Original Article

Effects of Acidic Electrolyzed Water and Peppermint Nanoemulsion in Gum Coatings on Eggshell Quality

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How to Cite This Article Ramezanipour-Laskokalayeh, M., Ataee, M., Khani, M., Anvar, S. A., & Haji-Seyed-Mohammad-Shirazi, R. (2026). Effects of Acidic Electrolyzed Water and Peppermint Nanoemulsion in Gum Coatings on Eggshell Quality. *Iranian Journal of Veterinary Medicine, 20*(4), 803-820. http://dx.doi.org/10.32598/ijvm.20.4.1005982

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ABSTRACT

Background: Slightly acidic electrolyzed water (SAEW) efficiently inactivates bacteria on the egg surface, but it may damage the eggshell's cuticle. A protective coating applied after disinfection can prevent moisture and carbon dioxide loss.

Objectives: This study examined the efficacy of SAEW (30 mg/L chlorine), peppermint essential oil nanoemulsion (PME-NE) (25 mg/mL), and tragacanth and zedo gums (1% each) alone and in combination, in enhancing antimicrobial, sensory, and structural qualities of eggshells stored for 1, 28, and 56 days at 25 ˚C.

Methods: The eggs were initially subjected to treatment with SAEW and later coated using solutions of the nanoemulsion and gum. The different analyses performed included minimum inhibitory and bactericidal concentrations, agar disc diffusion assay, microbiological counts, scanning electron microscopy, and sensory evaluation.

Results: Eggs treated with SAEW, followed by coating with tragacanth and zedo gums combined with peppermint essential oil nanoemulsion, resulted in the lowest counts of total viable microorganisms (5.39 log CFU/g), *Salmonella enteritidis* (4.41 log CFU/g), and *Staphylococcus aureus* (5.03 log CFU/g) on day 56. Microbial load showed an increasing trend over time ($P \leq 0.05$). Sensory evaluation revealed the highest scores for flavor, odor, color, texture, and overall acceptability of cooked eggs and raw egg odor. Scanning electron microscopy indicated that this treatment produced the thickest and most protective coating.

Conclusion: Applying SAEW followed by coating with tragacanth and zedo gums combined with PME-NE is the most effective method to enhance antimicrobial, sensory, and structural quality of eggshells at 25 ˚C.

Keywords: Antimicrobial efficacy, Food safety, Gum-based nanoemulsion, Natural preservatives, Shelf-life extension

Article info:
Received: 10 Feb 2026
Accepted: 25 Apr 2026
Publish: 01 Jul 2026

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Introduction

In an attempt to guarantee safety and quality, the food industry is undertaking all possible measures; yet, foodborne outbreaks still occur. Eggs can harbor a multitude of microorganisms capable of entering through the eggshell and thereby transmitting foodborne diseases to consumers. Thus, washing eggs before sale is considered an obligatory regulation in many countries. Some studies have suggested that traditional chemical cleaners are linked to drug residues and public health safety problems. Another factor affecting just about every cleaning or disinfecting agent is that their potential is reduced significantly in the presence of organic matter, like chicken manure, which is strongly adhered to the eggshell surface.

It becomes imperative to formulate a new eco-friendly cleaner and disinfectant for the destruction of bacteria and feces from egg surfaces (Tu et al., 2024; Sheng et al., 2021). An innovation in the control and prevention of microorganisms is slightly acidic electrolyzed water (SAEW, pH 5.0-6.5), as it is produced continuously by electrolysis using normal commercial equipment. SAEW has antimicrobial properties, is less surface corrosive, and is associated with reduced harm human health and the environment (Zhang et al., 2023a; Yuan et al., 2023; Nishihama et al., 2024; Yan et al., 2022). Zang et al. showed that SAEW can be used effectively as a sanitizer for *Salmonella enteritidis* on the shells of eggs. It was further discovered that SAEW successfully reduced the load with *S. enteritidis* and *Escherichia coli* on eggs, probably reinforcing their quality during storage of fresh eggs (Zang et al., 2019).

Coating materials, such as propolis, chitosan, proteins, oils, and starches have been employed to address problems encountered during the storage of food products. Certain coating materials prolong an egg's shelf-life by preventing the entrance of microorganisms through the shell and by curbing the evaporation of albumen water, thereby resulting in minimized economic losses (Sariyel et al., 2022).

Tragacanth gum (*Astraglus gossypinus*) is an anionic polysaccharide secreted from certain Asian species; its property to stabilize and lower tension makes it the basis for several industries. Further, its peculiar composition of monosaccharides is envisaged to offer applications in tissue engineering and drug delivery (Asadzadeh & Pirsa, 2020). Tragacanth gum mainly increases aqueous phase viscosity and decreases tension at the oil-water interface, whereas its stability in acidic conditions is the basis of its use as a thickener and stabilizer for the food industry (Goudar et al., 2020). Zedo gum (*Amygdalus scoparia*) is a polysaccharide obtained from *A. scoparia* Spach, which has both soluble and insoluble fractions, possesses maximum viscosity at pH 7.2 and 24 ˚C (Fadavi, 2014), and is used for emulsifying and suspending alongside tragacanth in the food sector (Pirouzifard et al., 2020).

The cuticle is destroyed by its contact with SAEW on the egg surface with an increase in eggshell permeability, thereby reducing egg quality during storage. The use of suitable coating compounds can actually reduce the permeability, thus forming a new membrane to hinder moisture and carbon dioxide loss through the damaged cuticle. Other antimicrobial agents may exhibit synergistic effects with acidic electrolyzed water regarding disinfection of eggs, especially essential oils when used in their nanoemulsified form.

Research commonly emphasizes disinfection against certain monoculture bacteria by SAEW-disinfecting *E. coli*, *Staphylococcus aureus*, *Salmonella*, *vibrio*, *Bacillus* spores, and *Leuconostoc* spp. (Zhang et al., 2023a; Yuan et al., 2023; Zhang et al., 2022; Luo et al., 2024; Mo et al., 2024). Nevertheless, the effectiveness of SAEW on its own declines with prolonged storage, while coating materials may not adequately compensate for cuticle damage if used alone. Combining SAEW with natural polymers and essential oils might synergistically provide an extra level of protection (Luo et al., 2024). Essential oil from peppermint (*Mentha piperita*) (PME) is famous for its antimicrobial activity for a variety of foodborne pathogens (Ashrafudoulla et al, 2023). However, this mode of application is limited due to PME's hydrophobicity, volatility, and instability during processing conditions. To overcome these limitations, peppermint oil can be formulated as a nanoemulsion (PME-NE) for improved stability, dispersion in aqueous systems, and penetration into microbial cell membranes for enhanced antimicrobial performance (Maurya et al, 2021; Prakash & Markose, 2025).

Therefore, the aim of this study was to investigate the antimicrobial properties, sensory evaluation, and morphological effects of SAEW, PME, tragacanth, and zedo gums on the shelf life of eggs stored at 25 ˚C.

Materials and Methods

Materials

Freshly laid chicken eggs were obtained from a local poultry farm in Tehran, Iran (n=400, each weighing 55–60 g). Only intact eggs were selected after visual examination, and cracked eggs were discarded. SAEW was prepared using potassium chloride (KCl, 1 mol/L) mixed with tap water. Tragacanth and zedo gums (10 g/L) were used as coating agents. Peppermint (*M. piperita*) leaves were obtained from a reputable local supplier for essential oil extraction. Tween 80 (20 g/L) was used as the emulsifier for nanoemulsion preparation. Bacterial strains, including *E. coli* O157:H7 (ATCC 43894), *S. aureus* (ATCC 13565), and *S. enteritidis*, were used for microbiological assays. All reagents and chemicals were of analytical grade and obtained from reputable suppliers. Ultrapure and deionized water were used all through the experimentation.

