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Research Article

Effect of chitosan combined coating on chicken and quail eggs for controlling *Escherichia coli* and *Salmonella enteritidis*

Esin Derelioğlu[1], Özlem Turgay[2]*

ABSTRACT

Objective:
In this study, we aimed to minimize crust fragility during transportation and to prevent economic loss and microbial contamination caused by breakage. Reduction of the initial microbial load was also aimed at the contribution of phenolic substances.

Conclusion:
The chitosan coating materials were prepared with different organic acids (acetic acid, lactic acid, and propionic acid) and phenolic compounds (gallic acid and caffeic acid). Quail and chicken eggs were covered with these combined coating materials. Coated and uncoated samples were contaminated with *Escherichia coli* and *Salmonella enteritidis*. The coating process inhibited the microorganisms and provided a safer environment. Chitosan - Lactic acid-Gallic acid and Chitosan-Lactic acid-Caffeic acid coating material was found potentially usable in the preservation of chicken eggs and quail eggs freshness.

Keywords: Chicken egg, Quail egg, *Escherichia coli*, *Salmonella enteritidis*.

TÜBİTAK ULAKBİM

1 Bioengineering, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey,
2 Food Engineering, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey
*Corresponding author. Tel.: +903443002083. E-mail address: ozlem@ksu.edu.tr
Esin DERELİOĞLU Orcid ID: 0000-0002-8014-3604 Özlem TURGAY Orcid ID: 0000-0003-2286-833X

Introduction

Chitosan is a functional biopolymer with antimicrobial and antioxidant properties and has high potential for biodegradable active food packaging material (Guoa et al., 2015). Chitosan, derived from chitin by deacetylation, is a linear polysaccharide consisting of beta (1.4) linked D-glucosamine residues with some N-acetyl-glucosamine units (Skorski et al., 2009). It is good for film forming and its structural properties and biodegradability are interesting for packaging applications and it can even be used as edible material (Dutta et al., 2009), and only soluble in acidic solutions which is the reason why it is often indicated as cationic polymer (Madeleine-Perdrillat, 2015).

Chitosan is known for its activity against a wide range of microorganisms. The most acceptable antimicrobial mechanism is found to include the presence of charged groups in the polymer backbone and their ionic interactions with bacteria wall constituents. This interaction offers the occurrence of a hydrolysis of the peptidoglycans in the microorganism wall, provoking the leakage of intracellular electrolytes, leading the microorganism to death (Goy et al., 2016). A lot of models recommended that the antimicrobial activity of chitosan is a result from its cationic nature (Goy et al., 2009). The electrostatic interaction between positively charged $R\text{-}N(CH_3)_3^+$ sites and negatively charged microbial cell membranes, is predicted to be responsible for cellular lysis and assumed as the main antimicrobial mechanism (Tripathi et al., 2008). Charged chitosan can also interact with essential nutrients therefore interfering on microbial growth. That polymers were consequently expected with higher charge densities resulted in an improved antimicrobial activity (Goy et al.,2016).Chitosan combines were were studied in aqueousacidic solutions such as acetic (Devlieghere et al., 2004), lactic (Papineau et al., 1991), ascorbic (Lee et al., 2003) acids. These acids improved bactericidal effect of chitosan (Gao et al., 2012; Raftari et al., 2012).

This study was evaluated the antimicrobial effect of chitosan against *E. coli* and *S. enteritidis* on chicken and quail eggs. Chitosan was dissolved in lactic, acetic and propionic acid and enriched phenolic compounds as gallic acid and caffeic acid.

Materials and methods

Materials

In this study, chicken eggs were obtained from Kahramanmaraş Sütçü Imam University Agricultural Faculty Research and Application Farm and quail eggs were obtained from Gazivet Animal Production Marketing Company. Prepared shrimp shell chitosan was obtained from the (deacetylation grade was 75%) Sigma (C3646). Coating materials were prepared using acetic acid (Sigma, 320099), lactic acid (Sigma, 69775) and propionic acid (Sigma, 402907). Gallic acid (Merck, 1.01347.0500) and caffeic acid (Acros, 114930250) were used in the coating formulations as phenolic materials. *E. coli* (ATCC 25922) and *S. enteritidis* (ATCC 13076) strains were obtained from Refik Saydam Hıfzıssıhha Institute.

