SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 22(1) (2024) 34-42, DOI: 10.24323/akademik-gida.1460979

Research Paper / Araştırma Makalesi

Decontamination of *Salmonella* Enteritidis on Eggshell: Assessment of Efficiency of a Bacteriophage and Levulinic Acid-Sodium Dodecyl Sulfate

Gamze Koçer Alaşalvar[1], Zeliha Yıldırım[2]

[1]Department of Food Processing, Bor Vocational School, Niğde Ömer Halisdemir University, Niğde, Türkiye
[2]Department of Food Engineering, Faculty of Engineering, Niğde Ömer Halisdemir University, Niğde, Türkiye

Received (Geliş Tarihi): 29.06.2022, Accepted (Kabul Tarihi): 17.03.2024
Corresponding author (Yazışmalardan Sorumlu Yazar): gkoceralasalvar@ohu.edu.tr (G. Koçer Alaşalvar)
+90 388 225 2354 +90 388 225 0112

ABSTRACT

The majority of food infections caused by consumption of egg and egg products are caused by *Salmonella enterica* subspecies *enterica* serovar Enteritidis. In this study, the inhibitory effect against *S.* Enteritidis was determined by dipping the eggshell into *Salmonella*-specific bacteriophage SE-P47 and levulinic acid (LVA) plus sodium dodecyl sulfate (SDS) solutions (0.5% LVA+0.05% SDS, 1% LVA+0.05% SDS and 2% LVA+0.5% SDS) separately for 10 minutes. The treatments of phage and 2% LVA+0.5% SDS reduced *S.* Enteritidis below the detectable level on eggshell (at 2.76, 3.22, 4.48 and 5.30 log CFU/cm^2 inoculum levels). After the treatment of 1% LVA+0.05% SDS, 1.94 and 0.89 log reductions were obtained at 4.48 and 5.30 log CFU/cm^2 inoculum levels, respectively, while *S.* Enteritidis decreased below the detectable number at 2.76, 3.22 log CFU/cm^2 inoculum levels. Although the lowest antibacterial activity was observed in the treatment of 0.5% LVA+%0.05 SDS, the decrease in the number of *S.* Enteritidis detected in all samples except 5.30 log CFU/cm^2 inoculum level was found to be significant compared to the control sample. The results indicated that the combination of LVA and SDS, and especially SE-P47 phage alone had good potential efficacy for *Salmonella* decontamination on eggshell.

Keywords: Bacteriophage, Decontamination, Eggshell, Levulinic acid, *Salmonella* Enteritidis

Yumurta Kabuğunda *Salmonella* Enteritidis'in Dekontaminasyonu: Bakteriyofaj ve Levülinik Asit-Sodyum Dodesil Sülfatın Etkinliğinin Değerlendirilmesi

ÖZ

Yumurta ve yumurta ürünlerinin tüketiminden kaynaklanan enfeksiyonların çoğu, *Salmonella enterica* subspecies *enterica* serovar Enteritidis kaynaklıdır. Bu çalışmada, yumurta kabukları *Salmonella*'ya özgü SE-P47 bakteriyofajı ve levülinik asit (LVA) ile sodyum dodesil sülfat (SDS) çözeltilerine (0.5% LVA+0.05% SDS, 1% LVA+0.05% SDS ve 2% LVA+0.5% SDS) ayrı ayrı 10 dk süreyle daldırılarak *S.* Enteritidis üzerindeki inhibitör etki belirlenmiştir. Faj ve %2 LVA+%0.5 SDS uygulamaları, yumurta kabuğu üzerinde *S.* Enteritidis'i tespit edilebilir seviyenin altına düşürmüştür (2.76, 3.22, 4.48 ve 5.30 log kob/cm^2 inokulum seviyelerinde). %1 LVA+%0.05 SDS uygulamasından sonra 4.48 ve 5.30 log kob/cm^2 inokulum seviyelerinde sırasıyla 1.94 ve 0.89 log azalma elde edilirken, 2.76, 3.22 log kob/cm^2 inokulum seviyelerinde *S.* Enteritidis, tespit edilebilir seviyenin altına düşmüştür. En düşük antibakteriyel aktivite %0.5 LVA+%0.05 SDS uygulamasında gözlemlenmesine rağmen, 5.30 log kob/cm^2 inokulum seviyesi hariç tüm örneklerde tespit edilen *S.* Enteritidis sayısındaki azalma, kontrol örneğine göre önemli bulunmuştur. Sonuçlar, yumurta kabuğunda *Salmonella* dekontaminasyonu için LVA ile SDS'nin kombinasyonu ve özellikle SE-P47 fajının tek başına iyi bir potansiyel etkinliğe sahip olduğunu göstermiştir.

Anahtar Kelimeler: Bakteriyofaj, Dekontaminasyon, Yumurta kabuğu, Levülinik asit, *Salmonella* Enteritidis

INTRODUCTION

Increasing urbanization and changes in consumer dietary trends (increased consumption of quality protein) lead to an increase in the demand for animal products and therefore more animal food products to be processed [1, 2]. The increase in production complicates food safety control and increases the risk of contamination of products with foodborne pathogens. Approximately 600 million cases of foodborne diseases and 420.000 deaths occur worldwide every year according to the World Health Organization. About 40% of foodborne diseases are particularly common among children under 5 years of age due to weak immune systems [3].

Insufficient food safety applications during production, packaging, transportation, and storage cause serious consequences such as foodborne illness and death, as well as socioeconomic and psychological problems in society. Unsafe food consumption is estimated to cause losses of 110 billion dollars each year, especially in low- and middle-income countries, through reductions in productivity, health expenditures, and mass destruction of food [4].

