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Effect of zein and zein-*Peganum harmala* extract coatings of eggshell on the internal quality of eggs and control of *Salmonella enteritidis*

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Abstract: The high nutritional value of egg makes it vital to the human diet. *Salmonella enteritidis* is one of the major global causes of foodborne enteritis in humans. The chicken intestine is the main source of *S. enteritidis*. Therefore, eggs play an important role in the transmission of salmonellosis. In this study, we evaluated the effectiveness of coatings made of zein either alone or in combination of hydro-alcoholic extract of *Peganum harmala* on the quality of eggs and control of *S. enteritidis* at 7°C during a period of 28 days. Results demonstrated that both types of the coating significantly improved the physicochemical properties of eggs including weight loss, Haugh unit, and yolk index compared to controls during storage. However, neither of coatings resulted in significant changes in yolk color and pH ($p < 0.05$). Both types of coating caused two log CFU/ml reductions in *S. enteritidis* population from the first day and eliminated the contamination at the end of the experiment (for 28 days). *Salmonella* elimination occurred at day 21 for zein-plant extract coating. Our findings demonstrate zein coating can be an appropriate approach for maintaining the quality of eggs during shelf life and an effective and economic strategy for control of *S. enteritidis* in eggs.

KEYWORDS
egg, internal quality, *Peganum harmala*, *Salmonella enteritidis*, zein coating

Practical Application: This study shows that the application of zein coating can preserve the internal quality and freshness of eggs during storage. Moreover, zein coating is a highly effective strategy in the control of *Salmonella*. This method can be used on a commercial scale for enhancing the safety and quality of eggs.

1 | INTRODUCTION

Eggs have a high nutritional value especially a high level of essential amino acids. Thus, they play a key role in the human diet (Ruxton et al., 2010; Wang et al., 2015). On the other hand, the high nutrient content of egg makes it perishable. Moreover, despite the protective role of eggshell, oxygen, CO_2, and water vapor can be exchanged through its pores because there is a positive correlation between number of pores and high age of egg. This phenomena results in loss quality and increase in penetration of microorganisms (Shebuski and Freier, 2009; Wardy et al.,

2013). *Salmonella* enterica is the major global causes of foodborne enteritis in humans. The main causative agents of foodborne *Salmonella* outbreaks in the United States and Europe are the serovars Typhimurium and Enteritidis. The chicken intestine is the main source of both of these serovars, and all reported cases of *S. enteritidis* infection in humans were associated with chicken (Tack et al., 2019). Therefore, egg as a commonly consumed food in daily life, which is sometimes used raw in desserts, should be considered as a vital source of human infection by *S. enteritidis*. Recently, due to the undesirable effects of chemical food preservatives on human health, applying natural antimicrobial strategies such as plant extracts for the control of foodborne pathogens has gained much attention (Mostafa et al., 2017). Edible films and coatings in food products can be a natural protective barrier against pathogens. Furthermore, they can control air transference and thus increase the shelf life of food products (Sothornvita & MKrochta, 2001). A suitable coating of eggs can preserve the quality of fresh eggs by reducing mass transfer, oxidation processes, and microbial growth. Edible films and coatings can be obtained from proteins, lipids, hydrocolloids, and their composites (Suppakul et al., 2010). Corn zein is one of the desirable protein biopolymers for coating due to its good barrier properties against moisture and oxygen (Caner & Yüceer, 2015). *Peganum harmala* extract has been identified to have antifungal and antibacterial substances. *P. harmala* seeds contain 2–6% biologically active alkaloids especially β-carboline alkaloids including harman, harmine, harmaline, and harmalol (Farouk et al., 2008). In a study, protein coating of eggs could preserve the quality characteristics of fresh eggs during storage and zein showed a good efficiency for maintaining eggshells (Caner & Yüceer, 2015). Using natural antimicrobial compounds such as organic acids, nisin, isothiocyanate, and lauric arginate ester in chitosan coating of eggs not only demonstrated inhibitory effect against *Salmonella* during storage at 4 and 7°C but also decreased weight loss of treated eggs (Jin et al., 2013). However, in another study, antimicrobial coating of eggs with pectin-alginate containing ethyl laurolyl arginate did not alter the physicochemical properties of *Salmonella* (Leo et al., 2018). *P. harmala* extract demonstrated antibacterial effect against *Escherichia coli*, *Salmonella* Typhimurium, and *Staphylococcus aureus* (Igwe and Okwu, 2013). In this study, we aimed to evaluate the effect of zein coating of eggs as well as its combination with hydro-alcoholic extract of *P. harmala* on the physicochemical properties of eggs during a 28-day storage period at 7°C. Moreover, the potential antibacterial effect of both types of coatings was determined against *S. enteritidis* contamination on the egg.

