Application of pectin-alginate and pectin-alginate-lauroyl arginate ethyl coatings to eliminate Salmonella enteritidis cross contamination in egg shells

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Abstract
This study highlights the potential application of pectin-alginate blend (PA) and pectin-alginate-LAE blend (PAL) coatings to eliminate Salmonella enteritidis 10,118 cross-contamination without changing the shelf-life of fresh eggs and their physico-chemical properties during storage at 7 °C for 42 days. Egg shells were dipped in a solution of Salmonella enteritidis 10,118 with a concentration of 7 x 10^6 cfu/ml to assess Salmonella cross-contamination. PA and PAL coatings did not have a significant effect on shelf-life based on physico-chemical properties. The egg shells treated with PA and PAL coatings had a significantly lower microbial population compared to the uncoated egg shells. PA and PAL coatings effectively inhibited the growth of Salmonella after 1 and 7 days of storage, respectively. In addition, no outgrowth was observed up to 42 days.

Practical applications
This study highlights the results of coating applications on eggs to enhance food safety. In the food industry, the only technology applied to eggs is brushing, however, this technique does not eliminate the safety risks such as Salmonella and other pathogenic bacteria. The coating enhances the shelf-life of eggs and their safety in terms of human consumption, by blocking the horizontal cross-contamination. Our results can be integrated with other studies to bring this technology from the lab to the egg industry.

1 | INTRODUCTION

Salmonella is one of the most common foodborne pathogens worldwide (Galis et al., 2013; Howard, O’Bryan, Crandall, & Ricke, 2012; Whiley & Ross, 2015). Consumption of contaminated food products is one of the most prevalent sources of Salmonella infection (Hur, Kim, Choi, & Lee, 2013; Pande, Gole, McWhorter, Abraham, & Chousalkar, 2015). According to the European Food Safety Authority (EFSA) and the European Center for Disease Prevention and Control report, in 2015 a total of 94,625 salmonellosis cases were reported by 28 EU countries at a rate of 21.2 cases per 100,000 population.

Food from animal sources, especially poultry and its derive products, including eggs, have been consistently involved in salmonellosis outbreaks (Bradon, 2006; EFSA, 2016; Jin, Gurtler, & Li, 2013; Luber, 2009). Egg-related salmonellosis represents a major risk for consumers and consequently plays an important public health role (Gole et al., 2014; Patrick et al., 2004). Egg-related salmonellosis is mainly caused by the two most commonly reported Salmonella serovars: Salmonella enteritidis and Salmonella typhimurium (EFSA, 2015; Howard et al., 2012). These serotypes are regarded as unrestricted, which means that they can cause infections in animals as well as in humans (Galiq et al., 2013; Whiley & Ross, 2015). S. enteritidis outbreaks occur relatively often in EU countries. With eggs as the most common source. Salmonella typhimurium outbreaks are relatively common in Australia and New Zealand (Greig & Ravel, 2009).

Salmonella is a member of the gram-negative Enterobacteriaceae family (D’Aoust & Maurer, 2007; Galiq et al., 2013; White, Baker, & James, 1997). Salmonella can survive, grow, and develop in a wide
Edible films and coatings can be derived from several sources, such as polysaccharides, proteins, and lipids (Caner, 2005). Generally, lipids are used to reduce water transmission, proteins provide mechanical stability, while polysaccharides are used to control oxygen and other gas transmissions. Among polysaccharides, pectin, and alginate have been reported as two of the main raw materials to obtain edible films and coatings because of their natural abundance, low cost, excellent film forming properties, and renewable components (Seol, Lim, Jang, Jo, & Lee, 2009; Valdés, Burgos, Jiménez, & Garrigós, 2015). The wide use of alginate and pectin in the food industry is enhanced by their lack of toxicity and allergenicity (Solak & Dyankova, 2014).

