Effect of Chitosan as a Biological Sanitizer for *Salmonella* Typhimurium and Aerobic Gram Negative Spoilage Bacteria Present on Chicken Skin

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Abstract: Two experiments were conducted to evaluate the effect of chitosan as a biological sanitizer on chicken skin during storage. For experiment 1 (two trials) five skin samples of equal size were dipped into a solution containing 10^6 cfu/mL of *Salmonella* Typhimurium (ST) for 30s. Skin samples were then removed and dipped into a solution containing PBS or 0.5% chitosan for 30s. In experiment 2, aerobic Gram negative spoilage bacteria were used as indicators instead of ST. In both experiments, all samples were placed in individual bags and kept at 4°C. In experiment 1, dipping ST contaminated skin samples in a solution of 0.5% chitosan reduced ($p<0.05$) the recovery of ST by 24 h. In experiment 2, 0.5% chitosan treatment solution reduced ($p<0.05$) the presence of spoilage-causing psychrotrophic bacteria below detectable levels. These results suggest that 0.5% chitosan has a potential for use in an intervention technology for the control of foodborne pathogens on the surface of chicken skin contaminated with bacteria during storage.

Key words: *Salmonella*, chitosan, chicken skin, sanitizer

INTRODUCTION

Chickens contain large numbers of microorganisms in their gastrointestinal tract and on their feathers and feet; therefore, storage quality of fresh chicken is partially dependent on the bacteria present on the integument prior to slaughter (Ramirez *et al*., 1997; Northcutt *et al*., 2003). Pathogenic microorganisms present in chicken carcasses after processing and throughout scalding and picking can contaminate equipment and other carcasses (Hargis *et al*., 1995; Byrd *et al*., 1998; Sarlin *et al*., 1998; Corrier *et al*., 1999b; Zhang *et al*., 2013). Pathogenic bacteria such as *Salmonella enterica* and *Campylobacter* spp. are able to attach to skin and penetrate in skin layers or feather follicles (Zhang *et al*., 2013), facilitating their presence on chicken skin and carcass during poultry processing (Chaine *et al*., 2013). Critical control point determination at broiler processing has become very important, especially because of the recent attention on Hazard Analysis and Critical Control Points (HACCP) for reduction of microbial contamination of meat and poultry (Rose *et al*., 2002). For all these reasons, strategies to reduce bacterial contamination on poultry carcasses are important. However, most of the bacterial reduction strategies for poultry comprise the use of antimicrobial chemicals in rinses or washes and their efficacy is reduced by the presence of organic matter (Zhao *et al*., 2009). Therefore, it grows the need of biological sanitizers in the processing plant to prevent carcass to carcass cross-contamination by pathogenic bacteria and to lower the potential of foodborne diseases.

Interest in chitosan, a biocompatible polymer derived from shellfish, as a biological sanitizer arises from reports showing several beneficial effects such as antimicrobial and antioxidative activities in foods (No *et al*., 2002; Friedman and Juneja, 2010). The use of chitosan in industry, agriculture and medicine is well described (Rabea *et al*., 2003; Senel and McClure, 2004; Friedman and Juneja, 2010). The antimicrobial activities of chitosan against foodborne pathogens has been broadly investigated in the food industry (Singla and Chawla, 2001; No *et al*., 2002; Senel and McClure, 2004; Petrovich *et al*., 2008; El-Hadrami *et al*., 2010; Kong *et al*., 2010; Vargas and Gonzalez-Martinez, 2010). Therefore, the objective of the present study was to evaluate the effect of chitosan as a biological sanitizer for *Salmonella* and aerobic Gram negative spoilage bacteria on chicken skin during storage at 4°C.

MATERIALS AND METHODS

Bacterial strain and chitosan: A poultry isolate of *Salmonella enterica* serovar Typhimurium (ST), selected for resistance to Nalidixic Acid (NA) (Catalog No. N-4382, Sigma, St. Louis, MO 63178), was used for all experiments. The amplification and enumeration protocol for the isolate have been previously described (Tellez *et al*., 1993). Briefly, ST was grown in tryptic soy broth (TSB, Catalog No. 22092, Sigma, St. Louis, MO

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63178) for approximately 8 h. The cells were washed three times with 0.9% sterile saline by centrifugation (1,864 x g) and the approximate concentration of the stock solution was determined spectrophotometrically at 625 nm. The stock solution was serially diluted and confirmed by colony counts of three replicate samples (0.1 mL/replicate) spread plated on brilliant green agar (BGA, Catalog No. 278820, Becton Dickinson, Sparks, MD 21152) plates containing 25 µg/mL novobiocin (NO, Catalog No. N-1628, Sigma, St. Louis, MO 63178) and 20 µg/mL NA.

