ABSTRACT

SYBIRTSEVA, IRYNA. Efficacy of Naturally Occurring Antimicrobials to Reduce *Salmonella* spp. Associated with Poultry at Various Stages of Processing. (Under the direction of Dr. Fletcher M. Arritt and Dr. Sophia Kathariou.)

Salmonellae remain one of the leading causes of foodborne illnesses worldwide with poultry being recognized as a primary vehicle for salmonellosis. Despite considerable attention from the media and diligence by the industry to prevent product contamination, poultry remains responsible for about 50% of common-vehicle outbreaks. The efficacy of oxalic and fumaric acids, as naturally occurring antimicrobial agents for poultry processing, was evaluated by simulating on-line or post-chill dipping, water-immersion chilling, and soft scalding for reduction of *Salmonella* spp. attached to raw chicken skin. Skin samples were collected at different time periods to account for the natural variation observed in the industry and processed using the modified skin attachment model and thin agar layer plating technique for acid-injured cells. On-line or post-chill dipping (22ºC) with oxalic (0.5 to 2.0%) and fumaric (0.25 to 0.5%) acids showed the least quantitative reductions in *Salmonella* spp. compared to other applications. The greatest *Salmonella* reductions were achieved with 2.0% oxalic acid and 1.5% fumaric acid when applied for 3 min in a soft scalder treatment (53ºC), reducing initial *Salmonella* inoculum by 2.43 and 1.53 log, respectively. Simulated water-immersion chilling (3ºC) with 0.5% oxalic acid and 0.25% fumaric acid for 60 min resulted in *Salmonella* reductions of 2.25 and 0.72 log, respectively. Mutations in the *fur, atp,* or *rpoS* loci did not impact the survival of *S.* Typhimurium attached to broiler skins when treated with oxalic acid during dipping and scalding. The bactericidal effectiveness of fumaric acid as an antimicrobial agent was limited by its low water solubility and resulted in less than a 2-log *Salmonella* reduction on raw chicken skins at all tested

applications. Fumaric acid's effectiveness in vitro was improved by the addition of acetic, oxalic, and lactic acids, and depended on the concentration of supplemented acid. The greatest antimicrobial effect was observed when 0.45% fumaric acid was coupled with 0.45 or 1.35% oxalic acid resulting in a 2.09-log and ≥6-log reduction in *Salmonella*, respectively. Variation was observed in the effectiveness of oxalic and fumaric acids to reduce *Salmonella* among three skin batches, even though no obvious difference was found among the batches that could explain variation in *Salmonella* reduction, including the variation in initial pH, fat and moisture content, thickness of epidermal layer as well as presence of channels and crevices on skins. This observation emphasized the importance of collecting samples from different flocks, days, and seasons for testing antimicrobial agents to best simulate the natural variation that occurs within the industry and more accurately assess the efficacy of antimicrobials.

Efficacy of Naturally Occurring Antimicrobials to Reduce *Salmonella* spp. Associated with Poultry at Various Stages of Processing

by
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BIOGRAPHY

Native to Ukraine, Iryna Sybirtseva, is a young scholar with a diverse professional and academic background. She earned her Master's Degree in Food Technology and Engineering in February 2003 from the Ukrainian State University of Food Technologies, Kiev, Ukraine. There, as a part of the research team, Mrs. Sybirtseva contributed to the creation of enriched grain products designed for the diabetes patients. She also worked as a Food Technologist and College Instructor before moving to the United States.

In the U.S. Mrs. Sybirtseva held the positions of Quality Assurance Technician and Quality Assurance Supervisor in the meat processing industry as well as developing a Safety and Sanitation Workshop before joining the Ph.D. program at North Carolina State University (NCSU). Her doctoral research was concentrated on efficacy of naturally occurring antimicrobials to reduce pathogenic microorganisms associated with poultry at various stages of processing.