Generation of slightly acidic electrolyzed water

Slightly acidic electrolyzed water (SAEW) was made by electrolyzing a 1 mol/L KCl solution mixed with tap water using the generator for SAEW (D35L, PERIC Hydrogen Technology, Japan) with a production rate of 2.05 L/min. From the pre-experiment analyses, the oxidation-reduction potential (ORP), pH, concentration of available chlorine, and UV-visible characteristics were determined in triplicate. The pH and ORP of SAEW were quantified with a pH/ORP meter (827, Metrohm, Switzerland). The available chlorine concentration was assessed with a chlorometer (Chlorometer Duo, Palintest Co., UK). Changes in the ACC speciation were analyzed via the spectrophotometric characteristics of SAEW with a spectrophotometer (UH5300, Hitachi, Japan) at 200 and 400 nm. Ultraviolet spectra were recorded at 25 ˚C in 1 cm quartz cells with tap water as the blank standard. Measurements of absorbance at 234 and 292 nm were used to determine concentrations of HClO and ClO^-, respectively (Wang et al., 2017).

Preparation of tragacanth and zedo gums for egg coating

Tragacanth and zedo gums (10 g/L) were powdered and kept in appropriate containers. Then, 50 g of each gum was taken in different flasks and filled with ultrapure water to a total volume of 1000 mL. Then, the mixtures were stirred with a magnetic stirrer at 500 rpm and at room temperature for a duration of 10 h, followed by overnight keeping in the refrigerator without stirring at 4 ˚C. The gum solutions were prepared one day before using (Sariyel et al., 2022).

Nanoemulsion preparation

Peppermint (*M. piperita*) essential oil was obtained by hydrodistillation using the Clevenger apparatus. Fresh peppermint leaves were bought from the local grocery store (Tehran, Iran). Accordingly, 150 g of dried leaves were placed in a flask of the Clevenger apparatus. Then, the apparatus was also filled with distilled water up to half the volume before being heated for 4 h. The cooling tubes of the Clevenger apparatus liquefied the vapors coming from the plant material, which were then collected in the receiver. The essential oil was dehydrated with dry sodium sulfate and filtered through a 0.45 µm microfilter. The prepared essential oil was transferred into sterile, dark glass containers to prevent photodegradation and stored in a refrigerator at 4 ˚C before experiments conducted (Yousef et al., 2018). Nanoemulsion preparations were performed by oil-to-water dilution (1:2 oil to water) using distilled water, and then the emulsifier Tween 80 (20 g/L) was mixed with the preparation. The obtained emulsion underwent sonication for about 20 min at a frequency 40 kHz employing an ultrasonic cleaner (make: WUC-DO3H, 100 W), and then sonicated for 1 minute with a high-energy ultrasonic probe (model VCX750, 750 W, 20 kHz), and resonicated for about 20 min with the ultrasonic cleaner under cooling conditions (Anuchapreeda et al., 2012; Yousef et al., 2018). Amplitude of 50% was set with a 1 s on/1 s off pulse mode during probe sonication, and the temperature was maintained below 30 ˚C using an ice bath (Gupta et al., 2016).

Treatments

According to Sheng et al. (2021), the study was carried out on eight groups of 50 eggs each. The treatments included SAEW (30 mg/L), tragacanth and zedo gums (together at a 50:50 ratio) and PME-NE at its minimal bactericidal concentration (MBC; 25 mg/mL), alone or in combination (Table 1). Eggs were immersed in SAEW for 4 min and dried, wherever applicable. After drying, batches of 10 eggs each were placed in a 1 L beaker, with respect to the above conditions. If applicable, the eggs were immersed in PME-NE (1 L) for 2 min and transferred to a laminar flow hood for drying for 1 h before immersion in the gum coating solution for 2 min. The tragacanth and zedo gum coating was prepared by mixing 10 g/L solutions of both gums in a 50:50 ratio. The samples were then stored in open-top sterile baskets at ambient conditions (25 ˚C) for qualitative and quantitative evaluation on days 1, 28, and 56 (Table 1).

Table 1. Experimental treatments and codes used in this study

Treatment Code	Tragacanth + Zedo Gums (1%, Coating)	PME-NE (25 mg/mL, Coating/Immersion)	SAEW (Immersion; ACC ≈ 30 mg/L)
C	–	–	–
SAEW	–	–	✓
PME-NE	–	✓	–
SAEW + PME-NE	–	✓	✓
ZTG	✓	–	–
PME-NE + ZTG	✓	✓	–
SAEW + ZTG	✓	–	✓
SAEW + PME-NE + ZTG	✓	✓	✓

Note: C=Control (no SAEW, no coating). SAEW=Slightly acidic electrolyzed water (pH 5.0–6.5; available chlorine concentration ≈ 30 mg/L). PME=Peppermint (*M. piperita*) essential oil. PME-NE=Peppermint essential oil nanoemulsion (presented here at 25 mg/mL). ZTG=Coating with tragacanth + Zedo gums (total 1% w/v). PME-NE + ZTG indicates simultaneous application of nanoemulsion and gum coating.

Antibacterial activity

Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the prepared nanoemulsion and peppermint essential oil

To determine MIC, the broth macro dilution method was performed. Overnight cultures of the gram-negative *E. coli* O157:H7 (43894) and gram-positive *S. aureus* (ATCC 13565) in the log phase were used to prepare cell suspensions of 10^6 CFU/mL in Mueller-Hinton Broth (MHB). The test sample was prepared by diluting it in 4% dimethyl sulfoxide (DMSO), and 100 µL of each dilution was added to the microtubes, which were then separately inoculated with 100 µL of the bacterial suspension. The inoculated microplates were kept at 37 ˚C for 24 h after thorough mixing. The MIC was defined as the lowest concentration that caused noticeable inhibition of microbial growth. The occurrence of turbidity indicated the presence of bacterial growth. To determine MBC, 100 µL of sample was taken from each test tube and incubated on Mueller-Hinton agar medium at 37 ˚C for 24 hours. It was then defined as the least concentration of the test samples that produced a 99.9% reduction in CFU/mL compared to the control. Gentamicin served as a positive control for antimicrobial efficacy (El Hamdaoui et al., 2018).

Agar disc diffusion assay

The antibacterial potency of the prepared PME-NE and PME was assessed using the agar disc diffusion assay. Dried surfaces of Mueller-Hinton agar plates were inoculated with 0.5 mL of broth culture standardized against *E. coli* O157:H7 43894 and *S. aureus* ATCC 13565. Thereafter, antimicrobial discs acquired commercially with streptomycin as the positive control and dimethyl sulfoxide (DMSO) as the negative control were pipetted onto the inoculated agar plate. Each disc was compressed firmly to allow for sufficient contact of the agar surface. The plates were placed in an incubator at 37 ˚C for 24 h without inversion. The diameter of inhibition zones was measured using a ruler in millimeters. Each test was performed in triplicates, and the mean values were calculated (Agbaje et al., 2023).

