Received: 14 March 2018 | Revised: 27 July 2018 | Accepted: 29 August 2018

DOI: 10.1111/jfs.12567

WILEY Journal of Food Safety

ORIGINAL ARTICLE

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

Riccardo De Leo[1] | Andrea Quartieri[1] | Hossein Haghighi[1] | Silvia Gigliano[1] | Elisa Bedin[1] | Andrea Pulvirenti[1,2]

[1]Department of Life Sciences, University of Modena and Reggio Emilia, Reggio Emilia, Italy

[2]Interdepartmental Research Centre BIOGEST-SITEIA, University of Modena and Reggio Emilia, Reggio Emilia, Italy

Correspondence
Andrea Pulvirenti, Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42122 Reggio Emilia, Italy.
Email: andrea.pulvirenti@unimore.it

Funding information
Academic Found for Research of the University of Modena and Reggio Emilia, Grant/Award Number: FAR 2015; PROT.N. 81705

Abstract

This study highlights the potential application of pectin-alginate blend (PA) and pectin-alginate-LAE blend (PAL) coatings to eliminate *Salmonella enteriditis* 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 (Galiş 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 derivate products, including eggs, have been consistently involved in salmonellosis outbreaks (Braden, 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 (Galiş 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; Galiş et al., 2013; White, Baker, & James, 1997). *Salmonella* can survive, grow, and develop in a wide

range of environmental conditions combined with a wide range of hosts, which make it difficult to control (White et al., 1997). Foods with low water activity can only stop the reproduction of *Salmonella* but not its survival (Al-Moghazy, Boveri, & Pulvirenti, 2014). This organism is heat-sensitive and can be readily destroyed at pasteurization temperature (Wybo et al., 2004).

Eggs can be contaminated with *Salmonella* by two pathways: vertical and horizontal transmission. In vertical transmission, eggs are infected from the spoiled reproductive tissues of hens during their formation in the hen's ovary and oviduct. In horizontal transmission, eggs are exposed to a contaminated environment, that is, in the presence of feces on the egg shell after being laid by the hen. Due to the humidity of the egg shell, storage at room temperature and shell damage, bacteria may be transferred through the egg shell and membranes and thus negatively impact on the egg's content (Braden, 2006; De Reu, Grijspeerdt, Messens, & Heyndrickx, 2006; Gole et al., 2014; Howard et al., 2012; Whiley & Ross, 2015).

Jin et al. (2013) found that more than 90% of the cases of foodborne salmonellosis due to *S. enteritidis* are caused by contaminated egg shells. The moment of breaking is another delicate practice where the bacteria can be transmitted from the shell to its content, thus causing potential direct or indirect cross-contamination to other foods (Botey-Saló, Anyogu, Varnam, & Sutherland, 2012; Luber, 2009).

Egg producers employ various procedures such as dry cleaning, washing with water, chilled storage, electrolyzed oxidizing water, ozone, ultrasound, microwaves, irradiation (from high-energy gamma rays, X-rays, and accelerated electrons), gas plasma, ultraviolet light, and pulsed light technology to decontaminate egg shells and diminish the risk of salmonellosis (Galiş et al., 2013; Howard et al., 2012; Upadhyaya et al., 2016; Whiley & Ross, 2015). However, these methods have not been accepted and implemented worldwide. Just to mention a few examples, egg washing is applied in the United States, Canada, Australia, and Japan, while in the EU, it is currently banned. This is because according to the EU's egg regulation it might compromise—partially or completely—the cuticle layers, which represent an effective and natural barrier against bacteria due to their antimicrobial properties. Thus, egg washing might encourage the transfer of harmful bacteria such as *Salmonella* from the outside to the inside of the egg. Furthermore, with these techniques eggs can no longer be classified as fresh (EFSA, 2015; Galiş et al., 2013).

