

PROCESSING AND PRODUCTS

Effects of subzero saline chilling on broiler chilling efficiency, meat quality, and microbial safety

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ABSTRACT The poultry industry has attempted to improve carcass chilling efficiency, meat quality, and product safety. The purpose of this research was to investigate the effects of subzero saline chilling on carcass chilling, breast fillet tenderness, and microbial safety. After evisceration, broiler carcasses were chilled using ice slurry control (0% NaCl/0.5°C) or subzero saline solutions (3% NaCl/−1.8°C and 4% NaCl/−2.41°C). Broiler carcasses in the subzero saline solutions were chilled efficiently and reduced the chilling time by 11% in 3% NaCl/−1.8°C and 37% in 4% NaCl/−2.41°C over the ice slurry chilling. The breast fillets of broiler carcasses in 4% NaCl/−2.41°C were

significantly tenderized than those in water control ($P < 0.05$), with an intermediate value observed in 3% NaCl/−1.8°C. Before chilling, broiler carcasses possessed mesophilic aerobic bacteria, *Escherichia coli*, and total coliforms for 3.81, 0.78, and 1.86 log cfu/g, respectively, which were significantly reduced after chilling in 3% NaCl/−1.8°C or 4% NaCl/−2.41°C solution over the water control ($P < 0.05$), except the mesophilic aerobic bacteria. Based on these results, chilling of boiler carcass in 4% NaCl/−1.8°C solution appears to improve carcass chilling efficiency, meat tenderness, and bacterial reduction for *E. coli* and total coliforms.

Key words: subzero saline chilling, chilling efficiency, meat tenderness, broiler carcass quality

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INTRODUCTION

Rapid chilling of broiler carcasses is an important step to prevent meat toughness and microbial growth. When an animal is slaughtered and chilled, the carcass stiffens as rigor mortis develops, influencing the tenderness of fresh meats and the quality of processed products (Wakefield et al., 1989; Dunn et al., 1993a). After the onset of rigor mortis, rigor shortening (or heat shortening) occurs when the muscle is exposed to temperatures of 20°C or higher, whereas cold shortening occurs when the muscle temperature is reduced to 12°C or lower, with a minimal shortening observed between 15°C and 20°C (Locker and Hagyard, 1963; Marsh and Leet, 1966; Honikel et al., 1983). Bilgili et al. (1989) and Dunn et al. (1993b) found that rigor (or heat) shortening at warm temperatures negatively influenced broiler meat quality over cold shortening at cold

temperatures. As a result, it is important to minimize both rigor and cold shortening during carcass chilling as meat tenderness is one of the most important quality parameters (Savell et al., 1989).

In addition to meat quality, it is important to improve product safety as poultry consumption has increased tremendously. Per capita consumption of broiler in the United States increased from 23.6 lbs in 1960 to 92.4 lbs in 2018, with the processing of 9 billion chickens in 2019 (NAMI, 2019; NCC, 2019). During processing, poultry carcasses are subjected to various bacteria contamination especially in scalding, defeathering, evisceration, chilling, and further processing (Arnold, 2007; Vihavainen et al., 2007; Warsow et al., 2008; Luber, 2009). Trembley and Ames (2014) reported that the number of microorganisms on carcasses was significantly reduced when poultry carcasses were rapidly chilled using very cold air (−50°C to −120°C). In a constant temperature at 0.6°C in water immersion chill, however, no practical impact on bacterial reduction was observed when the amount of chilling water was simply increased from 2.1 to 16.8 L/kg or the rinsing time was extended from 1 to 4 min (Northcutt et al., 2006; Hannah et al., 2008).

Subzero saline chilling has the potential to improve chilling efficiency, meat tenderness, and bacterial reduction. Recently, our laboratory chilled broiler carcasses

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using various subzero saline solutions (Metheny et al., 2019). When broiler carcasses were submerged in high saline and subzero temperature solutions of 4% NaCl/ -2.41°C and 8% NaCl/ -5.08°C , the carcasses were chilled significantly faster than those in water immersion chilling (0% NaCl/ 0.5°C) with tenderness improvement. When broiler carcasses were chilled in control or subzero saline solutions (0% NaCl/ 0.5°C , 1% NaCl/ -0.6°C , 2% NaCl/ -1.2°C , and 3% NaCl/ -1.8°C), the breast fillets were stepwise tenderized as the salt content increased from 0 to 3% and the solution temperature decreased from 0.5°C to -1.8°C .