Pectin is used as an edible film and coating because of its gelling and thickening properties and its ability to retard lipid migration and moisture loss (Moalemian, Ramaswamy, & Maftoonazad, 2012). It is especially suitable for low moisture foods (Dhanapal, Rajamani, Kavitha, Yazhini, & Banu, 2012). Alginate is used for edible films and coatings because of its unique colloidal properties and ability to form strong gels (Rojas-grau et al., 2008). However, it exhibits poor moisture barrier properties because of its hydrophilic nature (Dhanapal et al., 2012).

In this study, a composite coating formulation was produced by blending pectin and alginate, to achieve a synergistic effect from the combined features of pure components. Both pectin and alginate are natural anionic polysaccharides and undergo chain-chain association (da Silva, Bierhalz, & Kieckbusch, 2009).

 Lauroyl arginate ethyl (LAE) is considered to be one of the most powerful antimicrobial GRAS food additive substances (Muriel-Galet, Carballo, Hernández-Muñoz, & Gavara, 2016). The incorporation of LAE into edible coatings can enhance the activity of coatings in protecting food from microbial spoilage and therefore extending the postharvest life and quality (Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015).

Egg quality includes a number of phenomena related to the shell, albumin, and yolk, which can be subdivided into external and internal quality characteristics, such as moisture loss, albumin pH, yolk index, yolk color, egg shell color, and Haugh unit (H; Caner & Cansiz, 2007; Morsy, Sharoba, Khalaf, El-Tanahy, & Cutter, 2015). Morsy et al. (2015) studied the effects of pullulan coatings on the microbiological qualities, physical properties, and freshness parameters of fresh eggs. Pullulan coatings were shown to minimize weight loss (<1.5%) and preserved the albumen and yolk quality of eggs for 3 weeks longer than noncoated eggs at 25 °C. Upadhyaya et al. (2016) demonstrated that when phytochemicals are added to pectin and arabic-gum based coatings, they were effective in reducing S. enteritidis on egg shells. In addition, coating egg shells with chitosan preserves the internal quality and extending shelf-life of eggs and acts as a protective barrier against contamination from S. enteritidis (Hur et al., 2013).

The main objective of this study was to develop an edible egg coating based on a pectin-alginate blend (PA) with LAE as an antimicrobial compound to evaluate the effects of coating on the physicochemical properties of eggs during storage at 7 °C for 42 days. The effectiveness of the coating against S. enteritidis cross-contamination was also examined.
2 MATERIALS AND METHODS

2.1 Chemicals and medias

The coating contained low-methoxyl amidated pectin CF 010D (Herbstreith & Fox, Neuenburg, Germany) and sodium alginate (Reire, Reggio Emilia, Italy). The antimicrobial compound LAE was provided as MIRENAT-G (90% glycerol, 10% LAE) by Vedeqa (Terrassa, Barcelona, Spain). Brain Heart Infusion (BHI) agar was purchased from Bio- life (Milan, Italy) and Salmonella-Shigella (SS) agar was purchased from Lioflichem srl (Roseto degli Abruzzi, Italy). All the other chemicals were purchased from Sigma-Aldrich (Milan, Italy).

2.2 Experimental design

Conventional “AA” class, medium size eggs (53–63 g) were purchased from a local shop (Reggio Emilia, Italy). The eggs had been laid 8 days before being purchased. The eggs were individually marked and randomly assigned to each experiment. Two treatments were evaluated: a coating with PA and a coating with pectin-alginate-LAE blend (PAL). Uncoated eggs were used as controls (C). The first experiment monitored the physical and chemical features and the microbiological charge of the eggs every 7 days for up to 42 days (1, 7, 14, 21, 28, 35, and 42; Caner & Yüceer, 2015). In the second experiment, the egg shells were inoculated with S. enteritidis 10,118 (Zooprophylactic Institute of Palermo) before the coating treatments (Jin et al., 2013). The microbial charge was examined on the same days as the other analysis. Nine eggs per treatment were analyzed three times at each storage interval during the 42 days of analysis.

