Research Note

Sanitization of Chicken Frames by a Combination of Hydrogen Peroxide and UV Light To Reduce Contamination of Derived Edible Products

A. M. JONES-IBARRA,1 C. Z. ALVARADO,1 CRAIG D. COUFAL,2 AND T. MATTHEW TAYLOR3*

1Department of Poultry Science, Texas A&M University, College Station, Texas 77843-2472; 2Department of Poultry Science, Texas A&M AgriLife Extension, College Station, Texas 77843-2472; and 3Department of Animal Science, Texas A&M AgriLife Research, College Station, Texas 77843-2471, USA

ABSTRACT

Chicken carcass frames are used to obtain mechanically separated chicken (MSC) for use in other further processed food products. Previous foodborne disease outbreaks involving Salmonella-contaminated MSC have demonstrated the potential for the human pathogen to be transmitted to consumers via MSC. The current study evaluated the efficacy of multiple treatments applied to the surfaces of chicken carcass frames to reduce microbial loads on noninoculated frames and frames inoculated with a cocktail of Salmonella enterica serovar Enteritidis and Salmonella enterica serovar Typhimurium. Inoculated or noninoculated frames were left untreated (control) or were subjected to treatment using a prototype sanitization apparatus. Treatments consisted of (i) a sterile water rinse, (ii) a water rinse followed by 5 s of UV-C light application, or (iii) an advanced oxidation process (AOP) combining 5 or 7% (v/v) hydrogen peroxide (H2O2) with UV-C light. Treatment with 7% H2O2 and UV-C light reduced numbers of aerobic bacteria by up to 1.5 log CFU per frame (P, 0.05); reductions in aerobic bacteria subjected to other treatments did not statistically differ from one another (initial mean load on nontreated frames: 3.6 ± 0.1 log CFU per frame). Salmonella numbers (mean load on inoculated, nontreated control was 5.6 ± 0.2 log CFU per frame) were maximally reduced by AOP application in comparison with other treatments. No difference in Salmonella reductions obtained by 5% H2O2 (1.1 log CFU per frame) was detected compared with that obtained following 7% H2O2 use (1.0 log CFU per frame). The AOP treatment for sanitization of chicken carcass frames reduces microbial contamination on chicken carcass frames that are subsequently used for manufacture of MSC.

HIGHLIGHTS

• Chicken carcass frames were sanitized using an advanced oxidation process.
• Salmonella was reduced by 1.1 log CFU per frame with H2O2 and UV-C light.
• Aerobic bacteria were reduced by up to 1.5 log CFU per frame with 7% H2O2 plus UV-C light.
• Advanced oxidation processing produced greater reductions than water or UV-C light alone.

Key words: Advanced oxidative processing; Chicken; Hydrogen peroxide; Salmonella enterica; Sanitization; UV

According to the Centers for Disease Control and Prevention (Atlanta, GA), since 2011 there have been 11 outbreaks of human foodborne salmonellosis disease (non-typhoidal salmonellae) involving raw poultry products (chicken, turkey), including both intact and nonintact or comminuted products (3). In these events, Salmonella was transmitted through food vehicles including retail chicken, whole birds, ground poultry, and mechanically separated chicken (MSC). In 2013, after the development of a risk assessment motivated by multiple Salmonella outbreaks related to use of MSC, the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) conducted a 6-month sampling period targeting Salmonella prevalence in not-ready-to-eat comminuted poultry products, which included ground chicken and MSC (14). The USDA-FSIS reported 82.9% (n = 2,150) sampled MSC was Salmonella positive for samples collected between June 2013 and December 2014 (15). MSC has been previously implicated in the transmission of Salmonella to consumers and detection of foodborne disease outbreaks, potentially the results of raw material cross-contamination and improper processing or handling in the food service establishments handling the raw product (2, 9).

