Investigating the Effects of Functional Ice (FICE) on Food Safety, Shelf-life and Quality of Poultry Thigh Meat During Refrigerated Storage

by

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Abstract

Food Safety and microbiological spoilage are two major concerns during the extended periods of refrigerated storage and transportation. Antimicrobial interventions applied during the poultry processing may not have a prolonged antimicrobial activity and may fail to control pathogen and spoilage microorganism growth during the refrigerated storage and transportation. Hence, “Functional Ice” (FICE), a novel antimicrobial intervention, is developed to facilitate a “Sustained Release Mechanism” besides the cooling effect of regular ice, thereby improving the food safety and shelf-life of poultry meat. Research was conducted to investigate the effects of FICE, an innovation over the traditionally used regular water on food safety, yield, shelf-life and quality parameters of poultry thigh meat during refrigerated storage. FICE treatments were prepared by freezing aqueous solution of sodium tripolyphosphate (STPP) (2.5% and 5% w/v) and sodium lactate-sodium diacetate (SL-SD) (1% and 2.5% v/v). Ice made from tap water was utilized as a control treatment. In the first part of the study, the different FICE formulations were tested for their antimicrobial efficacy against *Salmonella* Typhimurium inoculated on thigh meat (n = 375) over a refrigerated storage of 48 h. Results indicated that FICE made of 5% STPP and 2.5% SL-SD lead to a reduction in *Salmonella* by more than 1 log as compared to the regular ice treatment ($p \leq 0.05$) at the end of 48 h storage. Weight pick-up % (yield) was recorded for the uninoculated thigh samples stored in FICE for 48 h and data obtained indicated that FICE with 5% STPP and 2.5% SL-SD lead to an increased yield of 7-8% as compared to regular ice at the end of 48 h of storage. Another part of the study investigated the effects of 48 h FICE (5% STPP

and 2.5% SL-SD) and regular ice storage on the subsequent shelf-life and quality attributes (color, texture and cook loss) of tray-packed poultry thigh meat for 8 days. Results indicated that FICE treatments significantly suppressed ($p \leq 0.05$) the growth of spoilage microorganisms and extended the shelf-life of tray-packed poultry thigh meat by 1-2 days. Cook loss % of thighs stored in 5% STPP FICE was significantly lower ($p \leq 0.05$) than other treatments without affecting the color of poultry meat. These results indicated that FICE has the potential to improve the food safety and shelf-life of poultry meat while maintaining the yields during refrigerated storage.

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APC	Aerobic Plate Count
CFU	Colony-Forming Unit
TSP	Trisodium phosphate
STPP	Sodium tripolyphosphate
SL-SD	Sodium lactate and sodium diacetate
FICE	Functional Ice
PSY	Psychrotrophic
LAB	Lactic Acid Bacteria
WB	Warner- Bratzler
PBS	Phosphate Buffered Saline
BHI	Brain Heart Infusion

CHAPTER I.

LITERATURE REVIEW

Salmonella-related foodborne infections have resulted in 1.2 million illnesses, 23,000 hospitalizations and 450 deaths annually in the United States (CDC, 2018), leading to an economic loss of $2600 million/year (Taskila et al., 2012). Consumption of *Salmonella*-contaminated food, especially raw poultry meat can cause bloody diarrhea, inflammation of intestines, abdominal cramps, vomiting and fever while it can be fatal to immunocompromised individuals (Hafez, & Rüdiger, 2015). *Salmonella* is a gram-negative bacteria living in the animals during their growth, slaughtering and packaging operations and therefore, is associated with the pork, beef and poultry products (Mani-lópez et al., 2012). To eliminate the pathogen, the poultry carcasses are being subjected to several physical and antimicrobial interventions at various steps of poultry processing (Rouger et al., 2017). Proper refrigeration temperatures are maintained using transportation and storage to prevent the multiplication of pathogens in poultry meat (Bolder, 1997). The objective of this review is to outline the different strategies to inhibit *Salmonella* in the raw poultry meat and products at various stages of poultry processing and post-processing.

Steps of Poultry Processing

This section briefly describes the sequence of steps involved in primary poultry processing (Barbut, 2015). The poultry slaughter begins with hanging the live birds on the over-head shackles. Next, the birds are stunned using an electric current or a gas which makes the birds unconscious followed by bleeding of the bird. The birds then enter the Scalder which involves immersing the birds in a tank of water (50-61 °C) which facilitates an easier removal of feathers. De-feathering of the carcass is achieved by using the mechanical pickers with rubber

fingers. After the removal of feathers, oil gland from the tail area and the feet are removed using a knife or a blade and the birds are moved to another line manually. The next step is the evisceration which involves the opening of the body cavity and removal of birds' internal organs. Following this, the birds are washed and inspected. Following evisceration, carcasses are chilled to a temperature of 4 °C within 2-6 hrs. of slaughter. After chilling, the carcasses are weighed and deboned. The deboned meat can then be transported under refrigeration to further processors or tray-packed for retail purposes.

The major processing steps which lead to *Salmonella* spp. cross contamination of carcasses are scalding, picking, evisceration and chilling (Lillard, 1989; Yang, Li, & Slavik, 1998). These steps receive significant attention to improve food safety and reduce overall bacterial load on the carcasses during primary poultry processing. However, food safety and microbial-reduction strategies during storage and transportation are limited to product cooling using refrigeration and usage of dry ice. It is known that *Salmonella* survives cooling and freezing, making the storage and transportation steps vulnerable to food safety (Bailey, Lyon, Lyon, & Windham, 2000; Pradhan et al., 2012). Various in-plant interventions are being conducted by the poultry processors to reduce the population of pathogen during processing, but simple and advanced interventions are still needed to improve the microbial safety of poultry meat during packaging and extended periods of refrigerated storage and transport.

Antimicrobial Interventions to Control *Salmonella* During Poultry Processing

The poultry industry employs several antimicrobial interventions to reduce or eliminate *Salmonella* at multiple steps during poultry processing. Antimicrobials such as chlorine (Mead, Adams, & Parry, 1975; Lillard, 1980), ozone (Fabrizio et al., 2018), tri-sodium phosphate (Yang et al., 1998; Lillard, 1994), Cetylpyridinium chloride (Slavik et al., 1995), acetic acid (Dickens,

Lyon, Whittemore, & and Lyon, 1994; Hwang & Beuchat, 1994) and peracetic acid (Bauermeister, Bowers, Townsend, & McKee, 2008) and lactic acid (Izat et al., 1990) have demonstrated efficacy against *Salmonella* on poultry carcasses when applied during spray washing or chilling or post chilling .

Scalding is the first step where the carcasses are dipped in water and exhibits a washing effect which removes the debris and bacterial load on the carcasses (Lillard, 1990). The hard scalding method which utilizes the water temperature of 60-66 °C, is commonly followed in the poultry industry and is more effective in reducing the *Salmonella* counts on carcasses during processing as compared to the lower temperatures of soft scalding (Buhr et al., 2014). Besides the high temperature of scald water, the use of additives such as sodium hydroxide (McKee, Townsend, & Bilgili, 2008) aids in reducing the bacterial load on the carcasses by altering the pH of the scald water. It is believed that the alkaline pH due to sodium hydroxide facilitates the destruction of bacteria by impairing the enzyme functioning in the bacterial cells (Humphrey, 1981).

On-line spray washers installed at multiple locations along the processing line, such as before and after scalding, pre and post Inside-Outside (I/O) wash, pre-chill and post chill spray, help in achieving a higher bacterial reduction (Berrang and Bailey, 2009). Berrang & Bailey, (2009) reported a reduction in the prevalence of *Salmonella* on the carcasses from 80% to 24% due to the cumulative effect of the I/O spray washers, pre-chill and post-chill spray washing. A study conducted by Northcutt and others reported a reduction in the level of *Salmonella* by 0.7 to 1.1 log CFU/mL using an Inside-Outside spray washing for 5 s at a temperature of 54.4 °C and suggested that addition of chlorine to the spray water did not result in any additional bacterial reduction (Northcutt et al., 2005). Yanbin, Slavik, Walker, & Xiong, (1997) reported that a pre-chill carcass spray with 10% trisodium phosphate at 827 kPa for 90 s resulted in the reduction of

Salmonella Typhimurium by 3.8 logs whereas a 10% TSP spray for 30 s at 827 kPa resulted in 1.82 log reduction of *Salmonella,* optimizing the contact time and pressure for the spray treatment. However, such high pressure could result in the cross-contamination of carcasses with the spray water and may also result in the deeper penetration of bacteria in the skin (Brashears et al., 2001). Hwang & Beuchat (1994) reported that washing the chicken skin with 1% TSP solution for 30 min resulted in the reduction of *Salmonella* by more than 3.4 log CFU/cm^2. Furthermore, Zaki, Mohamed, & El-Sherif (2015) suggested that the antimicrobial efficiency of organic acids in reducing *Salmonella* inoculated on the chicken skin can be increased when used in combination with sodium dodecyl sulphate (SDS). The results of this study reported that washing the chicken skin with a combination of lactic acid and SDS (2:1) for 3 min resulted in the reduction of *Salmonella* by 7.43 log CFU/cm^2 whereas treatment with lactic acid resulted in a reduction of 3.36 log CFU/cm^2. It is believed that SDS aids in the denaturation of proteins and dissolve fats of the skin follicles facilitating the further penetration of organic acids, resulting in the higher *Salmonella* reduction rates (Bales, Messina, Vidal, Peric, & Nascimento, 1998; Zaki et al., 2015).

The broiler carcasses undergo chilling to cool the carcasses after evisceration and bring down the temperature of carcasses to < 4°C as per the USDA standards (Barbut, 2015). The low temperature of carcasses can be attained using a pre-chiller and an immersion primary chiller in the poultry processing plant (Wideman, 2013). Several antimicrobials used in poultry chillers as chilling step involves the longest contact time of carcasses with the water or the antimicrobial solution. Immersion chilling with added antimicrobials is the most common method followed in the poultry industry to chill the carcasses and aid in the bacterial reduction due to the chill temperature and added antimicrobials (Chen, 2013).

Chorine was one of the most widely used antimicrobial in the poultry chillers and throughout the poultry processing plant (McKee, 2012). However, washing of the carcasses increases the organic load in the chiller as well as increases the pH thus reducing the efficiency of chlorine as a decontaminant (Byrd and McKee, 2005). Chlorine when added to water, hydrolyzes to form hypochlorous acid at a pH of 6.0, which is the active antimicrobial component of chlorine. However, higher pH results in the formation of hypochlorite ion which has lower efficacy as an antimicrobial (Odlaug, 1981).