Salmonella enterica (non-typhoidal) is one of the main causes of foodborne diseases, especially diarrheal diseases [3, 5]. The Centers for Disease Control and Prevention (CDC) estimates there are approximately 1.35 million diseases, 26,500 hospitalizations, and 420 deaths from *Salmonella* in the United States every year [6]. *Salmonella* is a member of the *Enterobacteriaceae* family and has two main species, *S. enterica* and *S. bongori*. Approximately 2,600 serotypes have been identified for *Salmonella* species, of which less than 100 are known to cause human infections. *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) and *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) are the most frequently reported serovars responsible for *Salmonella* infections worldwide [1, 7, 8].

Salmonella species are found in the intestinal microflora of humans, domestic, and farm animals [2]. *Salmonella* is usually transmitted to humans by consuming contaminated food or water but can also be transmitted through contact with infected animals [7]. The poultry, egg and egg products, pork, beef, dairy products, fruits, vegetables, seafood, and water are the reservoirs of *Salmonella* species [9, 10]. It is well known that one of the most common sources of *Salmonella* outbreaks is the consumption of poultry and eggs, however, the most common serotypes isolated from poultry and egg products are *S.* Enteritidis [2, 11-13].

Egg is a frequently preferred food for human nutrition because it is nutritious and cheap compared to other protein sources [14]. Various egg products consumed in the world can be listed as shell eggs, egg whites, egg yolks, liquid, frozen or dried forms [15, 16]. In addition, the egg is included in the composition of many products such as bakery products, noodles, mayonnaise, ice cream, and desserts, due to the functional properties of its various components such as emulsifying and foaming ability [16, 17].

The inner part of eggs obtained from healthy poultry is considered sterile, however, it is known that there are a large number of microorganisms in the eggshell. Eggshells can be contaminated with microorganisms during production, processing, preparation, and packaging in the food chain [18]. Contamination of eggshell with *Salmonella* occurs due to contact of the eggshell with contaminated feces during or after laying. Other sources such as farmers, pets, and rodents play a role in the contamination of eggshells with *Salmonella.* Contamination of egg contents with *Salmonella* occurs through transfer from the eggshell (horizontal contamination) or direct contamination of the egg as a result of infected ovaries or oviduct tissue before shell formation (vertical contamination) [15, 19].

Various methods have been investigated to reduce or prevent *Salmonella* contamination on eggshells, including washing with chlorine-based surface sanitizers [20], hydrogen peroxide and sodium dodecyl sulfate [21], ozone [22], lactic acid [23], plant extracts with antimicrobial properties [18, 24], X-ray irradiation, chlorine dioxide, and the synergistic effect of the combined treatment [25], hot air treatment (pasteurization) [26], ultraviolet light [27], pulsed UV light [28, 29], far infrared [30], atmospheric plasma treatment [31, 32].

Organic acids are known to have antimicrobial effects. Among organic acids, levulinic acid (LVA) stands out because it can be produced with high efficiency from renewable raw materials [33]. LVA is also used as a flavoring agent in addition to its antimicrobial effect in the food industry. On the other hand, sodium dodecyl sulfate (SDS) which has inhibitory and lethal effects against foodborne microorganisms is used as an all-purpose food additive and surfactant. Both LVA and SDS have been generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) [34, 35]. The bactericidal and virucidal activities of the combination of LVA with SDS are greater than that of LVA or SDS alone [36, 37]. Moreover, these antimicrobial activities can be achieved in lower LVA and SDS concentrations and shorter contact time. This is due to the synergistic effect between LVA and SDS. In previous studies, it has been shown that the combined use of LVA and SDS solution is effective in inactivating various pathogenic microorganisms in biofilms [37, 38], food contact surfaces [39], and food surfaces such as lettuce, poultry skin, cantaloupe, strawberries [33, 40, 41]. It was reported that the use of LVA with SDS is effective in the inactivation of microorganism groups such as bacteria, viruses, molds, and yeast, but not in foodborne parasites such as *Cryptosporidium* [36].

Bacteriophages (phages) are bacteria-specific viruses. Virulent phages infect bacterial cells and multiply intracellularly, causing host bacterial cells to lyse. Therefore, phages are potential biocontrol agents against foodborne pathogens [42]. They attract attention

with their host specificity and environmentally friendly characteristics when compared to chemical compounds. In addition, the advantages of using phages as biocontrol agents are that they can eliminate biofilms, are effective even at low doses and have a relatively cost-effective and simple production process [43, 44].

Studies on the use of bacteriophages as biocontrol agents in various foods have focused on *Salmonella* serovars, *Listeria monocytogenes*, *Escherichia coli*, *Shigella* spp., *Campylobacter jejuni*, and *Staphylococcus aureus* pathogenic bacteria [42, 45-49]. FDA has approved the use in certain foods of several phage-based preparations designed for the control of foodborne bacterial pathogens such *as E. coli* (Secure Shield E1), *E. coli* O157:H7 (EcoShield™), *L. monocytogenes* (ListShield™, PhageGuard Listex™), *Salmonella* spp. (SalmoFresh™, PhageGuard S™, SalmoPro®), *Shigella* spp. (ShigaShield™) [46, 50].

In this study, phage or different concentrations of LVA plus SDS solutions were used for decontamination of *S.* Enteritidis on the surface of eggshells. It was aimed to evaluate the inhibition effects of phage and LVA plus SDS treatments against *S.* Enteritidis on the eggshell.

MATERIALS and METHODS

Bacterial Strain and Bacteriophage

S. Enteritidis MET-S1-411 and SE-P47 phage specific for *S.* Enteritidis used in the present study were obtained from our bacterial and phage culture collection. *S.* Enteritidis MET-S1-411 was cultured in Brain Heart Infusion (BHI) broth (Lab M, United Kingdom) and stored in BHI broth containing 20% glycerol at −80°C. For the preparation of the SE-P47 phage, isolated and characterized in previous studies [51, 52], nutrient broth (Lab M, United Kingdom) was used and phage samples were stored in 30% glycerol (in total solution) at −80°C.