Balancing her academic endeavors, career and family, Mrs. Sybirtseva also serves on the board of the Center Miro-Priyatie, Inc. (CMP), a non-for-profit organization that facilitates educational cross-cultural exchanges between USA and Eastern European States. She is an active member of International Association for Food Protection (IAFP), Institute of Food Technologists (IFT), American Meat Science Association (AMSA), Phi Tau Sigma Honorary Society and NCSU Food Science Club. Other interests of Mrs. Sybirtseva include Statistical Analysis, Professional Leadership and Business Ethics.

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SALMONELLA CONTAMINATION OF POULTRY

Introduction

Salmonellae remain one of the leading causes of microbial foodborne illness worldwide, and poultry is recognized as a primary vehicle for salmonellosis *(44, 61)*. Despite considerable attention from the media, governmental oversight, and continuous effort by the industry to prevent product contamination, poultry remains responsible for about 50% of outbreaks *(44)*. In 2006, epidemiologic data on foodborne disease outbreaks in the U.S. identified poultry as the most common single food vehicle in outbreak-related cases *(35)*. Increases in the number of poultry associated foodborne diseases, particularly salmonellosis, may be attributed, at least in part, to increased poultry consumption from 27.4 lbs in 1997 to 59.2 lbs in 2004, per capita *(26)*.

Each year in the U.S., *Salmonella* causes an estimated 1.4 million illnesses, resulting in approximately 15,000 hospitalizations and 400 deaths *(153)*. Despite numerous interventions designed to prevent foodborne infections, *Salmonella,* with an incidence rate per 100,000 individuals of 16.2 in 2008 *(34)* and 15.19 in 2009 *(36),* remains one of the pathogens farthest from its Healthy People 2010 target, which was to have reduced the *Salmonella* incidence rate to 6.8 cases per 100,000 individuals *(34)*.

Contamination of poultry with *Salmonella* is expensive for the poultry industry and is taxing on the economy as a whole. The total cost includes: (i) expense associated with human illnesses, (ii) expenditures that processors face to meet regulatory requirements, (iii) medical costs caused by the consumption of contaminated poultry products, and (iv) various other

financial consequences of product contamination. *Salmonella* related losses in human productivity, medical expenses and increased production is estimated at $0.6 to $3.5 billion/annually in the U.S. alone *(61, 138)*. More specifically, in 2007 an annual economic cost of foodborne illness caused by nontyphoidal *Salmonella* was $227.76 million in Ohio *(129)*.

Efforts to reduce contamination of poultry on farms and at processing plants are ongoing. However, due to multistep automated and manual processes that involve carcass handling there are many points where cross-contamination may occur. In addition, U.S. Department of Agriculture, Food Safety and Inspection Service (USDA/FSIS) has proposed further reductions in the incidence of *Salmonella* on poultry carcasses from the current 20% to <7.5% *(16, 148)*. USDA/FSIS expects 90% of establishments to meet the new microbiological standard by the end of 2010 *(148)*, which may create a challenge for the processors.

Salmonella can infect a wide variety of hosts producing clinical disease, and in some instances result in a relatively asymptomatic intestinal carriage. Different *Salmonella* serovars detected in poultry are also associated with significant numbers of human illnesses. Controlling *Salmonella* contamination has become an important issue for the poultry industry from both public health and economic perspectives. To control this biological hazard during poultry production is to diminish the safety risk associated with poultry consumption and to reduce the economic losses related to foodborne illnesses and regulatory recalls. This research focused on *Salmonella* serovars that are associated with both poultry and human

illnesses in order to more effectively develop a control strategy for the industry to reduce human disease.

Salmonella spp.