Methods

Chitosan solutions were prepared by dissolving 3 g of chitosan in 100 ml distilled water that containing 1% of organic acids (AA: acetic acid, LA: lactic acid, PA: propionic acid) and 1g/chitosan phenolic compounds gallic acid (AA+GA, LA+GA, PA+GA), caffeic acid (AA+CA, LA+CA, PA+CA). The solution was heated (40°C) and agitated constantly for 45 min. Finally, polyethylene glycol added to the solution for elasticity (0.25 ml/g chitosan) and agitated 15 min (No et al., 2002).

One milliliter of the overnight *E. coli* and *S. enteritidis* culture was inoculated onto the chicken and quail eggs. The cultures were allowed to air dry on the egg samples at room temperature. The samples were then dipped into the different type of chitosan solution that given above for 60s and allowed to air dry for 1h at the room temperature. This process were done twice. After coating, all eggs were allowed to dry then placed in a small-end down position in aluminium foil on cardboard egg racks and stored at room temperature. The samples bacterial counts determined at days 0, 7, 14, 21, and 28. *E. coli* and *S. enteritidis* isolation procedures were based on FDA/BAM method (Food and Drug Administration/ Bacteriological Analytical Manual) (Anonymous, 2016).

E. coli: By adding (90 ml for chicken egg shells, 9 ml for quail egg shells) phosphate-buffered water samples were stomaching for 30 min and making serial dilutions. Plating the dilutions on to EMB agar diffusion method were prepared in petri dish and left to incubate at 37°C for 24-48 hours. Purple colored colonies were counted at the end of incubation.
S. enteritidis: By adding (90 ml for chicken egg shells, 9 ml for quail egg shells) Trypticase Soy Broth samples were incubate for 24 hours at 37°C for pre-enrichment. For a second pre-

enrichment, 5 ml of the samples incubated for 24 hours at 37°C were added to 25 ml of Selenite Cystine Broth in sterile sample pouches and incubated at 37°C for 24 hours. Plating the dilutions onto Salmonella-Shigella agar diffusion method prepared in petri dish, and incubated at 37°C for 24 hours. Yellowish-brown colored colonies formed in petri dishes were counted.

Statistical Analysis

The entire experiment was replicated on three separate occasions (three replicates in each independent experiment) and the results shown were means of data obtained. One-way analysis of variance of data was carried out using the SPSS for Windows software package. The difference between pairs of means was resolved by means of confidence intervals using Duncan's tests; the level of significance was set at $P < 0.05$.

Results and Discussion

Results

E. coli Analyses

Results were illustrated in Figure 1 and 2. Coating groups inhibited microorganism growth significant of chicken eggs for *E. coli* ($P < 0.05$). During the storage period, the control group values were significantly higher than the coating group values ($P < 0.05$). Chicken eggs coated with Ch-LA-CA indicated significant the highest decrease in number of *E. coli* at week ($P<0.05$). Coating formulations with added phenolic compound have better results than the others. This differences in antimicrobial activity may also be due the of phenolic substances.

Figure 2 presents that all treatments significantly inhibited microbial growth during storage ($P<0.05$). Inoculated *E. coli* on untreated quail eggs gradually decreased from 9.35 log CFU/g to 9.19 log CFU/g. Ch-LA-CA indicated the best inhibition data in the 4th and to be found statistically significant ($P<0.05$). Analyzed of coated quail eggs indicated a faster decline. This rapid decline was thought due to the antimicrobial effect of chitosan and phenolic materials.