2 | MATERIALS AND METHODS

2.1 | Materials

Zein powder (Sigma-Aldrich, Saint Louis, MO, USA), Muller Hinton Broth (MHB), and Muller Hinton Agar (MHA) were purchased from Sigma–Aldrich (Hamburg, Germany). Salmonella Shigella Agar (SSA) was obtained from Merck (MSD, Darmstadt, Germany). *Salmonella enteritidis* ATCC 1735 was obtained from the Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad.

2.2 | Methods

2.2.1 | Preparation of plant extract

Dried *Peganum harmala* seeds were purchased from a local market in Mashhad (Iran). The extract was prepared according to the method of (Mazandarani et al., 2012) with some modifications. Dried seeds (100 g) were mixed with 1 L ethanol 80% (1:10 w/v) and shaken for 24 h at room temperature. Then the solution was filtered using Whatman No. 1 filter papers and a rotary evaporator was used for removing the solvent. The extract was stored at 4°C until use.

2.2.2 | Determination of antimicrobial activity of the extract

Minimum inhibitory concentration (MIC) of the plant extract was determined against *Salmonella enteritidis* ATCC 1735 using the microbroth dilution method (Roshanak et al., 2020). *Salmonella enteritidis* was cultured in MHB and incubated overnight at 37°C. Bacterial culture was standardized according to McFarland 0.5 standard (1.5×10^8 CFU/ml) (M et al., 2016). A range of dilutions of 1, 2, 4, 8, 16, 32, 128, 256, and 512 mg/ml *P. harmala* extract was prepared in MHB in 96-well microplates. Twenty microliters of bacterial culture and 180 µl of each dilution of plant extract were inoculated into the microplates and incubated for 24 h at 37°C. Then, in order to assay the inhibitory effect of *P. harmala* extract, the optical density of cultures was measured at 630 nm by an ELISA reader (Bio Tek ELx808). The minimum concentration of the plant extract that inhibited the bacterial growth and demonstrated no turbidity was considered as MIC. To determine the minimum bactericidal concentration (MBC) of the plant extract, according to the results of MIC assay, the broth dilutions

without turbidity were subcultured to MHA agar plates and incubated for 24 h at 37°C. The lowest concentration of plant extract without bacterial growth on the plate is considered as MBC (Habibipour and Haghgou, 2015).

2.2.3 | Preparation of zein-coated eggs

Zein 8% (w/v) was dissolved in ethanol (80%, v/v) by shaking for 10 min at 60 ± 1°C. Then glycerol was added (0.15% dry zein weight) as a plasticizer and stirred for 10 min using a magnetic stirrer until it was completely dissolved. This solution is used for zein coating. For zein-plant extract combination, 7.68 g of dried hydro-alcoholic extract of *P. harmala* was also added to 100 ml of the zein solution (1.2 MIC) and stirred at 40°C to provide a homogenous mixture (Kashiri et al., 2016).

Average size eggs (56–67 g) were obtained from Partellae® (Kashmar, Khorasan Razavi, Iran) and kept under ambient conditions for 48 h before the test. The eggs were divided into three groups including two tests (zein coated and zein-coated containing *P. harmala* hydro-alcoholic extract), as well as uncoated eggs as negative control. The experiment was carried out in three repetitions. According to the method of De Leo et al. (2018) with some modifications, the coating solutions were separately sprayed on the test eggs from a 30 cm distance to avoid draining. After allowing the eggs to dry, spraying was repeated. The eggs were left at room temperature for 1 h to completely dry and stored at 7°C until the test.