2.3 Coating formulation

Distilled water was used as the solvent for preparing the film solutions. The pectin-alginate coating (PA) was formulated as follows: pectin 15 g/L, sodium alginate 10 g/L, glycerol 6.75 ml/L, sodium bicarbonate 2 g/L. Sodium bicarbonate was used to neutralize the pH of the coating and to prevent negative effects on the calcium carbonate of the egg shells. The compounds were mixed with constant stirring (750 rpm) at 40 °C until the polymers had been completely dissolved. The final coating solution was degassed under vacuum for 15 min. The same procedure was applied for the PAL, substituting glycerol with 7.5 ml/L of MIRENAT-G.

2.4 Coating application

Each egg was uniformly sprayed with the coating formulation, and any excess coating solution was drained off. The eggs were then reticulated with anhydrous CaCl2 solution (50 g/L). Each coated egg was picked up with beeswax-coated tweezers and placed on a petri dish with a diameter of 140 mm, covered with beeswax to avoid gel adhesion to the bottom of the petri dish. After drying at 25 °C for 1 hr under ventilation (30 m/s), the eggs were stored for 1 day at 7 °C before the analysis. Each egg was weighed before storage using a laboratory scale (BL 2002 XS BALANCE, China).

2.5 Physical and chemical analysis of the eggs

On each day of the analysis (1, 7, 14, 21, 28, 35, and 42), the following parameters were measured.

2.5.1 Moisture loss

The loss of water and the consequent weight loss were calculated by subtracting the final weight from the initial weight of the eggs divided by the initial weight for each day of the analysis. The percentage moisture loss was calculated by multiplying the moisture loss by 100. A European 1,700 technical scale was used for this measurement (Gibertini, Novate Milanese, Milan, Italy).

2.5.2 Shell and yolk color

The egg shell and yolk color were measured with a Minolta Chroma Meter Model CR-400 (Minolta Co., Ltd., Osaka, Japan). Three egg shells and yolks were analyzed in three different points and the measurements were averaged. The results were expressed as L value (Lightness), a value (redness), and b value (yellowness). ΔEab indicated the size of the color differences compared with the control and was calculated by the following equation (Caner, 2005):

\[ ΔEab^* = \sqrt{[\Delta L^*]^2 + [\Delta a^*]^2 + [\Delta b^*]^2} \]

where \( \Delta L^* = L \) coating – L control, \( \Delta a^* = a \) coating – a control, \( \Delta b^* = b \) coating – b control.

2.5.3 Haugh unit

The HU was measured before separating the yolk from the albumen. A digital caliper (CDJAAB 15, Borletti, Antegnate, Bergamo, Italy) was used to measure the height of the albumen placed on a glass surface. The height was the mean of three measurements in three different points of the albumen (Yüceer, Aday, & Caner, 2016). The HU was calculated with the following formula:

\[ HU = 100 \times \log (H - 1.7 \times G0.37 + 7.6) \]

where H is the height of the albumen (mm) and G is the weight of the eggs (g).

2.5.4 Albumen pH

After the eggs had been broken, the albumen was separated from the yolk with a glass pipette (50 ml) and the small volumes of albumen were homogenized for 20 s in a blender. The pH of homogenized albumen was measured with a pH meter (VWR, pH110, Milan, Italy).

2.5.5 Yolk index

The yolk index was measured after separating the yolk from the albumen, with a digital caliper (CDJAAB 15, Borletti, Antegnate, Bergamo, Italy) to estimate the height and width of the yolk placed on a glass surface. The percentage yolk index was calculated with the following formula:

\[ YI = \left( \frac{H_{yolk}}{W_{yolk}} \right) \times 100 \]

