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Benefits of pectin coating of cuticle damaged egg to prevent contamination with *S.* Enteritidis and *E. coli* during sanitization with slightly acidic electrolyzed water

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ABSTRACT

Ensuring the microbial safety of eggs is essential to reduce foodborne illnesses caused by *Escherichia coli* and *Salmonella* Enteritidis. This study aimed to evaluate the efficacy of slightly acidic electrolyzed water (SAEW) and SAEW combined with pectin coating (SAEW-p) in reducing bacterial survival on eggshells and egg contents during refrigerated storage over 7 days. Fresh cuticle-damaged eggs were artificially inoculated with *E. coli* and *S.* Enteritidis and subjected to SAEW or SAEW-p treatments. Bacterial counts were assessed on day 0 and throughout storage (days 1, 3, 5, and 7) at 4°C. On eggshells, SAEW-p treatment demonstrated significantly enhanced bacterial reduction compared to SAEW alone. By day 7, SAEW-p reduced *E. coli* and *S.* Enteritidis counts to 3.30 and 3.74 log CFU/ml, respectively, compared to 4.67 and 4.38 log CFU/ml for SAEW-treated eggs and 5.65 and 5.37 log CFU/ml for the control. In egg contents, SAEW-p eliminated *E. coli* by day 5 and maintained sterility through day 7, whereas SAEW alone achieved partial bacterial reduction. The superior efficacy of SAEW-p is attributed to the synergistic effect of SAEW's oxidative disinfection and the physical barrier provided by the pectin coating, which inhibited bacterial recontamination and moisture loss. These findings highlight the potential of SAEW-p as a safe, eco-friendly, and highly effective treatment to enhance microbial safety and extend the shelf life of eggs during storage. The combined approach offers a promising solution for the food industry to mitigate contamination risks and ensure food safety.

Introduction

Egg is one of the most devoured nourishments around the world due to its dietary quality; also, it has innovative properties that can be executed in the nourishment industry (Ramírez-Orejel and Cano-Buendía, 2022).

Pasteurization is a treatment alternative to diminish nourishment harming through egg utilization; in any case, warm treatment influences on egg physical properties (Ramírez-Orejel and Cano-Buendía, 2022). Thus, washing eggs with antimicrobial operators is one of the most critical measures to diminish the event of nourishment borne diseases (Cichoski *et al*., 2019). Therefore, creating profoundly viable residue-free disinfectants has been met with expanding intrigued (Yuan *et al*., 2023).

An elective treatment is the utilize of electrolyzed water (EW) as a disinfecting specialist, which has as of now been tried in distinctive nourishments. EW has created favorable comes about in terms of bacterial burden decrease, in the enhancement of physicochemical and useful properties of nourishment (Ramírez-Orejel and Cano-Buendía, 2022).

In comparison to customary cleaning specialists, EW is temperate, eco-friend, simple to utilize and emphatically compelling. EW is too utilized in its acidic frame, but it is noncorrosive to the human epithelium and other natural matter. The EW can be utilized in a differing run of nourishments; in this way, it is an appropriate choice for synergistic microbial control in the nourishment industry to guarantee nourishment security and quality without harming the organoleptic parameters of the nourishment (Rebezov *et al*., 2022). However, the nearness of natural matter such as feces or plumes on eggs may essentially diminish the bactericidal impact of the slightly acidic electrolyzed water (SAEW) (Zang *et al*., 2019).

In any case of which combined sanitization strategy is utilized, it has been appeared that when SAEW is utilized on the egg surface, the cuticle of the egg may be annihilated (Sheng *et al*., 2021). The harm to the cuticle may cause the trans-shell penetration of eggs with microscopic organisms, diminish the quality of eggs during storage and in this manner increment the security hazard to shoppers. In acknowledgment of this, advance efforts are required to disband the harmed cuticle caused by SAEW cleansing. Consequently, numerous considers combining SAEW with other sanitization strategies have developed to fathom this issue (Jiang *et al*., 2020; Koide *et al*., 2011). In this manner, a combination of SAEW sanitization with pectin (PT) coating was attempted in avoiding bacterial attack and quick weakening of egg content.

