez, Cháfer, González-Martínez, & Chiralt, 2015; Moreno, Díaz, Atarés, & Chiralt, 2016) and could be used for food packaging/coating uses. Nevertheless, the films are highly hydrophilic in nature and their properties are greatly affected by the water content. In this sense, starch oxidation through the hydroxyl groups in positions C-2 and C-3 of the anhydroglucose units, producing di-aldehyde starch (DAS) (Yu, Chang, & Ma, 2010), have been used to reduce the hydrophilic nature of starch, while allows for binding amino groups (e.g. from proteins), producing a reinforced crosslinked matrix (Wang et al., 2015), with improved mechanical properties and water resistance. DAS is also suitable for food contact purposes and could be used for food packaging applications (Martucci & Ruseckaite, 2009). Nevertheless, due to the lower thermo-stability of oxidized starch (Soliman, El-Shinnawy, & Mobarak, 1997), and the uncontrolled condensation reactions with the protein carbonyls at high temperature, thermoprocessing of oxidized starch-protein blends was not possible and casting methods would recommended to obtain DAS-G blend films or coatings.

S-G matrices could also be used as carriers of active compounds (e.g. antimicrobials) to obtain active films for food packaging applications. S-G matrices carrying antimicrobial compounds are suitable for food contact applications and could exert a controlled release of the antimicrobial towards the food surface. The application of antimicrobial packaging is especially interesting for the highly perishable meat products, where microbial contamination occurs primarily at the surface, due to post-processing handling

(Quintavalla & Vicini, 2002). Chicken meat is very popular in Europe, but it is highly perishable due to its characteristic composition, high water activity (a_w) and a high pH (Rodríguez-Calleja, Cruz-Romero, O'Sullivan, García-López, & Kerry, 2012). Then, the use of technologies, such as antimicrobial packaging, which can extend the shelf life of chicken breast fillets are very interesting for the poultry industry (Azlin-Hasim, Cruz-Romero, Morris, Cummins, & Kerry, 2015).

Of the current antimicrobials, N-α-lauroyl-l-arginine ethyl ester monohydrochloride, (LAE), is a cationic surfactant considered as GRAS (Generally Recognized As Safe) by the FDA, and accepted for use in meat products in Europe (E243) (Hawkins, Rocabayera, Ruckman, Segret, & Shaw, 2009; Higueras, López-Carballo, Hernández-Muñoz, Gavara, & Rollini, 2013). LAE has a wide spectrum of antimicrobial activity (Muriel-Galet, López-Carballo, Gavara, & Hernández-Muñoz, 2015), even at low concentrations. Higueras et al. (2013) reported a minimally inhibitory concentration (MIC) of 16 and 8 μg/mL for *Escherichia coli* and *Listeria monocytogenes*, respectively. LAE increases the permeability of the cell membrane, as a consequence of a membrane protein denaturation, causing cell growth inhibition or death (Rodríguez, Seguer, Rocabayera, & Manresa, 2004). This promising antimicrobial has been successfully applied on chicken (Higueras et al., 2013; Nair, Nannapaneni, Kiess, Mahmoud, & Sharma, 2014); but, to the best of our knowledge, neither the use of S-G matrix as a carrier of this antimicrobial nor the application of the these antimicrobial films for the purposes of extending the shelf life of chicken fillets was reported.

The aim of this study was to assess the effectiveness of antimicrobial starch-gelatin films containing LAE at extending the shelf life of chicken breast fillets. Antimicrobial layers were obtained by either the thermoprocessing of non-oxidized starch-gelatin blends or the casting of oxidised starch-gelatin solutions. In both cases, food contact with the films was promoted through the vacuum packaging of samples in commercial polyethylene/polyamide laminates.