Reproduction of Bacteriophage Sample

The phage samples were prepared with some modifications to the method of Yildirim et al. [51]. The phage sample (100 µL) and fresh culture of *S.* Enteritidis (300 µL) in 10 mL of nutrient broth were incubated at 37°C overnight at 120 rpm. Then, chloroform (50 µL/mL) was added to lyse the bacterial cells and the mixture was centrifuged at 7000×*g* for 15 minutes at 4°C. After the supernatant was filtered using a 0.45 µm pore size filter, the phage titer was determined by the double-layer agar plate method [53]. Briefly, 300 µL of host cells and 100 µL of phage dilution were added to nutrient soft agar (0.7% agar) at 45-50°C, mixed, and spread on petri dishes containing solidified nutrient agar (1.5% agar). After 24-48 hours incubation at 37°C, phage titer was expressed as a plaque forming unit per mL (PFU/mL). The activity of the phage used for decontamination was determined as 9.9 log PFU/mL.

Preparation of Levulinic Acid plus Sodium Dodecyl Sulfate Solutions

LVA (Merck, Germany) and SDS (≥98.5%, Sigma-Aldrich, USA) solutions were prepared with distilled water at concentrations of 0.5% LVA plus 0.05% SDS, 1% LVA plus 0.05% SDS, and 2% LVA plus 0.5% SDS before each experiment under aseptic conditions.

Determination of Antibacterial Activity of Treatment Solutions

The antibacterial activities of 0.5% LVA plus 0.05% SDS, 1% LVA plus 0.05% SDS, 2% LVA plus 0.5% SDS solutions, SE-P47 phage, and water against *S.* Enteritidis were determined using the disc diffusion method [54]. For this purpose, 20 µL of bacteria suspension (8 log CFU/mL) was spread on the soft nutrient agar (0.7% agar). Sterile filter paper discs (Oxoid, United Kingdom) with a diameter of 6 mm were immersed in washing solutions (1 mL) and left for 15 min to allow the solutions to penetrate the discs. Then, the paper discs were placed on the soft nutrient agar surface inoculated with *S.* Enteritidis. The diameter of the inhibition zones were measured after incubation at 37°C for 24-48 hours in aerobic conditions.

Preparation and Inoculation of Eggshell

Fresh eggs were purchased from a local market in Nigde, Turkey, and stored at 4°C to be used within a week. All of the eggs used in the study were of medium size (53-62 g). In the preparation of eggshells, the method of Rodriguez-Romo et al. [22] was used with some modifications. The shell parts were obtained by puncturing the tip of the eggshell and emptying the inside of the egg. Eggshells cut in 3x3 cm^2 dimensions were immersed in 70% ethanol solution and kept waiting for 5 minutes to disinfect. After the disinfection process, the shell pieces were washed with sterile distilled water and placed in sterile petri dishes and allowed to dry at room temperature under aseptic conditions (approximately 20 min).

For the preparation of inoculum solution, 100 µL of stock culture of *S.* Enteritidis was added to 5 mL of BHI broth and incubated at 37°C for 24 h. The absorbance of the bacterial solution was measured in a spectrophotometer (Evolution 300, Thermo Scientific, Waltham, USA) at 600 nm. When the inoculum solution had an optical density of approximately 0.3, the cell density was 7 to 8 log CFU/mL. The inoculum solution was diluted with 0.1% buffered peptone water (BPW) in the range of 4 to 8 log CFU/mL. The inoculum (100 µL) was spread on the eggshell (3x3 cm^2) using a pipette tip, and the samples were kept in the biosafety cabinet at room temperature for 20 min to ensure bacterial attachment onto the eggshell [55]. The count of the inoculated *S.* Enteritidis was determined by the spread plate method. A 100 µL of serial dilutions were spread on salmonella-shigella agar (1.5% agar) and colonies were counted after the incubation at 37°C for 24-48 h.

Determination of *S.* Enteritidis Inactivation on Eggshell

For each trial, five eggshell samples inoculated with *S.* Enteritidis were treated with LVA plus SDS solutions at three different concentrations, phage (9.9 log PFU/mL), and sterile distilled water. Briefly, the inoculated samples were immersed in washing solutions (20 mL) in sterile petri dishes. The inoculated eggshell surfaces were placed in direct contact with the washing solutions. The samples were kept in treatment solutions for 10 min at room temperature. Additionally, inoculated and untreated samples were used as positive control, and uninoculated and untreated samples were used as negative controls in each experiment. For bacterial count, eggshells were homogenized in a stomacher bag (VWR, West Chester, PA, USA) with 0.1% 10 mL BPW in a stomacher (IUL 707/470 Instruments, Spain) for 2 minutes. The bag fluid was serially diluted in 0.1% BPW and 100 µL from each dilution was plated in duplicate on salmonella-shigella agar (Merck, Germany) plates. After incubation at 37°C for 24-48 h, the colonies were counted and expressed as colony-forming units per cm^2 (CFU/cm^2).

Statistical Analysis

Samples were tested in triplicate for evaluating the inhibitory effect of the sanitizer washing on eggshells. The obtained data were analyzed using ANOVA-General Linear Model in MINITAB 17. Tukey's method was used to determine the mean significant differences between treatments at the 95% confidence interval ($p<0.05$).

RESULTS and DISCUSSION

Antibacterial Activities of Treatment Solutions

The antibacterial activity of SE-P47 phage (9.9 log PFU/mL), 0.5% LVA+ 0.05% SDS, 1% LVA+ 0.05% SDS, and 2% LVA+0.5% SDS solutions against *S.* Enteritidis was determined using disc diffusion method. In addition, it was examined whether sterile distilled water had an antibacterial effect when compared to the treatment solutions. The clear zones formed by the treatment solutions on the surface of the medium inoculated with *S.* Enteritidis are given in Figure 1. The clear zone diameters observed in 0.5% LVA+0.05% SDS, 1% LVA+0.05% SDS, and 2% LVA+0.5% SDS solutions were 6.72, 7.67, and 9.78 mm, respectively, and the difference between them was statistically significant ($p<0.05$) (Table 1). The clear zone formed by the use of SE-P47 phage was measured as 15.56 mm. As seen in Figure 1, a larger clear zone was obtained compared to other solutions and this value was statistically significant ($p<0.05$). A clear zone was not observed for the sterile distilled water.