2.2.4 | Percentage of coating

Percentage of coating on eggs was calculated according to the formula bellow:

(Nasiri et al., 2011):

$$Coating\ \% = \left[\frac{A-B}{B}\right] \times 100 \quad (1)$$

A = weight of samples after coating, B = weight of samples before coating

2.2.5 | Weight loss

The percentage of weight loss was calculated as below:

$$\left\{\begin{array}{l}(Initial\ weight\ of\ coated\ eggs\ (g)\ at\ day\ 0 - weight\ of\\ the\ coated\ eggs\ (g)\ at\ a\ certain\ day\ of\ storage)\\ /initial\ weight\ of\ coated\ eggs\ (g)\ at\ day\ 0\end{array}\right\} \times 100 \quad (2)$$

2.2.6 | Haugh unit

Haugh unit (HU) is used to indicate the height of albumen (internal quality) and was measured by using a digital caliper (1108-150, INSIZE CO., LTD, China). The HU was calculated according to the formula below:

$HU = 100 \log (H - 1.7\ W^{0.37} + 7.6)$, where H is the height of albumen (mm) and W is the mass of egg (g) (Eisen et al., 1962).

2.2.7 | Yolk Index

Egg albumen was separated from the yolk and the height and width of the yolk were then measured by a digital caliper. Yolk index was calculated as yolk height (mm) / yolk width (mm) (Stadelman, 1995).

2.2.8 | Yolk color

The yolk color of the eggs was measured with a Chroma Meter (Konica Minolta, Chroma Meter CR-410, Japan). The measurement was performed on two different points of eggs and assessed in terms of lightness (L value), redness (a value), and yellowness (b value), and ΔE_{ab}(total color difference in treated samples rather than control during storage) were calculated according to formulation below: (Leo et al., 2018).

$$\Delta\ E_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

Δa^* = a tested−a control, Δb^* = b tested–b control, ΔL^* = L tested−L control.

2.2.9 | Albumen pH

After separating eggs albumen from the yolk, the albumen was homogenized for 20 s in a blender. Albumen pH was measured by a pH meter (inoLab, WTW, pH 7310, Wilhelm, Germany).

2.2.10 | Microbial experiment

The effectiveness of coating against *S. enteritidis* was evaluated following the method of Jin et al. (2013) with some modifications. Briefly, the surface of eggshells was first sterilized by immersing in ethanol 70%. After drying, the eggs were experimentally contaminated by immersion in *S. enteritidis* ATCC 1735 suspension at a concentration of

7×10^6 CFU/ml for 1 min at room temperature, then were dried under a laminar flow biosafety cabinet. One group of the samples was coated with zein, and another group was coated with zein-extract. While control samples were *Salmonella*-contaminated eggs with no coating. All samples were stored at 7°C for 28 days and examined at days 1, 7, 14, 21, and 28. For counting bacteria at the above-mentioned days, each egg was added to 100 ml of PBS in a sterile blender bag and manipulated for 1 min. Serial dilutions were prepared and each dilution was cultured onto SSA plates. Then the plates were incubated at 30°C for 24 h (Leo et al., 2018; Yousef and Carlstrom, 2003).

2.2.11 | Statistical analysis

All experiments were repeated three times. Data were analyzed according to the analysis of variance (ANOVA) using SPSS version 21 software and Duncan's multiple range tests ($p < 0.05$) samples. The results were indicated as means ± SD.

3 | RESULTS AND DISCUSSION

3.1 | Antimicrobial activity extract against *Salmonella enteritidis*

MIC and MBC of *P. harmala* extract against *Salmonella enteritidis* ATCC 1735 were determined 64 and 265 mg/ml, respectively. Results obtained from studying Hadadi et al. (2020) showed a MIC as low as 1.56×10^{-3} mg/ml for the methanolic extract of *P. harmala* against *S. aureus* and *E. coli* and for its chloroformic extract against *P. aeruginosa*. Other studies reported MICs of 0.625 mg/ml (Darabpour et al., 2011) and 1.56 mg/ml for methanolic extracts of *P. harmala* against *E. coli* and *S.* Typhimurium respectively, and 0.78 mg/ml against *L. monocytogenes* (Zeinali et al., 2016). In other studies, MBCs of methanolic extracts of *P. harmala* had ranged between 3.12 µg/ml and 1.56 mg/ml against *E. coli* and *S.* Typhimurium, respectively (Hadadi et al., 2020; Zeinali et al., 2016). Different MIC and MBC values of *P. harmala* extract may be due to the variety in extraction methods, solvents, growth mediums and bacterial strains. Moreover, different parts of plants have various chemical ingredients and volatile nature that can affect antimicrobial activity (Darabpour et al., 2011; Mostafa et al., 2017). Tayel et al. (2014) reported MICs > 1000 µg/ml in olive leaf ethanolic extract against *S.* Typhimurium and *S. enteritidis* to disinfection of eggshell. Kothari et al. (2019) expressed that diet containing allium can improve high unit in the eggs during storage but high amounts of allium in the diet can be have negative effect on taste and poultry odor and decrease acceptance also, high dose (20 g/kg) of garlic in the diet of poultry resulted in an off-flavor.

