

# Effects of hot water spray and sub-zero saline chilling on bacterial decontamination of broiler carcasses

I. Kang,<sup>\*,1</sup> H. C. Lee,<sup>\*</sup> B. Adhikari,<sup>†</sup> S. D. Ha<sup>Ⓛ,‡</sup> and Y. M. Kwon<sup>†</sup>

<sup>\*</sup>Department of Animal Science, California Polytechnic State University, San Luis Obispo, CA 93407, USA;

<sup>†</sup>Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA; and <sup>‡</sup>Department of Food Science and Technology, Chung-Ang University, Ansung, Gyunggido 456-756, Republic of Korea

**ABSTRACT** Reduction of *Salmonella* on poultry carcasses is one way to prevent salmonellosis. The purpose of this research was to evaluate the effects of sub-zero saline chilling (SSC) with/without hot water spray (HWS) on broiler carcasses prior to chilling for bacterial reduction. Eviscerated broiler carcasses were subjected to water immersion chilling (WIC, 0% NaCl/0.5°C) or SSC (4% NaCl/−2.41°C) with/without prior HWS at 71°C for 1 min. Broiler carcasses in SSC were chilled faster than those in WIC, regardless of HWS. The combination of HWS and SSC resulted in the best reduction of mesophilic aerobic bacteria, *Escherichia coli*, and total coliforms on the carcasses

over the WIC, SSC, and HWS/WIC. No *Salmonella* was detected on the carcasses in SSC and HWS/SSC while *Salmonella* positive was observed on the carcasses chilled in WIC and HWS/WIC. A trace of Gram-negative genus was detected on carcasses in HWS/SSC while many other microbiomes were observed on those in WIC, SSC, and HWS/WIC when quantitative microbiota profiles of 16S rRNA gene sequences were evaluated. Based on these results, chilling of broiler carcasses in 4% NaCl/−2.41°C after HWS at 71°C for 1 min significantly reduced carcass chilling time and bacterial contamination over the control chilling.

**Key words:** bacterial decontamination, hot water spray, sub-zero saline chilling, rRNA gene profile

2022 Poultry Science 101:101688

<https://doi.org/10.1016/j.psj.2021.101688>

## INTRODUCTION

Poultry became the world's most consumed meat in 2019 after overtaking the consumption of pork but has received negative publicity due to the high incidences of foodborne illnesses, particularly for salmonellosis and campylobacteriosis (CDC, 2018; Chainé et al., 2013). In poultry processing, birds are chilled in cold water (water immersion chilling, WIC) in the United States (US) or cold air (air chilling, AC) in the European community (Kang and Kim, 2017). In WIC, broiler and turkey carcasses are usually immersed in a water tank for 1 to 2 h and 4 to 5 h, respectively. However, the tank may provide an environment for bacterial cross-contamination and subsequent growth if the chilling is conducted poorly or in warm conditions (Singh et al., 2016; Bilgili et al., 2002; Blank and Powell, 1995).

The primary purpose of carcass chilling is to reduce carcass temperature and bacterial growth to the levels that will maximize food safety and shelf-life for consumer's safety (Sams, 2001). Poor chilling will negatively influence product flavor, appearance, and processing efficiency. When carcasses are contaminated during processing, bacteria initially attach to the liquid film on the skin and then become tighter attached to the skin. Upon attaching to surfaces, bacteria showed a high resistance (up to 150 times) to disinfectants over the free cells and further resistance (up to 3,000 times) upon formation of a biofilm (LeChevalier et al., 1988).

Sub-zero saline chilling (SSC) of poultry carcasses has the potential to improve chilling efficiency, bacterial reduction, and overall cost savings. Recently, our laboratory developed a proprietary chilling method using sub-zero saline solutions in pre-/post-chilling using various saline concentrations and temperatures (Kang, 2021; Kang and Hurley, 2019). After chilling broiler carcasses from 0% NaCl/0.5°C to 8% NaCl/−5.08°C solutions, we found that the carcasses in 4% NaCl/−2.41°C and 8% NaCl/−5.08°C solutions showed the best bacterial reduction and meat tenderness than the carcasses in the solutions of lower salt concentrations and warmer temperatures, with no significant difference

© 2022 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received September 27, 2021.