2. Materials and methods

2.1. Materials

Film components: Corn starch (S) (Roquette Laisa España, S.A., Valencia, Spain); Bovine gelatin type A (G) (Sancho de Borja, S.L., Zaragoza, Spain); Sodium periodate (SP) (Fluka Analytical, Sigma–Aldrich Chemie GmbH, Steinheim, Germany); Ethyl lauroyl arginate (LAE) at 10 % w/v in ethanol (Vedeqsa, Lamirsa, Terrassa, Spain) and glycerol (Panreac Química S.A., Castellar de Vallès, Barcelona, Spain). Magnesium nitrate was supplied by Panreac Química S.A. (Castellar del Vallés, Barcelona, Spain). Polyamide/low density polyethylene (PA/LDPE) pouches (200x300 mm, water vapour transmission rate of 2.8 g/m^2 24 h and oxygen permeability rate of 50 cm^3/m^2 24 h) were supplied by Cryovac (Sealed AirW.R. Grace Europe Inc., Lausanne, Switzerland).

The microbiological media (Maximum Recovery Diluent, Plate Count Agar (PCA), M RS Agar, Brilliance™ *E.coli* / Coliform Selective Medium), were supplied by Oxoid (Oxoid Ltd., Basingstoke, England). Tryptic Soy Agar and Yeast extract granulate were supplied by Merck (Merck KGaA, 64271 Darmstadt, Germany).

2.2. Film preparation

Thermo-processed starch-gelatin films

Two thermo-processed (TP) film formulations were obtained based on a S:G blend (wt. ratio 1:1). To prepare the control formulation (TP_C), the dry components were mixed, and glycerol and water added in a polymer:glycerol:water mass ratio of 1:0.3:1.1. For the antimicrobial active formulation (TP_LAE), LAE was also added, in a polymer: LAE mass ratio of 1:0.1. Each formulation was hot-blended at 160 ºC and 8 rpm for 10 minutes on a two-roll mill (Model LRM-M-100, Labtech Engineering, Thailand). The pellets were conditioned at 53 % relative humidity (RH) for one week at 25 ºC on a desiccator

containing an oversaturated solution of Mg $(NO_3)_2$. The films were obtained by compression moulding using a hot-plate press (Model LP20, Labtech Engineering, Thailand). Four grams of the conditioned pellets were pre-heated for 5 min at 160 ºC in the press plate and then pressed at 3000 KPa for 2 min and 13000 KPa pressure for 6 min at 160 ºC. Thereafter, a cooling cycle to 6 ºC was applied for 3 min. The obtained films were 17 cm in diameter and 180 ± 0.014 μm thick.

Coated packaging with oxidized starch-gelatin blends

The corn starch was oxidized following the method described by Yu et al. (2010), using SP as oxidizing agent, with some modifications. Briefly, a 10 % (w/v) of S was dispersed in distilled water while gently stirred. SP was added in a molar ratio SP:Glucose unit of 1:1. The dispersion obtained was kept in the dark for four hours, under controlled conditions (35 ºC and pH 3.5). The oxidized starch (OS) was vacuum filtered (Vacuum / Pressure Station, Barnant Company, Barrington, Illinois, United States) and washed three times with distilled water to ensure the complete elimination of the reagent. The oxidised starch was re-dispersed in water at 8000 rpm for 30 seconds using an ultraturrax (DI25, Janke andKunkel, Germany)) and vacuum filtered. The obtained wet solids were used for film preparation, taking into account the water content, previously determined gravimetrically.

OS (6 % wt.) was dispersed in distilled water and gelatinized at 99 ºC in a thermostatic water bath (SW23, Julabo GmbH, 77960 Seelbach/ Germany) for 1 h under gentle agitation at 100 rpm. A 6 % wt. dispersion of G was also prepared at 40 ºC while being stirred at 450 rpm in a hot-plate for 30 min. Then, both dispersions were cooled down to room temperature and blended in a 1:1. mass ratio and glycerol was added at 25 % w/w of the total polymer mass (OC_C, control formulation). For the active formulation (OC_LAE), LAE was added in a polymer:LAE ratio of 1:0.1. All of the solutions were kept

under constant stirring for 30 min at 450 rpm until casting. Casting was carried out on a levelled inner polyethylene layer of PA/LDPE laminates using a Micron II film applicator (Gardco, FL, USA) and dried for 48 h at 20 ºC. The solid density on the surface of the PA/LDPE films was 840 g dry solids/m^2. To obtain pouches (102 x 177 mm), the edges of the films were heat-sealed using a Henkelman Polar 80 (Henkelman Vacuum System, Model Polar 80, 5221 CK 's-Hertogenbosch, The Netherlands) with a sealing time of 2.5 s.