S. enteritidis Analyses

Microbial analysis results indicated that the inhibition of microorganisms between weeks was statistically significant ($P<0.05$) (Figure 3). The highest inhibition was Ch-LA-GA with 9.18 log CFU/g at first week, Ch-PA-GA with 8.90 log CFU/g at second week, Ch-LA-GA with 8.85 log CFU/g at third week and 8 log CFU/g with Ch-LA-GA at fourth week. Coating formulations with added phenolic compounds had better antimicrobial activity because antimicrobial effect of phenolic compounds.

During storage, Ch-AA had the lowest inhibition rate in coating groups. There was no statistically significant difference ($P<0.05$) between 0th. and 7th. day in Ch-AA coated quails (Figure 4). The difference between the different coating formulations weekly was statistically significant ($P<0.05$). During storage, as seen in the other analysis results, the coating formulations with phenolic additive made better results than the control group and coating formulations prepared only with chitosan-organic acids.

Discussion

The highly deacetylated chitosan have good antimicrobial activity due to the fact that the solubility and charge density are increased (Dutta et al., 2009). Chitosan also diffuse toward the nuclei of the microorganisms and inhibition of DNA, mRNA and protein synthesis (Devlieghere et al., 2004). Chitosan coating and films showed antimicrobial effect of growth *E. coli* and *Staphylococcus aureus* (Torlak and Nizamoğlu, 2011).

Kim et al. (2011), investigated the microbial effect of chitosan on *Listeria monocytogenes*, *E. coli* O157:H7 and *Salmonella typhimurium* with agar diffusion method and reported that chitosan had an antimicrobial effect on *L. monocytogenes*, *E. coli* O157:H7 and *S. typhimurium*.Duan et al. (2007) reported that chitosan films showed effectively antimicrobial activity on *L. monocytogenes*, *E. coli* and *Pseudomonas fluorescens* at 10°C in Mozzarella cheese and the incorporation of 60% lysozyme into chitosan films greater antimicrobial effect than chitosan alone.

In this study chitosan films showed that antimicrobial activity against to *E. coli* and *S. enteritidis* and this antimicrobial effect sustain by incorporation phenolic compounds. Hisar et al. (2008), observed that the growth of *Enterobacteriaceae* and *Pseudomonas* were lower than the control group in *Sarda sarda* fillet covered with chitosan. Erdoğrul et al. (2002), investigated the presence of *S. enteritidis* in 123 quail eggs. *S. enteritidis* D1 sero group was isolated in 7 of the 123 quail

eggs. Zhang and Zhu (2013), found that 1% chitosan inhibited *E. coli* and *Staphylococcus aureus*. In the study, 3% chitosan was used and it was observed that chitosan had an inhibitory effect on *E. coli* and *S. enteritidis*. Leceta et al. (2013), reported that chitosan showed a bacteriostatic effect on *E. coli* and *L. plantarum*. Microbial activity on *L. monocytogenes*, *E. coli* O157: H7 and *S. typhimurium* was examined that chitosan films indicated to inhibit *L. monocytogenes*, *E. coli* O157: H7 and *S. typhimurium* (Kim et al., 2011).

In this study, coating groups prepared form of chitosan-organic acids and chitosan-organic acids-phenolic substances indicated significant inhibitory effect on *E. coli* and *S. enteritidis*. Var and Evliya (1995), screened *Salmonella* in chicken, duck and quail eggs. *Salmonella* found that chicken and duck eggs only shell but in quail eggs *Salmonella* found shell, yolk and albumen.

It was considered that the chitosan coating inhibited to *Salmonella*. In the present study, the highest inhibition for *E. coli* was obtained in Ch-LA-GA (8.71 log CFU/g) coating group in chicken eggs, and Ch-LA-CA (8.64 log CFU/g) coating group in quail eggs. The best inhibition against *S. enteritidis* was obtained in Ch-LA-GA (8.72 log CFU/g) coating group in chicken eggs and Ch-LA-CA (8.72 log CFU/g) coating group in quail eggs. The coating process that was done, inhibited the microorganisms and provided a safer environment.

In conclusion, Ch-LA-GA and Ch-LA-CA coating material was found potentially usable in the preservation of chicken eggs and quail eggs freshness. It was aimed to minimize crust fragility during transportation and to prevent economic loss and microbial contamination caused by breakage. Reduction of the initial microbial load is also aimed at the contribution of phenolic substances. Thus, public health and economic benefits may be provided.