Accepted December 24, 2021.

<sup>1</sup>Corresponding author: [ikang01@calpoly.edu](mailto:ikang01@calpoly.edu)

observed between the two solutions (Kang 2021; Lee et al., 2020; Kang and Hurley, 2019; Metheny et al., 2019).

Hot water treatment is one of promising intervention strategies that inactivate bacterial pathogens and improves product safety (Tompkins et al., 2008). Hot water exposure, which is approved for carcass decontamination in the US (1996) can be used to decrease the population of spoilage and pathogenic bacteria by 1–3 logs (Sofos and Smith, 1998). However, carcass exposure to 71°C water for 1 min led to a partially cooked appearance (Zhang et al., 2011; Cox et al., 1974), whereas no detrimental effect on skin color was found at 70°C for 40 s. James et al. (2007) reported that a single water treatment at 80°C for 20 s decreased *Campylobacter jejuni* populations by 2.0 log CFU/cm without extensive degradation of the carcass appearance.

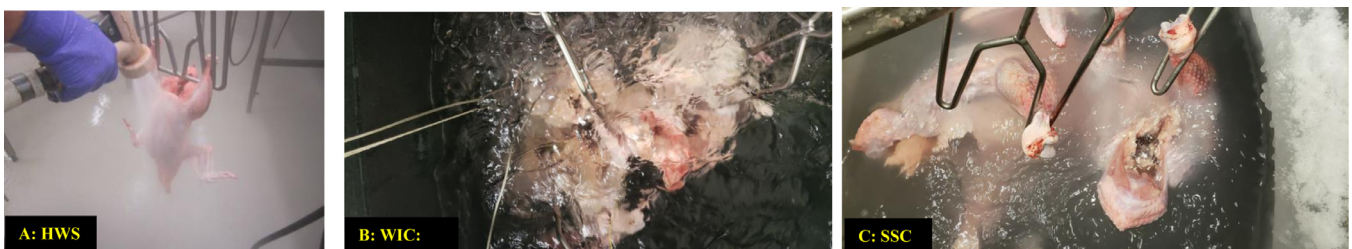
Combination of hot water spray (HWS) and SSC is expected to further reduce bacterial populations over a single application potentially due to the synergistic effects on broiler carcasses for heat-sensitive (hot water), cold-sensitive (cold water), and salt-sensitive (salt-water) bacteria. The purpose of this research was to evaluate the combined effects of HWS and SSC on broiler carcasses for chilling efficiency and bacterial reduction.

## MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee of California Polytechnic State University (Protocol #2010)

### Broiler Processing

A total of 16 commercial broilers in 6 wk old and live weights between 2.3 and 3.3 kg were obtained locally. These birds were electrically stunned for 3 s (40 mA, 60 Hz, 110V), bled for 120 s, scalded for 120 s at 56.7°C, and defeathered in a rotary drum picker for 120 s in the Meat Processing Center at California Polytechnic State University. After manual evisceration and washing, carcasses (4 replications, n = 4) were randomly assigned to one of 4 chilling treatments as explained below.



**Figure 1.** Hot water spray of carcasses (A), water immersion chilling (WIC, B), and sub-zero saline chilling (SSC, C). (A) HWS: hot water spray at 71°C for 1 min. (B) WIC: water immersion chilling in 0% NaCl/0.5°C. (C) SSC: Subzero saline chilling in 4% NaCl/−2.41°C.

### Preparation of Control and Sub-zero Saline Solutions

Prior to poultry processing, sub-zero saline solution (189 l) was prepared by dissolving 4% salt (w/w) in tap water using a 389-liter container. The solution was then placed in freezing room overnight at −12°C, and a target temperature (−2.41°C) of the saline solution was achieved while agitating continuously using a submersible pump (4E-34N, SupplyHouse.com). A control solution (0.5°C) was prepared with ice, and the target temperature was maintained in a refrigerated room while agitating continuously as explained above.