2.3. Chicken sample preparation and experimental Design

Fresh chicken breast fillets were purchased from a local supplier (Shannon Vale Foods Ltd. Clonakilty, Co. Cork, Ireland), kept in a chill room at 2 ºC and used within 24 h. To avoid cross contamination during sample preparation, all utensils and work surfaces were sanitized with 70 % ethanol and the TP and OC films were decontaminated by exposure to UV light for 15 min in a laminar flow (Airclean 600 PCR Workstation STARLAB, Airclean Systems, USA). Excess fat and cartilage were trimmed from the chicken breast fillets and immediately packaged using either control (TP_C or OC_C) or active (TP_LAE or OC_LAE) films with LAE. Chicken breast fillets (150 - 180 g, 1 cm of thickness) were individually wrapped in the sterilised TP films and placed individually into PA/LDPE pouches, or packaged in the coated PA/LDPE pouches. Afterwards, all pouches were vacuum sealed using a Henkelman Polar 80 vacuum system and stored at 4 ºC for 19 days. Three independent experimental series, with different reception days, were run for both TP and OC packaged samples. Three fresh chicken samples were vacuum packaged in the conventional PA/LDPE pouches for the initial characterization of the raw material (chicken control). All samples were kept packaged under refrigerated conditions (4 °C) throughout different storage times until 19 days and three samples from

each series (different packaging conditions) were analysed at 0 (2 h contact time) 2, 6, 9, 12 and 19 days.

2.4. Physicochemical characterization

Proximal analysis

The proximal composition (fat, moisture, protein and ash) of the chicken control and packaged samples was determined after 2 hours of contact with the packaging.

Fat and moisture contents were determined using the CEM Analysis System (CEM Corporation, Matthews, NC 28105, USA) as described by Bostian, Fish, Webb, & Arey, (1985). Protein content was determined using the Kjeldahl Method, following AOAC Procedures (1999) (method 981.10). Finally, the ash content was obtained by a gravimetric method, weighing the samples before and after incineration in a furnace (Nabertherm, Model L9/C6, Nabertherm, Germany) at 550 ºC. All the tests were run in duplicate for each series, and the reported values are the average of 6 replicates.

Determination of pH, lipid oxidation and colour

Throughout storage, the pH, lipid oxidation and colour changes in the packaged chicken breast fillets were monitored. For day 0, the reported values correspond to 2 h contact with the films. The pH was measured using a digital pH meter by direct insertion of the glass electrode probe into the fillet (Mettler-Toledo GmbH, Schwerzenbach, Switzerland). Four measurements were taken per each sample (12 replicates).

180 The lipid oxidation was assessed using the method described by Siu & Draper (1978).
181 The results were expressed as mg of malondialdehyde (MDA)/kg of sample (6
182 replicates).

183 Once the films were removed, the colour (CIE L* a* b* colour coordinates) of the fillets
184 was measured using D65 illuminant/10° observer, at ten random points on the sample
185 surface, using a portable Minolta CR-300 colorimeter (Minolta Camera Co., Osaka,
186 Japan), previously calibrated with a white ceramic plate (Y = 93.6, x = 0.3130, y =
187 0.3193). The total colour difference with respect to the fresh chicken throughout chilled
188 storage was calculated using Equation 1. The reported value is the average of 30
189 replicates.