Since PT is a common component of vegetables and fruits, it is a secure nourishment added substance (E440) with no decided constrain of day by day admissions (Khedmat *et al*., 2020). Besides, it is known as a great antibacterial and antifungal specialist (Salas *et al*., 2011). It can be utilized on eggs after SAEW sanitization to give an obstruction on the surface of the egg, avoiding the attack of outside microbes and at the same time preventing the loss of dampness from the egg through the harmed cuticle caused by SAEW disinfection. Therefore, the present study aimed to Investigate the impact of SAEW against Enteropathogenic *E. coli* and *S.* Enteritidis in cuticle damaged egg, and to assess the impact of pectin coating cuticle damaged eggs sanitized with SAEW as a barrier on the surface of the egg for avoiding the invasion of *E. coli* and *S.* Enteritidis through the damaged cuticle.

Materials and methods

Preparation of samples

A total of 120 Fresh cuticle damaged eggs were purchased from a local poultry farm (Assiut, Egypt), and transported to the laboratory (Animal Health Research Institute, Assiut Branch) within 1 h, weighing 55 to 59 g per egg.

Preparation of slightly acidic electrolyzed water (SAEW) according to Al-Haq *et al*. (2005); Hricova *et al*. (2008) and Athayde *et al*. (2018).

Slightly acidic electrolyzed water (pH, 6) was prepared through electrolysis of tap water with sodium chloride (NaCl) 0.2%. A current of 9-10 volt and 8-10 amber was passed through electrolysis chamber with two poles, anode (+) and cathode (-) for 10 min. The exchange of ions happened between two partitioned sides through a bridge. At the anode side, SAEW was shaped due to the generation of hypochlorous acid (HOCl), hypochlorite ions (OCl^-) and chlorine gas (Cl_2). The pH of the solution was assessed instantly before each experiment. The pH was measured by pH meter (AD11, Adwa, waterproof pH-Temp pocket tester with replaceable probe, Romania).

Bacterial strains

Salmonella Enteritidis (field strain was taken up from Isolate's Bank from animal health institute Cairo branch-Al- Dokki, Giza, Egypt) and *Escherichia coli* (ATCC25922) were obtained from Animal Health Research Institute, Dokki, Giza, Egypt. The strains were stored chilled on tryptic soy agar (TSA) slants and activated separately in 9 g of tryptic soy broth (TSB) at 37°C for 24 h prior to experiments. Then strains were streaked on xylose lysine deoxycholate (XLD) agar for *S.* Enteritidis and eosin methylene blue (EMB) agar for *E. coli* and kept at 37°C for 24 h.

Preparation of bacterial inoculums

Two to three separate colonies of each *S.* Enteritidis and *E. coli* strains from overnight culture on their selective solid media were transferred into separated 10 g brain heart infusion (BHI) broth and incubated at 37°C for 20 h. Each strain culture in BHI broth was diluted to approximately match 1.5×10^8 CFU/g (0.5 Mcfarland) and further confirmed by counting on agar plates.

Preparation of pectin coating solution

High methoxyl apple pectin (PT, food-grade, 65% esterification) was obtained from Qualikems Company (India). The PT coating film was prepared as follow: 100 g of PT was firstly mixed with 0.5% lactic acid and stirred by mixer (JYL-Y12H, Jiuyang Co., Ltd., Shandong, China) for 10 mins, then glycerol (2% g) was added to the mixture and was stirred for an additional 10 min. The PT coating film was prepared as described by Li *et al.* (2015) with modification.