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Figure 1. Effect of chitosan coatings on the growth of *E. coli* that inoculated to chicken eggshell (log CFU/g)

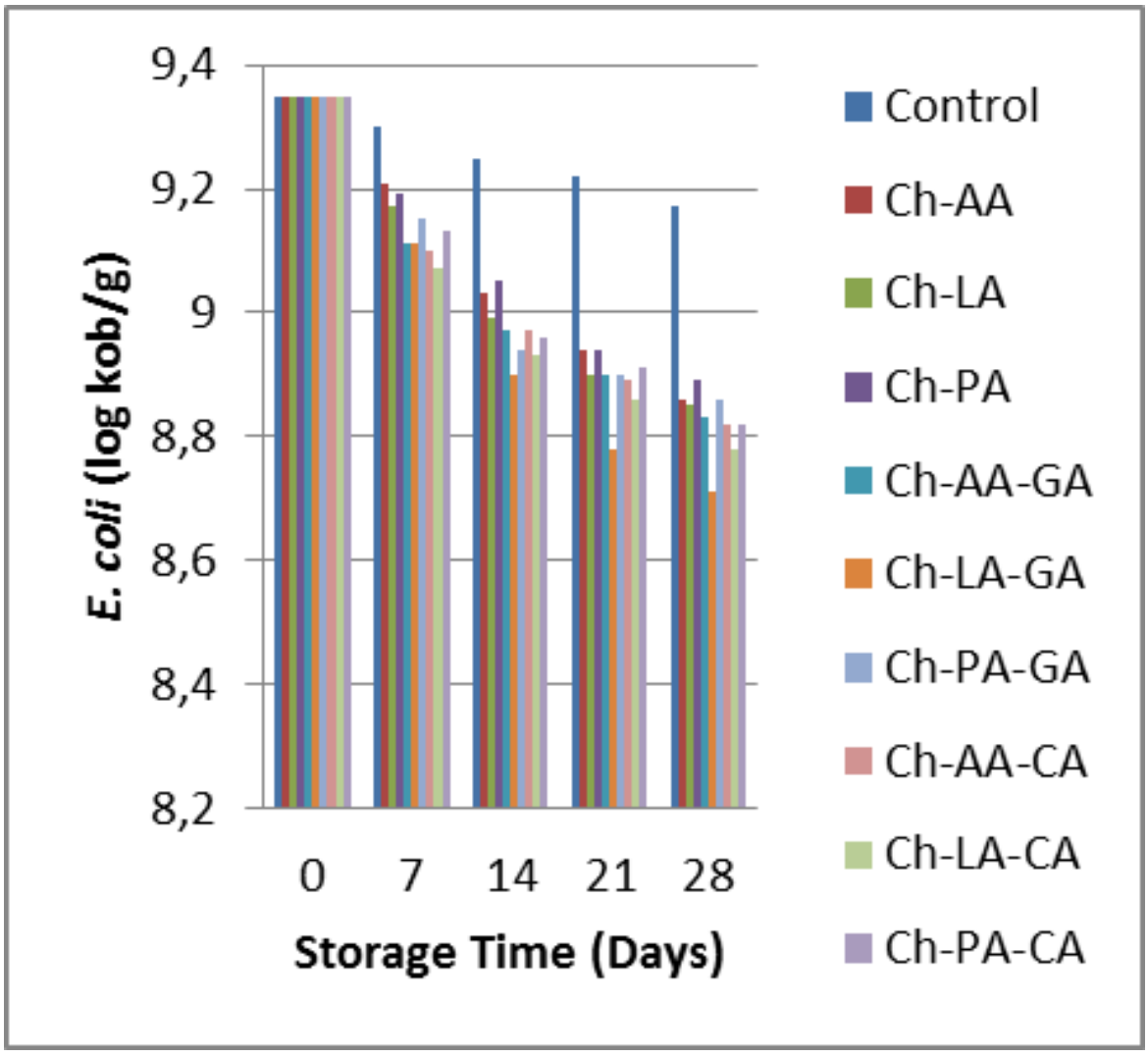

Figure 2. Effect of chitosan coatings on the growth of *E. coli* that inoculated to quail eggshell (log CFU/g)