3.2 | Percentage of coating

The percentage of coating was calculated in the eggs with both types of coatings (zein coating and zein extract) as seen in Figure 1. The average values of the three experiments were 0.108 ± 0.02% and 0.135 ± 0.03% for zein-coated and zein-extract coated samples, respectively. Significant difference in percentage of coating between samples coated with zein and zein-extract did not indicate coating using spray method was performed uniformly in the samples coated with zein and zein + extract.

3.3 | Effect of coatings on weight loss

The eggs were weighted once a week at certain time points (1, 7, 14, 21, and 28 days) during storage. The percentage of the weight loss is shown in Table 1. While weight loss in control samples was higher compared to both types of coated eggs, the zein-coated and zein-extract coated samples were not significantly ($p > 0.05$) different in weight loss throughout the experiment. Weight loss values in control group were from 0.12% to 1.85%, while in zein and zein + extract coatings ranged from 0.25% to 1.45% and from 0.16% to 1.53 %, respectively. After 14 days, weight loss significantly increased in the control and the test samples. At the end of 28 days, weight loss values reached 1.85% in control group, and 1.45% and 1.53% in test groups. Typically, prolonged storage causes weight loss in eggs due to the evaporation of water and losing carbon dioxide of albumen from pores of the eggshell (Bhale et al., 2003). Caner and Yüceer (2015) showed protein coatings including zein, shellac, and whey protein had significantly affected weight loss of eggs during storage compared to control samples at 24°C, but our studies showed no significant difference between coated samples and uncoated ones at 7°C during storage. Pires et al. (2019) investigated effect of rice protein coating on quality of eggs during storage at 20°C and their results showed protein coating had a significant difference in decreasing loss weight compared to control samples. The results showed that storage temperature had significant effect in prevention of weight loss of treated and untreated samples.

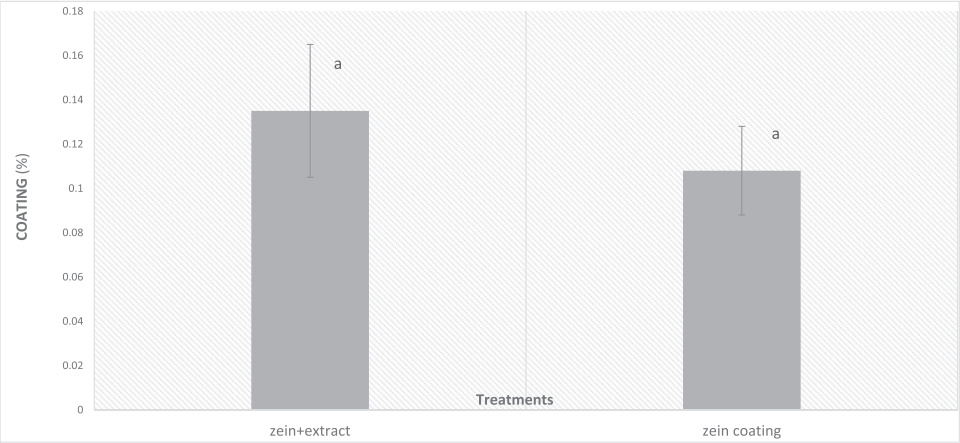

FIGURE 1 Comparison of percentage of coatings with zein and zein extract ($p < 0.05$)

TABLE 1 Weight loss (%) of values in eggs coated and uncoated during storage at 7°C for 28 days