The term advanced oxidation process (AOP) was first introduced in 1987 reporting studies of the effects of combining ozone (O3) or hydrogen peroxide (H2O2) with UV light to describe the processes in which the hydroxyl
radical, a powerful oxidizing agent, is generated (7). When H2O2 is exposed to UV-C light, homolytic cleavage of the H2O2 molecule occurs, yielding two hydroxyl radicals per photon absorbed (6). This reaction occurs more quickly with the application of UV-C light at wavelengths of 200 to 300 nm. Hydroxyl radicals quickly react with organic materials in a nonselective manner, resulting in damage to nucleic acids, proteins, and lipids. This leads to cell membrane integrity loss and genetic mutations that inhibit proper cell replication (5, 10). Advanced oxidation processes have been widely used to kill microorganisms and degrade organic pollutants in waste water treatment processing (6).

Wells et al. (18) reported that applying 3% H2O2 and UV light independent of each other on eggshell surfaces resulted in reductions in aerobic bacteria counts by up to 2 log CFU per egg. However, when 1.5% H2O2 application was followed by an 8-min UV light (254-nm) treatment, aerobic plate counts (APCs) were reduced by more than 3 log CFU per egg. Al-Ajeeli et al. (1) and Gottselig et al. (8) showed this process was capable of producing significant reductions in Salmonella enterica serovar Enteritidis and Salmonella enterica serovar Typhimurium numbers on eggshells with the use of H2O2 at 3.5 and 3%, respectively, in conjunction with a short UV light exposure time. These researchers determined there was not a difference in the reduction of Salmonella Typhimurium achieved by 3% H2O2 followed by 60-s UV-C exposure compared with 5.0-s UV-C application (8). In addition, researchers reported a reduction of 5.3 log CFU per egg in Salmonella Enteritidis with a twice-repeated application of 3.5% H2O2 plus 5 s of UV-C light (1); numbers of the pathogen were reduced to nondetectable counts. Finally, Gottselig et al. (8) also reported no statistical differences in numbers of surviving aerobic bacteria on eggs treated by the AOP incorporating differing UV-C light intensities, reporting rather that the content of H2O2 was the limiting parameter of the AOP with respect to microbial inactivation.

The use of Salmonella-contaminated MSC obtained from poultry carcass frames presents a food safety hazard risk. Use of an AOP sanitization technique on chicken frames before mechanical separation of meat could help reduce the risk of Salmonella presence in MSC product. A chicken carcass frame is the remainder from the commercial slaughter and cutting process, yielding edible meat and other by-products. It consists of the skeleton including ribs, keel, and sternum as well as neck and some skin and may also have remnants of other organs and tissues (e.g., muscle, gizzard). The primary objective of this study was to determine the effectiveness of H2O2 plus UV-C AOP application to reduce APC and inoculated Salmonella numbers on chicken frames as a sanitization process for carcass frames intended for MSC production. The secondary objective of this study was to determine the minimum concentration of H2O2 needed to achieve maximum reductions of Salmonella and APC on chicken frames.

MATERIALS AND METHODS

Bacterial culture preparation and maintenance. One isolate each of Salmonella Enteritidis (recovered from a commercial poultry slaughter facility) and Salmonella Typhimurium strain ATCC 13311 (Manassas, VA) were revived from cryo-storage (−80°C) in the Food Microbiology Laboratory culture collection (Department of Animal Science, Texas A&M University, College Station). Tryptic soy broth (TSB; BD, Sparks, MD)—containing test tubes were inoculated separately with each Salmonella isolate and incubated aerobically without agitation at 37°C for 24 h to revive cultures. Overnight cultures were loop inoculated into sterilized TSB in 15-mL sealable conical centrifuge tubes (VWR International, West Chester, PA) and then incubated aerobically without agitation at 37°C for 24 h to complete revival. Conical tubes were centrifuged at 2,500 × g in a biosafety level 2–compliant centrifuge for 10 min at ambient temperature to produce bacterial pellets. Supernatants were gently poured off, and pellets were individually suspended in 10 mL of phosphate-buffered saline (PBS; Millipore-Sigma Corp., St. Louis, MO). Centrifugation and wash procedures were repeated twice in an identical manner to wash cultures of excess microbial fermentate. Final pellets were diluted in 9 mL of PBS and vortexed to ensure complete suspension in diluent. Both strains were aseptically combined into a 50-mL conical vial and vortexed to homogenize to make an inoculum cocktail.