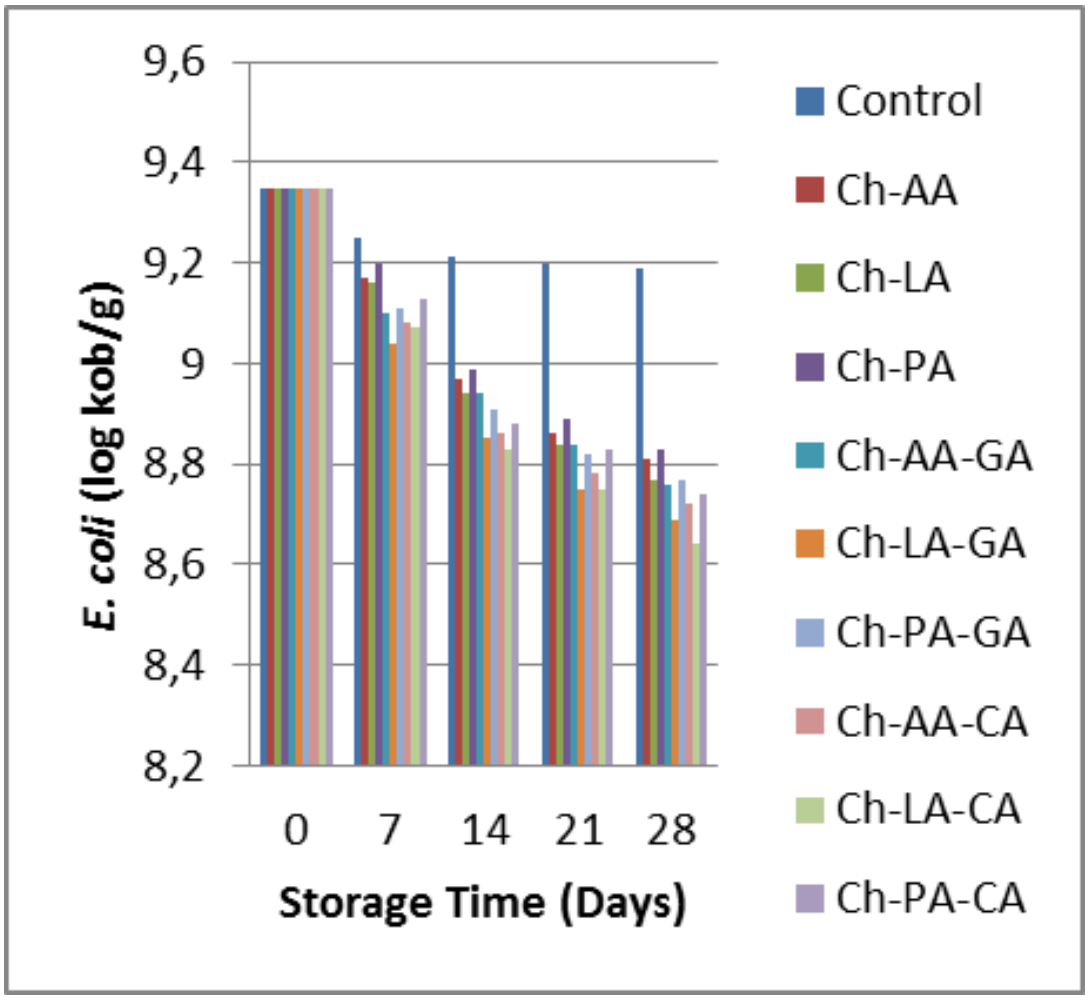

Figure 3. Effect of chitosan coatings on the growth of *S. enteritidis* that inoculated to chicken eggshell (log CFU/g)