As an alternative and innovative solution, the concept of edible films and coatings has received considerable attention in the egg industry. This is because of the advantages, including the capacity to improve the shelf-life of the egg, the preservation of the egg's internal quality, the minimization of weight loss, the reduction in breakage, and increase in shell strength. Edible films and coatings act as a semipermeable barrier against oxygen, carbon dioxide and moisture, thus reducing respiration, water loss, and oxidation rates. They can also be applied on the egg shell, thus acting as carriers of substances such as natural antimicrobial compounds aimed at preventing the growth of pathogenic bacteria such as *Salmonella* (Ali, Maqbool, Ramachandran, & Alderson, 2010; Biladeau & Keener, 2009; Bourtoom, 2008; Dhall, 2013; Rojas-grau, Tapia, & Martin-Belloso, 2008; Sánchez-Ortega et al., 2014).

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 (Moalemiyan, 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 (HU; 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 Vedeqsa (Terrassa, Barcelona, Spain). Brain Heart Infusion (BHI) agar was purchased from Biolife (Milan, Italy) and Salmonella-Shigella (SS) agar was purchased from Liofilchem 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. enteriditis* 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 $CaCl_2$ 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). Δ*Eab* indicated the size of the color differences compared with the control and was calculated by the following equation (Caner, 2005):

$$\Delta Eab^* = \sqrt{\left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]}$$

where ΔL^* = *L* coating – *L* control, Δa^* = *a* coating – *a* control, Δb^* = *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:

$$\mathrm{HU} = 100 \times \log\left(H - 1.7 \times G\,0.37 + 7.6\right)$$

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:

$$\mathrm{YI} = (H\,\mathrm{yolk}/W\,\mathrm{yolk}) \times 100$$

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.

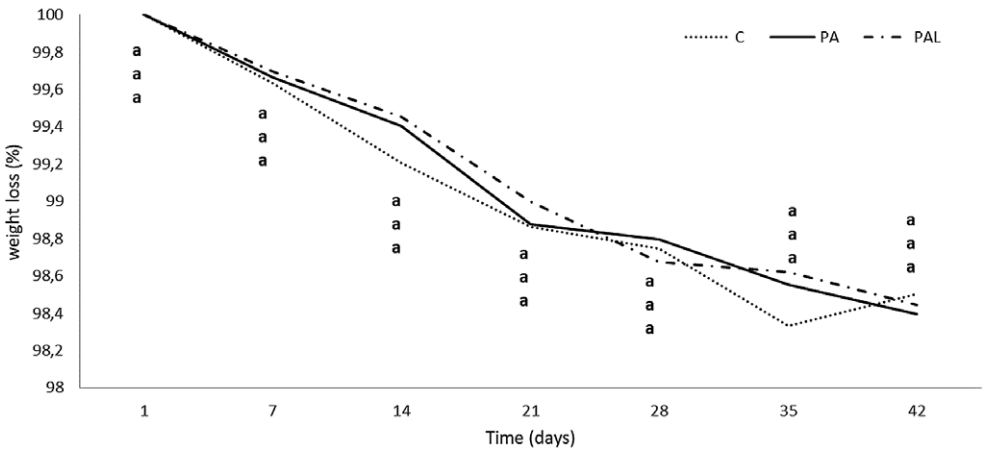

FIGURE 1 Moisture loss expressed in percentage of weight loss during the storage at 7 °C for 42 days. The values are relative to the weight of eggs in comparison with the starting weight. Different letters within a column indicate significant differences $p < .05$ (in this specific case there are no 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

	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
C	0	0	0	0	0	0	0
PA	2.49	4.48	11.45	2.08	0.93	4.06	0.94
PAL	4.98	3.48	1.40	1.22	2.91	1.56	5.84

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

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

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 protein quality, and its expression provides an indication of 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

Beside 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, CO_2 is released from the albumen to the external environment through the egg shell pores. This CO_2 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 3 Albumen pH values during storage at 7 °C for 42 days, for the control (C) and the coated eggs (PA and PAL)

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

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 Δ*E* calculated for each day of analysis between the treatments and the control. The table shows that a large variation is time dependent and Δ*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 the 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 during 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 Δ*Eab* during storage at 7 °C for 42 days

	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
C	0	0	0	0	0	0	0
PA	3.76	2.61	2.72	1.69	1.88	1.89	1.31
PAL	3.33	1.80	1.90	2.10	3.55	4.88	3.65

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

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

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

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

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

TABLE 7 Dimensions of pores (μm) during the storage of control eggs (C) and treated (PA and PAL) at 7 °C for 42 days