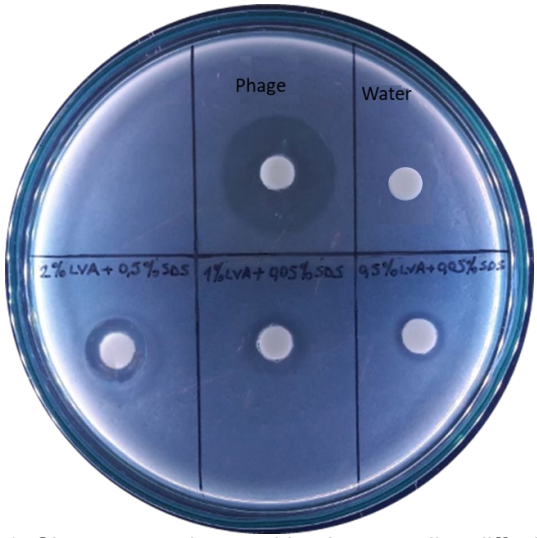

Figure 1. Clear zones observed by the agar disc diffusion method

Table 1. Diameters of clear zones obtained by disc diffusion method

Treatment solutions*	Clear zone diameter (mm)
Sterile distilled water	$0.00\pm0.00^{e**}$
0.5% LVA+0.05% SDS	6.72 ± 0.23^{d}
1% LVA+0.05% SDS	7.67 ± 0.28^{c}
2% LVA+0.5% SDS	9.78 ± 0.21^{b}
Bacteriophage SE-P47	15.56 ± 0.44^{a}

*LVA: levulinic acid, SDS: sodium dodecyl sulfate; **Different letters in the same column of treatment indicate statistically significant differences ($p<0.05$).

Inactivation of *S.* Enteritidis on Eggshell

Inoculum of different microbial concentrations was used to determine the antibacterial activity of LVA plus SDS solutions prepared at different concentrations and SE-P47 phage against *S.* Enteritidis on the eggshell surface. The *Salmonella* count of the inoculum solutions was determined separately as 4.7, 6.9, 7.88, and 8.3 log

CFU/mL. After inoculation, the *Salmonella* count on the eggshell was determined as 2.76, 3.22, 4.48, and 5.3 log CFU/cm^2, respectively. The microbial inactivation results are given in Table 2.

A 2-3 log difference was observed between the bacterial count of the *S*. Enteritidis inoculum solution and the bacterial count detected on the eggshell surface after inoculation. The fact that the bacterial count attached to the eggshell is less may be due to the physicochemical structure of the eggshell. The shell, the protective structure of the egg, contains the cuticle layer on the outside and the shell membrane on the inside. The cuticle layer is a proteinaceous layer covering the shell that has pores. On the other hand, the shell membranes consist of 3 different layers the inner membrane, the outer membrane, and the limiting membrane, and they are responsible for the bacterial defense system of the eggs [56]. In a study by Himathongkham et al. [57], the initial bacterial count of eggshells immersed in *S.* Enteritidis culture solution was approximately 7.5 log CFU/mL, while after 3 minutes incubation at 37°C and 30% RH, the *Salmonella* count decreased by about 2 logs. Himathongkham et al. [57] reported that a significant number of *S.* Enteritidis penetrated through the shell and on the shell membrane, based on the correlation between the bacterial count of the shell and the membrane. In a study investigating the penetration of *Salmonella* through the eggshell, it was reported that *Salmonella* translocated from the eggshell surface to the outer and inner membranes (shell membrane layers) [58]. Accordingly, in our study, the decrease count of *S*. Enteritidis on the eggshell after inoculation is attributed to bacterial penetration and displacement in/on the shell.

Table 2. Inhibitory effects of water, LVA plus SDS solutions, and bacteriophage treatments on *Salmonella* Enteritidis on the eggshell surface.

Treatments*	pH	*Salmonella* Enteritidis count on eggshell surface (log CFU/cm^2)			
SE	-	2.76±0.05^{a**}	3.22±0.03^{a}	4.48±0.07^{a}	5.30±0.21^{a}
Water	8.65±0.10	2.76±0.05^{a}	3.10±0.18^{ab}	4.15±0.17^{a}	5.19±0.24^{ab}
0.5% LVA+0.05% SDS	2.96±0.01	2.34±0.13^{b}	2.82±0.07^{b}	2.93±0.03^{b}	5.11±0.20^{ab}
1% LVA+0.05% SDS	2.76±0.02	<1^{c}	<1^{c}	2.54±0.16^{b}	4.41±0.19^{b}
2% LVA+0.5% SDS	2.69±0.01	<1^{c}	<1^{c}	<1^{c}	<1^{c}
SE-P47	7.94±0.16	<1^{c}	<1^{c}	<1^{c}	<1^{c}

*SE, the sample containing only *S.* Enteritidis MET-S1-411, control sample; <1, undetectable level, log CFU/cm^2 <1; LVA, levulinic acid; SDS, sodium dodecyl sulfate. **Different letters in the same column of treatment indicate statistically significant differences ($p<0.05$).