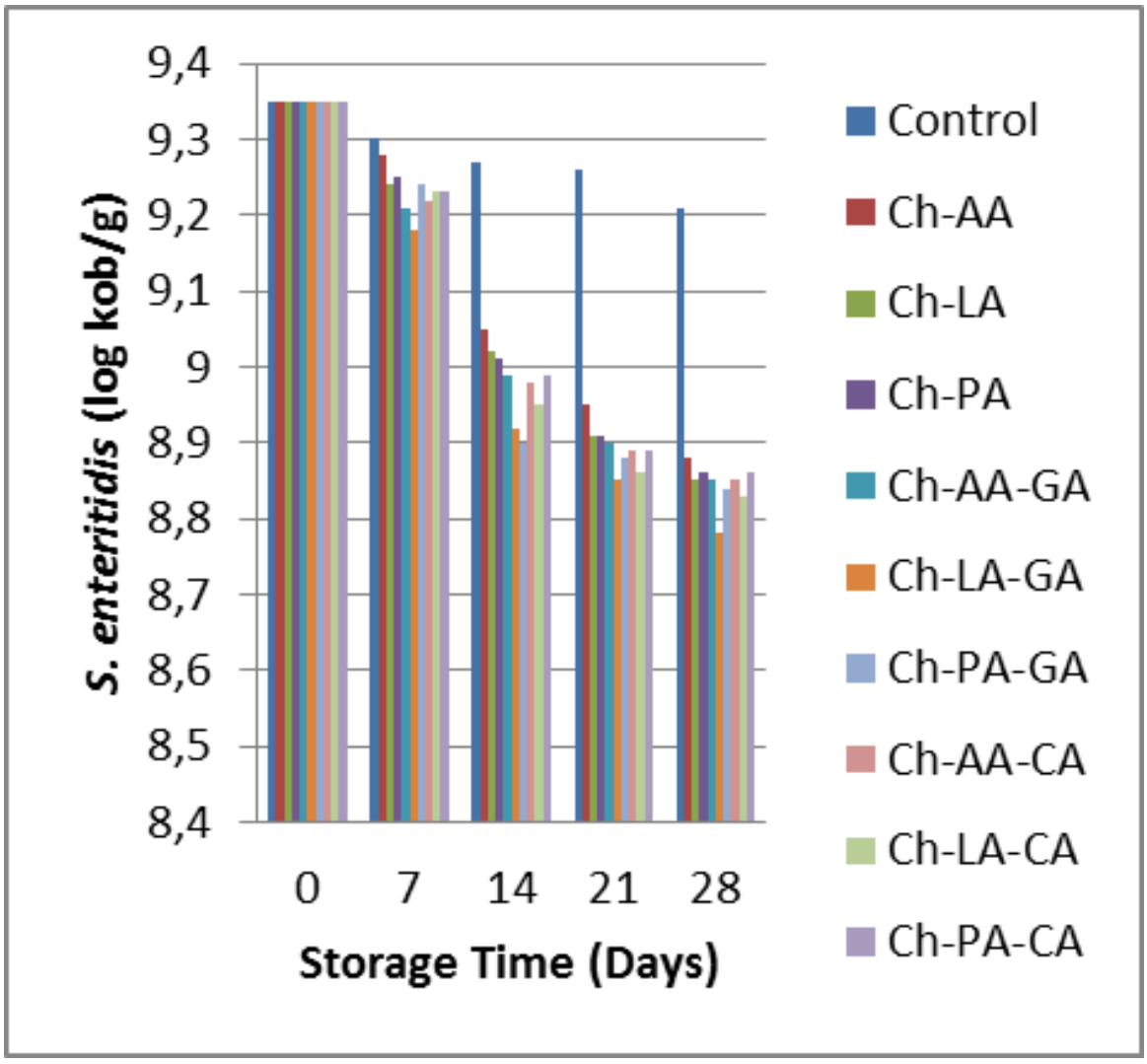

Figure 4. Effect of chitosan coatings on the growth of *S. enteritidis* that inoculated to quail egg shell (log CFU/g)