Treatments	Day 1	Day 7	Day 14	Day 21	Day 28
Control	$0.123 \pm 0.04^{b, C}$	$0.677 \pm 0.13^{a, C}$	$0.87 \pm 0.08^{a, BC}$	$1.47 \pm 0.46^{a, AB}$	$1.848 \pm 0.43^{a, A}$
Zein coating	$0.255 \pm 0.14^{a, C}$	$0.379 \pm 0.73^{ab, BC}$	$0.715 \pm 0.11^{a, B}$	$1.23 \pm 0.5^{b, C}$	$1.451 \pm 0.3^{a, A}$
Zein + extract	$0.165 \pm 0.09^{a, C}$	$0.256 \pm 0.12^{b, C}$	$0.820 \pm 0.04^{a, B}$	$1.063 \pm 0.07^{b, C}$	$1.53 \pm 0.14^{a, A}$

Note: Results are shown as means ± SD. Means with different small letters within a column express significant difference ($p < 0.05$). Means with different capital letters within a row express significant difference ($p < 0.05$).

TABLE 2 Haugh unit values in coated (zein and zein extract) and uncoated eggs during storage at 7°C for 28 days

Treatments	Day 1	Day 7	Day 14	Day 21	Day 28
Control	$93.85 \pm 1.52^{a, C}$	$90.46 \pm 0.3^{ab, C}$	$86.1 \pm 1.39^{ab, BC}$	$77.04 \pm 0.89^{b, AB}$	$77.78 \pm 1.94^{b, A}$
Zein coating	$88.81 \pm 5.39^{a, A}$	$92.43 \pm 1.35^{a, A}$	$86.7 \pm 0.07^{a, A}$	$85.1 \pm 1.99^{a, A}$	$86.34 \pm 3.4^{a, A}$
Zein + extract	$90.71 \pm 0.72^{a, A}$	$88.16 \pm 0.43^{b, B}$	$83.5 \pm 0.29^{b, D}$	$85.82 \pm 0.55^{a, C}$	$86.93 \pm 1.53^{a, BC}$

Note: Results are shown as means ± SD. Means with different small letters within a column express significant difference ($p < 0.05$). Means with different capital letters within a row express significant difference ($p < 0.05$).

3.4 | Effect of coatings on Haugh unit

HU indicates the quality of albumen and freshness of egg based on egg weight and albumen height (Caner, 2005). Lower HU demonstrates a reduction in albumen volume and egg weight, which can be an indicator for distinguishing old eggs (Chen et al., 2005; No et al., 2007; Yuceer and Caner, 2014). Table 2 shows changes in HU of eggs coated with zein and zein-plant extract as well as of uncoated control eggs at certain time points during 28 days storage at 7°C. Our results demonstrated that HU in control samples was lower than both types of coated samples during storage, because the coating can confine the evaporation of water and diffusion of carbon dioxide from albumen. In our study, HU value decreased from 93.85% to 77.78% in the control group, and from 88.81% to 86.34%, and from 90.71% to 86.93% in zein coating, and zein + extract coating groups, respectively (Table 2). According to our results, HU values in zein coating were higher compared with zein-extract coating ($p < 0.05$). Therefore, zein coating had a better effect in maintaining the quality of albumen and preventing weight loss. At days 7 and 14, HU values in zein-extract coating were lower than zein coating but in other days they were similar to zein coating ($p < 0.05$). Also at day 14, HU values in zein-extract coating were at their lowest during storage, that can be associated to their age; moreover, there is a direct relation between aging and enlargement of the pores in the eggshells (Bhale et al. 2003) because *P. harmala* extract has alkaloids with hydrophobic nature so it has suitable compatibility with zein (hydrophobic) (Fahmy et al., 2021). The decrease of HU value in prolonged storage is due to the liquefaction of albumen. This phenomenon occurs following the breakdown of ovomucin–lysozyme complex. Consequently, a reduction in carbohydrate levels of ovomucin and an increase in water loss and pH level occurs in long-term storage. Other studies confirm

TABLE 3 Yolk index in coated (zein and zein extract) and uncoated eggs during storage at 7°C for 28 days