Eggshell inoculation

Eggshell inoculation was done according to Ni et al. (2014) with some modifications. Before testing, the eggshells were brought to room temperature, washed with deionized water, and dried in a laminar hood for 1 h. To prepare for the inoculation of the desired pathogenic microbes, the respective strains were added to 10 mL of a suspension containing 10^9 CFU/mL of *S. enteritidis* and *S. aureus* into 1 L of ¼ Ringer's solution and agitated for 5 min. Each egg was then individually immersed in each of the prepared microbial solutions for 10 s and dried for 1 h at a room temperature of 20±2 ˚C under the laminar flow hood to allow for bacterial attachment.

Microbiological determination

This section describes the process of sampling, preparing, and isolating eggs following treatment and infection. Each egg sample was collected at each time interval to be individually transferred to a volume equal to ten times its weight of Ringer's solution and then stirred on a shaker for 10 min. The eggs were then taken out and serially diluted in a neutralization buffer solution of 1 g/L of sterile peptone water. Total plate count measurement was done following incubation at 37 ˚C for 48 h on agar plate count medium. Each group was represented by three eggs for microbial counts. To determine *S. enteritidis*, 0.1 mL of all the prepared dilutions was cultured on Salmonella Shigella agar plates, which were then incubated at 37 ˚C for a duration of 48 h. To determine *S. aureus*, the incubation in mannitol salt agar medium lasted 48 h. Counts were based on a log10 (log10 CFU/g) scale (Yenilmez et al., 2020).

Examining egg shell morphology

To compare the shell morphological characteristics of the egg samples on the last day, three eggs from each group were dried at 37 ˚C in an air-blast drying oven (DHG-9030A, China). The eggs were broken down by tweezers into small fragments between 3 and 5 mm and attached to the table with respect to the outer surfaces of the shell samples. The surface morphology and cross-section of the eggshell were studied using a scanning electron microscope (SEM; 5 kV). The eggshell morphology was examined on days 1 and 56 (Yuan et al., 2022). ImageJ processing software was used for the quantification of pore area as a percentage, visual assessment of particle size, and measurement of the coating thickness.

Sensory evaluation

The boiled eggs were cut into two pieces and analyzed for aspect, texture, odor, taste, and acceptability. The evaluators were semi-trained evaluators within the age group of 20-40 years who evaluated the raw and cooked forms using a 5-point hedonic test (score 1=very unpleasant, score 2=unpleasant, score 3=moderate, score 4=pleasant, and score 5=very pleasant). The evaluators provided the panelists with drinking water to rinse their mouths after the evaluation of individual samples. Sensory evaluation was undertaken on days 1, 28, and 56 for both raw and cooked samples of egg. It thus aimed at understanding the sensory perception of cooking and raw eggs (Hailemariam et al.; 2022).

Statistical analysis

The statistical analysis of results was carried out using SPSS software, version 26 (SPSS Inc., Chicago, Illinois, USA). Values are expressed as mean±standard error of the mean. Data were analyzed uisng analysis of variance (ANOVA), followed by Tukey's test to assess significant differences ($P<0.05$) between samples on different days.

Ethical considerations

This study was conducted on eggs obtained from a commercial poultry farm and did not involve any experimental interventions on live animals; therefore, animal ethics approval was not required. Regarding the sensory evaluation, all coating ingredients used (tragacanth gum, zedo gum, and peppermint essential oil) were food-grade and safe for consumption. Verbal informed consent was obtained from all semi-trained panelists prior to their participation in the sensory analysis.

Results

Antimicrobial activity of peppermint essential oil and its nanoemulsion: comparison of MIC, MBC, and inhibition zone diameters against *E. coli* and *S. aureus*

The comparative analysis of MIC, MBC, and inhibition zone of PME-NE and PME against *E. coli* and *S. aureus* bacteria is tabulated in Table 2. The essential oil

Table 2. Comparison of MIC, MBC, and inhibition zone of PME and PME-NE

Bacteria	Inhibition Zone (mm)		MIC (mg/mL)		MBC (mg/mL)	
	PME	PME-NE	PME	PME-NE	PME	PME-NE
E. coli	9.66 ± 0.33^{aA}	6.66 ± 0.44^{aB}	6.25 ± 0^{bA}	6.25 ± 1.44^{bA}	10.41 ± 0^{bB}	12.5 ± 1.44^{bA}
S. aureus	6.66 ± 0.4^{bA}	6.33 ± 0.5^{aA}	12.50 ± 1.44^{aB}	16.66 ± 1.44^{aA}	20.83 ± 1.44^{aB}	25 ± 1.44^{aA}

Note: Different lowercase letters in each column indicate significant differences between two microorganisms and different capital letters in each row indicate significant differences between PME and PME-NE for each factor ($P<0.05$).

and PME-NE had a very small MIC (6.25 mg/mL and 6.25 mg/mL respectivly) for *E. coli* compared to *S. aureus* (12.50 mg/mL and 16.66 mg/mL for essential oil and PME-NE, respectivly) (P≤0.05). Furthermore, the MBC of essential oil and PME-NE (10.41 mg/mL and 12.50 mg/mL, respectivly) was lower for *E. coli* compared to *S. aureus* (20.83 mg/mL and 25 mg/mL, respectivly) (P≤0.05). In the case of *S. aureus*, the MIC and MBC for PME-NE were significantly greater than PME (P≤0.05). PME and its nanoemulsion exhibited lower inhibition and lethal concentrations against *E. coli* compared to *S. aureus*. PME had a lower MIC and MBC against bacteria compared to the mint essential oil nanoemulsion, while the inhibition zone for *E. coli* was greater than that for *S. aureus*, and the inhibition zone diameter for PME was greater than that for its nanoemulsion. The inhibition zone of PME and PME-NE for *E. coli* was significantly greater than *S. aureus* (P≤0.05). Also, with respect to both pathogenic organisms, the inhibition zone diameter for the PME (9.66 mm and 6.66 mm for *E. coli* and *S. aureus*, respectivly) was significantly greater than for PME-NE (6.66 mm and 6.33 mm for *E. coli* and *S. aureus*, respectively) (P≤0.05).

Egg quality test results

Antimicrobial tests

The total microbial counts and *S. aureus* counts were highest in the control sample at all time intervals, while the SAEW + PME-NE + ZTG sample contained the lowest total microbial population (P≤0.05), so that on day 56, total microbial counts and *S. aureus* counts were 7.65 log CFU/g and 7.27 log CFU/g, respectively for the control sample and 5.39 log CFU/g and 5.03 log CFU/g, respectively for the SAEW + PME-NE + ZTG sample. All samples witnessed a significant increase in the total microbial count and *S. aureus* counts over time (P≤0.05) (Table 3). On day 1, there was no statistical significant difference in the *Salmonella* counts among all the different samples (P≥0.05); however, in the next time interval, the highest number of *Salmonella* was observed in the control sample, while the lowest number was observed for the SAEW + ZTG and SAEW + PME-NE + ZTG samples (P≤0.05), so that on day 56, the number of *Salmonella* was 7.31 log CFU/g for the control sample and 4.39 log CFU/g and 4.41 log CFU/g for the SAEW + ZTG and SAEW + PME-NE + ZTG samples, respectively. The *Salmonella* counts in all samples increased significantly over time (P≤0.05).