Chitosan: Deacetylated 95% food grade chitosan was obtained commercially (Paragon Specialty Products, LLC Rainsville, AL) and used in all experiments. The chitosan molecular weight was 350 kDa with viscosity of 800 mPas and particle size of 100 US mesh (sieve size 0.152 mm). Chitosan was prepared by dissolving it in a solution containing 0.5% (w/v glacial acetic acid (Catalog No. J41A08, Mallinckrodt Baker Inc, Phillipsburg, NJ 08865).

Chicken skin samples: As described by Sarlin *et al*. (1998), raw chicken skin was used as an alternative to other sampling methods (whole carcass rinse procedure, excised skin sampling, or skin swabs) in all experiments. Chicken thighs were purchased from a local super market and a strip of skin (approximately 2 by 2 cm) was aseptically collected using forceps and scissors.

Microbiological procedures:

- **Experiment 1:** Two trials were conducted. In each trial, skin samples (N = 20) were dipped into a phosphate buffered saline (PBS) solution containing 10^8 cfu/mL of ST for 30 seconds. Skin samples were then removed, drained off and dipped for an additional 30s into a solution containing PBS (control; N = 10) or 0.5% chitosan (N 1). Control and treated samples were placed in individual sample bags and kept in a refrigerator at 4°C. At one or twenty four hours, five control and five treated samples were removed from the refrigerator and cultured for ST recovery. Briefly, skin samples were homogenized within sterile sample bags using a rubber mallet. Sterile saline (5 mL) was added to each sample bag and hand stomached. Serial dilutions were spread plated on BGA plates containing 25 µg/mL of NO and 20 µg/mL of NA. Each sample was plated as triplicate. The plates were incubated at 37°C for 24 h then viable colonies were observed and enumerated.

- **Experiment 2:** Skin samples were dipped into a solution containing either PBS (control; N = 30) or 0.5% chitosan (N = 30) for 30s and drained off. Control and treated samples were placed in individual sample bags and kept in a refrigerator at 4°C. At 1 h, 24 h, 3, 6, 9 and 12 days, 5 control and 5 treated skin samples were homogenized within sterile sample bags using a rubber mallet. Sterile saline (5 mL) was added to each sample bag and hand stomached. Serial dilutions were spread plated on MacConkey agar (Becton, Dickinson and Co. Sparks, MD, USA). Each sample was plated as triplicate. The plates were incubated at 37°C for 24 h and then viable colonies were observed and enumerated respectively. The identification of individual colonies with different morphology on MacConkey agar was determined using the API-20E test kit for the identification of enteric Gram-negative bacteria (BioMerieux, Inc., Hazelwood, MO).

Statistical analysis: The Most Probable Number method was used to obtain the lowest possible detection limit: 0.5 log cfu/square cm in the enumeration of ST and aerobic Gram negative bacteria. Colony forming units of bacteria per square cm were converted to $\log_{10}$ numbers and analyzed using Analysis of Variance (ANOVA) with further separation of significantly different means using Duncan's Multiple Range test using SAS (SAS Institute, 2002). Significant differences were reported at ($p<0.05$).