Treatments	Day 1	Day 7	Day 14	Day 21	Day 28
Control	42.71 ± 1.2 $^{a, C}$	37.38 ± 1.81 $^{a, A}$	37.45 ± 0.04 $^{c, A}$	34.95 ± 0.86 $^{a, A}$	32.55 ± 1.03 $^{b, A}$
Zein coating	45.04 ± 4.22 $^{a, A}$	41.47 ± 1.76 $^{a, AB}$	42.3 ± 0.09 a, A	34.58 ± 0.11 $^{a, C}$	36.46 ± 0.43 $^{a, BC}$
Zein+extract	43.36 ± 4.75 $^{a, A}$	40.18 ± 1.64 $^{a, AB}$	39.6 ± 0.55 $^{b, AB}$	35.97 ± 2.07 $^{a, BC}$	32.64 ± 1.44 $^{b, C}$

Note: Results are shown as means ± SD. Means with different small letters within a column express significant difference ($p < 0.05$). Means with different capital letters within a row express significant difference ($p < 0.05$).

our results for example; shellac coating was very effective on the maintenance of egg quality at 4°C. The HU value in their study was 81.11% at the end of 30 days storage (Tilki and Saatci, 2004). At higher temperatures (24°C), the coating was also reported to be effective on the quality of eggs during 6 weeks shelf life. HU values were higher in zein and shellac coatings than whey protein coating (Caner and Yüceer, 2015).

3.5 | Effect of coatings on Yolk Index

Yolk index is a parameter based on the yolk height and width that indicates the quality and freshness of the eggs. In high-quality eggs, the yolk index value is ~0.45, and lower levels indicate old eggs (Yuceer and Caner, 2014). A reduction in yolk index value indicates the vitelline membrane is becoming weaker and the yolk begins liquefaction, which is a consequence of water diffusion from albumen (Obanu and Mpieri, 1984; Stadelman, 1995). In our study, the mean values of yolk index decreased during 28 days from 42.71 to 32.55 in controls, and from 45.04 to 36.46, and 43.36 to 32.64 in zein coating, and zein + extract coating, respectively (Table 3). At day 21th, the yolk index significantly decreased in all samples compared with earlier days. The lowest yolk index in our experiment was observed in controls and the highest value was observed in zein-coated samples. Therefore, zein coatings can reduce water loss and transfer gases from albumen through the eggshell. Consistent with our findings, beeswax and gelatin coatings were also effective in maintaining yolk quality at 30°C for 6 weeks (Mudannayaka et al., 2016). Pectin biofilm was also reported as an effective coating for yolk index and in preserving eggs quality during 35 days storage at 25 and 5°C (Oliveira et al., 2020).

3.6 | Effect of coatings on albumen pH

Albumen pH is another important factor that indicates the freshness and quality of the eggs. The values of albumen pH in fresh eggs range between 7.5 and 8.5. By extending the storage period, pH can rise to the value of 9. In a long-stored eggs, the carbonic acid of the egg white breaks down. Consequently, carbon dioxide spreads out and bicarbonate buffer system changes. This process results in a rise in pH albumen (Leo et al., 2018; Yuceer and Caner, 2014). However, in our study, as Table 4 shows, albumen pH did not change significantly in control (from 8.7 to 8.9) and test samples (from 8.4 to 8.6 in both tests) throughout the experiment. Moreover, neither of the coatings significantly influenced albumen pH compared to control samples (Table 4). In contrast to our results, although coating with pectin–alginate and pectin–alginate–laurolyl did not have a significant effect on the albumen pH during 42 days incubation at 7°C, pH in coated and control samples had an ascending trend throughout the experiment (from ~ 8.5 at day 1 to ~ 9.6 at day 28 in the coated and control samples). In a study, different types of protein-based coatings resulted in a significantly lower albumen pH compared to controls during 5 weeks of storage at 24°C. In their study, the albumen pH of 7.5 increased to the level of 8.78 in zein-coated eggs after 4 weeks storage, while in controls, pH reached to level of 9.38 (Caner and Yüceer, 2015). Our results demonstrate, although coatings can be effective in maintaining the quality of eggs, environmental temperature also plays a central role in the shelf life of eggs.