Scanning electron microscope analysis

Using scanning electron microscope (SEM), the surface microstructure and cross-sectional area of eggshells were analyzed affected by SAEW, PME-NE, and tragacanth and zedo gums (Figures 1A, 1B, 1C, 1D, and 1E). In Figure 1A, the control sample showed surface holes just after 24 hours, whose volume percent with respect to the complete structure was about 4.06%. After 56 days, these cracks enlarged to cover about 39.6% of the entire surface area. The cross-sectional images indicated the increase in roughness and porosity over time. The results presented in Figure 1B confirm the possibility of achieving porosity on the shell's surface through treatment using SAEW compared to the control sample, which had a porosity of 14.5%. After 56 days of treatment, deep and large cracks were observed in the eggshells with the volume percentage of pores and cracks increasing from a basic value of 13.66%. More pores were observable in both the cross-sectional area of the electrolyzed water-treated sample and the control sample, particularly at the upper end of the eggshell. However, over time, the number of pores increased. After one day of applying the PME-NE coating, the surface showed dense impressions containing several agglomerated nanoparticles, each ranging in size from 0.2 to 1 µm. The observation technique should employ FE-SEM, as the small nanoparticles cannot be visualized using conventional SEM, which lacks the magnification required for such observations.

Also, the surface porosity percentage in this sample was found to be approximately 46.4%, which is almost equal to that of the control sample readings. After 56 days, the surface roughness was observed to decrease, accompanied by a reduction in particle size and a narrowing of the particle size distribution. The results for cracks and porosity were 66.4%, which is significantly lower than those reported in Figures 1A and 1B.

As shown in Figure 1D, one day after coating the sample with essential oil nano-emulsions along with tragacanth and zedo gums, the surface appeared completely smooth and uniform, with only 0.32% of the surface exhibiting cracks and/or pores. At this stage, particles derived from the essential oil nano-emulsions were visible, measuring 0.3–2.5 µm in diameter. Over time, the percentage of cracks and pores increased to 2.99%, a notable figure compared with earlier samples.

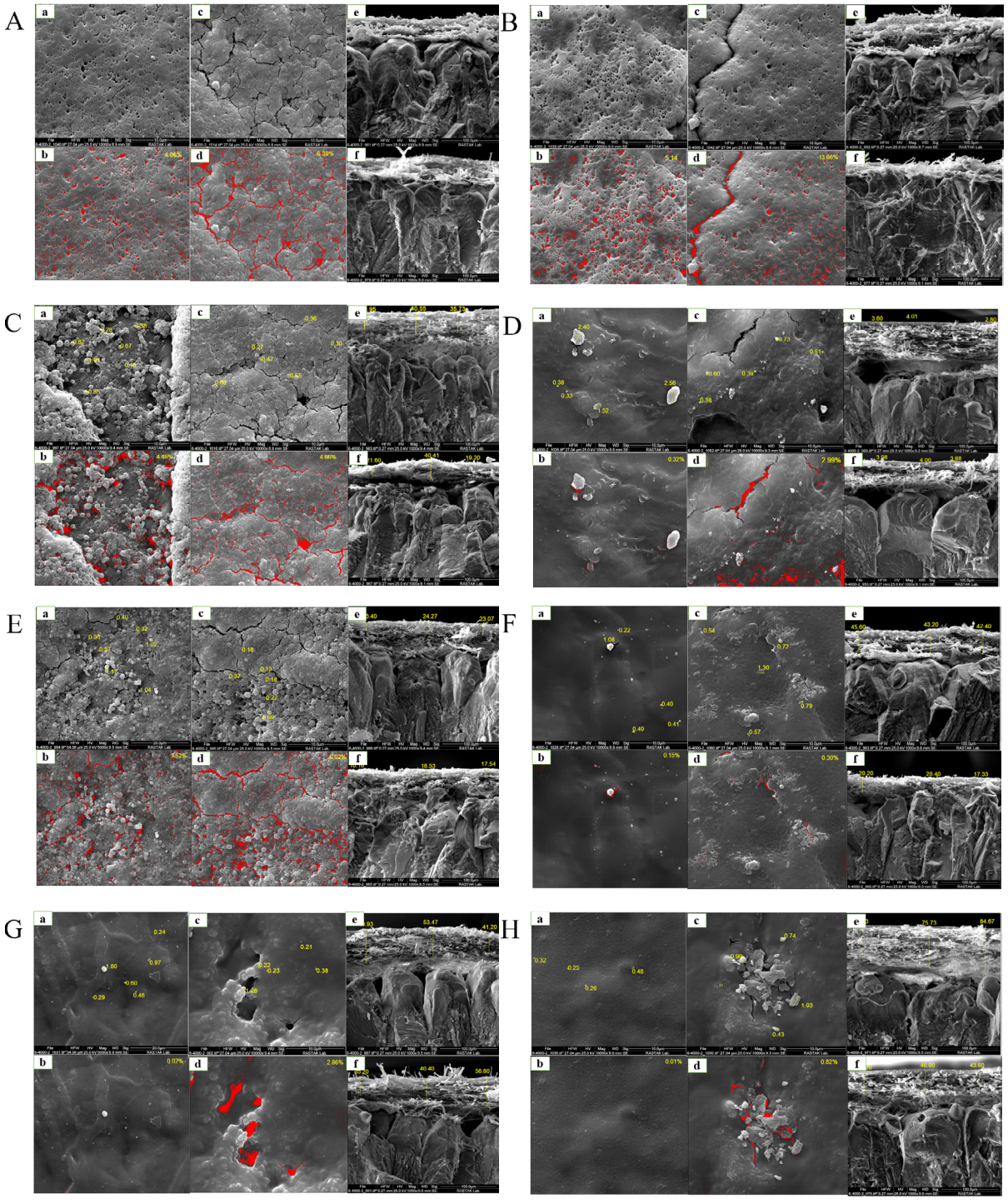

Figure 1. SEM analysis of the eggshell surface

A) Control sample, B) Sample treated with electrolyzed water, C) Sample coated with peppermint essential oil nanoemulsion, D) Sample coated with tragacanth and zedo gum, E) Sample treated with electrolyzed water and peppermint essential oil nanoemulsion, F) Sample treated with electrolyzed water and PME-NE, G) Sample treated with peppermint essential oil nanoemulsion and tragacanth and zedo gum, H) Sample treated with electrolyzed water and peppermint essential oil nanoemulsion, and tragacanth and zedo gum

Note: a and b: After 1 day; c and d: After 56 days; e: Cross-sectional micrograph after 1 day; f: Cross-sectional micrograph after 56 days. The red areas in the processed images correspond to areas of porosity and cracks on the surface of the eggshell.

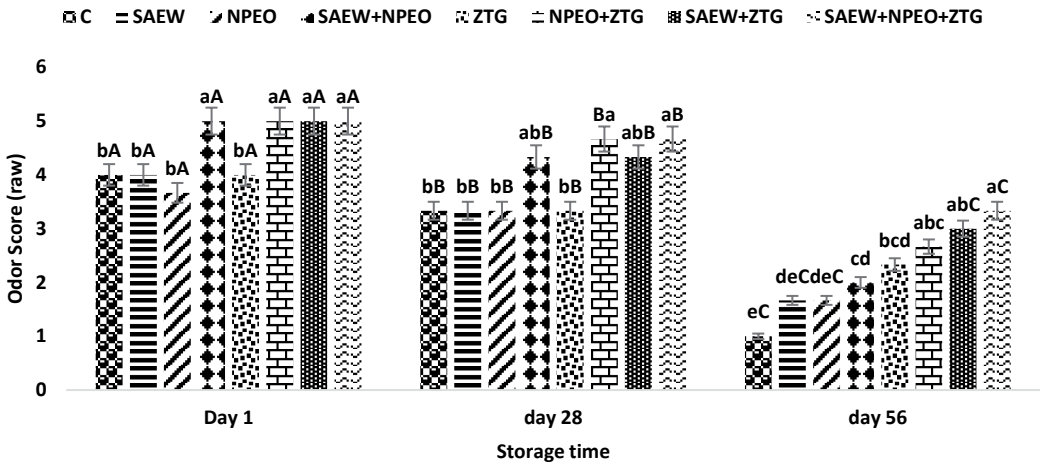

Figure 2. Changes in odor scores of raw egg samples coated with SAEW, zedo and tragacanth gums, and PME-NE at 25 ˚C during 56 days of storage.