Peroxyacetic acid (PAA) has emerged as the most commonly used processing aid to decrease *Salmonella* in poultry processing plant (McKee, 2012). Unlike chlorine, the presence of high organic loads does not affect the efficacy of PAA (Kitis, 2004). Bauermeister et al., 2008 reported that chilling the carcasses in 85 ppm mixture of peracetic acid mixture (15% peracetic acid + 10% Hydrogen peroxide) for 20 min decreased the prevalence of *Salmonella* by about 92% whereas 30 ppm of chlorine caused a reduction in the prevalence of *Salmonella* by 57%. Another study reported chilling carcasses in chill water containing 25 ppm of PAA in poultry chiller for 2 h resulted in 0.85 log higher reduction in *Salmonella* counts as compared to 30 ppm chlorine in the chiller water (Bauermeister, 2015). The lower efficacy of chlorine has been attributed to the higher pH and presence of organic matter in the chiller water. In addition to the immersion chillers, post-chill dip tanks are used to further lower the count of the pathogen when applied with other interventions in the processing plant (Russell, 2010). Peracetic acid has also been tested for its anti-*Salmonella* efficacy in post-chill dip tanks. Nagel, Bauermeister, Bratcher, Singh, & McKee (2013) reported that post-chill immersion of carcasses in 0.1% PAA (1000 ppm) resulted in *Salmonella* reduction on inoculated carcasses by 2.14 log CFU/ml in comparison to less than a log reduction attained when 40 ppm chlorine was utilized in the post-

chill dip tank. The antimicrobial activity of peracetic acid is assumed to be due to the oxidization of sulfhydryl and sulfur bonds present in the proteins, thereby disrupting the movement of ions across the cell membrane made of lipoproteins or rupturing the cell wall of bacteria (Kitis, 2004; Maris, 1995).

Trisodium phosphate (TSP) has also been investigated as an antimicrobial during poultry processing. Lillard (1994) showed that dipping the inoculated carcasses (10^8 CFU/carcass) in 10% solution of trisodium phosphate reduced the population of *Salmonella* by about 8 logs while a water dip resulted in about 0.30 log reduction in the pathogen count. The results proposed that the antibacterial efficacy of TSP could be due to the high pH (12.1) of the solution which results in lethal or sub-lethal injury to Salmonellae cells attached on the carcasses. Rodriguez de Ledesma, Riemann, & Farver (1996) reported that dipping the chicken wings in a 10% solution of TSP for 15 s reduced the *Salmonella* Typhimurium counts by 93.45%. Baysal & Ünlütürk, (2005) showed that the count of *Salmonella* on turkey breasts 2 h. after a 1 min dip in solution of 10% TSP and 10% Sodium tripolyphosphate (STPP) were 3.5 log CFU/fillet and 3.2 log CFU/fillet lower than that of the untreated control samples.

Anang, Rusul, Bakar, & Ling (2007) showed that dipping the chicken breast in a 2% lactic acid solution for 30 min resulted in a reduction of 1.71 log CFU/ml of *Salmonella* Enteritidis. The antibacterial action of lactic acid is believed to be due to the decrease in the intracellular pH of the cells causing the dissociation of the acids that acidify the cytoplasm of the cell in response to which much of the cells energy is utilized in maintaining a constant pH. Thus the growth of bacterial cells decrease due to insufficient energy (Shelef, 1994). Salts of organic acids such as lactates and acetates have been studied for their antimicrobial action mostly in ready-to-eat meats. The antimicrobial activity of a combination of sodium lactate and sodium

diacetate was studied in beef bologna by Mbandi & Shelef (2002). The results of the study indicated that the combination of 2.5% sodium lactate and 0.2% sodium diacetate (by weight) was most effective in rapidly declining the population of *Salmonella* in the inoculated bologna samples stored at 5 °C and 10 °C as compared to individual inclusion salts in the meat. While, the above-mentioned approaches involve spray or dip application of various antimicrobial solutions, the antimicrobial treatment can also be applied to processed chicken parts in the form of ice. Richter (2018) studied the antimicrobial efficacy of ice slurry composed of fresh water, salt and PAA against *Salmonella* Typhimurium inoculated on chicken wings and reported that immersion chilling of wings in ice slurry for 20 min reduced the bacterial count by 1.32 log CFU/ mL.

Even after utilizing various antimicrobial interventions during the processing of chicken, the processed carcasses might get contaminated with *Salmonella* during storage, packaging and transportation. During the transportation of processed poultry, the processed chicken might be subjected to temperature abuse, improper handling conditions or contamination from the environment which might result in an unsafe and low-quality product (USDA-FSIS, 2003). Therefore, dry ice i.e. solid CO_2 is commonly used during the transportation chicken to maintain low temperatures during transportation. Fratamico et al., (2012) investigated the antimicrobial efficacy of ozonated dry ice i.e. ALIGAL BLUE ICE (ABI) against *Salmonella* Typhimurium in the liquid released from chicken meat during the storage and transportation. The results of the study indicated that ozonated ice decreased the *Salmonella* count 1.8 log CFU/mL whereas dry ice storage resulted in about 1 log reduction in the bacterial count. The researchers reported that the higher efficacy of ABI is due to the enhanced cooling capacity of dry ice in combination with the antimicrobial activity of ozone incorporated into dry ice (Jeyasekaran, Ganesan, Shakila,

Maheswari, & Sukumar, 2004; Kim, Yousef, & Dave, 1999). Another antimicrobial ice formulation containing chlorine dioxide (ClO_2) was examined for its effect against *Salmonella* Typhimurium on the mackerel skin (Shin et al., 2004). The study showed that the storage of fish skin in antimicrobial ice containing 100 ppm of ClO_2 for 120 min resulted in a total reduction of 2.6 log CFU/cm^2 in the population of *Salmonella* Typhimurium on the fish skin whereas the storage in control ice after 120 min maintained a higher bacterial count of 5.2 log CFU/cm^2 . The researchers suggested that storage in antimicrobial ice containing ClO_2 exhibited a sustained release of ClO_2 on the surface of skin as the ice melted over the period of time (Shin et al., 2004).

While organic acids and trisodium phosphate have been studied extensively for their antimicrobial action against *Salmonella* during the processing, a lot of research is still needed to be done to explore the action of salts of organic acid and tripolyphosphates against the bacteria during storage and transportation.

Interventions to Improve the Quality and Shelf-life of Poultry Meat

Poultry meat makes a significant part of the present-day diets due to its relatively low cost of production, low fat content and the high nutritional value of poultry meat. Therefore, maintaining the microbiological quality of poultry is of utmost importance to the poultry producers. As poultry meat is a perishable commodity, it is always susceptible to deterioration or spoilage of meat even at refrigeration temperature (Mantilla et al., 2011). Higher levels of microorganisms on raw meat results in undesirable and unappealing surface changes making it objectionable for human consumption (Gram et al., 2002). Spoilage of poultry meat depends on various factors such as initial microbial level, physiological status of the chicken at the time of slaughter, contamination in the processing plant, temperature and storage conditions (Nychas et al., 2008). For fresh meat distribution and consumption, it is extremely important to monitor the

time/temperature conditions. To ensure both safety and overall meat quality, the vehicles for the transportation of meat must be equipped with a good refrigeration system. However, there are always chances of failure of refrigeration equipment, which would lead to the spoilage of the meat products (Gebresenbet and Bosona, 2012). The different storage conditions also influence the type of spoilage organisms. For instance, when chicken is stored in limited oxygen conditions or in the absence of oxygen, facultative anaerobes or anaerobic gram-positive microbiota dominate whereas when stored under aerobic conditions i.e. high oxygen conditions, aerobic or facultative anaerobic gram-negative bacteria will predominate (Doulgeraki et al., 2012). The metabolic activities of microorganisms responsible for spoilage comprise the primary spoilage mechanism and results in the production of off-odors (Dainty, 1996). Temperature of storage of the meat product determines its microbial spoilage by affecting the lag phase duration, maximum specific growth rate and final microbial count (Mataragas et al., 2006). Also, the freshly processed poultry will predominantly have the mesophilic bacteria which grow at an optimum temperature of 35 °C. During the storage of poultry meat at refrigeration temperatures, Psychrotrophic bacteria predominates the microbial population (Smaoui et al., 2011). Different storage temperature conditions influence the growth of different genera of microorganisms. For example, psychrotrophic bacteria belonging to gram positive genera such as Lactic Acid Bacteria (LAB) and gram-negative bacteria such as *Pseudomonas* spp. and *Enterobacteriaceae* prefer chilled temperature conditions to grow (Newton and Gill, 1978). *Pseudomonas* spp. primarily results in spoilage due to the formation of slime and malodorous sulfides, esters, acids and amines in meat stored at refrigerated conditions (Ercolini et al., 2007). Also, pseudomonads prefer aerobic atmosphere for their growth whereas the spoilage of vacuum packed is mainly caused by psychrotrophic LAB forming lactic acid and volatile fatty acids as spoilage end

products which impart dairy or cheesy odors to the vacuum-packaged meat (Borch, Kant-Muermans, & Blixt, 1996; Pothakos, Devlieghere, Villani, Björkroth, & Ercolini, 2015). Production of off-odors, off-flavors and formation of slime in meat make the product unpalatable and unacceptable to consumers. Therefore, upgrading the keeping quality, lowering or destroying the spoilage causing microorganism of chicken is one of the principal objectives of the poultry producers and food microbiologists.