where H is the height (mm) and W is the width of the yolk (mm).
2.5.6 | Pore number and dimension
An optical microscope (CHK model, Olympus, Japan) with 32x magnification was used to determine the number and dimension of the pores of the egg shells. Before the analysis, the egg shells were treated with nitric acid using a modified protocol (Tyler, 1953). Briefly, the egg shell was dipped in 51% nitric acid solution for 25 s, the reaction was stopped with NaOH solution 4 M to prevent excessive corrosion by the acid. The egg shells were then washed with double distilled water and examined under the microscope. The number and dimension of the pores were analyzed using the ImageJ (Image processing and analysis in java, wsr@nih.gov) after digitalizing the view field of the microscope. Three fragments were taken from different areas of the shell which were then analyzed for each egg. For eggs treated with the coating formulation, the coating was peeled off, before the acid treatment to prevent protection of the shell and a false result in terms of the real dimension of the pores.

2.5.7 | Determination of the mesophilic aerobic charge
Each egg was washed with 100 ml sterile saline solution (0.9% NaCl) inside a sterile blender bag, which was gently rubbed by hand for 60 s to resuspend the microorganisms. Appropriate dilutions of the suspension were plated onto BHI agar plates and incubated at 30 °C for 24 hr. The same samples were also inoculated onto SS agar plates to ensure the presence of Salmonella contaminants. Three eggs per group were analyzed on each sampling day.

2.5.8 | Salmonella challenge test
Eggs were sterilized by immersion in 90% ethanol, dried and contaminated by dipping in an aqueous solution containing 7 x 10^6 cfu/ml of S. enteritidis (Botey-Saló et al., 2012). Eggs from the treated group were coated as described in Section 2.4 and stored at 7 °C for 42 days. For each day of the analysis (1, 7, 14, 21, 28, 35, and 42), three eggs per group were rolled on an SS agar plate (140 mm diameter) to evaluate the horizontal cross-contamination of the eggs. The plates were incubated at 30 °C for 24 hr. Three eggs per group were analyzed on each sampling day.

2.5.9 | Statistical analysis
Analysis of variance was carried out on all the measured parameters of the control and coated eggs during the storage time (42 days at 7 °C). All the experiments were performed in triplicate. The results were statistically analyzed using arithmetic means, and SD. p-Values <.05 were considered as statistically significant. Statistix 9 (https://www.statistix.com/) was used for data analysis.

3 | RESULTS AND DISCUSSION

3.1 | Moisture loss
The weight loss of the control (C) and coated eggs (PA and PAL) during 42 days of storage at 7 °C is shown in Figure 1. The weight of the eggs decreased until 35 days in C, PA, and PAL groups. The highest weight loss was obtained for the C after 35 days of storage with 1.67%. The weight loss continued to decrease until 42 days in PA and PAL with 1.6 and 1.5%, respectively. No significant differences were found among C, PA, and PAL eggs during 42 days of storage at 7 °C.

Kim, No, and Prinyawiwatkul (2008) reported no significant differences in weight loss among chitosan-coated eggs with different plasticizer types after 5 weeks of storage. Jin et al. (2013) reported that during storage at either 7 or 4 °C eggs coated with chitosan lost approximately 4% of the moisture, while uncoated eggs lost approximately 6% of their weight, and all the coated eggs had significantly less weight loss than the uncoated eggs. The weight loss of the eggs during storage was caused by the evaporation of water and the loss of carbon dioxide from the albumen through the shells. This parameter can be used as an index for egg quality, and prevention of weight loss is important for maintaining egg quality (Caner, 2005; Jin et al., 2013). Differences in weight loss among studies may be due to the storage conditions, temperature, egg size, or shell porosity (Caner, 2005; Jo, Ahn, Liu, Kim, & Nam, 2011). This study showed that eggs coated with pectin-alginate did not negatively affect the evaporation process, and coated eggs showed similar trends as the controls after 42 days of storage.