The purpose of this research was to investigate the effects of 1 water control solution (0% NaCl/ 0.5°C) and 2 subzero saline solutions (3% NaCl/ -1.8°C , 4% NaCl/ -2.41°C) on broiler carcasses for chilling efficiency, meat tenderness, and microbial safety.

MATERIALS AND METHODS

All procedures were approved by the Institutional Animal Care and Use Committee of California Polytechnic State University (Protocol #1908).

Brine Chilling Solution and Brine Ice Preparation

Three chilling solutions (0% NaCl/ 0.5°C , 3% NaCl/ -1.8°C , and 4% NaCl/ -2.41°C) were prepared with/without dissolving salt (NaCl) using tap water (w/w). Both the saline solutions (3 and 4% NaCl) were stored at -23°C to reach target temperatures of -1.8°C and -2.41°C , whereas the control solution (0% NaCl) was kept in a cooling room at 0.5°C . Samples of the 3 solutions were frozen in Ziploc bags and used for maintaining the target temperatures of the chilling solutions during carcass chilling.

Broiler Carcass Processing and Chilling

A total of 48 broilers (Ross 708, approximately 45 D of age) were obtained from the poultry unit and conventionally processed in the Meat Processing Center at California Polytechnic State University (Cal Poly). The broilers were then processed using the method described by Metheny et al. (2019) and Jeong et al. (2011). Eviscerated carcasses (4 carcasses/treatment for each of 4 replications) were tagged on wing, weighed (2.0 kg for an average weight), and chilled in 1 of the 3 chilling solutions (0% NaCl/ 0.5°C , 3% NaCl/ -1.8°C , and 4% NaCl/ -2.41°C). Before chilling, 1 medium carcass per chilling treatment was selected for monitoring the internal breast temperature every 5 min until the carcass temperature reached $\sim 4^{\circ}\text{C}$, using a digital thermometer logger (ThermaData Thermocouple Logger KTC, ThermoWorks, American Fork, UT). During chilling, control and brine ices were added to maintain the target solution temperatures. After chilling, carcasses were hung on a shackle for 5 min, weighed, and placed in a cooler (1.1°C) before deboning at 3 h postmortem.

pH, R-Value, and Sarcomere Length

Breast fillets were removed at 3 h postmortem, and left fillets were portioned into cranial and caudal pieces that were frozen in liquid nitrogen. Right breast fillets were stored in a cooler (2.2°C) for 24 h; pH and R-value of the cranial portion (2.5 g) were measured as per the method described by Sams and Janky (1986) and Thompson et al. (1987), respectively. Sarcomere length of the caudal pieces was evaluated for the status of muscle contraction, using a laser diffraction method (Cross et al., 1981). Shear forces of right breast fillets were measured by the razor blade method described by Cavitt et al. (2004), using a texture analyzer (TAHDI; Texture Technologies Corp., Scarsdale, NY) calibrated with a 25-kg load cell.

Cooking Yield and Shear Force

The breast fillets were cooked using the method of Metheny et al. (2019). Briefly, the fillets were placed on stainless steel racks in stainless trays, covered in foil, and cooked to an internal temperature of 76°C in a convection oven (36S-Y1A Wolf Challenger XL Range; ITW Food Equipment Group LLC, Glenview, IL), in accordance with USDA, Food Safety and Inspection Services (2001) guidelines. The cooking yield was then calculated using the formula: $(\text{postcook weight})/(\text{precook weight}) \times 100$.