190 $$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$ Equation 1

191

192 **2.5. Microbiological analysis**

193 Microbiological analysis of the packaged chicken breast fillets was carried out at the
194 different storage times. A total of 10 g of meat sample was aseptically taken from both
195 the upper and bottom surface of the chicken breast fillets using sterile forceps and
196 scalpels, placed into a sterile stomacher filter bag (Seward, UK) to which 90 mL of sterile
197 Maximum recovery diluent (MRD) (Oxoid, UK) was added and thoroughly mixed for 3
198 min using a stomacher (Seward, UK) in order to obtain a primary 10-fold dilution. This
199 homogenate was then 10-fold serially diluted using MRD and used to enumerate total
200 viable counts (TVC), psychrotrophic bacteria (PB), *Lactic acid bacteria* (LAB), anaerobic
201 bacteria (AB), total coliforms (TC) and *Escherichia coli* (*E. coli*). TVC and PB were
202 enumerated in PCA plates, after incubation at 37 ºC or 4 ºC for 48 h or 7 days,
203 respectively. LAB was enumerated using overlaid MRS agar plates after incubation at
204 30 ºC for 72 h. Enumeration of AB was performed in TSA enriched with 0.6 % yeast
205 extract after 72 h incubation at 30 ºC under anaerobic conditions in an anaerobic jar

containing Anaerocult®. Finally, TC and *E. coli* were enumerated in the chromogenic medium Brilliance *E.coli* /Coliforms Selective Agar after incubation at 37 ºC for 24 h. The results of bacterial counts were converted to $\log_{10}$ colony-forming units per gram of sample (log CFU/g) prior to statistical analyses.

2.6. Statistical analysis

Analysis of variance was performed using general linear models, considering the effect of the following factors: time, packaging treatment, chicken trial (random factor) and the interaction time * packaging treatment. These tests were carried out, using Statgraphics Centurion XVI (Manugistics Corp., Rockville, MD). Fisher's least significant difference (LSD) at the 95 % confidence level was used to compare treatments.

3. Results and discussion

3.1. Physicochemical characteristics of initial chicken breasts.

Proximal composition

All of the chicken samples exhibited a similar proximal composition (Table 1), in the range of that previously reported for these kinds of samples (Azlin-Hasim et al., 2015). The small variations could be attributed to commercial breeds, diet formulation, housing and general management practices (Qiao, Fletcher, Northcutt, & Smith, 2002). Contact with both TP films significantly decreased the moisture content in the chicken samples, which can be attributed to the high water absorption capacity by the films. Moreno et al. (2016) reported a water uptake capacity of 600 g water/g dry film for these films, whereas OC films showed lower water uptake (100-300 g water/g dry film), due to their reduced

hydrophilic nature (Yu et al., 2010). Likewise, the protein content of samples packaged in TP films was significantly ($p<0.05$) higher, which mainly resulted from their reduced moisture content.

Colour, pH and lipid oxidation

Table 2 shows the colour coordinates of fillets after two hours in contact with the packaging. Significant differences ($p<0.05$) in the sample lightness (L*), redness (a*) and yellowness (b*) were observed for the fillets packaged in TP films. These samples became slightly darker, with small chromatic changes, which may be attributed to the significant reduction in the moisture content, which mainly occurred at the surface in contact with the TP films (more hydrophilic than OC film), where colour was measured. Water loss leads to changes in the selective light reflection at the sample surface, due to the changes in the refractive index of the material and the surface concentration of the pigments (Hutchings, 1999). However, the sample colour is in the range of that reported (Huang, Williams, Sims, & Simmone, 2011) considering the natural variability of the product by genetics and other factors (Lonergan, Deeb, Fedler, & Lamont, 2003).

The pH of the fillets ranged between 5.86 and 6.16, which is considered a typical pH value for poultry meat (Barbut, Zhang, & Marcone, 2005; Huang et al., 2011; Rodríguez-Calleja et al., 2012; Qiao et al., 2002). However, samples in contact with OC_C films exhibited a significantly ($p<0.05$) lower pH-value (nearer of the OC_LAE sample), as compared with the more homogenous pH of the rest of the samples.