	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
C	81 ± 10a	73 ± 20a	31 ± 6a	63 ± 27a	63 ± 14a	66 ± 19a	44 ± 8a
PA	30 ± 8b	48 ± 8a	32 ± 1a	63 ± 12a	52 ± 2a	47 ± 14a	39 ± 6a
PAL	45 ± 10b	51 ± 10a	23 ± 3b	57 ± 13a	58 ± 2a	64 ± 17a	53 ± 15a

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

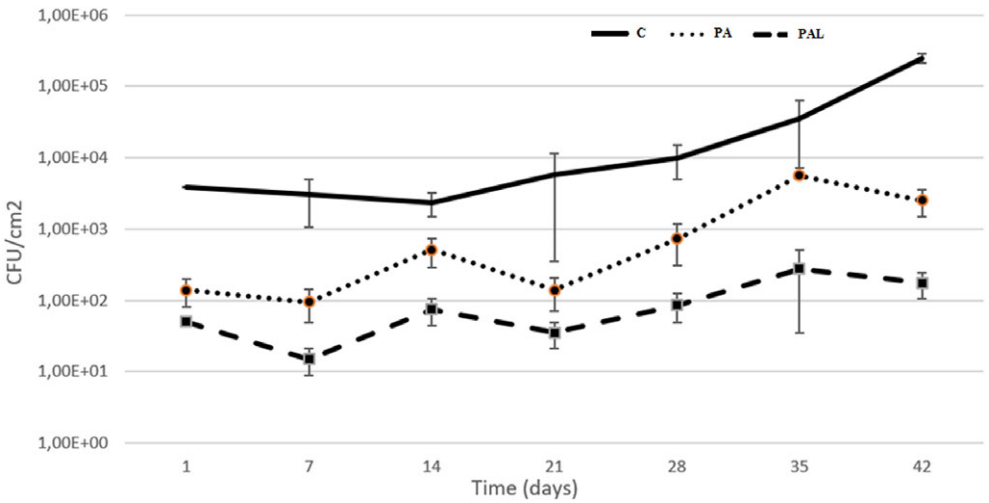

FIGURE 2 Development of total mesophilic aerobic bacteria population during the storage at 7 °C for 42 days

TABLE 8 Cross-contamination of *S. enteritidis* (cfu/egg) during storage of control eggs (C) and treated (PA and PAL) at 7 °C for 42 days

	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
C	849 ± 8	132 ± 9	120 ± 7	112 ± 6	110 ± 2	106 ± 4	107 ± 6
PA	11 ± 6	9 ± 3	0	0	0	0	0
PAL	9 ± 7	0	0	0	0	0	0

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.

ACKNOWLEDGMENT

This research was supported by the Academic Fund for Research (FAR 2015; PROT.N. 81705) of the University of Modena and Reggio Emilia.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ORCID

Andrea Pulvirenti http://orcid.org/0000-0002-8479-8878

REFERENCES

Ali, A., Maqbool, M., Ramachandran, S., & Alderson, P. G. (2010). Gum arabic as a novel edible coating for enhancing shelf-life and improving postharvest quality of tomato (*Solanum lycopersicum* L .) fruit. *Postharvest Biology and Technology*, *58*(1), 42–47. https://doi.org/10.1016/j.postharvbio.2010.05.005

Al-Moghazy, M., Boveri, S., & Pulvirenti, A. (2014). Microbiological safety in pistachios and pistachio containing products. *Food Control*, *36*(1), 88–93. https://doi.org/10.1016/j.foodcont.2013.07.030

Biladeau, A. M., & Keener, K. M. (2009). The effects of edible coatings on chicken egg quality under refrigerated storage. *Poultry Science*, *88*(6), 1266–1274. https://doi.org/10.3382/ps.2008-00295

Botey-Saló, P., Anyogu, A., Varnam, A. H., & Sutherland, J. P. (2012). Survival of inoculated Salmonella on the shell of hens' eggs and its potential significance. *Food Control*, *28*(2), 463–469. https://doi.org/10.1016/j.foodcont.2012.05.033

Bourtoom, T. (2008). Plasticizer effect on the properties of biodegradable blend from rice starch-chitosan. *Songklanakarin Journal of Science and Technology*, *30*(Suppl. 1), 149–155.