Several decontamination treatments such as physical or chemical or a combination of both has been studied by researchers to determine the efficacy of each treatment in reducing the spoilage microflora in chicken. In chemical decontamination treatments, antimicrobial activity of various food-grade chemicals, antimicrobial films, natural food preservatives have been evaluated in poultry meat. The shelf life of chicken legs was evaluated using several chemical compounds such as trisodium phosphate, acidified sodium chlorite, citric acid and peroxyacids and the results reported that TSP resulted in the greatest reduction of mesophilic, pyshcrotrophic and Lactic Acid Bacteria during the 5-day storage period (Del Río et al., 2005). In addition to the observed antimicrobial activity of TSP, appearance of darker, brownish color was reported in the TSP treated legs. Okolocha & Ellerbroek (2005) documented that dipping the chicken carcasses in 10% TSP for 6 s resulted in about 0.9 log reduction in *Lactobacillus* after 0-6 days of storage at 4 °C. Vareltzis, Soultos, Koidis, Ambrosiadis, & Genigeorgis (1997) found that dipping the chicken carcasses in 5% STPP (w/v) for 10 min effectively reduces the aerobic plate count on the carcasses. The lower APC in the samples treated with STPP could be attributed to the slight elongation in the lag phase of spoilage microorganisms (Molins et al., 1985). It is also believed that STPP facilitates the sequestration of metallic ions in the cell wall which prevents the cell

wall division, thus suppressing the growth of microorganisms (Firstenberg-eden, Rowley, & Shattuck, 1981; Tompkin, 1983)

In a research study conducted to compare the efficacy of different phosphates such as monopotassium phosphate (MKP), monosodium phosphate (MSP), sodium pyrophosphate (SPP) and TSP, it was observed that 5% TSP exhibited the greatest antimicrobial activity and extended the shelf life of chicken legs to 12 days (Kim & Marshall, 1999). In another study performed to evaluate the effectiveness of TSP on *Pseudomonas fluorescens* inoculated on chicken legs, it was reported that TSP successfully reduced the level of bacteria up to 0.9 logs after 5 days of refrigerated storage (Del Río et al., 2005). Very limited documentation is available which compares the antimicrobial action of sodium tripolyphosphates and trisodium phosphate. Elliott et al. (1964) also studied the effect of slush ice made of polyphosphates on the inhibition of *Pseudomonads* and reported that slush ice containing 3% polyphosphates extended the shelf life of chicken by two days. The study also observed that the ice containing 8% polyphosphate caused a 60% extension of shelf life of chicken fryers. It is believed that polyphosphates results in sequestrations of Mg^{2+} which leads to the inhibition of cell wall division, hence, loss of cell wall integrity (Lee et al., 1994a) . Polyphosphates also cause an increase in the lag phase of affected microorganisms (Elliott et al., 1964). The effect of sodium lactate on the shelf life of low-fat Chinese style sausage was evaluated by Lin & Lin (2002). The results reported that 3% sodium lactate resulted in comparatively lower APC throughout the refrigerated storage period. Sodium lactate at a concentration of 3% also had lower LAB and Psychrotrophic counts as compared to the TSP treatment. Lin and Lin suggested that sodium lactate act as a bacteriostatic agent which extend the lag phase of bacteria whereas TSP act as a surface antimicrobial agent. Steinhauer & Banwart (1963) observed that chicken carcasses chilled in 8% STPP solution and

8% commercial blend solution of tripolyphosphates resulted in lower average bacterial counts as compared to usual chilling in ice and water after several days of refrigerated storage. Combinations of 1.8% sodium lactate (SL) with 0.25% sodium diacetate (SD) have also been effective in reducing Lactic Acid Bacteria in pork bologna stored at 4 °C (Barmpalia et al., 2005). Akkara & Kayaardi (2013) determined the effect of dry ice blasting technique on the microbiological quality of chicken carcasses and stated that the chicken carcasses sprayed with and immersed in dry ice showed a significantly lower numbers of total mesophilic aerobic count as compared to the untreated samples. Oral, Gulmez, Vatansever, & Guven (2008) studied the effect of antimicrobial ice made from wild-thyme hydrosol on the shelf life of fish (*Capoeta capoeta capoeta*; Guldenstaedt, 1772). They found that the APC level of antimicrobial ice treated fish samples was less than 6 log CFU/g on day 20 of the refrigerated storage whereas the fish stored on ice made from tap water reached a count of 7.59 log CFU/g. Also, there were significant differences in the Psychrotrophic count between the treated and untreated samples on day 15 and 20 of storage. Thus, the results of this study proved that antimicrobial ice cubes delayed the spoilage in fish. Fratamico et al. (Fratamico et al., 2012) also studied the application of ABI and dry ice for extension of shelf life of pork riblets. The study showed that the APC count of ABI treated pork riblets was much lower than dry ice treated samples from day 8 until day 15 of refrigerated storage and lower Psychrotrophic count in ABI treated samples from day 8 until day 11. Smaoui et al., (2011) investigated the effect of sodium lactate and lactic acid on the shelf life of chicken thighs and found that 3% sodium lactate significantly increased the shelf life of marinated chicken by 3 days.

When buying chicken, appearance is the first factor which determines the choice of selection of poultry products for the consumers. Besides the appearance or color of the chicken,

the other factors which are of producers' concern and which might interest the consumes are drip loss, tenderness, juiciness, cook loss, pH etc. of the chicken (Allen et al., 1998). The color of poultry meat can be determined in terms of color reflectance which can be further measured using a colorimeter (Fletcher, 2002). The most common color scales to measure the reflectance is the Hunter Lab. The 'L' value depicts how lighter or darker the product is i.e. a value of 100 denotes pure white and a value of 0 denotes pure black. The 'a' value signifies the redness whereas the 'b' value symbolizes the yellowness of the product (Montgomery, 2007). The effect of different antimicrobial interventions on the color of poultry has been evaluated by various researchers. Bauermeister, Bowers, Townsend, & McKee, (2008) reported that chilling the carcasses in 0.02% PAA resulted in lighter color of carcasses, however, no negative impact on the appearance, flavor, texture and juiciness of breast fillets was observed. Young & Lyon (1997) studied the influence of sodium tripolyphosphate on color of cooked poultry meat and reported that STPP treatment in combination with NaCl did not significantly affect the L* and b* values but significantly reduced the a* values. Dickens et al., (1994) also determined the effect of acetic acid dip on the appearance of carcass (although, the means of evaluation was subjective) and observed that the carcasses treated with 0.6% acetic acid were darker in appearance as compared to the prechill water treated carcasses. Kim & Marshall (1999) also investigated the influence of 10% TSP dip treatment on the Hunter Lab values of chicken legs. Papadopoulos, Miller, Acuff, Vanderzant, & Cross (1991) showed that sodium lactate at higher concentrations tended to decrease the lightness and yellowness i.e. L* and b* values whereas, there was barely any effect on the a* value beyond the concentration of 1% of sodium lactate. Given the fact, that various processing aids and antimicrobials affect the appearance (color) of the meat, the type and concentration of the antimicrobial to be utilized during the processing

should be chosen accordingly in such a way that it does not compromise with the sensory attributes of the product.

The various processing aids also have an impact on the cooking quality and texture of the meat product. Froning (1965) reported that dipping poultry carcasses in 6% solution of sodium tripolyphosphate has lower cooking losses as compared to the ones dipped in water. The probable reason behind the lower cooking losses is the increased moisture retention in the chicken meat treated with STPP. Similar results were also obtained when a combination of 0.5% STPP and 1.5% NaCl was used in chicken patties (Young et al., 1987). Sen, Naveena, Muthukumar, Babji, & Murthy, (2005) reported lower cooking losses in the breast meat samples injected with 3% sodium bicarbonate and 3% tetrasodium pyrophosphate for 24 h. This is due to the potential impact of bicarbonates and phosphates on the moisture retention property of the meat, as the ions of bicarbonates and phosphates interact with the protein of chicken breast and enhance the hydration (Sen et al., 2005). Wynveen et al.,(2001) further explain the phosphate-protein interaction due to which the metal linkages between the proteins breaks and aids in the entry of water into the structure of muscle, hence increasing the moisture retention. The effect of sodium lactate (NaL) on the cooking yield of tray-packed broiler breast meat was studied by Williams & Phillips (1998). The study showed that (NaL) at a pH of 7.30 exhibited highest cooking yield followed by NaL at pH of 5.00, and further suggested that the lower cooking yield of NaL at pH 5.00 might be due to the moderate denaturation of surface proteins that were in the immediate vicinity of sodium lactate. Protein denaturation leads to decreased water holding capacity of proteins and eventually, lower cooking yields. Another quality attribute of meat is the texture or the tenderness of the cooked meat product. Texture of the cooked meat product, in general, is the force required to cut through the muscle fibers of the meat during chewing.

Warner-Bratzler (WB) shear analysis is the most commonly used method to determine the tenderness (texture) in the meat. Warner-Bratzler shear test measures the force required to shear a piece of meat (Ruiz De Huidobro et al., 2005). The test utilizes a V shaped blade attachment, where cut is in the lower edge, of varying thickness. The meat sample to be analyzed is placed under the V cut and the texture analyzer is run to allow the blade to cut through the meat piece. The texture analyzer measures the resistance of the meat sample to the shearing by the blade which is recorded in terms of the shear force. To improve the textural properties of the chicken meat, different food additives are being added to the meat, the most common ones being the phosphates (Zheng et al., 2001). A study was conducted to investigate the effects of injecting NaCl, STPP and sodium lactate on the WB shear force and the results indicated that there was no significant difference in the shear force values of injected top and inside round steaks when compared with the untreated control samples, though the shear force values of non-injected samples were higher than the injected ones (McGee et al., 2003). Young & Lyon (1997) showed that marinating the chicken breast with a 4% solution of STPP with a post chill ageing time of at least 2 h decreased the Warner Bratzler shear force values significantly resulting in more tender breast fillets. Zheng et al., (2001) further reported that injecting STPP in chicken breasts also decreased the shear force values, implying that STPP treated chicken is more tender as compared to other salts and untreated samples. Increased tenderness in STPP treated chicken can be attributed to the higher cooking yields and moisture retention ability of meat treated with STPP (Smith and Young, 2007). Alvarado & McKee, (2007) stated that salts like STPP increases the tenderness of meat by increasing the water holding capacity (WHC). Salts results in unfolding the myofibrillar proteins of meat, actin, myosin and actomyosin due to the electrostatic repulsions which aids in accommodating greater number of water molecules between the muscle

fibres of meat, thereby, increasing the WHC of meat, and eventually tenderness of meat.

The effect of food grade antimicrobial ingredients such as sodium tripolyphosphate and sodium lactate-diacetate on the quality of poultry meat have been determined in the previous studies. However, the use of these ingredients based on their multifunctionality in the meat has not been determined as components of ice used during the refrigerated storage of meat. Therefore, the potential of these food grade ingredients in the form of ice can be explored to improve the safety, shelf life and quality of poultry meat during the refrigerated storage which could eventually reduce foodborne illnesses and food wastage, hence, improving food security.

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CHAPTER II.

INVESTIGATING THE EFFECT OF FUNCTIONAL ICE (FICE) ON FOOD SAFETY, SHELF-LIFE AND QUALITY OF RAW POULTRY THIGH MEAT DURING REFRIGERATED STORAGE

Abstract

In meat processing, antimicrobial interventions applied during slaughter and deboning operations may not have a prolonged antimicrobial efficacy and may fail to control pathogen and spoilage microorganism growth during transportation and storage. “Functional Ice” (FICE), an innovation over traditional ice, was investigated for its effects on food safety, shelf life, and quality of raw poultry thigh meat during refrigerated storage. FICE was prepared by freezing aqueous solutions of sodium tripolyphosphate (STPP) (2.5% and 5% w/v) and sodium lactate-sodium diacetate (SL-SD) (1% and 2.5% v/v). Potable water was used to prepare ice for the control treatment. Thigh meat inoculated with *Salmonella* Typhimurium (10^8 CFU/sample) was placed in FICE treatments, stored at 4 °C and sampled at 0, 12, 24, 36 and 48 h (n = 375). Weight pick-up was recorded for the uninoculated thighs. Additionally, shelf life and quality were evaluated for 8 days on tray-packed thighs that were stored in FICE treatments for 48 h (STPP 5% and SL-SD 2.5%). Differences among treatments were determined using ANOVA with LSMeans ($p \leq 0.05$). Results indicated that inoculated thighs stored in STPP 5% and SL-SD 2.5% lead to a >1 log reduction in *Salmonella* Typhimurium compared to the control ($p \leq 0.05$) after 48 h of storage. FICE treated thighs showed higher yields, lower cook loss, and an extended shelf life of 1-2 days, without any color changes. FICE has the potential to improve food safety and shelf life while improving the yields and quality during storage and transportation of raw poultry meat.