### Carcass Chilling

Immediately after evisceration and washing, one carcass/treatment in medium size (~ 2.8 kg) was selected, and a digital thermometer/logger (THS 292-501, ThermoWorks, American Fork, UT) was inserted to the center of carcass breast. Carcasses were randomly assigned to one of the 4 chilling solutions with or without HWS at 71°C for 1 min using a nozzle type of spray system as follow: 1) WIC in 0% NaCl/0.5°C solution; 2) SSC in 4% NaCl/−2.4°C solution; 3) HWS/WIC – HWS followed by WIC; 4) HWS/SSC – HWS followed by SSC (Figure 1).

### Skin Sample Preparation

After chilling, 25 g of skin was aseptically taken from the breast and neck areas to place in sterile WhirlPak bag (Nasco, Modesto, CA). Each sample bag received 225 mL of sterile phosphate buffer saline (PBS) and was stomached for 1 min (Stomacher 400 Circulator, Seward, Worthing, UK) to assess bacteria populations of the skin.

### Microbiological Analysis

- Mesophilic aerobic bacteria (MAB): Serial 10-fold dilutions of the stomached samples were surface-plated (0.1 mL) in duplicate on Petrifilm™ Aerobic Count Plates (3M Microbiology Products, St. Paul, MiN) to enumerate MAB after incubation at 37°C for 24 h.
- *Escherichia coli* (*E. coli*) and coliforms: Serial 10-fold diluted samples were similarly plated (0.1 mL) on

Petrifilm™ *E. coli*/coliform count plates (3M Microbiology Products). All samples were incubated at 37°C for 24 h before enumeration.

- *Salmonella*: The presence of *Salmonella* was assessed by adding 30 mL of the stomached sample to 30 mL of buffered peptone water followed by 20 h of incubation at 37°C. The amount (30 mL) of the pre-enrichment solution was then transferred to 30 mL of 2 × Rappaport-Vassiliadis broth and incubated at 42°C for 20 h. A 120 µL aliquot of this enrichment was used to examine for *Salmonella* using Reveal™ *Salmonella* test kits (Neogen Corp, Lansing, MI). All positive samples were streaked onto brilliant green sulfur and xylose lysine Tergitol 4 agar, incubated at 37°C for 24 h, and inspected for typical *Salmonella* colonies to confirm the Reveal™ *Salmonella* results.

### Analysis of Survived Bacterial Populations Using 16S rRNA Gene Profiling

To investigate any changes in bacterial populations on chicken carcasses after chilling in different solutions, the 16S rRNA gene microbiota profiling was performed using bacterial colonies formed on Petrifilm™ APC films. Briefly, all colonies on each Petrifilm were collected using sterile razor blades and combined. Genomic DNA was extracted from the pooled bacterial colonies, and the V4 region of the 16S rRNA gene from the genomic DNA was amplified for preparation the 16S rRNA gene sequencing library according to the protocol of the Earth Microbiome Project (<http://www.earthmicrobiome.org>). The amplicons from each sample were pooled together, gel-purified, and sequenced with the Illumina MiSeq platform with paired-end 250 cycle options. The raw sequence reads were processed using QIIME 2 version 2018.8 (Bolyen et al., 2019) with Deblur plugin (Amir et al., 2017). The taxonomic assignment was performed using a Naive Bayes classifier pre-trained with Greengenes (Version 13.8) at a 99% cutoff level for OTUs (DeSantis et al., 2006). To have a more accurate view of the changes in the populations, the relative abundance information for each genus was calibrated using the CFU/mL data to generate quantitative microbiota profiles.