Braden, C. R. (2006). Salmonella enterica serotype Enteritidis and eggs: A national epidemic in the United States. *Food Safety*, *43*(8), 512–517.

Caner, C. (2005). The effect of edible eggshell coatings on egg quality and consumer perception. *Journal of the Science of Food and Agriculture*, *85*(11), 1897–1902. https://doi.org/10.1002/jsfa.2185

Caner, C., & Cansiz, O. (2007). Effectiveness of chitosan-based coating in improving shelf-life of eggs. *Journal of the Science of Food and Agriculture Food*, *87*(2), 227–232. https://doi.org/10.1002/jsfa

Caner, C., & Yüceer, M. (2015). Efficacy of various protein-based coating on enhancing the shelf life of fresh eggs during storage. *Poultry Science*, *94*(7), 1665–1677. https://doi.org/10.3382/ps/pev102

da Silva, M. A., Bierhalz, A. C. K., & Kieckbusch, T. G. (2009). Alginate and pectin composite films crosslinked with Ca2+ ions: Effect of the plasticizer concentration. *Carbohydrate Polymers*, *77*(4), 736–742. https://doi.org/10.1016/j.carbpol.2009.02.014

D'Aoust, J. Y., & Maurer, J. (2007). Chapter 10–Salmonella species. In M. P. Doyle & L. R. Beuchat (Eds.), *Food Microbiology–Fundamentals and Frontiers* (3rd ed., pp. 187–236). Washington, DC: American Society for Microbiology Press.

De Reu, K., Grijspeerdt, K., Messens, W., & Heyndrickx, M. (2006). Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella enteritidis*. *International Journal of Food Microbiology*, *112*, 253–260. https://doi.org/10.1016/j.ijfoodmicro.2006.04.011

Dhall, R. K. (2013). Advances in edible coatings for fresh fruits and vegetables: A review. *Critical Reviews in Food Science and Nutrition*, *53*(5), 435–450. https://doi.org/10.1080/10408398.2010.541568

Dhanapal, A., Sasikala, P., L. Rajamani, Kavitha, V., Yazhini, G., & Banu, M. S. (2012). Edible films from polysaccharides. *Food Science and Quality Management*, *3*, 9–17.

EFSA. (2015). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA Journal*, *13*, 1–165. https://doi.org/10.2903/j.efsa.2015.3991

EFSA. (2016). The Euroean Union summary report on trends and sources of zoonsoes, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal*, *14*(12), 1–231. https://doi.org/10.2903/j.efsa

Figueiredo, T. C., Assis, D. C. S., Menezes, L. D. M., Oliveira, D. D., Lima, A. L., Souza, M. R., & Cançado, S. V. (2014). Effects of packaging, mineral oil coating, and storage time on biogenic amine levels and internal quality of eggs. *Poultry Science*, *93*(12), 3171–3178. https://doi.org/10.3382/ps.2014-04268

Galiş, A. M., Marcq, C., Marlier, D., Portetelle, D., Van, I., Beckers, Y., & Théwis, A. (2013). Control of salmonella contamination of shell eggs-preharvest and postharvest methods: A review. *Comprehensive Reviews in Food Science and Food Safety*, *12*(2), 155–182. https://doi.org/10.1111/1541-4337.12007

Gole, V. C., Chousalkar, K. K., Roberts, J. R., Sexton, M., May, D., Tan, J., & Kiermeier, A. (2014). Effect of egg washing and correlation between eggshell characteristics and egg penetration by various *Salmonella typhimurium* strains. *PLoS One*, *9*(3), e90987. https://doi.org/10.1371/journal.pone.0090987

Greig, J. D., & Ravel, A. (2009). Analysis of foodborne outbreak data reported internationally for source attribution. *International Journal of Food Microbiology*, *130*(2), 77–87. https://doi.org/10.1016/j.ijfoodmicro.2008.12.031

Guerreiro, A. C., Gago, C. M. L., Faleiro, M. L., Miguel, M. G. C., & Antunes, M. D. C. (2015). The use of polysaccharide-based edible coatings enriched with essential oils to improve shelf-life of strawberries. *Postharvest Biology and Technology*, *110*, 51–60. https://doi.org/10.1016/j.postharvbio.2015.06.019