Note: Different uppercase and lowercase letters indicate significant differences (P<0.05) between storage days within each treatment and among treatments on the same storage day, respectively.

Cross-sectional images represented that the coating was not of the same thickness as that produced the PME-NE. After 1 and 56 days, the average thickness was approximately 35 µm, with no noticeable gain or loss over the 56-day period. The presence of nanoparticles ranged approximately from 0.3 to 1.1 µm, similar to the nanoparticle characteristics shown in Figure 1D and further illustrated in Figure 1E.

On day 1, the porosity percentage in this particular sample measured lower than that of the sample without electrolyzed water, at approximately 3.52%. After 56 days, many cracks had evolved in the structure, bringing the total porosity and crack percentage to 6.02%, which is higher than that of the sample without electrolyzed water. The average coating thickness for this sample on days 1 and 56 was approximately 21 µm and 17 µm, respectively.

Figure 1F shows that the smoothness of the sample surface was retained after a day, with only 0.15% of pores—reduced compared to the sample treated without electrolyzed water. Particles were also observed to agglomerate within the size range of 0.4–1.1 µm. The percentage of pores slightly increased during this period, reaching 0.30% by the 56th day, which remained lower than in all other samples, including the coatings of traga-

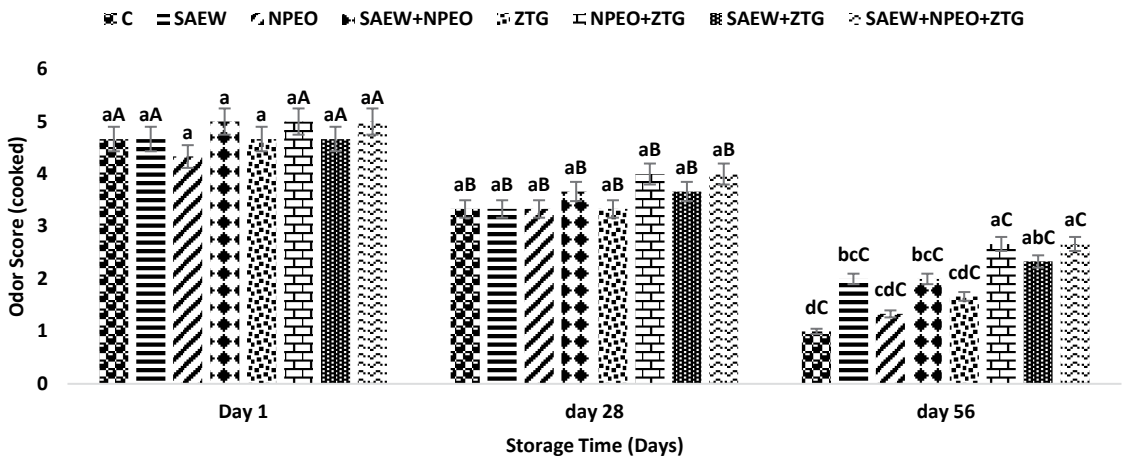

Figure 3. Changes in odor scores of raw egg samples coated with SAEW, zedo and tragacanth gums, and PME-NE at 25 ˚C during 56 days of storage.

Note: Different uppercase and lowercase letters indicate significant differences (P<0.05) between storage days within each treatment and among treatments on the same storage day, respectively.

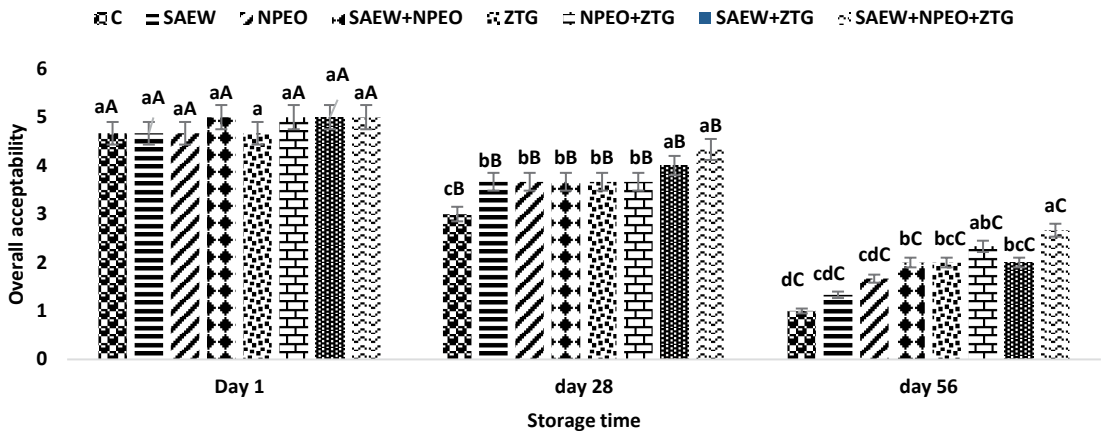

Figure 4. Changes in overall acceptance scores of raw egg samples coated with SAEW, zedo and tragacanth gums, and PME-NE at 25 ˚C during 56 days of storage

Note: Different uppercase and lowercase letters indicate significant differences (P<0.05) between storage days within each treatment and among treatments on the same storage day, respectively.

canth with zedo gum and those without electrolyzed water. After 1 and 56 days, the coating thicknesses were approximately 43 and 25 µm, respectively, representing a significant improvement in thickness compared to samples treated with tragacanth and zedo gum without electrolyzed water.

According to the results in Figure 1G, a coating formed after one day with an entirely smooth surface containing only 0.02% pores. The presence of some clearly visible nanoparticles in this coating was also noted; however, the pore percentage dramatically increased to about 86.2% by the 56th day. This value was lower than the 92.77% pore observed in samples made with single coatings of PME, tragacanth, and zedo gum, which demonstrated a comparatively better level of protection. The progressive loss of protective effectiveness in this coating over time, compared to that prepared with electrolyzed water, was clearly evident even upon visual inspection. The coating thickness after 1 and 56 days was approximately 52 µm and 46 µm, respectively, showing a slight decrease over time. However, this thickness remained much greater than that of coatings composed of individual components of these two materials.

The results presented in Figure 1H indicated that the coating achieved 0.01% porosity after 1 day, which increased to 0.82% after 56 days. The coating thicknesses at these two time points were approximately 79 µm and 47 µm, respectively—greater than those observed in the earlier samples. At both time points, this sample exhibited the presence of emulsion nanoparticles. The results suggest that this coating had the greatest thickness and offered the highest level of protective performance when used during egg incubation.