There was no reduction in the *S.* Enteritidis population on eggshells treated with sterile distilled water. This result supports that water does not form a clear zone in the agar disc diffusion method (Table 1). *S.* Enteritidis was inactivated ranged from 0.19 to 1.55 log CFU/cm^2 by treatment with 0.5% LVA+0.05% SDS solution. The difference between surviving *Salmonella* cells after the treatment of eggshells with 0.5% LVA+0.05% SDS and control samples is statistically significant, except inoculum level of 5.30 log CFU/cm^2 ($p<0.05$). However, there was no significant difference between water and 0.5% LVA+0.05% SDS treatments at inoculum levels of 3.22 and 5.30 log CFU/cm^2 ($p>0.05$). After treatment with 1% LVA+0.05% SDS solution, *S.* Enteritidis cell counts were undetectable at 2.76 and 3.22 log CFU/cm^2 inoculum levels. The *S.* Enteritidis population decreased by 1.94 and 0.89 log CFU/cm^2 at inoculum levels of 4.48 and 5.30 log CFU/cm^2, respectively ($p<0.05$). The highest log reductions in *S.* Enteritidis inactivation were achieved with 2% LVA+0.5% SDS and phage treatments at all inoculum levels, and viable cell counts were undetectable. The log reductions obtained in the 2% LVA+0.5% SDS and phage treatments were statistically significant compared to the control sample, water, 0.5% LVA+0.05% SDS, and 1% LVA+0.05% SDS treatments ($p<0.05$). It was observed that the decontamination results were consistent with the results obtained by the agar disc diffusion method (Table 1).

One of the microbiological criteria valid in many parts of the world for eggs defined as eggs in shell and egg products is the absence of *Salmonella* spp. in 25 g-mL [59, 60]. Also, according to the microbiological criteria of the Turkish Food Codex regulation, *Enterobacteriaceae* count should be less than 10^2 CFU/g-mL and *Salmonella* spp. should not be present in egg products (pasteurized and frozen eggs, egg powder, etc.) [61]. Since contamination of egg content with *Salmonella* can occur through transfer from the shell (horizontal contamination) [15], the microbiological safety of egg content and products is closely related to eggshells [62]. In our study, *S.* Enteritidis could not be detected in eggshells, especially after treatment with 2% LVA + 0.5% SDS or SE-P47 phage.

Levulinic acid causes the disruption of lipopolysaccharide in the outer membrane of Gram-negative bacteria. Depending on the increase in cell permeability, the absorption of both acid and SDS molecules into cells increases [36]. On the other hand, SDS can facilitate the contact of levulinic acid with bacterial cells by reducing the surface tension. It was reported that the effect of SDS to denature surface proteins and damage the cell membrane is higher between pH 1.5 and 3.0 [33, 36]. The average pH value of 0.05% SDS solution is 6.2. Therefore, the antimicrobial activity of SDS increases when used in combination with LVA. In this study, the pH values of LVA plus SDS solutions prepared at different concentrations decreased with increasing LVA concentration (Table 2). The reason for the lower log reductions in 0.5% LVA+0.05% SDS treatment compared to other LVA+SDS treatments can be attributed to the decrease in acid concentration, that is, the partial increase in pH value.

In a previous study, average log reductions in counts of influenza A H3N2 virus on eggshells individually treated with 0.5% LVA+0.5% SDS, 2% LVA+1% SDS, and 5% LVA+2 % SDS solutions for 1 minute at 21°C were 1.73, 1.90, and 2.33 log PFU/mL reductions, respectively [55]. The log reduction obtained in 0.5% LVA treatment was similar to our study. However, lower antimicrobial activity was observed in 2% LVA treatment compared to our study. This may be due to the diversity of the target microorganism and the short treatment time. Zhao et al. [33] reported that 0.5% LVA+0.05% SDS treatment (1 min) showed 4.4 and 4.5 log CFU/cm^2 reduction for *S.* Typhimurium and *E. coli* O157:H7 on the lettuce surface, respectively and 2.9 log CFU/cm^2 reduction for *S.* Enteritidis on chicken skin. After 5 minutes of treatment, approximately 7 log CFU/cm^2 reduction was obtained for the three pathogenic bacteria tested in both food samples. Maktabi et al. [21] investigated the inactivation of *S.* typhimurium on eggshell by immersion (5 min) in 1.5% SDS, 0.5% H_2O_2, and 1.0% citric acid solutions. After treatment with SDS, H_2O_2, and citric acid, the count of *S.* Typhimurium on eggshells decreased by 2.0, 2.1, and 0.4 log CFU/mL, respectively, compared to the control sample. They also reported that the antibacterial effect increased when citric acid or H_2O_2 combined with SDS.

Many studies have shown that the use of phages is effective in reducing the number of pathogenic bacteria in various food samples. However, there has been an increasing trend toward the use of phage and phage cocktails in eggs in recent years. To the best of our knowledge, there are limited studies that have applied phage to whole eggs or eggshells. Spricigo et al. [47] obtained 0.9 log CFU/cm^2 reduction for *S.* Enteritidis and *S.* Typhimurium in fresh eggs by spraying with the phage cocktail (10^{11} PFU/mL). In another study, approximately 3 log CFU/mL reduction was obtained in *S.* Typhimurium after 6 hours of phage cocktail (10^{10} PFU/mL) application on the eggshell while no viable cells could be detected in the samples after 24 hours of application [63]. In the same study, 1.7 log CFU/mL reduction was obtained after 72 hours of treatment of liquid egg with phage, and a lower antibacterial activity was observed compared to the eggshell sample. The difference in structure and composition between the eggshell and the liquid egg and the distribution of the microorganism on/in the sample can be effective on the results.

Controlling the colonization of pathogenic microorganisms in animals is one of the ways to prevent contamination of egg contents [15]. In the study of Henriques et al. [64], a phage cocktail (2×10^6 PFU/mL) was applied by aerosol spray on fertile eggs with *S.* Enteritidis to reduce horizontal contamination by *Salmonella*. Analysis of hatched chicks showed that the number of diseased chicks (arthritis and pasting) decreased. Furthermore, *S.* Enteritidis recovered from the chick ceca decreased after phage application to fertile eggs, while no significant reduction was observed for *S.* Enteritidis recovered from internal organs (pooled heart, liver, and spleen). This was attributed to the presence of high doses of bacteria by researchers. Therefore, the potential to use phages (phage therapy) to prevent pathogen colonization in poultry is thought to be quite high [64-66].