### Statistical Analysis

Mesophilic aerobic bacteria, *E. coli* and total coliforms counts per gram of skin from triplicate experiments were converted to log units for statistical analysis. Bacterial counts were compared to evaluate significant differences among treatments, using one way ANOVA and post hoc analysis via Tukey test at  $P < 0.05$  (IBM SPSS Statistic program, 2020). Prevalence differences for *Salmonella* were determined using the chi-squared test (FREQ procedure). A  $P$ -value  $< 0.05$  was considered significant (SPSS, 2021).

## RESULTS AND DISCUSSION

The time required for bird processing from stun to evisceration was about 15 to 20 min. The internal temperature of eviscerated carcasses gradually decreased from ~38 °C to 4.4°C during chilling that took 82 and 68 min in 0% NaCl/0.5°C and 4% NaCl/−2.41°C solutions, respectively, regardless of HWS. These results support the previous finding that broilers were chilled more effectively in sub-zero saline solutions of 1% NaCl/−0.6 °C to 8% NaCl/−5.08 °C than the control solution of 0% NaCl/0.5°C (Lee et al., 2020; Metheny et al., 2019).

After chilling, the populations of MAB on broiler carcasses were more reduced in combined treatments (HWS/WIC and HWS/SSC) than any single treatment (WIC and SSC). Out of the four treatments, HWS/SSC resulted in the best bacterial reduction followed by HWS/WIC, SSC, and WIC, with no difference observed between WIC and SSC (Table 1). The best decontamination of bacteria was expected due to the combined effect on broiler skin for the reduction of heat-sensitive bacteria by hot-water, cold-sensitive or Gram-negative bacteria by cold-water, and salt-sensitive bacteria by saline solution. The result of significant difference between WIC and SSC was in accordance with the previous report ( $P > 0.05\%$ ) (Lee et al., 2020). Microorganisms are inherently adapted to optimum function in their normal physiological environments, and any radical changes in environmental conditions inflict a stress on the organism (Bower and Daeschel, 1999). The environmental factors in a single treatment may not be as fatal to the cells as the combined factors of HWS and SSC. Many microorganisms can tolerate and adapt themselves to a certain environmental change over the time. The bacteria of psychrophiles, thermophiles, halophiles, acidophiles are known to have a better ability to survive than other bacteria in unfavorable conditions such as low/high temperature, high salt, and low pH, respectively (Beales, 2004; Hill et al., 1995; Prescott et al., 1993).

In case of *E. coli* and total coliforms, the best decontamination was observed in HWS/SSC among the four treatments. It has been known that Gram-negative bacteria are less tolerant to freezing, salting, and heating conditions than Gram-positive bacteria (Mai-

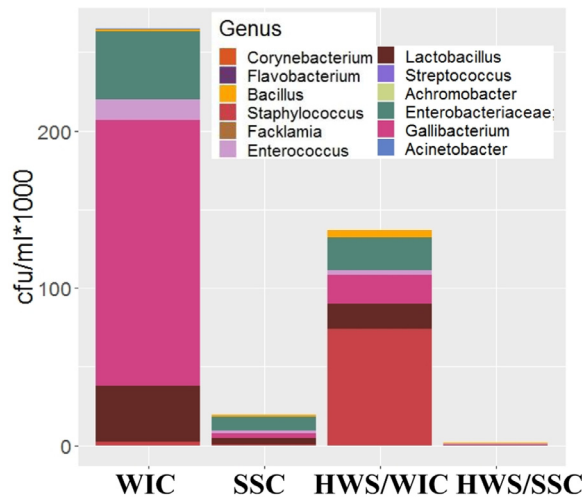
**Table 1.** Mean populations (log CFU/g) of mesophilic aerobic bacteria (MAB), *Escherichia coli* (*E. coli*), and coliform bacteria as well as prevalence of *Salmonella* on the chicken skin receiving different chilling methods.<sup>1</sup>