SEM analysis of the control eggshell surface showed increasing porosity and cracking with age over the 56-day period, while coated samples maintained varying degrees of surface integrity depending on the treatment. Surface roughness and porosity increased over time, gradually weakening the mechanical properties of the eggshell.

Sensory evaluation

Control sample sensory scores remained significantly lower across all time intervals, while the combined treatments attained the highest scores for all sensory parameters. In particular, SAEW + PME-NE + ZTG recorded the highest scores for texture, flavor, odor, color, and overall acceptance (Table 4), while PME-NE + ZTG also scored high in texture and odor. The addition of ZTG contributed to higher scores for color (P≤0.05). Conversely, the flavor and odor scores for single treatments (SAEW or PME-NE) were generally lower than those recorded for the combined treatments.

In general, sensory scores for all attributes tended to decrease significantly over time (P≤0.05). For raw eggs, odor followed the same trend, with the lowest scores observed in the control sample, while the highest scores occurred for SAEW + PME-NE + ZTG (Figure 2; P≤0.05). The boiled egg treatments failed to maintain sensorily acceptable characteristics after 56 days of storage; only SAEW + PME-NE + ZTG and SAEW + ZTG retained acceptable odor scores in the raw eggs.

Table 3. Changes in total viable microorganisms, *S. aureus* count (log10 CFU/g), and the number of *S. enteritidis* of egg samples coated with SAEW, zedo and tragacanth gums, and PME-NE at 25 ˚C during 56 days of storage

Treatments	Total Viable Microorganisms		
	Day 1	Day 28	Day 56
Control (C)	4.17±0.01^{aC}	5.36±0.02^{aB}	7.65±.01^{aA}
SAEW	2.86±0.03^{eC}	4.32±0.01^{cB}	5.98±00.03^{eA}
PME-NE	3.48±0.12^{bC}	4.92±0.03^{bB}	6.46±0^{bA}
SAEW + PME-NE	3.26±0.01^{cC}	4.25±0.01^{dB}	6.27±0^{cA}
ZTG	2.79±0.02^{efC}	4.2±0.01^{eB}	5.70±0.06^{fA}
PME-NE + ZTG	2.73±0.04^{eC}	4.07±0.02^{gB}	5.46±0^{gA}
SAEW + ZTG	2.96±0.01^{dC}	4.11±0^{fB}	6.20±0.01^{dA}
SAEW + PME-NE + ZTG	2.51±0.03^{gC}	3.80±0.02^{hB}	5.39±0^{hA}

***S. aureus* Count**			
Treatments	**Day 1**	**Day 28**	**Day 56**
Control (C)	4.74±0.06^{aB}	6.42±0^{aB}	7.27±0.08^{aA}
SAEW	4.38±0^{dB}	5.32±0.01^{eA}	5.32±1.01^{dA}
PME-NE	4.58±0.03^{bB}	6.15±0.02^{bA}	6.15±0.02^{bA}
SAEW + PME-NE	4.48±0.04^{cB}	5.94±0.01^{cA}	5.94±0.01^{cA}
ZTG	4.28±0^{eB}	5.27±0^{fA}	5.27±0^{deA}
PME-NE + ZTG	4.14±0.01^{fB}	5.22±0^{gA}	5.22±0^{eA}
SAEW + ZTG	4.70±0.07^{gB}	5.89±0^{dA}	5.89±0^{cA}
SAEW + PME-NE + ZTG	3.86±0.03^{hB}	5.03±0.01^{hA}	5.03±0.01^{fA}

***S. enteritidis* Count**			
Treatments	**Day 1**	**Day 28**	**Day 56**
Control (C)	4.41±0.4^{aC}	5.57±2.04^{aB}	7.31±0.03^{aA}
SAEW	4.74±0.28^{aC}	5.17±0.49^{aB}	6.76±1.01^{aA}
PME-NE	4.51±0.18^{aC}	5.13±0.22^{aB}	5.50±0.09^{bA}
SAEW + PME-NE	4.67±0.05^{aC}	5.32±0.12^{aB}	5.32±0.12^{bcA}
ZTG	4.48±0.11^{aC}	5.09±0.17^{aB}	5.09±0.17^{bcdA}
PME-NE + ZTG	4.25±0.16^{aC}	4.68±0.27^{bB}	4.68±0.27^{cdA}
SAEW + ZTG	4.22±0.26^{aC}	4.39±0.04^{bB}	4.39±0.04^{dA}
SAEW + PME-NE + ZTG	4.24±0.42^{aC}	4.41±0.09^{bB}	4.41±0.09^{dA}

Note: Different lowercase letters in each column indicate significant differences between egg samples on each day and different capital letters in each row indicate significant differences between days of storage in each sample (P<0.05).

Table 4. Changes in texture, color and flavor score of egg samples coated with SAEW, zedo and tragacanth gums, and PME-NE at 25 ˚C during 56 days of storage

Treatments	Texture		
	Day 1	Day 28	Day 56
Control (C)	5 ± 0^{aA}	2.33 ± 0.57^{cB}	1 ± 0^{cC}
SAEW	5 ± 0^{aA}	2.66 ± 0.57^{bcB}	1 ± 0^{cC}
PME-NE	5 ± 0^{aA}	3.33 ± 0.57^{abB}	1.66 ± 0.57^{bC}
SAEW + PME-NE	5 ± 0^{aA}	3.33 ± 0.57^{abB}	1 ± 0^{cC}
ZTG	5 ± 0^{aA}	4 ± 0^{aB}	2 ± 0^{abC}
PME-NE + ZTG	5 ± 0^{aA}	3.66 ± 0.57^{aB}	2 ± 0^{abC}
SAEW + ZTG	5 ± 0^{aA}	4 ± 0^{aB}	2 ± 0^{abC}
SAEW + PME-NE + ZTG	5 ± 0^{aA}	3.66 ± 0.57^{aB}	2.33 ± 0.57^{aC}
Color			
Treatments	**Day 1**	**Day 28**	**Day 56**
Control (C)	5 ± 0^{aA}	3.66 ± 0.57^{aB}	1 ± 0^{bC}
SAEW	5 ± 0^{aA}	3.66 ± 0.57^{aB}	1.33 ± 0.57^{abC}
PME-NE	5 ± 0^{aA}	3.66 ± 0.57^{aB}	1.66 ± 0.57^{aC}
SAEW + PME-NE	5 ± 0^{aA}	3.66 ± 0.57^{aB}	2 ± 0^{aC}
ZTG	5 ± 0^{aA}	4 ± 0^{aB}	2 ± 0^{aC}
PME-NE + ZTG	5 ± 0^{aA}	4 ± 0^{aB}	2 ± 0^{aC}
SAEW + ZTG	5 ± 0^{aA}	4 ± 0^{aB}	1.66 ± 0.57^{aC}
SAEW + PME-NE + ZTG	5 ± 0^{aA}	4 ± 0^{aB}	2 ± 0^{aC}
Flavor			
Treatments	**Day 1**	**Day 28**	**Day 56**
Control (C)	4.66 ± 0.57^{aA}	3 ± 0^{bB}	1 ± 0^{dC}
SAEW	4.66 ± 0.57^{aA}	3 ± 0^{bB}	1 ± 0^{dC}
PME-NE	4.66 ± 0.57^{aA}	3 ± 0^{bB}	1 ± 0^{dC}
SAEW + PME-NE	5 ± 0^{aA}	3.66 ± 0.57^{abB}	2 ± 0^{bcC}
ZTG	4.66 ± 0.57^{aA}	3 ± 0^{aB}	1.66 ± 0.57^{cC}
PME-NE + ZTG	5 ± 0^{aA}	3.66 ± 0.57^{abB}	2.33 ± 0.57^{abC}
SAEW + ZTG	5 ± 0^{aA}	3.66 ± 0.57^{abB}	2 ± 0^{bcC}
SAEW + PME-NE + ZTG	4.66 ± 0.57^{aA}	3 ± 0^{bB}	2.66 ± 0.57^{aC}

Note: Different lowercase letters in each column indicate significant differences between egg samples on each day and different capital letters in each row indicate significant differences between days of storage in each sample ($P<0.05$).