Lipid oxidation, one of the main factors causing flavour deterioration during the storage of meat and meat products (Azlin-Hasim et al., 2015; Rodríguez-Calleja et al., 2012) was evaluated through the TBARS assay, which provides an indicator of the secondary oxidation. The analyses of the initial samples (Table 2) showed low MDA levels, ranging between 0.05 and 0.33 mg MDA/kg of sample, which are typical values for fresh chicken

meat (Azlin-Hasim et al., 2015; Rodríguez-Calleja et al., 2012). Nevertheless, samples packaged with the starch films showed significantly ($p<0.05$) higher TBARS values, especially those in contact with the oxidized starch coatings.

Microbial counts

The initial microbial counts of the samples are shown in Table 3. The TVC counts of the all samples (3-5 log CFU/g) indicated the good microbiological quality of the chicken meat (Azlin-Hasim et al., 2015). Similar initial TVC and PB counts were previously reported for these kinds of products (Azlin-Hasim et al., 2015; Balamatsia, Paleologos, Kontominas, & Savvaidis, 2006; Rodríguez-Calleja et al., 2012), although slightly higher LAB counts were obtained. Significantly lower ($p<0.05$) counts of all bacteria, except Coliforms, were observed for fillets packaged in the OC_LAE system, where *E. coli* was not detected. In general, counts of the different bacteria were lower for samples packaged in films containing LAE, which indicates the fast action of this antimicrobial on the bacteria population.

3.2. Physicochemical changes during chilled storage

Lipid oxidation

Development of lipid oxidation during the chilled storage of the fillets are shown in Figure 1a for the different packaging systems. Samples wrapped in TP films exhibited very slow lipid oxidation throughout storage, whereas samples in contact with OC films exhibited a significant ($p<0.05$) increase in the TBARS values. This increase ($p<0.05$) in the lipid oxidation of the OC coated chicken samples, may be due to the presence of oxidant species formed during the oxidation process of starch, which can promote lipid oxidation in the fillets. In fact, Maillard reaction compounds produced in OC films due to the

281 carbonyl-amino condensation reaction (Moreno, Gil, Atarés, & Chiralt, 2017), generate
282 hydrogen peroxide (H_2O_2), which confer them antimicrobial action (Hauser, Müller,
283 Sauer, Augner, & Pischetsrieder, 2014). Samples packaged in OC_LAE films, underwent
284 significantly greater ($p<0.05$) lipid oxidation, suggesting a greater formation of Maillard
285 compounds and oxidant species when LAE was present in the films, probably due to the
286 presence of more reactive amino groups of this low molecular weight compound.

287 Melton (1983) reported that a TBARS value of 1.5 mg MDA/kg is regarded as the limit
288 beyond which chicken meat will normally develop objectionable odours/tastes. This limit
289 of acceptability in terms of TBARS was reached after 9 and 19 storage days in samples
290 packaged in OC_LAE and OC_C films, respectively. In contrast, the samples packaged
291 in TP films did not show significant differences in TBARS values, with respect to the fresh
292 chicken, throughout 19 days of chilled storage.

293

294 *pH and Colour*

295 Figure 1b and Figure 2 show the changes in the pH and colour of the fillets, respectively,
296 during the storage in the different packaging systems. The initial pH values of the
297 samples packaged in the different film formulations ranged between 5.86 and 6.16. This
298 range shows the variability expected for the raw material and, considering the fact that
299 different samples are analysed at each control time, only for samples packaged in films
300 with oxidized starch containing LAE, the pH value significantly decreased below the
301 lower limit at the longest storage time. The pH development in the chicken samples will
302 be affected by microbial growth and the oxidation process, which occurred to a different
303 extent in OC and TP packaged fillets. The different population of bacteria and the
304 subsequent lactic acid production or volatile amine and ammonia generation can alter
305 the sample pH promoting the sample differences (Azlin-Hasim et al., 2015; Cortez-Vega,
306 Pizato, & Prentice, 2012). Likewise, lipid oxidation is also associated with protein