Chilling	MAB	<i>E. coli</i> (log CFU/g)	Coliform	<i>Salmonella</i> (%)
WIC	4.70 <sup>a</sup>	2.60 <sup>a</sup>	2.61 <sup>a</sup>	100 (4/4) <sup>a,*</sup>
SSC	4.19 <sup>ab</sup>	1.78 <sup>b</sup>	2.12 <sup>a</sup>	0 (0/4) <sup>b</sup>
HWS/WIC	3.66 <sup>b</sup>	2.01 <sup>ab</sup>	1.91 <sup>a</sup>	100 (4/4) <sup>a</sup>
HWS/SSC	2.54 <sup>c</sup>	0.18 <sup>c</sup>	0.87 <sup>b</sup>	0 (0/4) <sup>b</sup>

<sup>a-c</sup>Means (n = 4) within a row within common letters within.

\*Four out of four samples were positive for *Salmonella* each column are significantly different ( $P < 0.05$ ).

Abbreviations: HWS, hot water spray; SSC, Subzero saline chilling; WIC, water immersion chilling.



**Figure 2.** Quantitative microbiome profiles after four different chilling treatments.

Prochnow et al., 2016; Dimitraki and Velonakis, 2007; Jay, 1992; Straka and Stokes, 1959). When the outer membrane of Gram-negative cells (thinner than Gram-positive cells) is damaged, the cells lose essential metabolic processes such as sensitization of cells to bile salts, lysozyme, or hydrophobic antibiotics (Mackey, 2000; Tsuchido et al., 1995). It is presumed that Gram-negative cells receive more heat-damage than Gram-positive ones during the hot water exposure. As a result, the physically damaged bacteria become more susceptible to the temperature and saline changes during chilling using the sub-zero saline solution.

*Salmonella* is one of most common bacteria that cause diarrhea and foodborne-related deaths (Eat right, 2019). No *Salmonella* positive was observed on the broiler carcasses after SSC chilling, regardless of HWS, whereas *Salmonella* positive was seen on the carcasses after control chilling, regardless of HWS (Table 1). These results support the previous results of *E. coli* in HWS/SSC and SSC chilling (Table 1; Lee et al., 2020).

To investigate the changes in bacterial population on chicken carcasses after the four treatments, we performed 16S rRNA gene profiling analysis using the pool of bacterial colonies recovered on Petrifilm APC films. In alliance with the results of bacterial enumeration (Table 1), the combination of HWS and SSC eliminated most genus, with no detection of Gram-negative bacteria, whereas more bacterial genera were detected in SSC, followed by WSH/WIC and WIC (Figure 2). The sensitivity of bacteria to cold stress is dependent on several factors including the rate of cooling and freezing (Berry and Foegeding, 1997; Gounot, 1991). The rapid change of temperature from 71°C to -4.1°C in this study might induce a synergistic effect on the bacteria on broiler carcasses, especially Gram-negative cells.

## CONCLUSIONS

In poultry processing, carcass chilling is one of major steps to prevent bacterial contamination and growth by

reducing carcass temperatures, whereas the same processing can provide the potential for cross-contamination and tight attachment if the chilling is not conducted rapidly or performed in warmed water. Addition of food preservatives, reduction of environmental temperatures, and lowering of water activity are popular methods to inhibit bacterial growth. The combination of hot water spray and sub-zero saline chilling may induce unfavorable conditions to the bacteria on carcasses with no addition of chlorine and/or peracetic acids. No use of chlorine in poultry chilling can bring positive results including employee safety, consumer health, and penetration of European markets. However, additional research is required to evaluate bacterial populations on poultry carcasses after subzero saline chilling in different conditions, salt pick-up by carcasses, visual discoloration (or partial cooked appearance) after HWS, recycling of the red water after saline chilling, salt issue in the wastewater treatment etc.

## ACKNOWLEDGMENTS

The authors thank the USDA-NIFA (2020-67017-31272) and the Agricultural Research Institutes (ARI) of the California State University system for funding this research. The data that support the findings of this study are available from the corresponding author upon reasonable request.

## DISCLOSURES

The authors have no conflicts of interest.