Preparation of cuticle damaged eggs and groups design

Cuticle damaged eggs were sanitized carefully by 70% ethanol and air dried inside a biosafety cabinet for 1 h then divided into 8 groups; each group represented by 15 eggs; half of them (4 groups =60 eggs) represented studying *S.* Enteritidis and the other half for *E. coli* studying. Each half comprises control positive (15 eggs), control negative (15 eggs), SAEW treatment (15 eggs) and SAEW-pectin coating treatment (15 eggs).

Cuticle damaged eggs inoculation and treatment application

Each egg was surface inoculated with the target bacterium (*S.* Enteritidis and *E. coli*) by dipping for 10 mins in calculated inoculums of 10^6 CFU/g of bacterial suspensions. Following inoculation, eggs were permitted to dry inside a biosafety cabinet for 60 mins at 25°C to permit bacterial attachment (Bing *et al.*, 2019). After that, as a group design, intended groups for SAEW treatment were dipped separately for 10 mins in freshly prepared EW at room temperature (22.0±3.0°C). For the SAEWP coating groups, eggs were immersed in the prepared PT coating solution for 2 mins, at that point set in a biosafety cabinet to dry. Whereas controls groups were dipped in sterile phosphate buffer saline. dipped eggs were dried at that point separately bundled in polyethylene packs.

All groups were stored at refrigerated temperature (4°C). Assessment of treatments and controls was carried through counting survivors of *S.* Enteritidis and *E. coli* on shell and in egg substance to assess the impact of treatments in preventing *S.* Enteritidis and *E. coli* from attacking cuticle damaged eggs instantly after treatments (day 0) and at the storage intervals 1, 3, 5. 7 days.

Eggshells were examined by surface rinsing as described by Moats (1980). Egg contents were prepared and evacuated according to Salfinger and Tortorello (2015).

Statistical analysis

Three eggs per treatment at every sampling time point during the 7 days were included in all three independent replicated experiments. One-way analysis of variance was performed using the SPSS program (SPSS Inc., Chicago, IL, USA) to determine the statistical significance of differences within the samples.

Results

The results of the *E. coli* (log CFU/g) on eggshells treated with slightly acidic electrolyzed water (SAEW) and SAEW combined with pectin coating (SAEW-p) were monitored immediately after treatment (day 0) and over a storage period of 7 days at 4°C. The control positive group, which received no treatment, showed a gradual reduction in *E. coli* during storage but remained consistently higher than the treated groups (Figure 1). On day 0, *E. coli* count in the control group was 8.14 log CFU/g, while eggs treated with SAEW and SAEW-p had *E. coli* of 8.30 and 8.12 log CFU/g, respectively. This indicates minimal initial reduction in bacterial load after treatment. By day 1, a significant reduction in *E. coli* count was observed in the SAEW group (6.01 log CFU/g), while the SAEW-p group maintained a similar count as day 0 (6.12 log CFU/g). In contrast, the control group showed a slower decline to 7.09 log CFU/g.

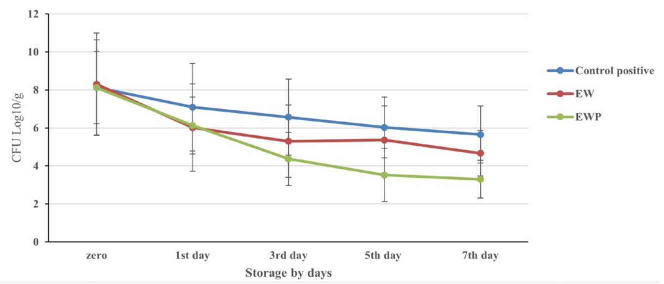

Fig. 1. Effect of EW and EWP coating on survival of *E.coli* on eggshell over storage time.