FIGURE 1  Moisture loss expressed in percentage of weight loss during the storage at 7 °C for 42 days. The values are relative to the weight of eggs in comparison with the starting weight. Different letters within a column indicate significant differences p < .05 (in this specific case there are no significant differences between means). C = control; PA = pectin-alginate blend; PAL = pectin-alginate-LAE blend

TABLE 1  Difference between the yolk color of the control (C) and the coated eggs (PA and PAL; expressed as ΔEab during storage at 7 °C for 42 days

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PA</td>
<td>2.49</td>
<td>4.48</td>
<td>11.45</td>
<td>2.08</td>
<td>0.93</td>
<td>4.06</td>
<td>0.94</td>
</tr>
<tr>
<td>PAL</td>
<td>4.98</td>
<td>3.48</td>
<td>1.40</td>
<td>1.22</td>
<td>2.91</td>
<td>1.56</td>
<td>5.84</td>
</tr>
</tbody>
</table>

Means ± SD of 3 measurements on 3 eggs. Different letters within a column indicate significant differences (p < .05).

3.2 | Shell color

The ΔE between the control color at Day 1 and all the treatments (control included) in the subsequent analysis times are shown in Table 1: the ΔE was calculated for each day of the analysis among C, PA, and PAL. The results obtained are in agreement with previous studies demonstrating that coating treatments can change the color of the eggs due to the difference in the diffraction of light due to the coating. ΔE values lower than 3 cannot be detected by the human eye, while values higher than 3 can be attributed to the glossiness of the coating (Kim, Daeschel, & Zhao, 2008).

3.3 | Haugh unit

The HU measures the egg protein quality and is often measured based on the height of the albumen and the egg weight. A fresh and good quality egg has a HU index of around 80 which decreases physiologically with the aging of the egg (Caner & Yüceer, 2015). The initial value of HU represents the main marker to evaluate the egg shelf-life as well as the storage conditions (Figueiredo et al., 2014). Changes in the HU of the C, PA, and PAL groups are shown in Table 2. HU at day 1 was 83.5, 87.34, and 90.04 for C, PA, and PAL, respectively. Coated eggs had a significantly higher HU value than the control eggs at Day 1. HU decreased for all groups during the storage time of 42 days, which is in agreement with previous studies (Caner, 2005; Caner & Yüceer, 2015; Morsy et al., 2015). The HU of coated eggs ranged from 73.71 to 78.34 after 42 days of storage; HU did not show significant differences in comparison with the control eggs (74.91). The present study showed that the coating of eggs with the PA and PAL blend did not influence the egg protein quality.

3.4 | Albumen pH

Aside the moisture loss and HU, albumen pH can also be used as an indicator of egg quality (Caner & Yüceer, 2015; Kim, Daeschel, et al., 2008; Morsy et al., 2015; Nongtaodum et al., 2013). Changes in albumen pH of the C, PA, and PAL groups during 42 days of storage at 7 °C are shown in Table 3. After 42 days of storage, the albumen pH of the control eggs increased from an initial value of 8.48–9.61, while those of the PA and PAL coated eggs increased from 8.55 and 8.58 to 10.02 and 9.93, respectively. No significant differences in pH values among C, PA, and PAL were observed throughout the 42 days of storage.

Morsy et al. (2015) observed that the albumen pH of noncoated eggs increased after 5 weeks of storage at room temperature from an initial value of 8.02–8.48, while those of coated eggs with pullulan and pullulan containing nisin increased to 8.15 and 8.14, respectively. Caner and Yüceer (2015) reported that a protein-based coating using whey protein isolate (WPI), whey protein concentrate (WPC), zein, and shellac had a significant effect on albumen pH. The albumen pH for uncoated eggs ranged from initially 7.50–9.50 at the end of 5 weeks of storage at 24 °C, while for coated eggs, albumen pH values reached 9.33 (WPC), 9.31 (WPI), 8.90 (Zein), and 8.83 (shellac). During the shelf-life of eggs, CO2 is released from the albumen to the external environment through the egg shell pores. This CO2 loss increases the albumen pH during storage. The carbon dioxide loss from the breakdown of carbonic acid in albumen results in changes in the bicarbonate buffer system; which consequently causes an increase in the albumen pH (Biladeau & Keener, 2009; Nongtaodum et al., 2013; Yüceer et al., 2016). Integrated with other parameters such as HU, YI, the numbers and dimension of the pores, the pH value can be used to evaluate the shelf-life of eggs.