Chicken frame sample collection. Chicken frames were obtained from a commercial chicken processing facility and refrigerated (4 to 5°C) until ready for use at the Poultry Science Microbiology Laboratory in the Poultry Science Research Center (Department of Poultry Science, Texas A&M University). Frames were received directly from the supplier and were tested for the incoming counts of Salmonella on frames; all were below the limit of detection (10 CFU per frame). Noninoculated carcass frames were first individually weighed, and the appropriate amount (1.81 kg/400 mL) of chilled buffered peptone water (BD) was placed into a poultry carcass rinse bag (14). Frame rinsate fluid samples were collected in accordance to the Raw Chicken Parts Sampling protocol (17). Samples were refrigerated (1.6°C) until serially diluted in PBS and plated on 3M Petrifilm aerobic count plates films (enumeration of aerobic bacteria) and xylose lysine Tergitol 4 (XLT4, BD) agar in petri plates for presumptive Salmonella enumeration. Petrifilms were incubated aerobically for 48 h at 37°C before enumeration; XLT4 petri plates were incubated 36 h at 37°C before enumeration of Salmonella-typical colonies.

Salmonella inoculation onto chicken frames. Flame-sterilized poultry shears were used to butterfly each frame at the sternum-keel bone. One noninoculated control frame was immediately placed into a labeled polyethylene poultry carcass rinse bag (38.1 by 50.8 cm [15 by 20 in.], 12-L capacity; VWR International) to determine the initial APC or determine Salmonella presence. For Salmonella decontamination trials, frames were individually placed into poultry rinse bags and inoculated with 0.1 mL of Salmonella cocktail (10⁶ CFU per frame). The bag was twisted to seal and hand massaged for 30 s; the time was determined by preliminary data that showed massaging longer than 30 s resulted in breaking of small bones and loss of meat integrity (data not shown). After massaging, each frame was aseptically removed from its bag and placed onto sanitized grills and allowed to rest for at least 15 min to allow for inoculum attachment. After the rest period, three frames were separately placed into poultry rinse bags to enumerate the counts of Salmonella cocktail inoculum on the frames.

Description of frames treatment. The AOP equipment used for chicken frame treatment and its design was described...
Enumeration of surviving microbes after frames treatment. Serial dilutions of frame rinsates were completed in sterilized PBS for the enumeration of inoculated Salmonella surviving cells. Aerobic bacteria were enumerated using PetriFilm aerobic count plates, and Salmonella were enumerated using XLT4 agar petri plates as described above. All petri plates were incubated at 37°C, and colonies were counted after 48 h (APC) or 36 h (XLT4). Countable CFUs for Salmonella Typhimurium colonies were smooth, round, and clear with black centers, whereas Salmonella Enteritidis colonies were smooth, round, red or yellow colonies with black centers.

Statistical analysis of data. Three trials were completed in identical manner with three independent replicates for each treatment per trial (n = 9). Means of log-transformed plate counts were statistically analyzed to detect differences in Salmonella or APC reductions as a function of the antimicrobial treatment compared with untreated control. Data were analyzed by treatment using one-way analysis of variance with JMP 14.0 (SAS Institute Inc., Cary, NC). Means were then separated using Student’s t test (P = 0.05).