CONCLUSION

In this study, the effectiveness of LVA plus SDS and SE-P47 phage treatments was tested in the biocontrol of *S.* Enteritidis on eggshell. At all inoculum levels, the highest bactericidal effect was obtained with 2% LVA+0.5% SDS and phage treatments. Treatment of 1% LVA+0.05% SDS was very effective in preventing the growth of *S.* Enteritidis on the eggshells at lower inoculum levels. By reducing the microbial count on the eggshell, it is expected that the probability of horizontal contamination will also decrease. In other words, the egg content will be protected against contamination. Moreover, it will reduce the risk of cross-contamination of other foods in case of contact with whole eggs. In conclusion, it was demonstrated that levulinic acid plus SDS and SE-P47 phage can be effectively used decontamination of eggshell against the foodborne pathogen *S.* Enteritidis. In future studies, it will be very useful to investigate the effect of these treatments on the quality characteristics of eggs and eggshells.

REFERENCES

[1] Dhama, K., Rajagunalan, S., Chakraborty, S., Verma, A.K., Kumar, A., Tiwari, R., Kapoor, S. (2013). Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. *Pakistan Journal of Biological Sciences*, 16(20), 1076–1085.

[2] Heredia, N., García, S. (2018). Animals as sources of food-borne pathogens: A review. *Animal Nutrition*, 4(3), 250–255.

[3] WHO, (2015). WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. In World Health Organization. Available from: https://apps.who.int/iris/handle/10665/199350 (Accessed 25 May 2022).

[4] Jaffee, S., Henson, S., Unnevehr, L., Grace, D., Cassou, E. (2018). The safe food imperative: Accelerating progress in low-and middle-income countries. Evidence on the Burden of Unsafe Food in Low- and Middle- Income Countries. Washington, D.C.: World Bank. pp. 27-67. ISBN 978-1-4648-1346-7 (online).

[5] Scallan, E., Hoekstra, R.M., Mahon, B.E., Jones, T.F., Griffin, P.M. (2015). An assessment of the human health impact of seven leading foodborne pathogens in the United States using disability adjusted life years. *Epidemiology & Infection*, 143(13), 2795–2804.

[6] CDC, (2022). Centers of Disease Control and Prevention. *Salmonella* Homepage. Available from: https://www.cdc.gov/salmonella/general/index.html (Accessed 25 May 2022).

[7] Eng, S.K., Pusparajah, P., Ab Mutalib, N.S., Ser, H.L., Chan, K.G., Lee, L.H. (2015). *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science*, 8(3),