TABLE 2  Haugh unit values during the storage of treated (PA and PAL) and control eggs (C) at 7 °C for 42 days

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>83.50 ± 2.58a</td>
<td>81.95 ± 1.60a</td>
<td>81.49 ± 0.69a</td>
<td>81.28 ± 1.94a</td>
<td>76.34 ± 3.32a</td>
<td>75.60 ± 2.97a</td>
<td>74.91 ± 5.35a</td>
</tr>
<tr>
<td>PA</td>
<td>87.34 ± 2.92b</td>
<td>86.89 ± 4.15a</td>
<td>81.62 ± 2.90a</td>
<td>80.89 ± 1.83a</td>
<td>80.03 ± 2.14a</td>
<td>79.48 ± 1.33a</td>
<td>78.34 ± 7.29a</td>
</tr>
<tr>
<td>PAL</td>
<td>90.04 ± 2.07c</td>
<td>82.92 ± 5.54a</td>
<td>81.94 ± 2.79a</td>
<td>78.60 ± 7.792a</td>
<td>78.81 ± 2.72a</td>
<td>76.07 ± 5.90a</td>
<td>73.71 ± 3.58a</td>
</tr>
</tbody>
</table>

Means ± SD of 3 measurements on 3 eggs. Different letters within a column indicate significant differences (p < .05).

TABLE 3  Albumen pH values during storage at 7 °C for 42 days, for the control (C) and the coated eggs (PA and PAL)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>8.48 ± 0.05a</td>
<td>8.74 ± 0.04a</td>
<td>8.75 ± 0.03a</td>
<td>9.04 ± 0.06a</td>
<td>9.55 ± 0.02a</td>
<td>9.60 ± 0.67a</td>
<td>9.61 ± 0.02a</td>
</tr>
<tr>
<td>PA</td>
<td>8.55 ± 0.06a</td>
<td>8.96 ± 0.06a</td>
<td>8.99 ± 0.05a</td>
<td>9.00 ± 0.01a</td>
<td>9.64 ± 0.04b</td>
<td>9.64 ± 0.03a</td>
<td>10.02 ± 0.09a</td>
</tr>
<tr>
<td>PAL</td>
<td>8.58 ± 0.11a</td>
<td>8.88 ± 0.01a</td>
<td>8.99 ± 0.04a</td>
<td>9.03 ± 0.01a</td>
<td>9.61 ± 0.02b</td>
<td>9.63 ± 0.07a</td>
<td>9.93 ± 0.06a</td>
</tr>
</tbody>
</table>

Means ± SD of 3 measurements on 3 eggs. Different letters within a column indicate significant differences (p < .05).
The results of this study indicated that coating eggs with PA and PAL blends did not influence the carbon dioxide release through the shell, thus providing evidence that the egg quality and consequently the egg shelf-life did not change after coating.

3.5 | Yolk color

Table 4 shows the $\Delta E$ calculated for each day of analysis between the treatments and the control. The table shows that a large variation is time dependent and $\Delta E$ increased with time in all the treatments, due to the aging of the eggs (Figueiredo et al., 2014). Conversely, the color variation was not significant among the different treatments. The values shown in Table 4 did not display a decreasing linear trend, and the differences among C, PA, and PAL were not statistically significant. A significant difference was evident at Day 42, due to the end of the shelf-life period, in line with the pH trend described in Section 3.4.