3.7 | Effect of coatings on yolk color

Yolk color was measured in the two types of coated (zein and zein-extract) samples and control eggs and assessed in terms of lightness, redness, and yellowness. Changes in yolk color are time dependent, and ΔE_{ab} has a direct correlation with time because it is influenced by time. Color changing occurs due to oxidation of lipids during storage (Moraleco et al., 2019). As Table 5 shows, significant difference was not observed in yolk color during 28 days storage at 7°C. The values of yolk color did not have a linear trend with respect to time in the coated samples and is constantly changing. In agreement with our results, the use of pectin–alginate and pectin–alginate–laurolyl coatings on eggshells at 7°C did not have a significant effect on yolk color during a period of 42 days (Leo et al., 2018).

TABLE 4 pH values of albumen in coated (zein and zein extract) and uncoated eggs during storage at 7°C for 28 days

Treatments	Day 1	Day 7	Day 14	Day 21	Day 28
Control	8.78 ± 0.84 $^{a, B}$	8.96 ± 0.06 $^{a, A}$	9.01 ± 0.06 $^{a, A}$	9.06 ± 0.01 $^{a, A}$	8.93 ± 0.49 $^{a, A}$
Zein coating	8.4 ± 0.11 $^{b, B}$	8.6 ± 0.02 $^{a, AB}$	8.56 ± 0.02 $^{a, AB}$	8.76 ± 0.09 $^{b, A}$	8.68 ± 0.24 $^{a, AB}$
Zein + extract	8.41 ± 0.13 $^{b, A}$	8.32 ± 0.38 $^{a, A}$	8.54 ± 0.32 $^{a, A}$	8.46 ± 0.07 $^{c, A}$	8.64 ± 0.05 $^{a, A}$

Note: Results are shown as means ± SD. Means with different small letters within a column express significant difference ($p < 0.05$). Means with different capital letters within a row express significant difference ($p < 0.05$).

TABLE 5 Yolk color values in coated (zein and zein extract) and uncoated eggs during storage at 7°C for 28 days

Treatments	Day 1	Day 7	Day 14	Day 21	Day 28
Control	0 ± 0^{b}	0 ± 0 ^{b}	0 ± 0 ^{b}	0 ± 0 ^{b}	0 ± 0 ^{b}
Zein coating	2.72 ± 0.66 $^{a, A}$	2.15 ± 1.05 $^{a, A}$	2.67 ± 0.26 $^{a, A}$	3.98 ± 1.05 $^{a, A}$	2.5 ± 0.17 $^{a, A}$
Zein + extract	1.89 ± 0.71 $^{a, A}$	4.03 ± 2.35 $^{a, A}$	1.67 ± 0.52 $^{a, A}$	4.11 ± 0.85 $^{a, A}$	4 ± 0.89 $^{a, A}$

Note: Results are shown as means ± SD. Means with different small letters within a column express significant difference ($p < 0.05$). Means with different capital letters within a row express significant difference ($p < 0.05$).