284–293.
[8] Lamas, A., Miranda, J.M., Regal, P., Vázquez, B., Franco, C.M., Cepeda, A. (2018). A comprehensive review of non-enterica subspecies of *Salmonella enterica*. *Microbiological Research*, 206, 60–73.
[9] Ferrari, R.G., Rosario, D.K.A., Cunha-Neto, A., Mano, S.B., Figueiredo, E.E.S., Conte-Juniora, C.A. (2019). Worldwide epidemiology of *Salmonella* serovars in animal-based foods: A meta-analysis. *Applied and Environmental Microbiology*, 85(14), e00591-19.
[10] Gut, A.M., Vasiljevic, T., Yeager, T., Donkor, O.N. (2018). *Salmonella* infection – prevention and treatment by antibiotics and probiotic yeasts: a review. *Microbiology*, 164(11), 1327–1344.
[11] Antunes, P., Réu, C., Sousa, J.C., Peixe, L., Pestana, N. (2003). Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. *International Journal of Food Microbiology*, 82(2), 97–103.
[12] Pande, V.V., Devon, R.L., Sharma, P., McWhorter, A.R., Chousalkar, K.K. (2016). Study of *Salmonella* Typhimurium infection in laying hens. *Frontiers in Microbiology*, 7, 203.
[13] Wright, A.P., Richardson, L., Mahon, B.E., Rothenberg, R., Cole, D.J. (2016). The rise and decline in *Salmonella enterica* serovar Enteritidis outbreaks attributed to egg-containing foods in the United States, 1973–2009. *Epidemiology & Infection,* 144(4), 810–819.
[14] Upadhyaya, I., Yin, H.B., Nair, M.S., Venkitanarayanan, K. (2017). Natural approaches for improving postharvest safety of egg and egg products. Producing safe eggs: Microbial Ecology of *Salmonella*, Edited by S.C. Ricke, R.K. Gast, Academic Press, Amsterdam, 391–420p.
[15] Howard, Z.R., O'Bryan, C.A., Crandall, P.G., Ricke, S.C. (2012). *Salmonella* Enteritidis in shell eggs: Current issues and prospects for control. *Food Research International*, 45(2), 755–764.
[16] Ricke, S.C., Birkhold, S.G., Gast, R.K. (2001). Eggs and egg products. Compendium of Methods for the Microbiological Examination of Foods, Edited by F.P. Downes, K. Ito, American Public Health Association, Washington, D.C. 473–479p.
[17] Baker, R.C., Bruce, C. (1994). Effects of Processing on the Microbiology of Eggs. Microbiology of the Avian Egg, Edited by R.G. Board, R. Fuller, Chapman & Hall, London, England, 153–173p.
[18] Tayel, A.A., El-Sedfy, M.A., Ibrahim, A.I., Moussa, S.H. (2018). Application of *Quercus infectoria* extract as a natural antimicrobial agent for chicken egg decontamination. *Revista Argentina de Microbiología*, 50(4), 391–397.
[19] Latimer, H.K., Jaykus, L.A., Morales, R.A., Cowen, P., Crawford-Brown, D. (2002). Sensitivity analysis of *Salmonella* Enteritidis levels in contaminated shell eggs using a biphasic growth model. *International Journal of Food Microbiology,* 75(1-2), 71–87.
[20] Lombardi, M.E., Ladman, B.S., Alphin, R.L., Benson, E.R. (2008). Inactivation of avian ınfluenza virus using common detergents and chemicals. *Avian Diseases*, 52(1), 118-123.
[21] Maktabi, S., Zarei, M., Rashnavady, R. (2018). Effect of sequential treatments with sodium dodecyl sulfate and citric acid or hydrogen peroxide on the reduction of some foodborne pathogens on eggshell. *Iranian Journal of Veterinary Research,* 19(2), 113-117.
[22] Rodriguez-Romo, L.A., Yousef, A.E. (2005). Inactivation of *Salmonella enterica* serovar Enteritidis on shell eggs by ozone and UV radiation. *Journal of Food Protection*, 68(4), 711–717.
[23] Li, Z., Guo, R., Wang, F., Geng, S., Kang, X., Meng, C., Gu, D., Jiao, X., Pan, Z. (2019). Inactivation of *Salmonella* Enteritidis on eggshells by lactic acid spray. *Food Control*, 104, 201–207.
[24] Abdel-Salam, B., Nader, M., Emam, R. (2018). Decontamination of eggshell contaminated with *Salmonella* Typhimurium using natural plant extracts. *International Journal of Pharmaceutical Research & Allied Sciences*, 7(3), 10–19.
[25] Park, S.Y., Jung, S.J., Ha, S. Do. (2018). Synergistic effects of combined X-ray and aqueous chlorine dioxide treatments against *Salmonella* Typhimurium biofilm on quail egg shells. *LWT - Food Science and Technology*, 92, 54–60.
[26] Manfreda, G., Cevoli, C., Lucchi, A., Pasquali, F., Fabbri, A., Franchini, A. (2010). Hot air treatment for surface decontamination of table eggs experimentally infected with *Salmonella*, *Listeria*, and *Escherichia coli*. *Veterinary Research Communications*, 34(1), 179–182.
[27] Turtoi, M., Borda, D. (2014). Decontamination of egg shells using ultraviolet light treatment. *World's Poultry Science Journal*, 70(2), 265-278.
[28] Keklik, N.M., Demirci, A., Patterson, P.H., Puri, V.M. (2010). Pulsed UV light inactivation of *Salmonella* Enteritidis on eggshells and its effects on egg quality. *Journal of Food Protection*, 73(8), 1408–1415.
[29] Lasagabaster, A., Arboleya, J.C., De Marañón, I.M. (2011). Pulsed light technology for surface decontamination of eggs: Impact on *Salmonella* inactivation and egg quality. *Innovative Food Science & Emerging Technologies*, 12(2), 124–128.
[30] Alkaya, G.B., Erdogdu, F., Halkman, A.K., Ekiz, H.I. (2016). Surface decontamination of whole-shell eggs using far-infrared radiation. *Food and Bioproducts Processing*, 98, 275–282.
[31] Dasan, B.G., Yildirim, T., Boyaci, I.H. (2018). Surface decontamination of eggshells by using non-thermal atmospheric plasma. *International Journal of Food Microbiology*, 266, 267–273.
[32] Wan, Z., Chen, Y., Pankaj, S.K., Keener, K.M. (2017). High voltage atmospheric cold plasma treatment of refrigerated chicken eggs for control of *Salmonella* Enteritidis contamination on egg shell. *LWT - Food Science and Technology*, 76, 124–130.
[33] Zhao, T., Zhao, P., Doyle, M.P. (2009). Inactivation of *Salmonella* and *Escherichia coli* O157:H7 on lettuce and poultry skin by combinations of levulinic acid and sodium dodecyl sulfate. *Journal of Food*