3.6 | Yolk index

The yolk index is an indicator of freshness, obtained by the measurements of the yolk height and width. A yolk index decrease indicates a gradual deterioration of the vitelline membrane and liquefaction of the yolk, caused by water diffusion from the albumen (Yüceer & Caner, 2014). Table 5 shows the changes in the yolk index of C, PA, and PAL during 42 days of storage at 7°C. The yolk index of the control eggs was lower than PA and PAL during storage. After 42 days of storage, the yolk index of C decreased from 39.96 to 30.99%. The yolk index of PA and PAL decreased from 38.45% and 39.06% to 33.2% and 32.72%, respectively. No significant differences were observed among C, PA, and PAL. However, the yolk index value of coated eggs was higher than the control eggs.

Nongtaodum et al. (2013) reported that the yolk index values of non-, glycerol-, and oil-coated eggs decreased from the initial value of 0.45–0.21, 0.23, and 0.34–0.36, respectively, after 5 weeks of storage at 25°C. The yolk index values of that study showed no differences among all the oil-coated eggs but were significantly higher than the non- and glycerol-coated eggs throughout the 5 weeks of storage. In the present study, the eggs had an initial yolk index of 39.96, 38.45, and 39.06% for C, PA, and PAL, respectively, without statistical differences. After 42 days of storage, the yolk index decreased to 30.99, 33.2, and 32.72% for C, PA, and PAL, respectively, without statistical differences during the period. Only on day 35 were statistically significant differences ($p < .05$) recorded, probably due to the different initial quality of the eggs. It is interesting that on Day 42, all the eggs maintained an optimal yolk index value, in contrast with the pH and the HU values recorded during the experiment. A plausible explanation could be the storage conditions of the eggs. The nature of the yolk is more stable at a refrigerated temperature (7°C) compared with the albumen pH.

3.7 | Pore numbers and sizes

Tables 6 and 7 show the number and size respectively of the pores on the shell eggs of 42 days of storage at 7°C. After 42 days of storage, the mean number of pores in the control eggs increased from 3,729 to 11,072, while pore numbers in PA and PAL increased from 4,697 and 4,957 to 9,845 and 10,754, respectively. No significant differences were observed among the control and coated eggs. In contrast, Leleu et al. (2011) reported that the numbers of pores in shell eggs were significantly reduced by chitosan coatings.

### Table 4
Difference between the yolk color of the control (C) and the coated eggs (PA and PAL) expressed as $\Delta E_{ab}$ during storage at 7°C for 42 days

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PA</td>
<td>3.76</td>
<td>2.61</td>
<td>2.72</td>
<td>1.69</td>
<td>1.88</td>
<td>1.89</td>
<td>1.31</td>
</tr>
<tr>
<td>PAL</td>
<td>3.33</td>
<td>1.80</td>
<td>1.90</td>
<td>2.10</td>
<td>3.55</td>
<td>4.88</td>
<td>3.65</td>
</tr>
</tbody>
</table>

### Table 5
Yolk index values during storage at 7°C for 42 days of the control (C) and the coated eggs (PA and PAL)

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>39.96 ± 0.86a</td>
<td>38.55 ± 0.61a</td>
<td>37.43 ± 1.68a</td>
<td>36.00 ± 1.76a</td>
<td>34.63 ± 2.02a</td>
<td>32.49 ± 1.42a</td>
<td>30.99 ± 1.35a</td>
</tr>
<tr>
<td>PA</td>
<td>38.45 ± 0.62a</td>
<td>38.39 ± 0.84a</td>
<td>35.85 ± 1.10a</td>
<td>35.54 ± 2.16a</td>
<td>34.17 ± 0.77a</td>
<td>33.93 ± 1.33b</td>
<td>33.20 ± 4.4a</td>
</tr>
<tr>
<td>PAL</td>
<td>39.06 ± 2.51a</td>
<td>36.85 ± 1.24a</td>
<td>36.81 ± 0.17b</td>
<td>35.05 ± 0.02a</td>
<td>34.44 ± 4.41a</td>
<td>33.72 ± 1.5c</td>
<td>32.73 ± 1.88a</td>
</tr>
</tbody>
</table>

Means ± SD of 3 measurements on 3 eggs. Different letters within a column indicate significant differences ($p < .05$).