Howard, Z. R., O'Bryan, C. A., Crandall, P. G., & Ricke, S. C. (2012). *Salmonella enteritidis* in shell eggs: Current issues and prospects for control. *Food Research International*, *45*(2), 755–764. https://doi.org/10.1016/j.foodres.2011.04.030

Hur, S. J., Kim, Y. C., Choi, I., & Lee, S. K. (2013). The effects of biopolymer encapsulation on total lipids and cholesterol in egg yolk during in vitro human digestion. *International Journal of Molecular Sciences*, *14*(8), 16333–16347. https://doi.org/10.3390/ijms140816333

Jin, T. Z., Gurtler, J. B., & Li, S.-Q. (2013). Development of antimicrobial coatings for improving the microbiological safety and quality of shell eggs. *Journal of Food Protection*, *76*(5), 779–785. https://doi.org/10.4315/0362-028X.JFP-12-460

Jo, C., Ahn, D. U., Liu, X. D., Kim, K. H., & Nam, K.-C. (2011). Effects of chitosan coating and storage with dry ice on the freshness and quality of eggs. *Poultry Science*, *90*(2), 467–472. https://doi.org/10.3382/ps.2010-00966

Kim, K. W., Daeschel, M., & Zhao, Y. (2008). Edible coatings for enhancing microbial safety and extending shelf life of hard-boiled eggs. *Journal of Food Science*, *73*(5), 227–235. https://doi.org/10.1111/j.1750-3841.2008.00776.x

Kim, S. H., No, H. K., & Prinyawiwatkul, W. (2008). Plasticizer types and coating methods affect quality and shelf life of eggs coated with chitosan. *Journal of Food Science*, *73*(3), S111–S117. https://doi.org/10.1111/j.1750-3841.2007.00650.x

Leleu, S., Herman, L., Heyndrickx, M., De Reu, K., Michiels, C. W., De Baerdemaeker, J., & Messens, W. (2011). Effects on Salmonella shell contamination and trans-shell penetration of coating hens' eggs with

chitosan. *International Journal of Food Microbiology*, *145*(1), 43–48. https://doi.org/10.1016/j.ijfoodmicro.2010.11.023

Luber, P. (2009). Cross-contamination versus undercooking of poultry meat or eggs - which risks need to be managed first? *International Journal of Food Microbiology*, *134*(1–2), 21–28. https://doi.org/10.1016/j.ijfoodmicro.2009.02.012

Moalemiyan, M., Ramaswamy, H. S., & Maftoonazad, N. (2012). Pectin-based edible coating for shelf-life extension of Ataulfo mango. *Journal of Food Process Engineering*, *35*(4), 572–600. https://doi.org/10.1111/j.1745-4530.2010.00609.x

Morsy, M. K., Sharoba, A. M., Khalaf, H. H., El-Tanahy, H. H., & Cutter, C. N. (2015). Efficacy of antimicrobial pullulan-based coating to improve internal quality and shelf-life of chicken eggs during storage. *Journal of Food Science*, *80*(5), M1066–M1074. https://doi.org/10.1111/1750-3841.12855

Muriel-Galet, V., Carballo, G. L., Hernández-Muñoz, P., & Gavara, R. (2016). Chapter 24-Ethyl Lauroyl Arginate (LAE): Usage and potential in antimicrobial packaging. In J. Barros-Velazquez (Ed.), *Antimicrobial food packaging* (1st ed., pp. 313–318). London: Elsevier Science. https://doi.org/10.1016/B978-0-12-800723-5.00024-3

Nongtaodum, S., Jangchud, A., Jangchud, K., Dhamvithee, P., No, H. K., & Prinyawiwatkul, W. (2013). Oil coating affects internal quality and sensory acceptance of selected attributes of raw eggs during storage. *Journal of Food Science*, *78*(2), S329–S335. https://doi.org/10.1111/1750-3841.12035

Pande, V. V., Gole, V. C., McWhorter, A. R., Abraham, S., & Chousalkar, K. K. (2015). Antimicrobial resistance of non-typhoidal Salmonella isolates from egg layer flocks and egg shells. *International Journal of Food Microbiology*, *203*, 23–26. https://doi.org/10.1016/j.ijfoodmicro.2015.02.025