Introduction

Salmonella is a major foodborne pathogen commonly associated with raw poultry and poultry products causing 1.2 million illnesses, 23,000 hospitalizations and 450 deaths, annually in the United States (CDC, 2018). To reduce the prevalence of *Salmonella*, poultry processors apply antimicrobial interventions at various steps of poultry processing (Rouger et al., 2017). However, refrigeration is the only major intervention during storage and transportation to improve food safety. However, *Salmonella* can survive at refrigeration temperature on raw chicken meat (Dominguez & Schaffner, 2009; Pintar et al., 2007) and further temperature abuse can initiate pathogen growth leading to food safety concerns (Palumbo, 1986). Additionally, spoilage-causing psychrotrophic microorganisms can grow and spoil meat under refrigerated storage and transportation leading to food waste and food loss (Lambert, Smith, & Dodds, 1991; Rouger et al., 2017). There is a need to develop intervention strategies to improve food safety and reduce spoilage during storage and transportation.

Ice is a common refrigeration medium utilized during storage and transportation of foods as it reduces the product temperature, resulting in a reduced rate of microbiological and biochemical degradation (Graham et al., 1992). In addition to ice, commercial poultry processors use dry ice for the packaging and shipment of raw poultry meat (Fratamico et al., 2012). Dry ice provides a greater cooling effect (-78.5 °C) than the regular ice (0-1 °C), thereby exhibiting a bacteriostatic effect on the microorganisms on the fresh meat (Jeyasekaran et al., 2004). Fratamico et al. (2012) developed another antimicrobial intervention, ALIGAL Blue Ice (ABI) for the packaging and transportation of food. ABI is an ozonated dry ice which incorporates both the enhanced cooling capacity of dry ice and antimicrobial action of ozone to suppress the growth of pathogens and improve shelf life (Fratamico et al., 2012). However, dry ice, either

used alone or in combination with ozone, has some potential hazards such as chances of explosion, suffocation, and contact hazards during transportation (Bragg, 2003). It requires great handling skills, which might limit its use. In addition, dry ice does not facilitate the improvement of meat quality parameters that are important to processors and consumers, such as yield of raw meat, color, texture, and cooking yields during the storage and transportation.

Another intervention, peracetic acid ice also known as frozen biocidal-active ice was invented to be used for the storage and transportation of poultry products commercially to inhibit the growth of spoilage and pathogenic microorganisms (Harvey and Howarth, 2007). Despite, the reported antimicrobial effect of peracetic acid, several occupational hazards have been reported due to the high reactivity of peracetic acid (Pechacek et al., 2015). There is limited published literature on the effect of peracetic acid ice on the microbial growth and quality characteristics of poultry meat during storage and transportation which restricts its application on a commercial scale.

Hence, there is a need for the development of a simple, feasible and safe antimicrobial ice which could effectively control the spoilage and pathogenic organisms while preserving the quality aspects of poultry meat during storage and transportation. An innovative product known as “Functional Ice” (FICE) was developed by freezing food-grade ingredient solutions. Functional ice acts as a “Sustained Release Mechanism” for the ingredients as FICE melts, it would ideally serve the following functions: 1. improve food safety by actively eliminating foodborne pathogens; 2. increase shelf life by actively eliminating spoilage microorganisms; 3. provide lower cooling temperatures thus reducing food safety and spoilage risks due to temperature abuse during storage and transportation; and 4. maintain quality and yield as they are highly important for the processors and consumers.

The ingredients selected for the study were sodium tripolyphosphate (STPP) and sodium lactate + diacetate (SL-SD) based on their multi-functional properties of eliminating microorganisms, while, at the same time maintaining meat quality and yield. These two ingredients are generally recognized as safe (GRAS) and are commonly used in meat and poultry products. (USDA-FSIS, 2000). The antibacterial mechanism of STPP has been reported to be due to the sequestration of metallic ions in the cell wall resulting in the loss of cell wall integrity, thereby inhibiting the growth of microorganisms (Lee, Hartman, Stahr, Olson, & Williams, 1994; Elliott, Straka, & Garibaldi, 1964). The organic acid salts, sodium lactate, and diacetate are believed to delay the growth of microorganisms by extending the lag phase; (Zeitoun & Debevere, 1992; Williams, Rodrick, & West, 1995). Food grade sodium tripolyphosphate functions as follows: 1. exhibits antimicrobial activity; 2. increase the water holding capacity of meat; 3. reduces the cook loss %; 4. increases the yield; and 5. improve the textural properties of meat (Alvarado & McKee, 2007; Smith & Young, 2004). Similarly, sodium lactate and sodium diacetate have been reported to give higher cooking yields and improved textural properties of meat (Williams & Phillips, 1998; Papadopoulos, Miller, Acuff, Vanderzant, & Cross, 1991). These ingredients have demonstrated antimicrobial and other functional properties in poultry meat, but there is limited research on their use in the form of ice. Therefore, the objective of the current research was to develop FICE using food grade ingredients and to evaluate the antimicrobial efficacy of different FICE formulations compared to traditional ice made with water in suppressing the growth of *Salmonella* Typhimurium on inoculated poultry meat and to investigate its effects on the yield, shelf life, and quality parameters of raw poultry meat under refrigerated storage.

Materials and Methods

Experiment 1: *Salmonella* Survival Study

Preparation of FICE

All the FICE treatments were prepared in the research kitchen at the Department of Poultry Science, Auburn University. The four FICE treatments were (1) STPP 2.5% (w/v) (Brifisol®, ICL Food Specialties, St. Louis, MO, U.S.A. (2) STPP 5% (w/v) (Brifisol) (3) Sodium lactate and sodium diacetate (SL-SD) 1% (v/v) (Opti. SD4, Corbion Purac, NE, U.S.A.) (4) Sodium lactate and sodium diacetate (SL-SD) 2.5% (v/v) (Corbion). Ice made from potable water served as a control treatment. FICE solutions were prepared by completely dissolving the ingredients in individual containers of potable water using a hand blender (Bella Immersion Blender, #HB1908KB-ET). The pH of all the treatments was recorded using a pH meter (Hach, Model No. H170G, Loveland, CO, U.S.A.). The pH of the solutions were 9.04, 9.11, 6.0, 5.94 and 6.04 for the STPP 2.5%, STPP 5%, SL-SD 1%, SL-SD 2.5% treatments and control, respectively. FICE solutions were poured into the ice cube trays (Sterilite, 29.97 × 12.06 × 4.32 cm) and frozen in a walk-in freezer (-20 °C) for 24-48 h. Frozen FICE cubes of each treatment were taken out of the ice trays and stored in the individual plastic bags in the freezer (-20 °C) until further use.

Preparation of Inoculum

A nalidixic acid resistant strain of *Salmonella* Typhimurium (isolated from the Auburn University Poultry Research Farm and selected for resistance to Nalidixic acid) was cultured in Brain-Heart Infusion broth (BHI; Acumedia Manufacturers, Lansing, MI, U.S.A.) for 24 h at 37 °C. Further, a loopful of culture was streaked on Xylose-Lysine-Tergitol 4 agar (XLT4; Acumedia Manufacturers, Lansing, MI, U.S.A.) containing 35µL/mL of Nalidixic acid (Sigma-

Aldrich, St. Louis, MO, U.S.A.) and incubated at 37 °C for 24 h. Typical, isolated *S.* Typhimurium colonies were inoculated in fresh BHI broth (with nalidixic acid 35 µL/mL) and incubated for 20-24 h at 37 °C. One mL of the *Salmonella* culture was sub-inoculated in 99 mL BHI media flasks and incubated for 12 h. After 12 h, the cultures were centrifuged at 8000 rpm for 10 min, the supernatant was decanted and the pellet was resuspended with 1% phosphate buffered saline, Fisher Scientific, Fair Lawn, NJ, U.S.A.). The centrifugation steps were repeated two times and the final pellet suspension in PBS (10^9 CFU/mL) was used to inoculate chicken thighs.

Inoculation of Thighs with Salmonella

Raw, boneless, skinless chicken thighs were obtained from a local commercial poultry processing facility and maintained at 4 °C prior to inoculation. For each replication, individual thighs were inoculated with 10 µL of the stock culture of *Salmonella* Typhimurium (10^9 CFU/mL). The inoculum was evenly spread on the surface of thighs with a sterile spreader and placed in the aluminum pans, covered and placed in the refrigerator (4 °C for 60 min) to allow bacterial attachment on the surface of thighs.

Ice Treatment

Inoculated thighs were placed in the ice coolers (Igloo 48-Qt Island Breeze Cooler; 64.92 × 35.71 × 35.86 cm) containing different ice treatments. The FICE:Meat ratio was maintained at 2:1 w/w with alternate layers of FICE and meat. The coolers were placed in a walk-in refrigerator maintained at 4 °C.

Microbiological Analysis

Samples were analyzed for the survival of *Salmonella* Typhimurium on XLT4 agar supplemented with 35 µL/mL of nalidixic acid at 0, 12, 24, 36 and 48 h of refrigerated storage.

At each sampling time, three thighs per treatment were randomly chosen and placed into separate Whirl-Pak® bags (15.24 × 22.86 cm, 710 mL, Whirl-Pak, Nasco, Fort Atkinson, Wisconsin, U.S.A.), 1% PBS (30 mL) was added into each bag and the samples were shaken manually for 1 min. After rinsing, all the thighs were removed from the bags using sterile tongs and returned to the ice cooler. Each thigh that had been sampled was placed in a red plastic mesh to make sure that a new sample was chosen at each sampling point. Serial dilutions were prepared from the rinsate, spread plated (0.1 mL) on duplicate XLT4 agar plates containing nalidixic acid (35µL/mL) and the plates were incubated at 37 °C for 24 h. Viable colonies showing typical *Salmonella* colony morphology were counted and reported as log CFU/mL of the rinsate. Experimental design for the study was as follows: 5 treatments × 5 sampling time points × 3 samples/treatment × 5 trials.