Protection, 72(5), 928-936.
[34] FDA, (2022a). U.S. Food and Drug Administration. CFR - Code of Federal Regulations Title 21. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=172.822 (Accessed 25 May 2022).
[35] FDA, (2022b). U.S. Food and Drug Administration. CFR - Code of Federal Regulations Title 21. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.515 (Accessed 25 May 2022).
[36] Zhou, M., Doyle, M.P., Chen, D. (2019). Combination of levulinic acid and sodium dodecyl sulfate on inactivation of foodborne microorganisms: A review. *Critical Reviews in Food Science and Nutrition*, 60(15), 2526-2531.
[37] Chen, D., Zhao, T., Doyle, M.P. (2015). Single- and mixed-species biofilm formation by *Escherichia coli* O157:H7 and *Salmonella*, and their sensitivity to levulinic acid plus sodium dodecyl sulfate. *Food Control*, 57, 48-53.
[38] Zhao, T., Zhao, P., Cannon, J.L., Doyle, M.P. (2011). Inactivation of *Salmonella* in biofilms and on chicken cages and preharvest poultry by levulinic acid and sodium dodecyl sulfate. *Journal of Food Protection*, 74(12), 2024–2030.
[39] Chen, D., Zhao, T., Doyle, M.P. (2014). Transfer of foodborne pathogens during mechanical slicing and their inactivation by levulinic acid-based sanitizer on slicers. *Food Microbiology*, 38, 263–269.
[40] Webb, C.C., Erickson, M.C., Davey, L.E., Doyle, M.P. (2015). Effectiveness of levulinic acid and sodium dodecyl sulfate employed as a sanitizer during harvest or packing of cantaloupes contaminated with *Salmonella* Poona. *International Journal of Food Microbiology*, 207, 71–76.
[41] Zhou, Z., Zuber, S., Cantergiani, F., Butot, S., Li, D., Stroheker, T., Devlieghere, F., Lima, A., Piantini, U., Uyttendaele, M. (2017). Inactivation of viruses and bacteria on strawberries using a levulinic acid plus sodium dodecyl sulfate based sanitizer, taking sensorial and chemical food safety aspects into account. *International Journal of Food Microbiology*, 257, 176–182.
[42] Greer, G.G. (2005). Bacteriophage control of foodborne bacteria. *Journal of Food Protection*, 68(5), 1102–1111.
[43] Loc-Carrillo, C., Abedon, S.T. (2011). Pros and cons of phage therapy. *Bacteriophage*, 1(2), 111–114.
[44] Sillankorva, S.M., Oliveira, H., Azeredo, J. (2012). Bacteriophages and Their Role in Food Safety. *International Journal of Microbiology*, 2012.
[45] Aydin Demirarslan, Ö., Alasalvar, H., Yildirim, Z. (2021). Biocontrol of *Salmonella* Enteritidis on chicken meat and skin using lytic SE-P3, P16, P37, and P47 bacteriophages. *LWT - Food Science and Technology*, 137, 110469.
[46] Moye, Z.D., Woolston, J., Sulakvelidze, A. (2018). Bacteriophage applications for food production and processing. *Viruses,* 10(4), 205.
[47] Spricigo, D.A., Bardina, C., Cortés, P., Llagostera, M. (2013). Use of a bacteriophage cocktail to control *Salmonella* in food and the food industry. *International Journal of Food Microbiology,* 165(2), 169–174.
[48] Şanlıbaba, P. Uymaz, B. (2015). Gıdalarda *Listeria monocytogenes*'in biyokontrolünde faj uygulaması. *Akademik Gıda*, 13 (1), 81-88.
[49] Ergin, F., Yıldız, G., Çomak Göçer, E.M. Küçükçetin, A. (2017). Bakteriyofajların antibakteriyel ajan olarak kullanımı. *Akademik Gıda*, 15 (2), 172-181.
[50] Cooper, I.R. (2016). A review of current methods using bacteriophages in live animals, food and animal products intended for human consumption. *Journal of Microbiological Methods*, 130, 38–47.
[51] Yildirim, Z., Sakin, T., Çoban, F. (2018). Isolation of lytic bacteriophages infecting *Salmonella* Typhimurium and *Salmonella* Enteritidis. *Acta Biologica Hungarica*, 69(3), 350–369.
[52] Yildirim, Z., Sakin, T., Akçelik, M., Akçelik, N. (2019). Characterization of SE-P3, P16, P37, and P47 bacteriophages infecting *Salmonella* Enteritidis. *Journal of Basic Microbiology*, 59(10), 1049–1062.
[53] Adams, M.H. (1959). Methods of study of bacterial viruses. *Bacteriophages*.
[54] Bhat, R. S., & Al-Daihan, S. (2014). Phytochemical constituents and antibacterial activity of some green leafy vegetables. *Asian Pacific Journal of Tropical Biomedicine*, *4*(3), 189-193.
[55] Aydin, A., Cannon, J.L., Zhao, T., Doyle, M.P. (2013). Efficacy of a levulinic acid plus sodium dodecyl sulfate (SDS)-based sanitizer on inactivation of influenza a virus on eggshells. *Food and Environmental Virology*, 5(4), 215–219.
[56] Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T.J., Van Immerseel, F. (2009). Mechanisms of egg contamination by *Salmonella* Enteritidis. *FEMS Microbiology Reviews*, 33(4), 718–738.
[57] Himathongkham, S., Riemann, H., Ernst, R. (1999). Efficacy of disinfection of shell eggs externally contaminated with *Salmonella* Enteritidis: Implications for egg testing. *International Journal of Food Microbiology*, 49(3), 161–167.
[58] Miyamoto, T., Horie, T., Baba, E., Sasai, K., Fukata, T., Arakawa, A. (1998). *Salmonella* penetration through eggshell associated with freshness of laid eggs and refrigeration. *Journal of Food Protection*, 61(3), 350–353.
[59] EFSA, (2014). EFSA Panel on Biological Hazards (BIOHAZ). Scientific Opinion on the public health risks of table eggs due to deterioration and development of pathogens. *EFSA Journal*, 12(7), 3782–3929.
[60] UNECE Standards, (1986). Hens Egg Products For Use in the Food Industry. 63. Available from: https://unece.org/fileadmin/DAM/trade/agr/standard/eggs/e/63produc.pdf (Accessed 7 March 2022).
[61] Anonymous, (2011). Regulation on Turkish Food Codex microbiological criteria. Law of Authorization: 5996. Official Gazette of Publication, No. 28157. Available from:

https://www.tarimorman.gov.tr/Belgeler/ENG/Legislation/regulation_microbiological_criteria.pdf (Accessed 25 May 2022).

[62] Akarca, G., Istek, Ö., Tomar, O. (2021). The effect of resin coating on the quality characteristics of chicken eggs during storage. *Journal of Food Science*, 86(4), 1243-1257.

[63] Sritha, K.S., Bhat, S.G. (2021). In vitro efficiency evaluation of phage cocktail for biocontrol of *Salmonella* spp. in food products. *Archives of Microbiology,* 203(9), 5445–5452.

[64] Henriques, A., Sereno, R., Almeida, A. (2013). Reducing *Salmonella* horizontal transmission during egg incubation by phage therapy. *Foodborne Pathogens and Disease,* 10(8), 718–722.

[65] Clavijo, V., Baquero, D., Hernandez, S., Farfan, J.C., Arias, J., Arévalo, A., Donado-Godoy, P., Vives-Flores, M. (2019). Phage cocktail SalmoFREE® reduces *Salmonella* on a commercial broiler farm. *Poultry Science*, 98(10), 5054–5063.

[66] Waseh, S., Hanifi-Moghaddam, P., Coleman, R., Masotti, M., Ryan, S., Foss, M., MacKenzie, R., Henry, M., Szymanski, C.M., Tanha, J. (2010). Orally administered P22 phage tailspike protein reduces *Salmonella* colonization in chickens: prospects of a novel therapy against bacterial ınfections. *PLos One*, 5(11), e13904.
.