The difference in the efficacy of treatments became more pronounced as the storage period progressed. By day 3, *E. coli* count for the SAEW-p group decreased to 4.37 log CFU/g, demonstrating a significantly enhanced reduction compared to the SAEW group (5.30 log CFU/g) and the control group (6.57 log CFU/g). By day 5, *E. coli* count in the SAEW and SAEW-p groups were 5.37 and 3.52 log CFU/g, respectively. The control group exhibited a count of 6.03 log CFU/g. Finally, on day 7, the SAEW-p group achieved the lowest *E. coli* of 3.30 log CFU/g, outperforming both the SAEW group (4.67 log CFU/g) and the control group (5.65 log CFU/g)

The results in Figure 2 revealed that SAEW-pectin (SAEW-p) coating was highly effective in reducing *E. coli* count in egg content during refrigerated storage compared to SAEW treatment alone and the untreated control. On day 0, bacterial counts were 8.64, 8.76, and 8.25 log CFU/g for the control, SAEW, and SAEW-p groups, respectively. By day 1, SAEW-p significantly reduced *E. coli* count to 3.69 log CFU/g, while SAEW and the control recorded 4.82 and 5.70 log CFU/g, respectively. On day 3, SAEW-p maintained lower bacterial counts (3.67 log CFU/g) compared to SAEW (4.52 log CFU/g) and the control (4.57 log CFU/g). Remarkably, SAEW-p eliminated *E. coli* by day 5, achieving 0 log CFU/gm, whereas

SAEW showed a count of 4.30 log CFU/g, and the control exhibited a count of 4.82 log CFU/gm. This sterility in SAEW-p-treated eggs was sustained through day 7, while SAEW further reduced bacterial count to 2.87 log CFU/g, and the control recorded 4.52 log CFU/g. These findings highlight the superior efficacy of SAEW-p coating in eliminating *E. coli* from egg content, ensuring microbial safety during storage.

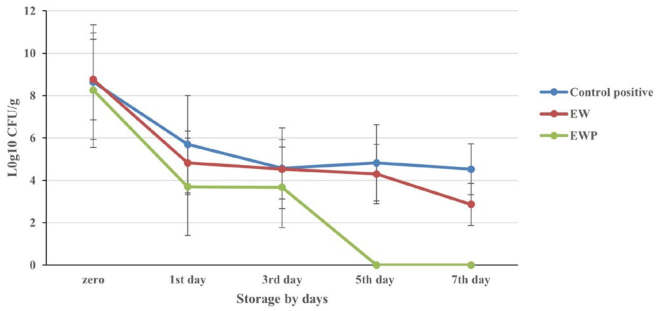

Fig. 2. Effect of EW and EWP coating on survival of *E. coli* in egg content over storage time.

The results demonstrated the effectiveness of SAEW and SAEW-pectin (SAEW-p) treatments in reducing *S.* Enteritidis survival on the eggshell (Figure 3) during refrigerated storage compared to the untreated control. On day 0, bacterial counts were 6.76, 6.65, and 6.70 log CFU/g for the control, SAEW, and SAEW-p groups, respectively. By day 1, the SAEW-p treatment significantly reduced the bacterial count to 5.80 log CFU/g, while SAEW and the control recorded 6.43 and 6.48 log CFU/g, respectively. On day 3, the SAEW-p group continued to demonstrate greater efficacy with a reduction to 5.37 log CFU/g, compared to 5.68 log CFU/g for SAEW and 6.43 log CFU/g for the control. By day 5, the bacterial counts decreased to 4.53 log CFU/g in the SAEW-p group, compared to 4.48 log CFU/g for SAEW and 5.52 log CFU/g for the control. On day 7, the SAEW-p treatment showed the greatest reduction, achieving a bacterial count of 3.74 log CFU/g, compared to 4.38 log CFU/g for SAEW and 5.37 log CFU/g for the control. These results highlight the superior efficacy of SAEW-p treatment in reducing *S.* Enteritidis on the eggshell, providing enhanced microbial safety during storage.