RESULTS AND DISCUSSION

Salmonella is one of the most widespread bacterial species in poultry and it is often associated with foodborne illness (Bailey *et al*., 2002; Lynch *et al*., 2006). Cross-contamination by *Salmonella* in birds and carcasses may occur during transportation and processing (Cason *et al*., 1997; Corrier *et al*., 1999a). Therefore, the poultry industry has the challenge of monitoring and controlling *Salmonella* at all production levels (Hargis *et al*., 1995; Corrier *et al*., 1999a; Mikolajczyk and Radkowski, 2002). In the present study, dipping ST contaminated skin samples for 30 s in a solution of 0.5% chitosan was able to significantly reduce the recovery of ST cfu/square cm after 24 h in both trials (Table 1). The presence of spoilage bacteria in food products is an important economic problem. Therefore, an inexpensive and safe treatment to prevent spoilage is needed. Chitosan has been shown to be an effective antimicrobial, especially antibacterial. As shown in Table 2, 0.5% chitosan was effective in reducing total aerobic mesophilic Gram negative bacteria (spoilage bacteria) to undetectable levels. The primary spoilage bacteria in the control group of experiment 2 were identified as *Escherichia coli*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa* using the API-20E test kit for enteric Gram-negative bacteria (bioMerieux, Inc., Hazelwood, MO). The concentration of *P. aeruginosa* in the control group increased from 7.5×10^6 - 1.5×10^8

Table 1: *Salmonella* Typhimurium (log_{10} cfu±standard error)/square cm of chicken skin treated with 0.5% chitosan solution in experiment 1

	Trial 1		Trial 2	
Dipping treatment	1 h	24 h	1 h	24 h
Control	6.57±0.11[a]	6.03±0.02[a]	6.78±0.06[a]	7.36±0.06[a]
Chitosan (0.5%)	6.23±0.03[a]	5.81±0.06[b]	7.06±0.08[a]	6.6±0.17[b]

Values within columns with different lowercase superscripts differ significantly ($p<0.05$)

Table 2: Aerobic Gram negative bacteria (log_{10} cfu±standard error)/square cm of chicken skin treated with 0.5% chitosan solution in experiment 2

Sampling time	Control	Chitosan (0.5%)
1 h	1.31±0.83[a]	Undetectable levels
24 h	1.20±0.73[a]	Undetectable levels
3 days	4.70±0.31[a]	Undetectable levels
6 days	6.25±0.21[a]	Undetectable levels
9 days	7.12±0.11[a]	Undetectable levels
12 days	8.15±0.11[a]	Undetectable levels

Values within columns with different lowercase superscripts differ significantly ($p<0.05$)

cfu/square cm from 6-12 days stored at refrigeration temperatures (data not shown). The decreased growth as shown in Table 2 indicates that chitosan was very effective in controlling this and possible other spoilage bacteria. These results are in agreement with those published by Darmadji and Izumimoto (1994) who described the effectiveness of chitosan on storage stability of minced beef. Solutions of chitosan at 0.5-1.0% were able to inhibit the growth of spoilage bacteria on red meat after 10 days of storage at 4°C (Darmadji and Izumimoto, 1994). The antimicrobial activity and film-forming characteristic of chitosan makes it a potential source of food preservative, increasing quality and shelf life of different types of foods (Darmadji and Izumimoto, 1994; Ouattar *et al*., 2000; No *et al*., 2007; Friedman and Juneja, 2010; Suman *et al*., 2010; Vargas and Gonzalez-Martinez, 2010). The mechanism of the antimicrobial activity of chitosan has not yet been fully elucidated; nevertheless different hypotheses have been proposed. The most realistic hypothesis is that chitosan is able to change cell permeability due to interactions between the positive charges of its molecules and the negative charges of the bacterial cell membranes (No *et al*., 2007; Friedman and Juneja, 2010). Other hypotheses include the chelation of metals and essential nutrients, inhibiting bacterial growth (Rabea *et al*., 2003). Zheng and Zhu (2003) had also suggested that high molecular weight chitosan could be able to form a polymer membrane around the bacterial cell, preventing it from receiving nutrients. On the other hand, Zheng and Zhu (2003) also proposed that the low molecular weight chitosan could enter the bacterial cell through pervasion, disrupting the physiological activities of the bacterium.

Conclusion: The results of these experiments suggest that dipping raw chicken skin in a 0.5% solution of chitosan can reduce populations of *Salmonella* Typhimurium, thus enhancing general food safety and maybe shelf life of chicken meat. Moreover, these results also suggest that a solution of 0.5% chitosan can extend the shelf life of chicken meat as well as cause decreased growth of Gram negative spoilage bacteria. Future research will be directed at determining the effect of these organic compounds on the texture, color, oxidative stability, pH and consumer acceptance of chicken meat with treatment combinations that exhibited the most effective antibacterial activity.

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