Patrick, M. E., Adcock, P. M., Gomez, T. M., Altekruse, S. F., Holland, B. H., Tauxe, R. V., & Swerdlow, D. L. (2004). *Salmonella enteritidis* infections, United States, 1985-1999. *Emerging Infectious Diseases*, *10*(1), 1–7. https://doi.org/10.3201/eid1001.020572

Rojas-grau, M. A., Tapia, M. S., & Martin-Belloso, O. (2008). Using polysaccharide-based edible coatings to maintain quality of fresh-cut Fuji apples. *LWT - Food Science and Technology*, *41*, 139–147. https://doi.org/10.1016/j.lwt.2007.01.009

Sánchez-Ortega, I., García-Almendárez, B. E., Santos-López, E. M., Amaro-Reyes, A., Barboza-Corona, J. E., Regalado, C., & Regalado, C. (2014). Antimicrobial edible films and coatings for meat and meat products preservation. *The Scientific World Journal*, *2014*, 1–18. https://doi.org/10.1155/2014/248935

Seol, K. H., Lim, D. G., Jang, A., Jo, C., & Lee, M. (2009). Antimicrobial effect of κ-carrageenan-based edible film containing ovotransferrin in fresh chicken breast stored at 5 °C. *Meat Science*, *83*(3), 479–483. https://doi.org/10.1016/j.meatsci.2009.06.029

Solak, A. O., & Dyankova, S. M. (2014). Composite films from sodium alginate and high methoxyl pectin—Physicochemical properties and biodegradation in soil. *Ecologia Balkanica*, *6*(2), 25–34.

Tyler, C. (1953). Studies on egg shells. A method for marking and counting pores. *Journal of the Science of Food and Agriculture*, *4*(6), 266–272. https://doi.org/10.1002/jsfa.2740040603

Upadhyaya, I., Yin, H. B., Surendran Nair, M., Chen, C. H., Lang, R., Darre, M. J., & Venkitanarayanan, K. (2016). Inactivation of *Salmonella enteritidis* on shell eggs by coating with phytochemicals. *Poultry Science*, *95*, 2106–2111. https://doi.org/10.3382/ps/pew152

Valdés, A., Burgos, N., Jiménez, A., & Garrigós, M. (2015). Natural pectin polysaccharides as edible coatings. *Coatings*, *5*(4), 865–886. https://doi.org/10.3390/coatings5040865

Whiley, H., & Ross, K. (2015). Salmonella and eggs: From production to plate. *International Journal of Environmental Research and Public Health*, *12*, 2543–2556. https://doi.org/10.3390/ijerph120302543

White, P. L., Baker, R., & James, W. O. (1997). Strategies to control Salmonella and Campylobacter in raw poultry products. *Revue Scientifique et Technique (International Office of Epizootics)*, *16*(2), 525–541.

Wybo, I., Potters, D., Plaskie, K., Covens, L., Collard, J. M., & Lauwers, S. (2004). *Salmonella enterica* subspecies houtenae serotype 44:Z4, z 23: As a rare cause of meningitis. *Acta Clinica Belgica*, *59*, 232–234.

Yüceer, M., Aday, M. S., & Caner, C. (2016). Ozone treatment of shell eggs to preserve functional quality and enhance shelf life during storage. *Journal of the Science of Food and Agriculture*, *96*(8), 2755–2763. https://doi.org/10.1002/jsfa.7440

Yüceer, M., & Caner, C. (2014). Antimicrobial lysozyme-chitosan coatings affect functional properties and shelf life of chicken eggs during storage. *Journal of the Science of Food and Agriculture*, *94*(1), 153–162. https://doi.org/10.1002/jsfa.6322

How to cite this article: De Leo R, Quartieri A, Haghighi H, Gigliano S, Bedin E, Pulvirenti A. Application of pectin-alginate and pectin-alginate-laurolyl arginate ethyl coatings to eliminate *Salmonella enteritidis* cross contamination in egg shells. *J Food Saf*. 2018;e12567. https://doi.org/10.1111/jfs.12567