Discussion

The use of nanoemulsions as novel antimicrobial agents is captivating. Nanoemulsions enhance the antimicrobial activity of essential oils by increasing their solubility, stability, and ability to interact with microbial cell membranes. The small droplet size allows better penetration and fusion with the lipid bilayer of bacterial cells, leading to membrane disruption, leakage of intracellular contents, and ultimately cell death. The electrostatic attraction between the cationic charge of our nanoemulsion and the anionic charge found in the pathogens makes nanoemulsions thermodynamically favorable for fusion with lipid membranes, resulting in cell lysis and death of the pathogen. Bolouri et al., (2022) state that nanoemulsions destroy bacterial layers non-specifically. Importantly, these factors have not prevented the development of resistant strains, suggesting that nanoemulsions can be considered significant antibacterial agents. The MIC is the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism, and the MBC is the lowest level that kills it. The observed reduction is not attributed to the lethal effect of the extract but mainly occurs because microorganisms enter the death phase and decline in number. In other words, these levels indicate the presence of the microorganism in the environment without reproduction (Trisha et al., 2024; Van de Vel et al., 2019).

Essential oils and plant extracts are widely used in the food industry due to their effectiveness against a broad spectrum of microorganisms, owing to their antimicrobial nature (Bolouri et al., 2022). There are differing views on how essential oils and plant extracts act on microorganisms. The accompanying chemical groups of the constituent components of the essential oil and plant extracts may not directly indicate a specific antimicrobial mechanism, as these compounds can affect multiple cellular targets (Van de Vel et al., 2019).

In the present study, the MIC and MBC values of the nanoemulsion were significantly higher than th o se of the essential oil. This suggests that the active ingredients were retained longer over time, and with the s1ow release of these compounds, the nanoemulsion exhibited a greater antimicrobial role compared to the extract. This can be explained by the higher concentration of the extract compared to the nanoemulsion.

Likewise, Azizkhani et al. (2021) revealed that the MIC and MBC of tarragon essential oil nano-emulsion were higher than the free version in both gram-positive and gram-negative bacteria. They noted that a greater MIC and MBC characterize all cases, with potentially increased values for the nanoemulsion compared to the free form (Azizkhani et al., 2021). Dabowl et al. also revealed that agarwood extract in its nanoemulsion form had greater MIC activity than in its free form, with *Listeria monocytogenes* being more sensitive than *E. coli* in this instance (Dabowl et al., 2021). Moghimi et al. showed that the MIC and MBC of rosemary essential oil nanoemulsion were about four times higher than those of the free form. They attributed this to the active binding of the bioactive substances of the essential oil with the applied surfactant, which prevented the essential oil from approaching the bacterial cell membrane (Moghimi et al., 2016).

Spearmint essential oil nanoemulsion was also evaluated by Mehran et al. for its antimicrobial activity and was reported to exhibit desirable activity against *E. coli* (Mehran et al., 2023). In a similar vein, when comparing the antimicrobial properties of rosemary essential oil nanoemulsion to its free form against various food pathogens, Hassanzadazar et al. showed that the diameter of the growth inhibition zone for rosemary essential oil was superior to that of its nanoemulsion form. They attributed this to the coating of the essential oil in the nanostate by a liquid phase containing the surfactant (Hassanzad azar et al., 2019).

Thus, the treatment of eggs with SAEW and coating with a solution of tragacanth and zedo gum along with PME-NE significantly reduced microbial loads. For total viable microorganisms, reductions ranged from 1.5–1.8 log CFU/g; for *S. aureus*, from 1.39–2.62 log CFU/g; and for *S. enteritidis*, from 1.16–2.9 log CFU/g on the 28th and 56th days of storage, compared to the control group.

Compared to our results, Kim et al. studied the disinfectant effect of SAEW against pure cultures of *E. coli*, *S. enteritidis, Typhimurium* sp., *S. aureus*, and *Bacillus cereus* spores. They reported that pathogenic vegetative cells were completely inactivated within 1 minute of treatment with SAEW at 20 mg/L (Kim et al., 2019).

The effect of synergistic bactericidal treatment with SAEW and UV-C light (ultraviolet lamp, λ 254 nm) on the inactivation of *S. enteritidis* on artificially inoculated eggshells was studied by Bing et al. They noted complete inactivation of *S. enteritidis* with SAEW + UV at a 20 mg/L available chlorine concentration (ACC). The combination of SAEW and UV rays appeared to yield better bactericidal activity for eggshells than other combined UV and SAEW methods. Overall, results indicate that this treatment, SAEW + UV, is a novel method for enhancing the microbial safety of eggshells (Bing et al., 2019).

Nishihama et al. evaluated the bactericidal activity of SAEW against Streptococcus mutans and confirmed the above observation, indicating that SAEW has high antibacterial action against oral bacteria. s suggests its clinical applicability in the dental field for disinfection, oral care, and the sterilization of devices used in dentistry (Nishihama et al., 2023). The aforementioned bactericidal activity can be attributed to the property of available chlorine to generate and invade chloride ions, thereby establishing osmotic pressure within bacterial cells.

The study by Chelliah et al. confirmed a significantly higher antimicrobial activity of the combination of essential oil and electrolyzed water with Thymus vulgaris nano-emulsion (NE) against foodborne pathogens compared to their individual activities. The findings indicated that the nano-emulsion enhances SAEW's ability to kill microbes, suggesting a complexation between SAEW and TV through hydrophobic interaction (Chelliah et al., 2023).

It is almost certain that their average weight is reduced to about 14.5% when surface-treated with SAEW. This suggests a short-term destruction of the egg, according to previous studies, due to the damaging effect of electrolyzed water on the cuticle of the maturing eggshell (Yuan et al., 2022). On the other hand, Favier et al. also reported that eggs with surface damage from treatment with water or chlorine solution showed an increase in the depth and width of cracks on their surfaces for both SAEW and control groups as storage time increased Cracked eggshells can release moisture and carbon dioxide (Favier et al., 2000).

The cuticle is an outer protective cover composed of hydroxyapatite crystals, polysaccharides, lipids, and glycoproteins, which contribute to mechanical strength and protect against bacterial penetration into the eggshell. After 56 days, the development of fine cracks was found in the eggshells, with the volume percentage of porous and cracked areas increasing to 13.66%. The upper part of the eggshells is filled with pores, which increase over time compared to the control sample. Gole et al. also reported that either water or chlorine solutions could damage the eggshell surface, and that coating could cover and crack the surface due to the application of SAEW treatment (Gole et al., 2014).