RESULTS AND DISCUSSION

Reduction of aerobic bacteria by AOP application. The mean APC on noninoculated chicken frames not subjected to sanitizing treatment (control) was 3.6 ± 0.1 log CFU per frame; AOP treatment with either 5 or 7% H$_2$O$_2$ in combination with UV-C light resulted in significant reductions of aerobic bacteria counts (P = 0.0025) on noninoculated frames of 0.8 or 1.5 log CFU per frame, respectively (Table 2). For frames treated with 7% H$_2$O$_2$ and UV-C light, differences in aerobic plate counts were significantly reduced (P < 0.05) compared with the control. In addition, small, nonsignificant reductions in APCs, ranging from 0.1 to 0.6 log CFU per frame, were observed for water-rinsed and UV-C-treated frames, compared with the control. Moore et al. (11) inoculated chicken frames with Salmonella enterica serovar Heidelberg and then dipped them into solutions of USDA-approved antimicrobials before blending the frames. No statistically significant

| TABLE 1. Experimental design groups for Salmonella-inoculated or noninoculated frames$^a$ |
| Treatment | AOP device use | H$_2$O$_2$ Final manual UV Frames/group (n) |
| Control | – | – | – | 9 |
| Manual H$_2$O rinse | – | – | + | 9 |
| H$_2$O$_2$+UV | + | 0 | + | 9 |
| 5.0% H$_2$O$_2$+UV | + | 5 | + | 9 |
| 7.0% H$_2$O$_2$+UV | + | 7 | + | 9 |
| Total | 45 |

$^a$ Control, no treatment; manual H$_2$O rinse, no treatment, final hand H$_2$O spray; H$_2$O$_2$+UV, device used with water, UV light, and final hand H$_2$O spray, no H$_2$O$_2$; 5% H$_2$O$_2$+UV, device used with 5% H$_2$O$_2$, UV light, and final manual H$_2$O spray; 7% H$_2$O$_2$+UV, device used with 7% H$_2$O$_2$, UV light, and final manual H$_2$O spray; + and – use or nonuse of indicated treatment application conditions per each treatment.

| TABLE 2. Least-squares mean reductions in counts of aerobic bacteria and Salmonella from treated chicken frames$^b$ |
| Treatment$^b$ | Aerobic bacteria (log CFU/frame) | Salmonella cocktail (log CFU/frame) |
| Manual H$_2$O rinse | 0.1 AB | 0.5 B |
| H$_2$O$_2$+UV-C | 0.6 AB | 0.7 C |
| 5.0% H$_2$O$_2$+UV-C | 0.8 AB | 1.1 D |
| 7.0% H$_2$O$_2$+UV-C | 1.5 B | 1.0 D |
| P < F | 0.0025 | <0.0001 |
| SEM | 0.23 | 0.11 |

$^a$ Numerical values present means calculated from three independently completed trials, with triplicate identically handled replicate samples per trial (n = 9) ± 1 sample standard deviation. Values within a column not sharing letters differ by Student’s t test at P = 0.05. Mean initial APC (noninoculated) and Salmonella (inoculated) were 3.63 ± 0.12 and 5.61 ± 0.22 log CFU per frame, respectively.

$^b$ Manual H$_2$O rinse, no treatment, final H$_2$O spray; H$_2$O$_2$+UV-C, device used with H$_2$O, UV-C light, and final H$_2$O spray, no H$_2$O$_2$; 5% H$_2$O$_2$+UV-C, device used with 5% H$_2$O$_2$, UV-C light, and final manual H$_2$O spray; 7% H$_2$O$_2$+UV-C, device used with 7% H$_2$O$_2$, UV-C light, and final manual H$_2$O spray.

$^c$ SEM, pooled standard error of the mean.
differences were found in the numbers of surviving aerobic bacteria after treatment with 0.1% peracetic acid (pH 2.8), 0.6% cetylpyridinium chloride (pH 6.7), 0.3% propionic acid (pH 3.5), or 1.5% lactic acid (pH 2.3) compared with the untreated control. Chen et al. (4), conversely, dipped skin-on chicken breasts and thighs into various antimicrobials, rinsed the treated samples, and then ground the tissue. They reported the use of peracetic acid at 0.07 or 0.1% significantly lowered ($P \leq 0.05$) aerobic bacteria counts compared with APCs surviving on samples treated by 0.35 or 0.6% cetylpyridinium chloride and 0.003% chlorine. They concluded the use of peracetic acid on cut chicken pieces before grinding would produce an extension of shelf life compared with other treatments. The current study demonstrates the ability of the AOP to reduce aerobic bacteria on frames before continuing to further processing, potentially improving shelf life of derived MSC.