Experiment 2: Determination of % Weight Pick-Up

Fresh, boneless, skinless raw poultry thighs were obtained from the same commercial poultry processor. The meat was stored at 4 °C for approximately 2-3 h until treatments were applied by packing the thighs in coolers with FICE. The thighs were tagged to provide a unique identification number to each thigh and to keep track of their weight throughout the 48 h study. At 0 h sampling time point, the weights of all individual thighs were recorded and then placed (n = 15/treatment/trial) in individual coolers (Igloo 48-Qt Island Breeze Cooler; 64.92 × 35.71 × 35.86 cm) with FICE treatments and stored as stated in the previous section. Sampling was conducted at 12, 24, 36 and 48 h of storage. At each sampling time, all the thighs were removed from the coolers with different FICE treatments, weighed and placed back into the respective coolers. The difference in the weights of the thighs before and after treatment at each sampling

time was calculated and reported as percent weight pick-up. The experimental design for the study was 5 treatments × 5 sampling points × 15 samples/treatment × 3 replications.

Experiment 3: pH and Temperature Study

Freshly deboned thighs (n = 45/treatment × 3 trials) were placed in different FICE treatments and traditional ice in coolers and placed in walk-in refrigerator (4 °C) for 48 h. Thigh samples from each treatment (n = 3 thighs/treatment/sampling time/trial) were analyzed for pH (Hach, Model No. H170G & PHW57-SS, Loveland, CO, U.S.A.) at 0 and 48 h while temperature was recorded using a digital thermometer (Taylor 1442 Critical Care Digital Thermometer with Dual Probes # 6081442, Lancaster, PA, U.S.A.) at 0, 4, 6, 8, 10, 12, 24, 30, 36 and 48 h of storage. These experiments were repeated in three separate trials.

Experiment 4: Shelf life of FICE-treated Tray-packed Thigh Meat

Based on the results from Experiment 1 (*Salmonella* survival study), STPP 5% and SL-SD 2.5% FICE treatments and control ice were selected for this experiment. Fresh boneless, skinless chicken thighs were obtained from the commercial poultry processor and stored at 4 °C until further treatment. Thigh meat (n = 180/treatment × 3 trials) was stored in respective FICE and ice treatments in a cooler (Coleman, 52-Quart Xtreme 5-Day Heavy-Duty Cooler; 68.83 × 38.1 × 44.20 cm) (n = 45 thighs/cooler; FICE: Meat: 2:1) for 48 h to simulate the storage period in a processing plant. After 48 h, the thighs were removed from their respective ice treatments using sterile stainless-steel tongs (30.48 cm) and packaged in the Styrofoam trays (23.50 × 18.41 × 6.98 cm; CKF Inc., Hantsport, NS, Canada) (4 pieces/tray) with two absorbent pads(12.7 × 17.78 cm.; tite-dri Industries, Boynton Beach, FL, U.S.A.) saran wrapped and stored in a walk-in refrigerator (4 °C).

Sampling was conducted on the freshly obtained thighs from the processor to establish a baseline, immediately after 48 h of FICE storage when the thighs were tray-packed (day 0), and every 2-days (day 2, 4, 6 and 8) until the tray-packed samples reached the aerobic plate count limit of 10^7 CFU/mL of rinsate. Samples were analyzed for microbiological and quality parameters. All the experiments were repeated in three separate trials.

Microbiological Parameters

Freshly procured raw poultry thigh samples (n = 30 samples × 3 trials) and tray-packed thighs (n = 2 thighs/tray × 5 trays/treatment/sampling time/trial × 3 trials) were analyzed for aerobic plate count (APC), psychrotrophic plate count (PSY) and Lactic Acid Bacteria (LAB). Individual thigh samples were aseptically placed in a Whirl-Pak® bag and rinsed with 1% phosphate buffered saline (30 mL), serially diluted in PBS and spread plated in duplicate on standard methods agar (PCA, Acumedia manufacturers Inc.,) for APC and PSY, and De Man, Rogosa and Sharpe agar (MRS) (Acumedia Manufacturers Inc.,) for LAB. PCA plates were incubated at 37 °C for 24 h and at 4 °C for 7-8 days for the estimation of APC and PSY, respectively. The MRS plates were placed in AnaeroPack rectangular jars (28.0 × 21.3 × 11.2 cm.; Mitsubishi Gas Chemical America, Tokyo, Japan) with three anaerobic packs (AnaeroPack® System, Mitsubishi Gas Chemical Company, Inc., New York, NY, U.S.A.) per container and incubated for 48 h at 37 °C. Viable colonies were counted and reported as log CFU/mL of rinsate.

Quality Parameters

Freshly procured raw thigh samples (25 samples × 3 trials) and tray-packed thighs (n = 1 thigh/tray × 5 trays/treatment/sampling time/trial × 3 trials) were analyzed for color, cook loss and texture. The objective analysis for color (3 measurements/ thigh) was conducted using a

Minolta colorimeter (Minolta Corp., model CR-300 / DP301, Ramsey, NJ, U.S.A.) using the CIE: L* (lightness), a* (redness), and b* (yellowness) color spectra (Fletcher, 2002). The colorimeter was calibrated with the white calibration plate, with a diffuse illuminant (D65) and 0° viewing geometry and had an 8 mm measurement area. Cook loss is expressed as weight loss after cooking the thigh relative to its initial weight. Briefly, individual thighs were weighed, placed on a raised stainless steel wire rack in a stainless-steel pan (53.02 × 32.54 × 10.16 cm; Vollrath Co., LLC, Sheboygan, WI, U.S.A.), covered with aluminum foil and cooked in a pre-heated (176.6 °C) forced air convection oven (Vulcan HEC5D, Troy, OH, U.S.A.) to an internal temperature of 74 °C measured using a stainless-steel digital thermometer (Taylor 1470FS Digital cooking thermometer and Kitchen Timer, Las Cruces, NM, U.S.A.). After cooking, the thighs were cooled to room temperature (22 ± 2 °C) in the covered pans and then reweighed. Cook loss was calculated using the following formula:

$$\text{Cook loss (\%)} = 100 \times \frac{(\textit{Initial weight of thigh} - \textit{cooked weight of thigh})}{(\textit{Initial weight of thigh})}$$

After recording the post-cook weight, the thighs were stored in resealable plastic bags overnight in the walk-in refrigerator at 4 °C for further analysis. The following day, the cooked thighs were brought to room temperature (22 ± 2 °C) for texture analysis. A Warner- Bratzler (WB) knife with guillotine block (TA-7, Stable Micro Systems, Hamilton, MA, U.S.A.) was utilized to shear the samples perpendicular to the muscle fibers. The tenderness of the thighs was evaluated by average peak force using the TA.XTPlus Texture Analyzer (Texture Technologies Corp., Hamilton, MA/Stable Micro Systems, Godalming, Surrey, UK) connected to a computer for obtaining data and analysis via Texture Expert software. The texture analyzer was calibrated using a load cell of 50 kg and at a crosshead speed of 20 mm/sec. From each thigh (*M. Iliotibialis lateralis)* two strips of approximately 2.5 × 0.5-0.7 cm were cut (long axis parallel to

muscle fibers) using a knife and placed under the 'V' slot of WB blade. Average peak force (kg) was measured and utilized as a measurement of thigh tenderness. The entire experiment was conducted in three separate trials.

Statistical Analysis

Data analysis was performed using the general linear model of SAS (SAS 9.4 Institute, Inc.). Statistical differences between treatments for the various parameters under study were reported as least square means and significance was reported at a level of $p \leq 0.05$. For statistical analysis of the microbial data, values of 0 were replace with the minimum detection limit of 5 CFU/mL.

Results and Discussion

Experiment 1

The first objective of the study was to validate the efficacy of different FICE treatments against *Salmonella* Typhimurium inoculated on poultry thigh meat during a 48 h storage period (Table 1). The initial population of *Salmonella* Typhimurium in the inoculated thigh meat samples was 6.8 to 6.9 log CFU/mL of rinsate. Storage of thigh meat samples in the control ice and STPP 2.5% FICE resulted in a 0.9 log reduction in *Salmonella* levels at the end of 48 h refrigerated storage. Comparatively, 0.9 log *Salmonella* reduction was observed at 12 h on thighs stored in STPP 5% FICE and 1.21 log reduction was observed at the end of the 48 h refrigerated storage, suggesting enhanced *Salmonella* inhibitions due to storage in STPP 5% ($p \leq 0.05$). Results further indicated that irrespective of the sampling time, STPP 5% FICE had lower ($p \leq 0.05$) *Salmonella* counts compared to other treatments (Table 1). The antimicrobial properties of phosphates can be attributed to (1) ability to sequester divalent metallic cations from the cell wall itself or the nutrient medium making them unavailable for physiological processes; (2) disruption

of proteins involved in cell division; and (3) causing cell lysis leading to cell death (Lee, Hartman, Olson, & Williams, 1994; Buňková, Pleva, Buňka, Valášek, & Kráčmar, 2008). In contrast to the current study, Baysal & Ünlütürk (2005) reported that dipping the breast meat samples in 5% STPP solution for 1 min did not give a reduction in *Salmonella*. The level of decline in bacterial population depends on variables such as contact time between the chicken meat and antimicrobials, concentration or the amount of antimicrobials used, and the method of application (Hill and Ivey, 1988). In the current study, the thigh meat samples were in constant contact with the antimicrobial ice containing polyphosphates which might have resulted in greater reduction whereas in the other study mentioned, the samples were dipped in 5% STPP aqueous solution for a minute. Chilling the carcasses in 1% and 1.5% Brifisol ice water solution has been found to reduce the incidence of *Salmonella* Typhimurium on inoculated carcasses by 100% and 97.5% respectively, whereas, chilling in only ice water reduced *Salmonella* incidence by 57.5% (Rathgeber and Waldroupl, 1995). Foster & Mead (1976) reported a reduction in the survival of *Salmonella* to 0.003-0.24% in the chicken breast muscle injected with 5% polyphosphate (w/v) (Puron 604) stored at -2 °C as compared to the survival of approx. 4% in the non-injected control samples. In another study conducted by Richter (2018), ice slurry media made of ice particles combined with salt and PAA solution was tested for its antimicrobial efficacy against *Salmonella* Typhimurium on the inoculated wings and reported that chilling the wings along with constant agitation in ice slurry for 20 min provided a reduction of 0.250 log CFU/mL more than chilled water alone, the higher reductions being attributed to the scrubbing or abrasive effect of ice slurry in constant agitation. However, in the current study, the ice/FICE and meat system was in the stationary phase and no agitation was involved.