## REFERENCES

- Amir, A., D. McDonald, J. A. Navas-Molina, E. Korylova, J. T. Morton, X. Z. Zech, E. P. Kightley, L. R. Thompson, E. R. Hyde, A. Gonzalez, and R. Knight. 2017. Deblur rapidly resolves single-nucleotide community sequence patterns. *mSystems* 2:e00191-16.
- Beales, N. 2004. Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. *Compr. Rev. Food Sci. Food Saf.* 3:1-20.
- Berry, E. D., and P. M. Foegeding. 1997. Cold temperature adaptation and growth of microorganisms. *J. Food Prot.* 60:1583-1594.
- Bilgili, S. F., A. L. Waldroup, D. Zelenka, and J. E. Marion. 2002. Visible Ingesta on prechill carcasses does not affect the microbiological quality of broiler carcasses after immersion chilling. *J. Appl. Poult. Res.* 11:233-238.
- Blank, G., and C. Powell. 1995. Microbiological and hydraulic evaluation of immersion chilling poultry. *J. Food Prot.* 58:1386-1388.
- Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, et al. 2019. QIIME 2: reproducible, interactive, scalable, and extensible microbiome data science. *Nat. Biotechnol.* 37:852-857.
- Bower, C. K., and M. A. Daeschel. 1999. Resistance responses of microorganisms in food environments. *Int. J. Food Microbiol.* 50:33-44.
- CDC (Center for Disease Control and Prevention). 2019. Morbidity and Mortality Weekly Report (MMWR). Preliminary incidence and trends of infections with pathogens transmitted commonly through food — foodborne diseases active surveillance network, 10 U.S. Sites, 2015-2018. <https://www.cdc.gov/mmwr/volumes/68/wr/mm6816a2.htm>. Accessed May 2021.