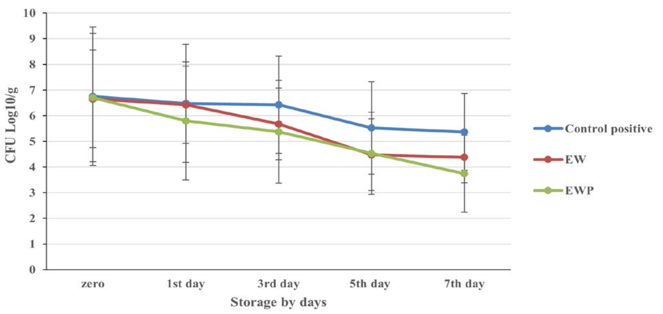

Fig. 3. Effect of EW and EWP coating on *S.* Enteritidis survival on eggshell over storage time.

The results in Figure 4 showed the efficacy of SAEW and SAEW-pectin (SAEW-p) treatments in reducing *S.* Enteritidis survival in egg content during refrigerated storage compared to the untreated control. On day 0, bacterial counts were 6.54, 7.32, and 6.64 log CFU/g for the control, SAEW, and SAEW-p groups, respectively. By day 1, SAEW-p reduced the bacterial count to 5.39 log CFU/g, while SAEW and the control recorded 4.80 and 5.52 log CFU/g, respectively. On day 3, SAEW-p showed greater reduction with a bacterial count of 3.43 log CFU/g compared to 4.48 log CFU/g for SAEW and 4.70 log CFU/g for the control. By day 5, SAEW-p further reduced *S.* Enteritidis to 2.52 log CFU/g, while SAEW and the control recorded 3.85 and 4.56 log CFU/g, respectively. By day 7, SAEW-p treatment achieved the most significant reduction, with a bacterial count of 1 log CFU/g, compared to 3.60 log CFU/g for SAEW and 4.43 log CFU/g for the control. These findings confirm the superior efficacy of SAEW-p in reducing *S.* Enteritidis in egg content, ensuring improved microbial safety during storage.

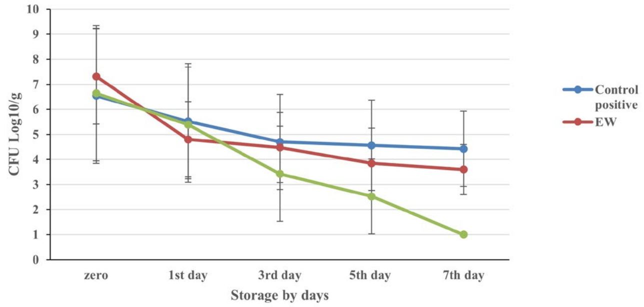

Fig. 4. Effect of EW and EWP coating on *S.* Enteritidis survival in egg content over storage time.

Discussion

The integration of slightly acidic electrolyzed water (SAEW) with pectin-based coatings has been investigated to enhance the microbial safety and quality of eggs during storage. SAEW serves as an effective disinfectant, reducing bacterial load on eggshells. However, its application can damage the egg's cuticle, potentially leading to bacterial invasion and quality degradation. To mitigate this, pectin coatings have been employed to restore the cuticle's protective barrier, thereby preserving internal egg quality.

The marginal reduction in bacterial count on day 0 for SAEW (8.30 log CFU/ml) and SAEW-p (8.12 log CFU/ml) compared to the control (8.14 log CFU/ml) indicates that SAEW alone may not achieve substantial immediate bacterial reductions. This is consistent with findings by Huang *et al.* (2008), who reported that SAEW requires adequate exposure time to disrupt bacterial membranes. The addition of pectin (SAEW-p) did not initially enhance the antimicrobial effect but appeared to provide a barrier for long-term inhibition.