The treatments SL-SD 1% and SL-SD 2.5%, reduced *Salmonella* by approximately 1 log after 48 h of storage with no differences between the two concentrations of SL-SD used for the study ($p > 0.05$). Similarly, Mbandi & Shelef (2001) reported bactericidal activity of SL and SD in beef emulsions incorporated with 2.5% SL and 0.2% SD during storage at 10 °C for 20 d. Shelef (1994) also stated the antimicrobial activity of the lactates could be attributed to the lowering of water activity and pH. However, in the current study, no differences were observed in the surface pH of the control and SL-SD FICE treated thigh meat ($p > 0.05$). Another theory stated that the antimicrobial activity of salts of organic acids could be correlated with the acidification of the microbial cells due to the dissociation of undissociated forms of weak lipophilic acids into the microbial cells (Salmond et al., 1984). However, there is not much published literature on the antimicrobial activities of a combination of sodium lactate and sodium diacetate against *Salmonella* in raw poultry meat.

Experiment 2

Yield is a very important factor for poultry processors. The meat can lose water through drip loss during storage and transportation thus leading to reduced yield. It is important to evaluate FICE to determine its effects on the % pick up of the thigh meat over 48 h of observation. The pick-up percentages of control and FICE treatments are presented in Table 2. The control and SL-SD 1% exhibited the lowest pick up (%) whereas the highest pick up (%) was observed in the SL-SD 2.5% and STPP 5% FICE ranging from 7.43 to 7.65% ($p < 0.05$). Lin & Lin (2002) conveyed a higher water holding capacity in the low fat Chinese-style sausage samples injected with 3% solution of sodium lactate, which could support the pick-up results. However, there has been very limited information on the increased water uptake due to the salts of organic acids in the poultry meat. Zheng et al. (2001) reported an increased weight pick up of

9.5% in the chicken breast when marinated with a higher concentration of STPP (38.0 g STPP + 70.0 g Salt). Sen, Naveena, Muthukumar, Babji, & Murthy (2005) observed an improved water binding capacity after injecting the breast fillets with 3% tetrasodium pyrophosphate post-chilling. The improved water holding capacity can be attributed to the elevated pH due to the addition of phosphates (Young & Lyon, 1997). Phosphates increase the ionic strength, increasing the protein-ion interactions and hydration, allowing more water to penetrate into the muscle structure (Wynveen et al., 2001; Froning, 1965).

Experiment 3

The pH of fresh, raw poultry thigh meat was 6.55 ± 0.22 (Table 2). The pH of control and SL-SD samples (1% and 2.5%) was not different after 48 h storage ($p > 0.05$). Similar findings were reported by Lin & Chuang, (2001) when the pork chops were dipped in a 10% solution of sodium lactate, suggesting a constant pH even at higher concentrations. The surface pH of the samples stored in STPP treatments resulted in an increase ($p \leq 0.05$) in the pH of meat to 7.45 (2.5% STPP FICE) and 7.84 (5% STPP FICE; Table 2). The increase in the pH of thighs stored in STPP can be attributed to the alkaline nature of STPP used (Cheng and Sun, 2008). Moreover, the higher concentrations of STPP result in a higher surface pH compared to lower concentrations of STPP (Zheng et al., 2001).

Figure 1 shows the temperatures of thigh meat samples stored in different ice treatments at different time points 0-48 h of refrigerated storage. The results obtained showed that there was an immediate drop in the temperature of thighs stored in different treatments within 2 h of storage, with thighs in STPP 5% FICE showing the lowest temperature (-1.14 °C; $p \leq 0.05$). After 2 h of storage, the temperature in the control samples slowly increased until 30 h of storage where the temperature held constant around 0-1 °C. On the other hand, compared to control, the

FICE treatments exhibited lower temperatures ($p \leq 0.05$) of -0.94 to -1.55 °C at the end of 48 h of storage. When the chicken thighs were stored on ice, the heat flowed from the meat to the ice, hence the temperature of meat stored in ice started decreasing. The different FICE ingredients further resulted in a depression in the freezing point of the ice, enhancing the cooling capacity of the FICE and thereby increasing the rate of heat transfer and lowering the temperature of meat (Rowe, 2016). By the end of the 48 h storage, the sub-zero temperature of FICE resulted in frozen thighs. Prout & Mission, (2004) also reported that temperature of haddock stored in crushed particulate ice (2% brine solution + 40 % ice) had a lower temperature 0.3 °C as compared to the solid ice (3 °C) after 9 h of storage and suggested that the concentration of brine affected the cooling capacity of the ice.

Experiment 4

Microbiological Analyses

The effect of refrigerated storage of thigh meat in STPP 5%, SL-SD 2.5% and control ice treatment for 48 h, on the microbiological (APC, PSY, and LAB) shelf life of tray-packed product was monitored for 8 d. The thigh meat samples were regarded as unacceptable when APC and PSY levels reached 10^7 CFU/mL of the rinsate.

APC, PSY, and LAB are common food spoilage indicators. Freshly procured thigh meat had an APC and PSY of approx. 3-4 log CFU/mL of rinsate while the LAB was approx. 2-3 log CFU/mL of rinsate (Figures 2, 3, and 4, respectively). As expected, FICE treated samples, especially STPP 5%, had lower APC, PSY and LAB counts compared to the control samples. The control samples reached the APC (Figure 3) and PSY (Figure 2) spoilage limit 1-2 days sooner than the FICE treated samples. The increase in shelf life of the FICE treated samples by 1-2 days can be attributed to the reduction in microbial load during storage and potential

antimicrobial effect of residual STPP and SL-SD in meat. Similar shelf life enhancement effects of polyphosphates were reported by Vareltzis, Soultos, Koidis, Ambrosiadis, & Genigeorgis (1997) in chicken carcasses dipped in 5% STPP (w/v) solution for 10 min. However, Baysal & Ünlütürk, (2005) reported that dipping the chicken samples in 5% STPP for 1 min did not improve the shelf life. This indicating that the antimicrobial efficacy of polyphosphates greatly depends on the method of polyphosphate application and contact time (Elliott et al., 1964). Smaoui, Hlima, Salah, & Ghorbel, (2011) reported a 3 d extension in the shelf life of chicken thighs marinated with 3% sodium lactate. Williams, Rodrick, & West (1995) also reported lower levels of APC for catfish fillets when a higher concentration of SL was used.

Quality Analyses

The color of poultry meat greatly influences the consumer selection of the product (Fletcher, 2002), therefore, FICE was investigated for its effect on the color of tray-packed thigh meat for 8 d after refrigerated storage in FICE treatments for 48 h. The mean L*, a* and b* values for the raw thigh samples before and after 48 h of FICE storage are presented in Table 3. After the 48 h storage treatment (day 0), the L* (lightness) and b* (yellowness) values increased while, the a* (redness) values decreased for all the ice treatments under study. The L* values in the control and SL-SD samples were higher than STPP 5% treatment until day 4 ($p \leq 0.05$), while on day 6 and 8, no differences were observed ($p > 0.05$). The lower L* value for STPP is correlated with the pH and water binding capacity of the meat (Fletcher et al., 2000). STPP exhibited higher pH and water uptake which resulted in the lower scattering of light by the meat resulting in darker samples (Sen et al., 2005). There were no differences ($p > 0.05$) in the a* values of control and SL-SD samples throughout the tray-pack storage for 8 d (Carroll, Alvarado, Brashears, Thompson, & Boyce, 2007; Shafit & Williams, 1996), indicating no adverse impact

on the redness of the samples. Lower a* values were observed for STPP 5% samples on day 6 and 8 indicating decreased redness of the samples, however, these differences were small and would likely not be noticeable to a consumer (Kim & Marshall, 1999; Smith & Young, 2004). The b* values for the control and SL-SD samples were fairly similar throughout the tray-packed storage ($p > 0.05$) and higher (indicating yellower samples) than STPP 5% samples until d 8 ($p \leq 0.05$). Thus, the tray-pack storage of different ice treated samples denoted a similar L* and b* value ($p > 0.05$), whereas, a comparatively lower a* (less red) value was observed for STPP 5% treatment by the end of the study. This change would not produce any negative quality defects in the tray-packed thigh meat.

The cook loss of the fresh meat procured from the poultry processor was 29.15 ± 4.7% which increased after a 48 h storage in control ice and SL-SD 2.5% FICE (Table 4), however, it was lower in samples stored in the STPP 5% FICE treatment. Cook loss of 48 h STPP 5% FICE treated samples was lower (26.53 ± 4.70 %; $p \leq 0.05$) than other treatments indicating the importance of FICE on the quality of raw poultry meat during storage. Cooking losses for control and SL-SD 2.5% samples were comparable throughout the study ($p > 0.05$) except for day 8 when the control exhibited the highest cook loss. By the end of study (d 8) STPP 5% exhibited the lowest cook loss (14.86 ± 2.18 %; $p \leq 0.05$) in the tray-packed meat. It has been reported widely that sodium tripolyphosphate results in reduced cooking losses in chicken meat (Young & Lyon, 1997; Young, Lyon, Searchy, & Wilson, 1987; Carroll, Alvarado, Brashears, Thompson, & Boyce, 2007). The reduced cooking losses in STPP treated samples is due to the increased water retention of the meat as the muscle proteins unfold, exposing more charged sites for water to bind (Cheng & Sun, 2008; Smith & Young, 2004; Froning, 1965).

The tenderness of thigh meat (shear force) increased over time as indicated by the Warner-Bratzler shear force analysis (Table 4). The shear force for control and SL-SD samples was higher than the STPP treatment until d 2 of tray-packed storage ($p \leq 0.05$) (Zheng et al., 2001). By the end of storage, there were no differences between the treatments ($p > 0.05$) (Williams and Phillips, 1998). McGee, Henry, Brooks, Ray, & Morgan (2003) also observed lower shear force values in beef injected with a combination of STPP, SL and sodium chloride. The reduced shear force can be attributed to the increased water holding capacity and reduced cooking losses (Alvarado and McKee, 2007).

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Conclusion and Future Works

Functional ice made with STPP 5% proved highly effective against *Salmonella* Typhimurium during storage and had a significant impact on improving yield, quality, and shelf life of raw thigh meat. SL-SD 2.5% was the second most effective FICE treatment but was not as effective as the STPP treatment. FICE could be applied during storage and transportation of raw poultry to not only provide an additional hurdle to ensure food safety and shelf life extension but also improve the quality characteristics of poultry meat.

From the current research study, it has been proved that FICE made with sodium tripolyphosphate, through its 'Sustained Release Mechanism' can potentially improve the food safety, shelf life and quality parameters of poultry meat during prolonged periods of refrigerated storage. However, the effect of FICE has not been tested on the sensory attributes of poultry meat. Knowing consumer acceptability by conducting the sensory analysis of FICE treated meat could further give an idea about the overall acceptability of the poultry meat stored in FICE. Additionally, analyzing the level of phosphates being absorbed into the meat will further aid in labelling the product with accurate information. Follow up research to investigate the efficacy of FICE under the conditions of temperature abuse would be beneficial to determine its potential usage during storage and transportation.