### Table 6
Number of pores during the storage of treated (PA and PAL) and control eggs (C) at 7°C for 42 days

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 35</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>3,729 ± 1123a</td>
<td>7,378 ± 2274a</td>
<td>7,771 ± 475a</td>
<td>8,903 ± 891a</td>
<td>9,304 ± 2748a</td>
<td>10,403 ± 2139a</td>
<td>11,072 ± 1191a</td>
</tr>
<tr>
<td>PA</td>
<td>4,697 ± 795a</td>
<td>6,439 ± 1509a</td>
<td>6,650 ± 495a</td>
<td>6,710 ± 964a</td>
<td>7,261 ± 1490a</td>
<td>8,386 ± 1145a</td>
<td>9,845 ± 3952a</td>
</tr>
<tr>
<td>PAL</td>
<td>4,957 ± 874a</td>
<td>5,875 ± 1109a</td>
<td>6,593 ± 1680a</td>
<td>6,997 ± 696a</td>
<td>7,790 ± 1687a</td>
<td>10,041 ± 1274a</td>
<td>10,754 ± 2372a</td>
</tr>
</tbody>
</table>

Means ± SD of 3 measurements on 3 eggs. Different letters within a column indicate significant differences ($p < .05$).
3.8 | Determination of mesophilic aerobic charge

Total mesophilic aerobic bacteria counts after 24 hr of incubation are shown in Figure 2. Coating formulations decreased the microbial load by one and two orders of magnitude for PA and PAL on each day of the analysis, respectively. A significant difference among C, PA, and PAL was maintained throughout the whole test period, despite some variations probably due to a different initial charge on each individual egg.

The results of the total mesophilic count of control on eggs are in agreement with those reported by Leleu et al. (2011), Morsy et al. (2015), and Upadhyaya et al. (2016). The effects of the coatings on the egg shell bacteria population showed the same decreasing trend in the coated eggs among the studies, while the absolute values of the count were different, due to the different polymer and antimicrobial content within the coating formulation.

3.9 | Salmonella enteritidis challenge test

As shown in Table 8, colonies on eggs from the C group were two orders of magnitude higher than PA and PAL. The S. enteritidis load decreased during 42 days of storage in the control and coated eggs in all the groups. After 42 days, 107 colonies were still found in the control group. In the C group, cross-contamination occurred for up to 42 days, when 107 colonies migrated from the egg shells to the Petri dishes. In the PA group, cross-contamination was blocked at Day 14. Finally, with PAL from Day 7 no colonies were detected on the Petri dishes. Thus, PAL blocks cross-contamination earlier than the other two treatments.

It is, therefore, possible to confirm that the coating does not allow Salmonella cells to arrive from the eggs to the surfaces; in general, the coating blocks any cross-contamination. In the formulation with LAE, this blocking is enhanced due to the strong antimicrobial activity of LAE (Jin et al., 2013).

4 | CONCLUSIONS

The aim of this study was to develop an edible egg coating to protect against Salmonella cross-contamination without changing the shelf-life of the fresh egg and its properties. This work demonstrated that the coatings with the PA did not negatively affect the quality parameters or the shelf-life of the eggs. In Europe, any treatment related to the food safety of eggs (apart from brushing) is strictly forbidden, due to the potential damage to the shell structure that will affect all the other
parameters. However, brushing does not guarantee protection against *Salmonella* contamination. The development of technologies such as an antimicrobial coating is, therefore, an effective alternative for the food sector to ensure the quality and safety of the food product. The polymer structure and pH of the coatings described in this study did not damage the structure of the shell. Regarding the microbial charge, the coatings significantly reduced the total aerobic mesophilic population, thus providing a higher level of safety for the consumer. Finally, the cross-contamination test showed positive results in the control of *S. enteritidis*, as it drastically reduced cross-contamination, which is one of the main causes of salmonellosis in Europe.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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