Shear force was determined by the razor blade method of Cavitt et al. (2004), using a texture analyzer (TAHDI, Texture Technologies Corp.) calibrated with a 25-kg load cell. The razor blade (height, 24 mm; width, 8 mm) was set at 10 mm/s and penetrated to the depth of 22 mm as the test was triggered by a 10-g contact force. Shear values (N) were recorded as the maximum force was obtained during the shear. Two shear forces were recorded in the quarter portion.

Microbiological Analysis Before and after carcass chilling, 25 g of skin was aseptically taken from the breast area and placed in a sterile Whirl-Pak bag. Each sample bag received 225 mL of sterile PBS and was stomached for 1 min.

Mesophilic Aerobic Bacteria Serial 10-fold dilutions of the stomached samples were surface plated (0.1 mL) in duplicate on Petrifilm Aerobic Count Plates (3M Microbiology Products) to enumerate mesophilic aerobic bacteria after incubation at 37°C for 24 h.

Escherichia coli and Coliforms Serial 10-fold dilutions of the stomached 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.

Statistical Analysis

The experiment was conducted with 3 replications for raw meat qualities (pH, R-value, and sarcomere length) or 4 replications for shear force and microbial counts. Four birds were randomly distributed to each chilling

treatment. The data of microbial counts were converted to cfu per milliliter (cfu/mL). All data were statistically analyzed by one-way ANOVA using PASW 18 statistic program (SPSS, 2011). Mean differences among treatments were determined by Duncan's multiple range test at $P < 0.05$ in a post hoc analysis (SPSS, 2011).

RESULTS AND DISCUSSION

During chilling, the internal temperatures of eviscerated carcasses continuously reduced from $\sim 40^\circ\text{C}$ to 4.3°C – 4.5°C in 90, 80, and 55 min in 0% NaCl/ 0.5°C , 3% NaCl/ -1.8°C , and 4% NaCl/ -2.4°C solutions, respectively (Figure 1). Compared with the water control (0% NaCl/ 0.5°C), 3% NaCl/ -1.8°C and 4% NaCl/ -2.4°C chilling solutions reduced the chilling time by 11 and 37%, respectively, presumably owing to the lower solution temperature with higher salt content. Previously, Metheny et al. (2019) reported that the chilling time of broiler carcasses was reduced similarly when 3% NaCl/ -1.8°C solution was used over a water control.

During the time of carcass chilling, meat tenderness is largely affected by the degree of rigor shortening (or heat shortening) and cold shortening, which are likely to occur during the carcass temperature reduction from 40°C to 20°C and from 20°C to 4°C , respectively (Locker and Hagyard, 1963; Marsh and Leet, 1966; Honikel et al., 1983). In the control (0% NaCl/ 0.5°C) and subzero (4% NaCl/ -2.41°C) solutions, the carcass temperatures were reduced from 40°C to 20°C in 30 and 17 min, respectively, and from 20°C to 4.4°C in 60 and 38 min, respectively. As a result, the combined time for rigor and cold shortening might be 90 (30 + 60) min in the control solution and 55 (17 + 38) min in the saline solution, indicating that carcasses in subzero saline chilling were exposed to 17 min for rigor shortening over the 30 min of control carcasses and 38 min for cold shortening over the 60 min of control carcasses (Figure 1).

Shear force values were measured as means of meat tenderness, using cooked breast fillets from broiler carcasses in different chilling solutions. Breast fillets from the carcasses in 4% NaCl/ -2.4°C solution showed significantly lowered shear force (8.4 N) than those (12.64 N) of broilers in water control, with the intermediate (10.15 N) in 3% NaCl/ -1.8°C solution ($P < 0.05$) (Figure 2). These results are in accordance with the trend of the previous research, indicating that breast fillets were tenderized as the salt content increased and the solution temperature decreased from 0% NaCl/ 0.5°C to 8% NaCl/ -5.1°C (Metheny, 2018). These results of meat tenderness were supported by sarcomere lengths that were significantly longer in subzero saline solutions than those in water solution ($P < 0.05$) (Table 1). No significant difference was found for pH and R-value except the R-value in water control (Table 1).