**Salmonella** reduction by antimicrobial treatments. Reducing the prevalence and numbers of *Salmonella* on chicken frames that are used for MSC production can reduce the risk of human foodborne salmonellosis. The mean *Salmonella* count on nontreated control frames was 5.6 ± 0.2 log CFU per frame; all antimicrobial treatments reduced numbers of inoculated *Salmonella* compared with the control ($P < 0.0001$; Table 2). The manual water rinse showed a significant ($P < 0.05$) decrease of 0.5 log CFU per frame from the control that indicated the manual water rinse likely mechanically removed loosely attached *Salmonella* cells. Treatments using the AOP system produced modest decreases in pathogen numbers compared with the control. Resulting *Salmonella* reductions from treatments were 0.7 log CFU per frame (H$_2$O+UV), 1.1 log CFU per frame (5.0% H$_2$O$_2$+UV), and 1 log CFU per frame (7% H$_2$O$_2$+UV; Table 2). Observed reductions in *Salmonella* with the AOP treatments align with existing data wherein use of H$_2$O$_2$ in the AOP, versus application of H$_2$O$_2$ or UV light alone, produced the greatest reductions in microbes (18). Regarding the two concentrations of H$_2$O$_2$ (5 and 7%), there was no difference in observed *Salmonella* reductions ($P \geq 0.05$), indicating 7% H$_2$O$_2$ did not produce any additional effect over 5% H$_2$O$_2$ (Table 2). This was a similar outcome as that reported by Wells et al. (18) for AOP usage to decontaminate eggshell surfaces from aerobic bacteria. The mechanical action of the sprayers was able to remove *Salmonella* with water alone, indicating the ability to remove *Salmonella* cells that had loosely attached onto the meat, bone, cartilage, or skin of the frames. Treatment with H$_2$O$_2$ and UV-C light likely damaged nucleic acids and other critical physiological components (membrane, lipid, and proteins) of the *Salmonella* cells, thus destroying their ability to sustain homeostasis and ultimately inactivating pathogen cells. Although the potential for *Salmonella* to become injured was likely produced by the use of the AOP, MSC is not produced for sale to consumers but rather is used in the manufacture of fully cooked poultry products.

Currently, the USDA-FSIS routinely tests raw comminuted chicken products for the presence of *Salmonella*, with a maximum acceptable performance standard of 25% (16). However, there is no prescribed performance standard for *Salmonella* for MSC. In this study, AOP treatment was able to reduce aerobic bacteria counts by up to 1.5 log CFU per frame on deboned chicken frames. Numbers of inoculated *Salmonella* were reduced up to 1.1 log CFU per frame by AOP treatment. Contamination of chicken carcasses and carcass frames by microorganisms raises concerns over resulting food shelf life and microbiological safety, which are often addressed by antimicrobial interventions placed throughout the animal harvest and carcass-cutting processes (12, 19). Findings from this study also indicate that 5% H$_2$O$_2$ is as effective as 7% H$_2$O$_2$ when applied in combination with UV-C light for the reduction of *Salmonella* on poultry frames. Further research is needed to identify suitable *Salmonella* surrogate microorganisms, *Campylobacter* surrogate microorganisms, or both, to facilitate commercial validation challenge trials to be used in hazard analysis and critical control point plan development or revision. In addition, research designed to determine the impact of AOP application on moisture retention, flavor, and color for products containing MSC obtained from treated chicken frames would enhance this technology’s usefulness within the poultry industry for producing safe, wholesome poultry products.

**ACKNOWLEDGMENT**

Funding for this research was provided by Texas A&M AgriLife Research, Department of Poultry Science, College Station, TX.

**REFERENCES**