Tables and Figures

Table 1. Effect of different FICE[1] Treatments and Control Ice[2] on the survival of Salmonella Typhimurium (log CFU/mL of rinsate) inoculated on thigh meat (n= 75/ trt.) over 48 h of refrigerated storage at 4 °C (mean; SEM: standard error)

Treatments	Storage time (h)					SEM
	0	12	24	36	48	
Control	$6.90^{ba,A}$	$6.36^{a,B}$	$6.26^{ba,B}$	$6.10^{ba,C}$	$5.96^{a,D}$	0.045
STPP 2.5%	$6.87^{ba,A}$	$6.03^{c,B}$	$5.95^{c,B}$	$5.93^{b,B}$	$5.93^{a,B}$	0.068
STPP 5%	$6.81^{b,A}$	$5.84^{d,B}$	$5.74^{d,B}$	$5.69^{c,B}$	$5.69^{b,B}$	0.061
SL-SD 1%	$6.97^{a,A}$	$6.22^{ba,B}$	$6.27^{a,B}$	$6.22^{a,B}$	$5.85^{ba,C}$	0.055
SL-SD 2.5%	$6.87^{ba,A}$	$6.17^{bc,B}$	$6.10^{bc,CB}$	$5.94^{b,CD}$	$5.85^{ba,D}$	0.063
SEM	0.047	0.055	0.055	0.066	0.069	

$^{a-e}$Means with the different letter within a column indicate the significant differences ($p \leq 0.05$) between different treatments at each sampling time point

$^{A-D}$Means with the different letter within the same row indicate the significant differences ($p \leq 0.05$) within the treatment at different sampling time point

[1]FICE Treatments: sodium tripolyphosphate (STPP 2.5% and STPP 5%); sodium lactate-sodium diacetate (SL-SD 1% and SL-SD 2.5%)

[2]Control Ice: Regular Ice made of Tap Water

Table 2. % Weight pickup and Surface pH of Raw Thighs Stored in FICE[1] Treatments and Control Ice[2] over 48 h of Refrigerated Storage at 4 °C (mean; SEM: standard error)

Treatment	Raw, meat pH (untreated)	Final pH (after 48 h)	Pick-Up (%)				SEM
			0 to 12 h	0 to 24 h	0 to 36 h	0 to 48 h	
Control	6.55	6.69^{c}	-0.54^{e,C}	-0.28^{d,C}	0.94^{d,B}	2.47^{c,A}	0.199
STPP 2.5%		7.457^{b}	1.06^{c,D}	1.82^{c,C}	3.07^{c,B}	3.99^{b,A}	0.172
STPP 5%		7.84^{a}	1.48^{b,D}	2.96^{b,C}	5.46^{b,B}	7.43^{a,A}	0.204
SL-SD 1%		6.89^{c}	0.59^{d,C}	1.44^{c,A}	0.96^{d,BC}	1.09^{d,BA}	0.169
SL-SD 2.5%		6.85^{c}	2.79^{a,C}	4.48^{a,B}	6.88^{a,A}	7.65^{a,A}	0.283
SEM		0.196	0.147	0.175	0.226	0.270	

$^{a-e}$Means with the different letter within a column indicate the significant differences ($p \leq 0.05$) between different treatments at each sampling time point

$^{A-D}$Means with the different letter within the same row indicate the significant differences ($p \leq 0.05$) within the treatment at different sampling time point

[1]FICE Treatments: sodium tripolyphosphate (STPP 2.5% and STPP 5%); sodium lactate-sodium diacetate (SL-SD 1% and SL-SD 2.5%)

[2]Control Ice: Regular Ice made of Tap Water

Table 3. Changes in color L*, a*, and b* values of thighs (n= 75/trt.) stored in FICE[1] treatments and Control Ice[2] for 48 h, transferred to tray-pack and stored for 8 days at 4 °C (mean; SEM: standard error)

Treatment	Parameter	Fresh, raw thigh meat (untreated)	Storage time (day)					SEM
			0	2	4	6	8	
Control			$59.23^{a,A}$	$57.07^{a,B}$	$54.12^{ba,C}$	$53.61^{a,C}$	$52.97^{a,C}$	0.709
STPP 5%	**L***	51.43	$52.75^{c,BA}$	$51.79^{c,B}$	$52.87^{b,BA}$	$53.50^{a,BA}$	$53.81^{a,A}$	0.703
SL-SD 2.5%			$56.67^{b,A}$	$55.52^{b,BA}$	$55.51^{a,BA}$	$53.81^{a,B}$	$53.83^{a,B}$	0.671
SEM			0.861	0.523	0.861	0.559	0.585	
Control			$2.70^{a,A}$	$2.57^{a,A}$	$2.77^{a,A}$	$2.22^{ba,A}$	$2.96^{a,A}$	0.296
STPP 5%	**a***	4.29	$2.67^{a,A}$	$2.45^{a,BA}$	$1.96^{a,BC}$	$1.65^{b,C}$	$1.41^{b,C}$	0.206
SL-SD 2.5%			$2.24^{a,B}$	$2.51^{a,BA}$	$2.53^{a,BA}$	$2.61^{a,BA}$	$3.01^{a,A}$	0.259
SEM			0.274	0.235	0.333	0.212	0.205	
Control			$6.92^{a,A}$	$7.28^{a,A}$	$5.15^{b,B}$	$6.73^{a,BA}$	$5.12^{a,B}$	0.573
STPP 5%	**b***	3.52	$4.96^{b,A}$	$3.90^{b,A}$	$4.88^{b,A}$	$4.57^{b,A}$	$5.63^{a,A}$	0.636
SL-SD 2.5%			$6.28^{a,A}$	$6.23^{a,A}$	$7.29^{a,A}$	$6.24^{a,A}$	$6.32^{a,A}$	0.578
SEM			0.676	0.592	0.612	0.479	0.604	

$^{a-c}$Means with the different superscript within the same column and parameter indicate the significant differences ($p \leq 0.05$) between different treatments on the same sampling day

$^{A-C}$Means with the different superscript within the same row indicate the significant differences ($p \leq 0.05$) within the treatment on different sampling days

[1]FICE Treatments: sodium tripolyphosphate (STPP 5%); sodium lactate-sodium diacetate (SL-SD 2.5%)

[2]Control Ice: Regular Ice made of Potable Water

Table 4. Cook Loss % and Peak Force (kg) values of thighs (n= 75/trt.) stored in FICE[1] treatments and Control Ice[2] for 48 h, transferred to tray-pack and stored for 8 days at 4 °C (mean; SEM: standard error)

Treatment	Parameter	Fresh, raw thigh meat (untreated)	Storage time (day)					SEM
			0	2	4	6	8	
Control	**Cook loss (%)**	29.15	$33.02^{a,A}$	$32.10^{a,A}$	$27.12^{a,B}$	$27.96^{a,B}$	$27.09^{a,B}$	0.765
STPP 5%			$26.53^{b,A}$	$23.01^{b,B}$	$18.32^{b,C}$	$19.06^{b,C}$	$14.86^{c,D}$	0.650
SL-SD 2.5%			$35.89^{a,A}$	$31.44^{a,B}$	$27.19^{a,C}$	$27.51^{a,C}$	$21.94^{b,D}$	1.136
SEM			1.006	1.272	0.617	0.719	0.548	
Control	**Peak force (kg)**	1.5	$1.13^{ba,A}$	$1.08^{a,BA}$	$0.91^{a,BC}$	$0.82^{b,C}$	$0.75^{a,C}$	0.070
STPP 5%			$0.97^{b,A}$	$0.91^{a,BA}$	$0.91^{a,BA}$	$1.03^{ba,A}$	$0.77^{a,B}$	0.064
SL-SD 2.5%			$1.24^{a,A}$	$1.10^{a,A}$	$0.78^{a,CB}$	$0.89^{a,B}$	$0.69^{a,C}$	0.058
SEM			0.078	0.071	0.059	0.069	0.034	

$^{a-c}$Means with the different superscript within the column and parameter indicate the significant differences ($p \leq 0.05$) between different treatments on the same sampling day

$^{A-D}$Means with the different superscript within the same row indicate the significant differences ($p \leq 0.05$) within the treatment on different sampling days

[1]FICE Treatments: sodium tripolyphosphate (STPP 5%); Sodium lactate-sodium diacetate (SL-SD 2.5%)

[2]Control Ice: Regular Ice made of Tap Water

Figure 1. Temperature change recorded in the thighs stored in Control Ice and different FICE treatments: sodium tripolyphosphate (STPP 2.5%; STPP 5%); sodium lactate-sodium diacetate (SL-SD 1%; SL-SD 2.5%) and over 48 h of refrigerated storage at 4 °C.