The PME-NE coating's numerical values indicated an agglomerated nanoparticle with 46.4% porosity on the surface. Surface roughness, particle size, cracks, and porosity decreased by 66.4% after 56 days. The coating thickness after 1 and 56 days was reduced to 35-30 µm and only 5 µm, respectively, after two months. The treatment of a sample with tragacanth and zedo gum nanoemulsion resulted in a smooth surface with 0.32% of cracks and pores. Over time, the percentage of cracks and pores increased to 2.99%, providing better protection than PME. Despite its lesser thickness, the tragacanth and zedo gum coating exhibited enhanced density and durability and performed more effectively.

Sheng et al. studied the effect of SAEW and chitosan coating on egg quality, and their results showed that eggs from the CSC and SAEW + CS groups were very smooth, whereas the control and SAEW groups showed surface cracks. In contrast, after SAEW exposure, compared to the control group, eggs stored on days 0 and 21 had cracks that were deeper and wider. This shows that SAEW disinfection adversely affected the integrity of the eggshells. Eggs in the SAEW group showed cracks on their surface, while in the SAEW + CS eggs group, the coating likely made the surface smooth and masked the cracks on the eggshells (Sheng et al., 2021).

When the protective properties of PME-NE and the sample of electrolyzed water were compared, it was found that with one day of treatment, the percentages of porosity were lower due to the nanoparticles. However, higher cracks occurred after 56 days. The average coating thickness was respectively 21 and 17 micrometers. Therefore, the long-term sustainability of electrolyzed water appears not to be feasible, nor does water-free PME-NE appear to be sustainable.

A day later, the sample treated with electrolysis -tragacanth- and zedo gum-based nano-emulsions possessed a smooth surface, showing as low as 0.15% pores, which signifies a better protection against the external environment. The percentage of pores increased within 56 days to slightly 0.3%. The thickness of the coating increased to 43 and 25 µm, indicating that it offered better protection than those which were not treated; although the protection may not sustain much over these long periods.

The results above indicated that PME-NE, tragacanth, and zedo gum formed a smooth coating only after one day. Over 56 days, porosity percentages increased to 86.2%, which offered better protection than a single coat. However, even if the thickness reduced, it still superseded the performance of a single coating. After 56 days, a sample with electrolyzed water, PME-NE, and tragacanth and zedo gum formed a protective layer with a porosity percentage of 0.01% and 0.82%. The emulsion nanoparticles were responsible for maximum thickness.

The impact of SAEW and chitosan and pectin coatings on egg quality was experimentally tested by Yuan et al. SEM images of the surface and cross-sections of all eggs stored for 0, 28, 56 days were studied. It was observed that the eggs in the coating groups (SAEW + PT, SAEW + CS, and SAEW + PT + CS) displayed a smooth surface, while cracks were observed in the control and SAEW eggs during the entire storage duration. It was also noted that with an increase in storage duration, the depth and width of eggshell cracks of both SAEW and control groups extended, while the coating thickness in the coating groups reduced. On day 28, membranes of the combined PT + CS-treated group were somewhat thicker compared to the separate treatment groups. By day 56, the egg surfaces of the SAEW + PT and SAEW + CS groups exhibited irregularities, while the surface of the eggs from the SAEW + PT + CS group remained smooth. Application of a pectin and chitosan coating leads to better physical properties than the individual use of either compound, again corroborating the findings of the present study (Yuan et al., 2022).

Storage-related decline in sensory attributes is probably related to moisture loss, oxidation of lipid constituents, and weakening of the cuticle layer, which in turn affect odor, color, and surface texture. The improvement of sensory attributes is attributed to the synergetic effect of SAEW and peppermint essential oil nanoemulsion. This combination prevents microbial spoilage and delays oxidative changes that are responsible for off-flavor formation (Bolouri et al., 2022; Mehran et al., 2023; Parra et al., 2024).

Recent studies support the above findings. Parra et al. (2024) showed that encapsulating electrolyzed acidic water (EAW) in polymeric coatings significantly enhances egg surface protection by reducing albumen liquefaction. This preserves sensory characteristics, with a panelist acceptance of approximately 85% for coated eggs compared to 57% for uncoated ones. These observations align with the general trend established by previous studies on essential oil-based coatings, where the incorporation of natural bioactives enhanced sensory acceptability by masking undesirable odors and improving gloss and texture.

Our results differ from those obtained by Tajik et al., who studied the effect of coating eggs with zein and chitosan biopolymers and oregano essential oil. They found that the sensory properties of the eggs were highly influenced by the incorporation of essential oil into the chitosan and zein immersion solution, which effectively promoted consumer acceptance of coated eggs (Tajik et al., 2010). According to Safavi and Javanmard, the application of whey protein edible coatings containing Shirazi thyme extract and rice bran oil on eggs resulted in positive improvements regarding egg quality. Of all sensory flavors, the coated eggs were found to be more acceptable than the control. Among the treatments, those with a lower percentage incorporation of rice bran oil in the coated eggs also resulted in higher acceptance from the consumers' perspective (Safavi & Javanmard, 2016).

Pujols et al. (2014) used alpha-chitosan, beta-chitosan, and soybean oil, either individually or in combination (chitosan-soybean oil emulsion), for egg coating applications. These authors further stated that since beta-chitosan is more swollen and reactive than alpha-chitosan, the percentage loss in weight is less under this type of coating. Moreover, it is found that beta-chitosan imparts a better shine to the eggshell surface, making it more customer-friendly. The integration of SAEW, peppermint essential oil nanoemulsion, and natural hydrocolloid gums appears to be a promising strategy for ensuring desirable sensory characteristics of ambient-stored eggs.

Conclusion

The present study aims to test the antimicrobial effectiveness, sensory characteristics, and morphological efficacy of SAEW and PME-NE, zedo gum, and tragacanth gum on eggshells at 25 ˚C. The results showed that eggs treated with SAEW, as well as those coated with tragacanth and zedo gums and with a PME-NE, had the least total microbial population, which includes *S. enteritidis* and *S. aureus*. Sensory evaluation indicated that boiled eggs received the highest scores for flavor, odor, color, texture, and overall acceptability, while raw eggs scored highest for odor. Also, the coating that showed maximum thickness exhibited desirable protective virtues, as per SEM analysis. It is suggested that the effect of using other disinfectant compounds or methods, along with SAEW, on the quality characteristics and shelf-life of eggs be investigated.

Ethical Considerations

Compliance with ethical guidelines

This study did not require animal ethics committee approval as it involved the evaluation of commercially sourced eggs without any interventions on live animals. For the sensory evaluation involving human panelists, all utilized materials (tragacanth gum, zedo gum, and peppermint essential oil) were food-grade and safe for human consumption. Furthermore, verbal informed consent was obtained from all panelists prior to their participation in the sensory analysis.

Funding

The study was extracted from the PhD dissertation of Masoud Ramezanipour-Laskokalayeh, approved by the Department of Veterinary Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

Conceptualization: Maryam Ataei and Masoud Ramezanipour-Laskokalayeh; Methodology: Masoud Ramezanipour-Laskokalayeh, Mohammadreza Khani, and Amirali Anvar; Investigation, data curation, and writing the original draft: Masoud Ramezanipour-Laskokalayeh; Formal Analysis and validation: Reza Haji-Seyed-Mohammad-Shirazi and Maryam Ataei; Supervision and project administration: Maryam Ataei; Resources: Mohammadreza Khani, Amirali Anvar, and Reza Haji-Seyed-Mohammad-Shirazi; Review & editing: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to acknowledge the Science and Research Branch, Islamic Azad University, Tehran, for the scientific support of this research.

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