By day 1, SAEW alone significantly reduced bacterial count to 6.01 log CFU/ml, compared to 6.12 log CFU/ml for SAEW-p and 7.09 log CFU/ml for the control. This aligns with Hricova *et al.* (2008), who found SAEW effective in reducing bacterial loads on fresh produce surfaces. SAEW-p's slightly slower reduction may indicate pectin's initial role in coating rather than direct bacterial inactivation. By day 1, SAEW alone significantly reduced bacterial count to 6.01 log CFU/ml, compared to 6.12 log CFU/ml for SAEW-p and 7.09 log CFU/ml for the control. This aligns with Hricova *et al.* (2008), who found SAEW effective in reducing bacterial loads on fresh produce surfaces. SAEW-p's slightly slower reduction may indicate pectin's initial role in coating rather than direct bacterial inactivation. On day 3, the bacterial count in SAEW-p-treated eggshells (4.37 log CFU/ml) was significantly lower than SAEW alone (5.30 log CFU/ml) and the control (6.57 log CFU/ml). This result suggests that pectin coating provides a physical barrier, preventing recontamination and reducing bacterial proliferation, as supported by Li *et al.* (2015). By day 5, SAEW-p-treated eggs showed a bacterial count of 3.52 log CFU/ml, which further decreased to 3.30 log CFU/ml on day 7. In comparison, SAEW-treated eggs retained a higher bacterial count (5.37 log CFU/ml on day 5 and 4.67 log CFU/ml on day 7). The control group showed the least reduction, maintaining 5.65 log CFU/ml on day 7. The superior performance of SAEW-p agrees with Yuan *et al.* (2023), who demonstrated that pectin-based coatings provide prolonged antimicrobial effects through moisture retention and the creation of unfavorable conditions for microbial growth.

SAEW's efficacy aligns with studies like Forghani *et al.* (2015), which demonstrated its broad-spectrum bactericidal activity on food surfaces. Pectin coating's ability to enhance microbial control over time has been highlighted in Ciriminna *et al.* (2022), who described its film-forming properties and its role in reducing bacterial invasion and moisture loss. The synergistic effect of SAEW and pectin coating is novel compared to the individual application of coatings or disinfectants, indicating a promising approach for the egg industry to enhance microbial safety during storage.

The combination of SAEW and pectin coating (SAEW-p) outperforms SAEW alone and untreated controls in reducing bacterial counts on eggshells over storage time. SAEW provides immediate microbial inactivation, while the pectin coating acts as a protective barrier, sustaining microbial inhibition and enhancing storage safety (Tu *et al*., 2024). These findings suggest that SAEW-p could be an effective, eco-friendly approach for improving the microbial safety of eggs, consistent with previous research on natural antimicrobial coatings and electrolyzed water.

The complete elimination of *E. coli* by SAEW-p on day 5 highlights the synergistic effects of slightly acidic electrolyzed water and pectin. SAEW provides an initial antimicrobial effect by generating hypochlorous acid, which damages bacterial cell walls and disrupts cellular functions (Huang *et al*., 2008). The addition of pectin enhances this effect by forming a physical coating, which creates an additional barrier to bacterial survival and potential recontamination. These results align with studies like Li *et al*. (2015), which demonstrated the extended antimicrobial efficacy of bio-coatings on food products.

SAEW treatment showed significant *E. coli* reduction in egg content but did not achieve sterility. On day 7, the residual bacterial count in SAEW-treated samples was still measurable. This is consistent with findings by Hricova *et al*. (2008), who reported that while SAEW effectively reduces bacterial populations, its efficacy may diminish over time without additional protective measures, such as coatings. The control group exhibited a persistent bacterial presence throughout the storage period, underlining the importance of implementing effective antimicrobial treatments for food safety. The absence of any intervention in the control group allowed for natural bacterial proliferation or survival, which poses a potential risk to consumer health. This observation supports previous research, such as Yoon *et al*. (2021), which highlighted the limitations of untreated eggs in microbial safety during storage.

Studies like Forghani *et al*. (2015) have emphasized the antimicrobial efficacy of electrolyzed water in reducing bacterial loads on food surfaces. However, the novel combination with pectin further enhances the long-term sterility, as shown in this study.