307 oxidation, which increases the number of carboxyl groups and decreases that of
308 sulfhydryl groups (Soyer, Özalp, Dalmış, & Bilgin, 2010). Therefore, the significantly
309 ($p<0.05$) higher oxidation level of the OC_LAE packaged fillets during storage could be
310 associated their lower pH. In samples packaged with OC films, the pH could be more
311 affected by the progress of the oxidation process, since they had lower microbial counts,
312 whereas for samples in TP systems the pH would mainly governed by the action of
313 bacteria that grow to a greater extent.

314 Figure 2 shows the changes in the L*, a* and b* values of fillets during chilled storage at
315 4 ºC. L* values increased during storage in the samples, indicating that the fillets became
316 paler. This could be due to the generalised pH decrease in the samples throughout
317 storage time. Previous studies reported that the pH significantly affects the lightness of
318 meat products (Barbut et al., 2005; Lonergan et al., 2003; Qiao et al., 2002). Low pH can
319 lead to protein solubilisation and denaturation and a paler meat. The sample redness (a*
320 values) was significantly ($p<0.05$) lower for those packaged in films containing LAE,
321 throughout the storage time. This suggests that LAE could interact with the meat
322 pigments, thus affecting redness. In contrast, the sample yellowness (b* values)
323 increased to a greater extent in fillets packaged in OC films. This significantly ($p<0.05$)
324 higher yellowness values could be related with the greater progression of lipid oxidation.
325 In fact, samples packaged in OC_LAE films (Figure 2c) exhibited the highest oxidation
326 levels and the lowest pH (Figure 1) at the end of storage. Figure 2d shows the total colour
327 difference quantified for the packaged samples throughout chilled storage at 4 ºC with
328 respect to the initial fresh chicken. A ΔE value of 1.5 was quantified between the different
329 fresh samples, which is at the limit of the visual perception of colour difference ($\Delta E \sim 1$ in
330 the CIEL*a*b* space; Hutchings, 1999). Most of the packaged samples exhibited colour
331 differences with respect to fresh chicken of under 5, which correspond to a reasonable
332 tolerance as regards the colour difference in food products (Hutchings, 1999). The
333 samples that were newly packaged in TP systems showed greater ΔE values due to their

surface dehydration, as commented on above, but this difference was mitigated throughout storage in line with the progressive water diffusion from the inner part of the breast. After 19 storage days, the samples packaged in the OC_LAE system also exceeded 5 ΔE units, which is attributable to the great oxidation progress and the pH associated change. Therefore, only samples packaged in the OC_LAE system exhibited an unacceptable colour change after 19 storage days, while they also presented excessive oxidation levels.

Microbial growth

The microbial quality of poultry meat is used as an indicator of freshness, since the growth of spoilage microorganisms can cause the development of unacceptable off-odours and off-flavours (Balamatsia et al., 2006). The recommended limits of acceptability for raw chicken are: m = 10^6 CFU/g for aerobic plate counts (acceptable limit) and M = 10^7 CFU/g (unacceptable limit) (EC, 2007). Thus, a value of 6 log CFU/g of meat for TVC was set as the maximum limit of acceptability.

Changes in the microbial counts of TVC, PB, LAB, AB and total coliforms in the samples are shown in Figure 3. Regardless of the packaging system, the PB counts were the highest, being the main spoilage microorganism, according to that reported for chilled meat (Murphy, O'Grady, & Kerry, 2013). LAB counts were also higher than the TVC counts and AB exhibited a similar behaviour, although the low O_2 concentration in the package could favour their growth (Rodríguez-Calleja et al., 2012). The initial counts of *E. coli* were below the detection limit (<1 log CFU/g) (data not shown) and a slight increase in the *E. coli* counts was noticed during the storage period in samples packaged in the LAE free films, reaching a level of 2.1 log CFU/g at the end of storage. TP_LAE or OC_LAE films were effective at maintaining the numerical presence of *E. coli* below the

359 detection limit, indicating the effectiveness of LAE as an antimicrobial with which to
360 preserve chicken breast fillets.