- Chaine, A., E. Arnaud, A. Kondjoyan, A. Collignan, and S. Sarter. 2013. Effect of steam and lactic acid treatments on the survival of *Salmonella* Enteritidis and *Campylobacter jejuni* inoculated on chicken skin. *Int. J. Food Microbiol.* 162:276–282.
- Cox, N. A., A. J. Mercuri, J. E. Thomson, and D. W. Gregory. 1974. Quality of broiler carcasses as affected by hot water treatments. *Poult. Sci.* 53:1566–1571.
- DeSantis, T. Z., P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G. L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* 72:5069–5072.
- Dimitraki, P., and E. Velonakis. 2007. The survival of pathogens in frozen food as a health risk. *Arch. Hell. Med.* 24:432–439.
- Eat Right. 2019. Most common foodborne pathogens. <https://www.eatright.org/homefoodsafety/safety-tips/food-poisoning/most-common-foodborne-pathogens#:~:text=Salmonella%20is%20the%20name%20of,foodborne%2Drelated%20hospitalizations%20and%20deaths>. Accessed Apr. 2021.
- Gounot, A. M. 1991. Bacterial life at low temperature: physiological aspects and biotechnological implications. *J. Appl. Bacteriol.* 71:386–397.
- Hill, C., B. O'Driscoll, and I. Booth. 1995. Acid adaption and food poisoning microorganisms. *Int. J. Food Microbiol.* 28:245–254. Accessed January 18, 2022. <https://uspto.report/patent/app/20210289799>.
- IBM SPSS Statistic program, version 27, 2020. Accessed January 2022. <https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-27>
- James, C., S. J. James, N. Hannay, G. Purnell, C. Barbedo-Pinto, H. Yaman, M. Araujo, M. L. Gonzalez, J. Calvo, M. Howell, and J. E. L. Corry. 2007. Decontamination of poultry carcasses using steam or hot water in combination with rapid cooling, chilling or freezing of carcass surfaces. *Int. J. Food Microbiol.* 114:195–203.
- Jay, J. M. 1992. High temperature food preservation, and characteristics of thermophilic microorganisms. Pages 341–362 in *Modern Food Microbiology*. 4th ed Chapman & Hall, New York, NY.
- Kang, I. 2021. Meat processing methods (Provisional – Application # 17/249,914).
- Kang, I., and S. Hurley. 2019. Meat processing methods (US 2019/0008174 A1).
- Kang, I., and H. B. Kim. 2017. Enhancing texture and tenderness in poultry meat. Pages 291–319 in *Achieving Sustainable Production of Poultry*. Burleigh Dodds Science Pub, Cambridge, UK.
- LeChevallier, M. W., C. D. Cawthon, and R. G. Lee. 1988. Inactivation of biofilm bacteria. *Appl. Environ. Microbiol.* 54:2492–2499.
- Lee, H. C., M. M. Metheny, S. Vilianni, D. C. Bennett, S. Hurley, and I. Kang. 2020. Effects of subzero saline chilling on broiler chilling efficiency, meat quality, and microbial safety. *Poult. Sci.* 99:5158–5162.
- Mackey, B. M. 2000. Injured bacteria. Pages 315–341 in *The Microbiological Safety, and Quality of Food*. M Lund, T. C. Baird-Parker and G. W. Gould, eds. Aspen Publisher, Gaithersburg, MD.
- Mai-Prochnow, A., M. Clauson, J. Hong, and A. B. Murphy. 2016. Gram positive and Gram-negative bacteria differ in their sensitivity to cold plasma. *Sci. Rep.* 6:38610.
- Metheny, M., M., H. C. Lee, S. Vilianni, D. Bennett, S. Hurley, and I. Kang. 2019. Improvement of chilling efficiency and meat tenderness of broiler carcasses using sub-zero saline solutions. *Poult. Sci.* 98:4190–4195.
- Prescott, L. M., J. P. Harley, and D. A. Klein. 1993. *Microbiology*. 2nd Edition Wm. C. Brown Publishers, Dubuque, 588–591.
- Sams, A. R. 2001. First processing: slaughter through chilling. Pages 19–34 in *Poultry Meat Processing*. A. R. Sams, ed. CRC Press, LLC, Boca Raton, FL.
- Singh, P., M. F. Silva, E. T. Ryser, S. D. Ha, and I. Kang. 2016. Recovery of associated and internalized *Salmonella* in broiler skin by stomaching and grinding. *Food Control* 73:883–888.
- Sofos, J. N., and G. C. Smith. 1998. Non-acid meat decontamination technologies: model studies and commercial applications. *Int. J. Food Microbiol.* 44:171–188.
- SPSS. 2021. PASW Statistics 18 for Windows. SPSS Inc., Chicago, IL.
- Straka, R. P., and J. L. Stokes. 1959. Metabolic injury to bacteria at low temperatures. *J. Bacteriol.* 78:181–185.
- Tompkins, N. M., J. S. Avens, P. A. Kendall, and M. D. Salman. 2008. Effect of boiling water carcass immersion on aerobic bacteria counts of poultry skin and processed ground poultry meat. *Zoonoses Public Health* 55:235–241.
- Tsuchido, T., N. Katsui, A. Takeuchi, M. Takano, and I. Shibasaki. 1995. Destruction of the outer membrane permeability barrier of *Escherichia coli* by heat treatment. *Appl. Environ. Microbiol.* 50:298–303.
- USDA-FSIS. 1996. Notice of policy change; achieving the zero tolerance performance standard for beef carcasses by knife trimming and vacuuming with hot water or steam; use of acceptable carcass interventions for reducing carcass contamination without prior agency approval. United States Department of Agriculture, Food Safety and Inspection Service. Accessed January 2020. <https://www.federalregister.gov/documents/1996/04/04/96-7938/notice-of-policy-change-achieving-the-zero-tolerance-performance-standard-for-beef-carcasses-by>
- Zhang, L., J. Y. Jeong, K. K. Janardhanan, E. T. Ryser, and I. Kang. 2011. Microbiological quality of water immersion-chilled and air-chilled broilers. *Poult. Sci.* 74:1531–1535.