Pectin coatings have been shown to improve the shelf life and microbial safety of various food products by forming a semi-permeable layer that inhibits bacterial penetration (Ciriminna *et al*., 2022). The ability of SAEW-p to eliminate *E. coli* from egg content and maintain sterility over storage is a significant advancement in food safety technology (Tu *et al*., 2024). This treatment addresses two critical concerns: Immediate bacterial inactivation to ensure consumer safety and Long-term protection during storage, reducing spoilage and contamination risks (Nan *et al*., 2010).

The findings highlight the efficacy of slightly acidic electrolyzed water (SAEW) and SAEW combined with pectin (SAEW-p) in reducing *Salmonella* enteritidis survival on eggshells during refrigerated storage. Both treatments significantly outperformed the untreated control, with SAEW-p demonstrating superior effectiveness in bacterial reduction. On day 0, the bacterial counts were similar across the groups, with 6.76 log CFU/ml for the control, 6.65 log CFU/ml for SAEW, and 6.70 log CFU/ml for SAEW-p. These results suggest that the initial treatment had a limited immediate effect, as the counts for SAEW and SAEW-p were only slightly lower than the control. By day 1, SAEW-p significantly reduced the bacterial count to 5.80 log CFU/ml, while SAEW and the control recorded higher counts of 6.43 and 6.48 log CFU/ml, respectively. The addition of pectin in the SAEW-p treatment likely enhanced its bactericidal effect by creating a protective barrier that inhibited bacterial survival, consistent with findings by Li *et al*. (2015). On day 3, SAEW-p continued to outperform SAEW, with bacterial counts reduced to 5.37 log CFU/ml compared to 5.68 log CFU/ml for SAEW. The control group remained significantly higher at 6.43 log CFU/ml. This progressive reduction aligns with studies like Huang *et al*. (2008), which reported that electrolyzed water treatments maintain antimicrobial activity over time. By day 5, SAEW-p demonstrated a reduction to 4.53 log CFU/ml, compared to 4.48 log CFU/ml for SAEW and 5.52 log CFU/ml for the control. The comparable performance of SAEW and SAEW-p at this point suggests that the pectin coating may contribute more to extended efficacy rather than immediate bacterial inactivation. On day 7, SAEW-p achieved the most significant reduction, with a bacterial count of 3.74 log CFU/ml, compared to 4.38 log CFU/ml for SAEW and 5.37 log CFU/ml for the control. The sustained reduction observed in the SAEW-p group highlights its enhanced ability to inhibit bacterial survival during storage. This result is consistent with Yoon *et al*. (2021), which noted the long-term benefits of combining electrolyzed water with coating agents for food safety. Studies such as Forghani *et al*. (2015) demonstrated the efficacy of SAEW in reducing microbial contamination on food surfaces. However, the current results emphasize that combining SAEW with pectin significantly enhances its antimicrobial effect. Pectin coatings, as reported by Ciriminna *et al*. (2022), create a semi-permeable barrier that prevents bacterial adherence and protects against recontamination, corroborating the superior performance of SAEW-p in this study.

The results underline the potential of SAEW-p as a superior treatment for ensuring microbial safety on eggshells during storage. The combination of SAEW's strong bactericidal action and pectin's physical barrier properties offers a dual mechanism for bacterial control. This approach could be particularly valuable for the egg industry in reducing the risk of *S.* Enteritidis contamination, a leading cause of foodborne illnesses.

Conclusion

Both SAEW and SAEW-p reduce bacterial counts on eggshells compared to the untreated control. SAEW-p consistently demonstrates superior bacterial reduction in eggshells throughout the storage period. SAEW-p treatment eliminates *E. coli* from egg contents by day 5 and maintained sterility through day 7, highlighting its exceptional effectiveness. SAEW alone is less efficient in reduction of bacterial count in egg contents compared to SAEW-p. This study underscores the importance of combining advanced sanitation methods like SAEW with natural coating agents such as pectin to achieve enhanced bacterial control. Future research could explore the application of SAEW-p on other food products and under varying storage conditions to further validate its efficacy and scalability.

Conflict of interest

The authors have no conflict of interest to declare.

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