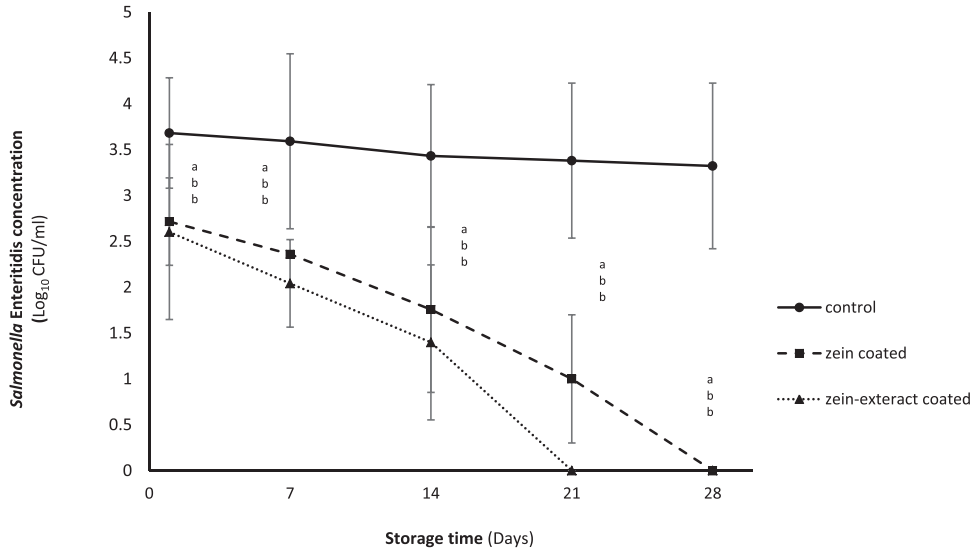

FIGURE 2 *Salmonella enteritidis* concentration on eggshell for control, zein, and zein extract-coated eggs for 28 days at 7°C. Different letters on different time points show significant difference between treated groups and nontreated group in the same day ($p < 0.05$). Results are shown as means ± SD

3.8 | Effect of coatings on the concentration of *Salmonella enteritidis*

Antibacterial effect of zein and zein-extract coatings was studied on the eggs experimentally contaminated with *Salmonella enteritidis* at a dose of 7×10^6 CFU/ml. All treated and control samples were stored at 7°C and examined once a week for 4 weeks. Figure 2 shows the *S. enteritidis* concentrations on the treated and control samples during incubation. A reduction of by 2 log-unit was observed in both types of coated (zein coating, zein-extract) samples compared to controls from day 1. At day 14 in comparison with day 1, a significant reduction of 1 log CFU/ml and 1.2 log CFU/ml was observed in zein and zein-extract coatings, respectively. At day 28, *Salmonella* populations in both types of coatings were eliminated, while no significant (0.3 log CFU/ml) reduction occurred in control. The elimination was observed in zein-extract coating at day 21 and earlier than zein-coated samples. The antibacterial activity of plant extracts is mostly attributed to their chemical characteristics. Polyphenols such as tannins and flavonoids as epigallocatechin, catechin, myricetin, quercetin, and luteolin are substantial antibacterial ingredients of plant extracts. A high level of phenolic groups, which can bind to certain proteins and enzymes, results in alteration in the equilibrium of bacterial enzymes

(Shan et al., 2007; Tulin et al., 2009). *P. harmala* comprises harmine, harmane, harmalol, harmaline, vasicine, vasicinon, and peganine. Harmane is a highly aromatic planar alkaloid that has antibacterial activity through interchelating with DNA. Therefore, the antibacterial mechanism of *P. harmala* can be attributed to harmane (Darabpour et al., 2011). In agreement with our results, the alcoholic extract of *P. harmala* demonstrated high antibacterial activity against pathogens such as *B. cereus* and *S. aureus* and *E. coli* (Mohsenipour and Hassanshahian, 2016). As Figure 2 shows, zein coating had a significant effect on the reduction of bacterial load on the eggs. This result could be due to the usage of ethanol 80% for the same amount of time as solvent in the coating and low temperature because zein coating alone did not show antimicrobial effect, and according to control group, only low temperature showed no significant difference on population of bacterial during shelf life at 7°C. *Salmonella* has serovars and strains, which are sensitive to the temperature and need minimum temperature for growth and survival. Our results are in agreement with the results of experiments (Tarlak et al., 2020) that investigated the effect of different temperatures on growth of *Salmonella* serovars in lettuce.

4 | CONCLUSION

This study demonstrates that zein-edible coating can enhance the internal quality of eggs during 4 weeks storage at 7°C. Moreover, zein coating either alone or in combination with *P. harmala* extract could eliminate *Salmonella enteritidis* contamination from the eggshell. However, the antimicrobial activity of plant extract accelerated the activity of zein coating against *S. enteritidis* contamination. Therefore, zein coating on the surface of eggs alone or in combination with other antimicrobials can be an economic natural strategy for controlling foodborne salmonellosis. Considering eggs are commonly stored and sold in shelves in some countries, further studies are needed for evaluating the effectiveness of zein coating against other pathogens on the egg at different temperatures.

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AUTHOR CONTRIBUTIONS

Asma Entezari: formal analysis; investigation; methodology; project administration; writing - original draft. **Sahar Roshanak:** methodology. **Golshan Shakeri:** writing - original draft. **Nasser Sedaghat:** supervision.

CONFLICT OF INTEREST

All authors have seen and agree with the contents of the manuscript. The authors declare that there is no conflict of interest.

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