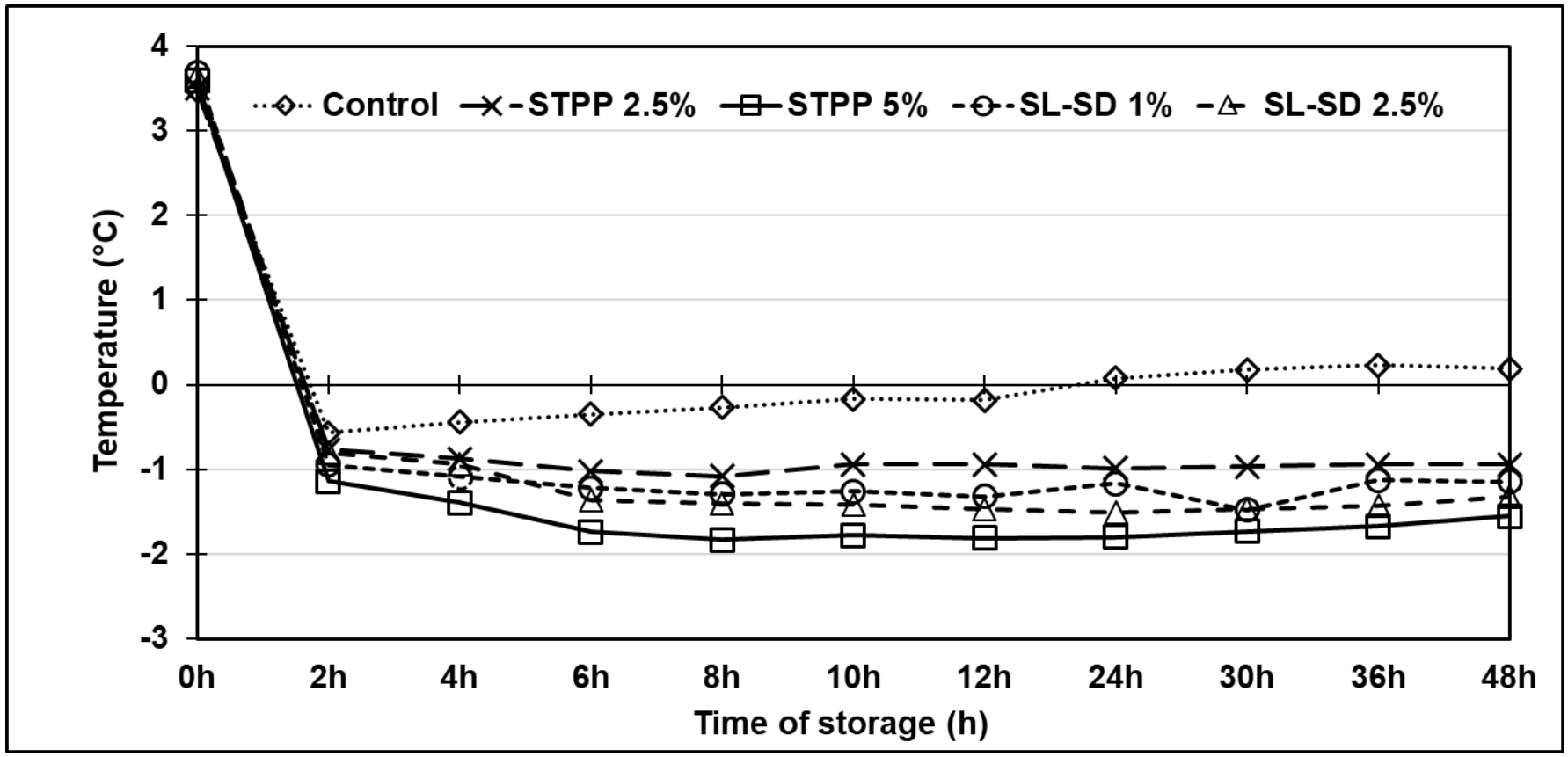

Figure 2. Psychrotroph levels (log CFU/mL of rinsate) stored in Control Ice and FICE treatments: sodium tripolyphosphate (STPP 5%) and sodium lactate-sodium diacetate (SL-SD 2.5%) for 48 h; then tray-packed and sampled for 8 days of storage at 4 °C (untreated thigh meat PSY: 3.49 ± 0.62)

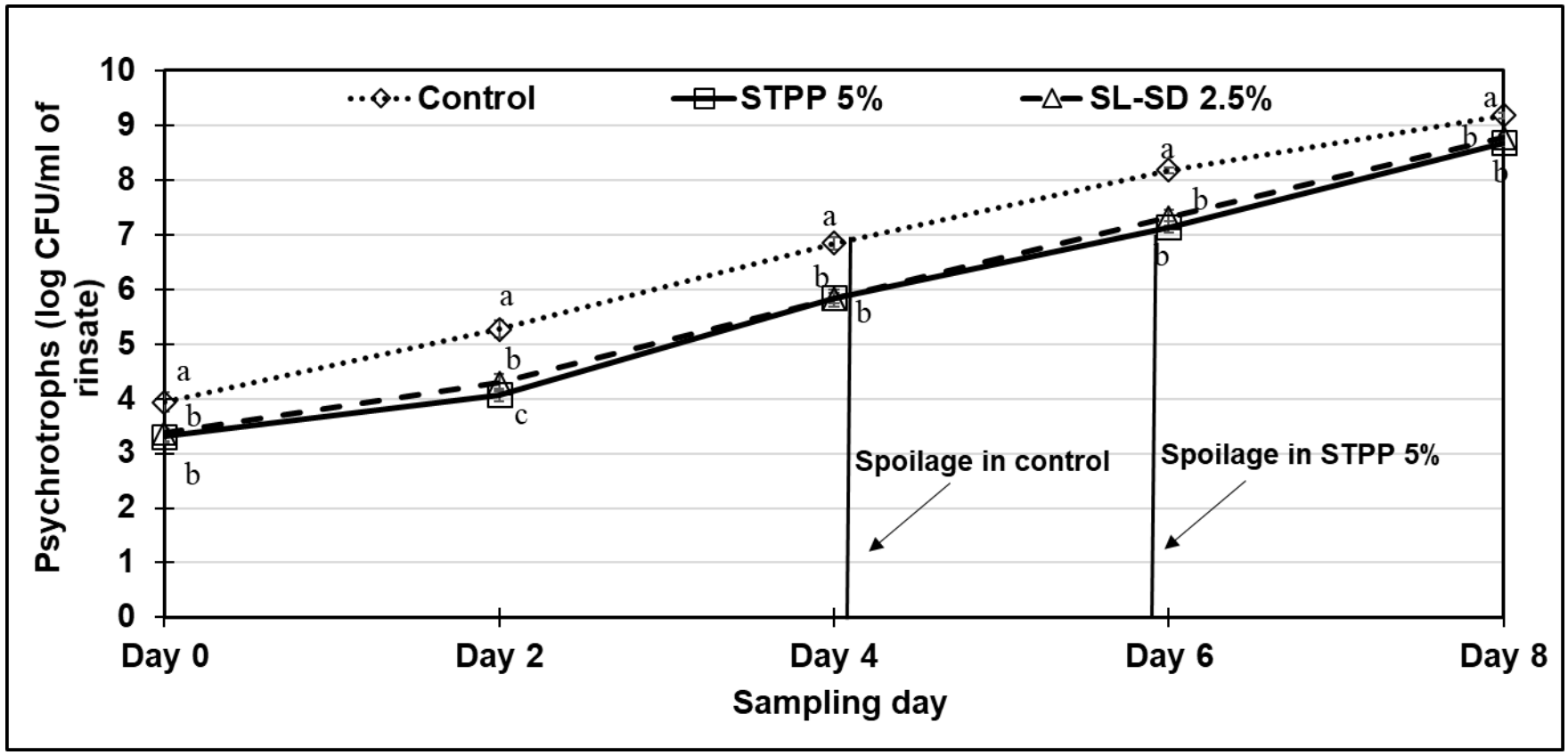

Figure 3. Aerobic Plate Count (log CFU/mL of rinsate) of stored in Control Ice and FICE treatments: sodium tripolyphosphate (STPP 5%) and sodium lactate-sodium diacetate (SL-SD 2.5%) for 48 h; then tray-packed and sampled for 8 days of storage at 4 °C (untreated thigh meat APC: 3.47 ± 0.531)

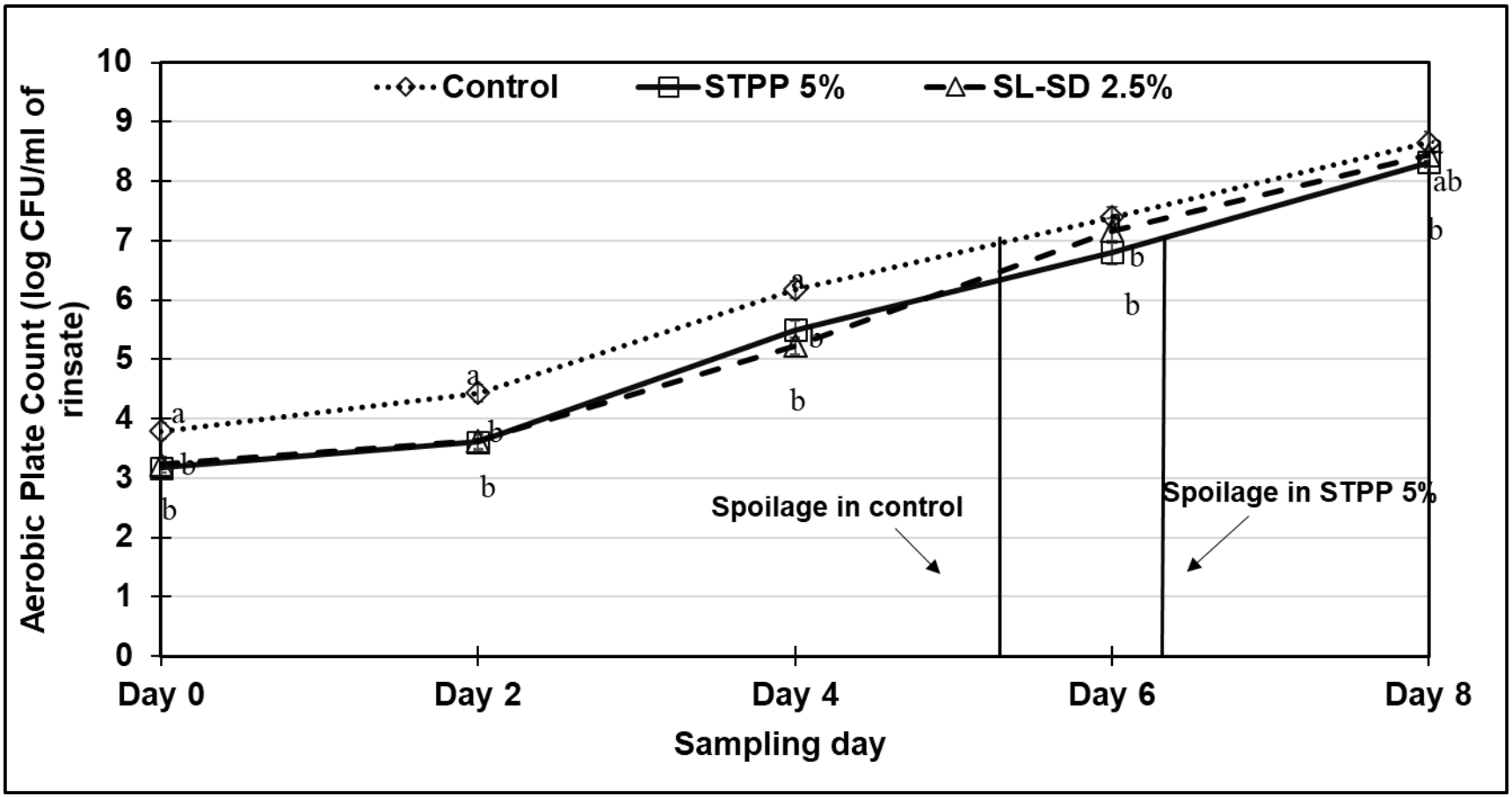

Figure 4. Lactic Acid Bacteria count (log CFU/ml of rinsate) of thighs (n= 150/ trt.) stored in Control Ice and FICE treatments: sodium tripolyphosphate (STPP 5%) and sodium lactate-sodium diacetate (SL-SD 2.5%) for 48 h; then tray-packed and sampled for 8 days of storage at 4 °C (untreated thigh meat LAB: 2.63 ± 0.697)