When hot-boned beef sirloins were immediately chilled to -1.5°C in a propylene glycol water bath at -20°C , the muscle was tenderized more than the control

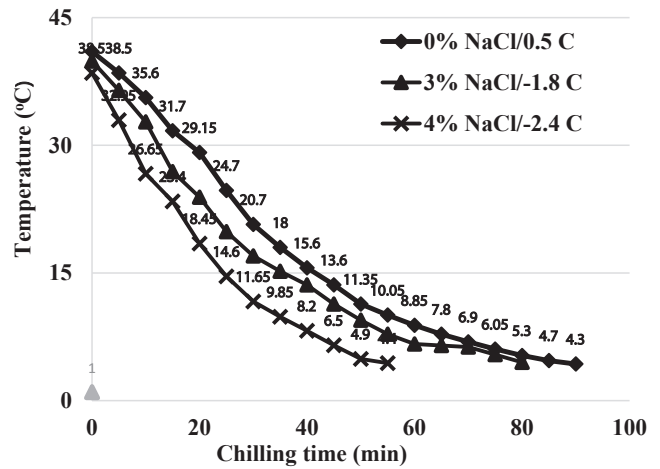


Figure 1. Temperature change profiles of broiler fillets during chilling in water and brine solutions. 0% NaCl/ 0.5°C , carcass chilling in ice slurry (0% NaCl) at 0.5°C ; 3% NaCl/ -1.8°C , carcass chilling in 3% NaCl at -1.8°C ; 4% NaCl/ -2.4°C , carcass chilling in 4% NaCl at -2.4°C .

chilling or delayed chilling, which was supported by the results of transmission electron microscopy for larger space between fibers (Roberts, 2005; Sikes et al., 2017). It is suggested that meat tenderization in subzero chilling is obtained owing to the expansion of water between fibers by osmotic pressure and ice formation at the freezing temperatures (Farouk et al., 2013). Regarding chilling time, Jacob et al. (2012) reported that subzero temperatures must be reached before the rigor development for accelerated tenderization. The mechanism for meat tenderization in subzero chilling was presumed because of the structural expansion between fibers rather than fiber fragmentation through super contractions or proteolytic degradation (Nakai et al., 1995; Hopkins et al., 2000; Sikes et al., 2017).

To minimize carcass contamination, poultry plants have used various chemicals such as chlorine, trisodium phosphate, ozone, and organic acids during carcass washing and chilling (Fabrizio et al., 2002; James et al., 2006; Møretro et al., 2012). For the last 20 to

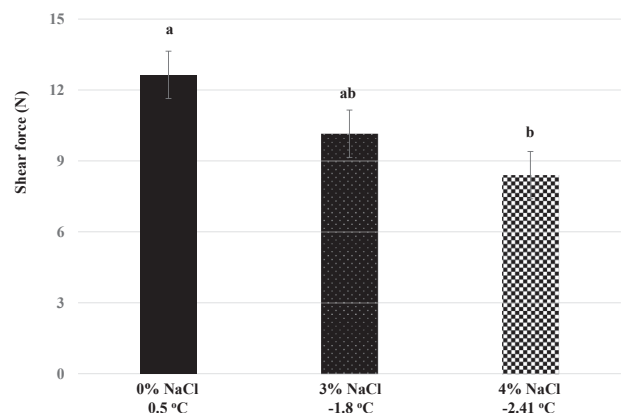


Figure 2. Effects of broiler chilling temperature and salt content on shear force (SF) of cooked broiler breast fillets. Chilling conditions as in Figure 1. Number of observation, $n = 16$. Bar, standard errors.

Table 1. Evaluation of pH, R-value, and sarcomere length after chilling carcasses in 3 different chilling solutions.

Parameter	After chilling		
	0% NaCl/0.5°C	3% NaCl/−1.8°C	4% NaCl/−2.4°C
pH	5.94 ^a (0.06) ¹	5.85 ^a (0.14)	5.90 ^a (0.13)
R-value	1.00 ^a (0.11)	1.15 ^a (0.17)	1.14 ^a (0.20)
Sarcomere length	1.57 ^b (0.15)	1.97 ^a (0.09)	2.02 ^a (0.07)

^{a,b}Means within a row with no common superscripts are different ($P < 0.05$).