361 The counts of all bacteria increased during storage, but samples packaged in films
362 containing LAE (TP_LAE or OC_LAE) exhibited a delayed bacteria growth. Likewise,
363 lower counts were found in samples packaged in OC LAE-free films when compared to
364 those coated by LAE-free TP films. This difference in the fillet bacterial load reflect the
365 antimicrobial action of the Maillard compounds formed in the OC films, as previously
366 reported by Moreno et al. (2017) for marinated salmon samples packaged in similar films.
367 The greatest effects were observed in TVC and LAB. Similarly, OC_LAE films were more
368 effective than TP_LAE films at delaying the growth of most bacteria, which also points to
369 a combined effect of LAE and the Maillard compounds in the OC films. Moreno et al.
370 (2017) reported antilisterial activity for oxidized starch-gelatine films with and without
371 LAE, while they extend the self-life of marinated salmon in terms of microbial growth.

372 Therefore, in terms of microbial growth, the use of films containing LAE significantly
373 extended the shelf life of chicken breast filets. The limit of microbial acceptability (6 log
374 CFU/g) for TVC was reached after 12 days for samples packaged in the control TP_C
375 films, whereas this limit was reached after 16 days for fillets packaged in TP_LAE or
376 OC_C films and it was not reached throughout the 19 storage days in fillets in contact
377 with OC_LAE films. It is remarkable that the antimicrobial effect of LAE was more
378 effective in OC films than in TP films due to the combined action of the active compounds.
379 However, the shelf life of chicken samples packaged in OC_LAE was limited by lipid
380 oxidation, as previously commented on.

381 It is remarkable that OC_LAE films seem to lose effectiveness after 6 storage days when
382 the cell growth resumed in the samples. This may be due to the partial recovery and
383 growth of the bacteria in response to the stress induced by the antimicrobial compounds.
384 Nevertheless, a slower growth was also observed after 13 storage days. Likewise, the

obtained results indicated that LAB (Gram positive bacteria) were more sensitive to the antimicrobial action of LAE than total coliforms (Gram negative bacteria), which agrees with that reported in previous studies (Higueras et al., 2013; Muriel-Galet et al., 2015).

4. Conclusions

Films of S-G containing LAE greatly enhanced the shelf life of chicken breast fillets. The microbiological limit of acceptability for TVC was reached after 16 or 12 storage days for fillets packaged in TP films containing or not LAE, respectively, which represented a notable increase in the shelf life of the fillets. Those films containing oxidised starch (OC) without LAE, also extended the microbiological shelf life of the fillets by 4 days while OC_LAE films were the most effective at controlling microbial growth, but the presence of pro-oxidant compounds in OC films promoted lipid oxidation, which, in turn, affected the sample colour. Therefore, in samples packaged in OC films, the critical parameter to define the shelf life of the chicken breast fillets was the lipid oxidation and they are not recommended as packaging material of oxidation-sensitive foodstuffs. Starch-gelatin TP films containing LAE have the potential to be used as antimicrobial packaging material in order to increase the shelf life of chicken breast fillets.

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501

Table 1 Composition of the breast samples of both fresh control chicken and after two hours of contact with the packaging*.