Number of observation, n = 12.

¹SE, standard error.

30 yr, however, the incidence of human foodborne illness from *Salmonella* has been remained unchanged, and *Campylobacter* is one of the most commonly identified sources of bacterial foodborne illness in the United States (Scallan et al., 2011). Before chilling, the skin of broiler carcass naturally possessed mesophilic aerobic bacteria, *E. coli* (*E. coli*), and total coliforms for 3.81, 0.78, and 1.86 log cfu/g, respectively, which were significantly reduced when the carcasses were chilled in 3% NaCl/−1.8°C or 4% NaCl/−2.41°C over the water control ($P < 0.05$), except the mesophilic aerobic bacteria (Table 2). The combination of subzero temperatures and unfavorable condition of 4% NaCl might induce a synergistic effect and reduce the bacterial populations on carcasses in subzero saline solutions.

During the carcass chilling in subzero saline solutions, bacterial activity and their attachment to carcasses are expected to drop to a minimal level owing to less skin swelling in subzero temperatures. It has been reported that traditional chilling of broiler carcasses caused skin swelling because of water absorption, which aided the opening of deep crevices and channels on the skin surface for more bacterial attachment and penetration (Thomas and McMeekin, 1982; Singh et al., 2015; Singh et al., 2017).

Microbes can adapt to elevated salt levels by accumulating potassium, amino acids, or sugars to prevent a massive influx of sodium and outflow of water from the cells. However, this process requires energy, and thus, the growth of the microbes can be slowed down (Roberts, 2005; Wu et al., 2014). Other strategies that bacteria can use are increasing the activity of sodium efflux, change of cell morphology, and production of specific stress proteins, which require extra energy (Duche et al., 2002; Lado and Youserf, 2007). In a broth culture at pH 6/10°C, salt significantly increased the generation time of pathogens such as *E. coli*, *Salmonella*

spp., and *Clostridium botulinum* from 5 h at 0.5% salt to 20–27 h at 9.5% salt (Dole and Glass, 2010).

Most disease-causing bacteria do not grow well at 10% NaCl or water activity (**Aw**) of 0.94, although most mold and halophilic bacteria can grow under high salt and low Aw conditions. For example, *Staphylococcus aureus* can grow at 37°C/Aw 0.86 but only produces enterotoxin if Aw is at least 0.90 or higher (Baird-Parker, 1990). Salt addition may bring a synergistic effect in subzero chilling solutions so that it improves product safety and extends shelf life after chilling.

CONCLUSIONS

Improvement of processing efficiency, bacterial safety, and meat tenderness after poultry chilling is ideal for the poultry processor and poultry consumers. Chilling of broiler carcasses in subzero saline solution at 4% NaCl/−2.41°C markedly reduced carcass chilling time due to the subzero temperatures, bacterial contamination due to the unfavorable environment (chlorine at low temperatures), and meat toughness due to the reduction of hot (or rigor) shortening. Additional research is required to evaluate the overall processing cost savings and the potable/wastewater savings.

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Table 2. Mean population (log cfu/g) of mesophilic aerobic bacteria (MAB), total coliforms, and *Escherichia coli* on broiler skin after chilling.

Parameter	Before chilling		After chilling	
	None	0% NaCl/0.5°C	3% NaCl/−1.8°C	4% NaCl/−2.4°C
MAB	3.81 ^a (0.09) ¹	3.62 ^{a,b} (0.13)	3.49 ^{a,b} (0.14)	3.34 ^b (0.15)
Total coliforms	1.86 ^a (0.12)	1.34 ^b (0.15)	0.37 ^c (0.12)	0.63 ^c (0.13)
<i>E. coli</i>	0.78 ^a (0.20)	0.60 ^a (0.13)	0.02 ^c (0.02)	0.23 ^c (0.09)

^{a-c}Means within a row with no common superscripts are different ($P < 0.05$).

Number of observation, n = 16.

¹SE, standard error.

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