Sample	Moisture %	Fat %	Protein %	Ashes %
Control Chicken	73.74 ±0.04^{b}	2.59 ±0.08^{ab}	23.7 ±0.3^{ab}	1.26 ±0.04^{a}
OC_C	73.5 ±0.6^{b}	2.4 ±0.7^{a}	23.7 ±0.3^{a}	1.24 ±0.06^{a}
OC_LAE	73.0 ±0.5^{b}	3.1 ±0.3^{b}	23.4 ±0.5^{a}	1.24 ±0.05^{a}
TP_C	72.1 ±0.7^{a}	3.15±0.10^{b}	24.9 ±1.3^{b}	1.25 ±0.02^{a}
TP_LAE	71.8 ±0.9^{a}	3.2 ±0.6^{b}	24.7 ±0.7^{b}	1.24 ±0.03^{a}

* All values are means ± standard deviations of duplicate data from three independent experiments (n=6).

OC: oxidized starch coating; TP: thermo-processed.

$^{a, b}$ Different superscripts letters in the same column indicate significant differences ($p<0.05$)

508 **Table 2** Values determined for CIE Lab* coordinates (L*, lightness; a*, redness; b*,
509 yellowness), lipid oxidation expressed as mg of MDA / kg of sample, and pH, for fresh
510 chicken and samples after two hours in contact with the different packaging systems*.

Sample	L*	a*	b*	pH	TBARS (mg MDA/kg sample)
Chicken control	56 ±3^{c}	1.29 ±0.14^{a}	6.4 ±1.9^{c}	6.07 ±0.09^{bc}	0.05 ±0.03^{a}
OC_C	55 ±2^{bc}	2.7 ±1.3^{c}	5.3 ±1.8^{b}	5.86 ±0.19^{a}	0.27 ±0.06^{cd}
OC_LAE	55 ±2^{b}	1.6 ±0.9^{ab}	5.3 ±1.8^{b}	6.00 ±0.17^{b}	0.33 ±0.09^{d}
TP_C	50 ±2^{a}	2.0 ±0.6^{b}	5.7 ±1.3^{bc}	6.10 ±0.11^{bc}	0.17 ±0.07^{b}
TP_LAE	50 ±2^{a}	1.8 ±1.1^{ab}	4.4 ±1.0^{a}	6.16 ±0.09^{c}	0.19 ±0.12^{bc}

511 * All values are means ± standard deviations of duplicate data from three independent experiments (n=6).

512 OC: oxidized starch coating; TP: thermo-processed.

513 $^{a, b, c, d}$ Different superscripts letters in the same column indicate significant differences ($p<0.05$).

514

515 **Table 3** Microbial counts for TVC, PB, LAB, AB, Coliforms and *E.coli* of the fresh chicken
516 breasts and samples after two hours in contact with the different packaging system.
517 Results expressed as log CFU/g of sample.

Sample	TVC	PB	LAB	AB	Coliforms	*E. coli*
Chicken control	4.3 ±0.3^{b}	5.1 ±0.1^{bc}	4.2 ±0.4^{bc}	4.6 ±0.4^{bc}	2.0 ±0.3^{ab}	1.49 ±0.16^{a}
OC_C	4.2 ±0.3^{b}	5.0 ±0.3^{bc}	4.3 ±1.2^{c}	4.8 ±0.8^{c}	2.7 ±0.3^{bc}	1.6 ±0.5^{a}
OC_LAE	3.0 ±0.3^{a}	4.2 ±0.6^{a}	2.3 ±1.3^{a}	3.5 ±0.7^{a}	1.5 ±0.5^{a}	ndg**
TP_C	5.1 ±0.5^{c}	5.5 ±0.5^{c}	4.4 ±0.4^{c}	4.9 ±0.3^{c}	3.0 ±0.7^{c}	2.1 ±0.7^{a}
TP_LAE	4.0 ±0.9^{b}	4.7 ±0.7^{b}	3.5 ±0.5^{ab}	4.1 ±0.7^{ab}	2.3 ±0.8^{b}	1.4 ±0.3^{a}

518 * All values are means ± standard deviations of duplicate data from three independent experiments (n=6).

519 OC: oxidized starch coating; TP: thermo-processed.

520 **ndg, no detected growth under the limit of detection.

521 $^{a, b, c}$ Different superscripts letters in the same column indicate significant differences ($p<0.05$)

522