Salmonella are rod-shaped, Gram-negative, non-spore forming, predominantly motile bacteria within the family *Enterobacteriaceae (18).* The *Salmonella* genus contains 2,463 serotypes which are divided into two species: *S. enterica* and *S. bongori (115).* The former includes six subspecies that are differentiated by biochemical characteristics and genomic relatedness. The majority of human infections are associated with serotypes belonging to subspecies I (*Salmonella enterica* subs. *enterica*) *(22).*

Most *Salmonella* species are ubiquitous, causing enteric disease in a wide range of host species. Others are host-adapted or prevalent in one particular host, but capable causing disease in other host species *(73).* For instance, *S.* Dublin and *S.* Choleraesuis are able to infect many mammalian hosts, but are generally associated with severe systemic disease in cattle and pigs, respectively. Some *Salmonella* species are restricted and almost exclusively associated with a specific host. Host-restricted species can be confined to humans (Typhi, Paratyphi A, B and C, and Sendai) causing enteric (typhoid) fever, to poultry (Gallinarum and Pullorum) causing fowl typhoid and pullorum diseases, or to swine (Typhisuis) causing chronic paratyphoid *(151).*

The primary habitat of salmonellae is the gastrointestinal tract of warm and cold blooded animals *(18).* The ubiquity of the pathogen in natural environments and its

physiology has hampered efforts to control environmental contamination and reduce transmission of the microorganism. Transmission routes to humans are mainly from person-to-person via the fecal-oral route and from animals or water via the food supply. Poultry, pork, and beef products, in decreasing order of importance, are identified as the principal reservoirs and transmission vehicles of foodborne salmonellosis *(46)*.

Human salmonellosis can lead to either enteric (typhoid) fever following infection with typhoid or paratyphoid strains, or to gastroenteritis with possible progression to a more serious infection. The infectious dose varies from a few cells to a million cells depending on the immunological heterogeneity within the human population, virulence of infecting strain, and/or chemical composition of the incriminated food vehicle *(47)*.

There is increasing concern over prevalence of multidrug-resistant (MDR) *Salmonella* serotypes in animals and humans, and their association with more severe illness compared to susceptible isolates *(49)*. Common poultry MDR serotypes (e.g. Typhimurium, Newport, Heidelberg, Schwarzengrund, Virchow, Kentucky, Hadar, and Mbandaka) are resistant to several antibiotics, including ampicillin, streptomycin, ceftiofur, kanamycin, nalidixic acid, sulfisoxazole, or tetracycline *(49, 84, 167)*. The levels and degree of resistance vary, and are influenced by usage of antibiotics in veterinary and human medicinal practices as well as geographical variations in the epidemiology of *Salmonella* infections *(47)*. Antibiotic resistance in salmonellae is attributed to the acquisition of foreign genes, residing on mobile genetic elements (e.g. plasmids, transposons, integrons), that encode enzymes to

destroy or inactivate the drug or provide an alternative pathway to the one targeted by the antibiotic *(47)*.

Salmonella spp. are resilient microorganisms that readily adapt to extreme environmental conditions. They are facultative anaerobes and can grow well under both aerobic and anaerobic conditions. The optimum temperature to support growth is 37°C, but they can grow over a range of 2 to 54°C and within a pH range of 4.5 to 9.5, with an optimum pH 7.0. Foods with water activity values (a_w) of less than 0.93 do not support the growth of salmonellae, but the pathogen is able to survive for up to several months depending on the menstruum *(47)*.

Salmonella serotypes in poultry and human salmonellosis

Although 2,463 serotypes of *Salmonella* have been identified, less than 10% are isolated from poultry, and an even smaller subset can cause enteric illness in humans *(115, 133)*. Surveys of *Salmonella* isolates from poultry yield diverse results, because the distribution of serotypes varies geographically and changes over time. Nevertheless, several serotypes are consistently found at higher incidence in both broiler chicken carcasses and raw chicken products at retail *(133)*. According to the report from USDA/FSIS, the most commonly identified *Salmonella* serovars in broilers from 1998 to 2007 in the United States were Kentucky, Heidelberg, Typhimurium, Enteritidis, Hadar, Montevideo, Schwarzengrund, Thompson, and Newport *(52, 147)*.

The connection between poultry and human reservoirs of salmonellae is apparent in distribution similarities of the serotypes reported from these sources. Generally, of the 10 most common serotypes responsible for human infection, *S.* Typhimurium, *S.* Enteritidis, *S.* Newport, and *S.* Heidelberg have been the most common serotypes since 1995 *(33)*. Combined these serotypes accounted for 49.1% of human infections in 2008 *(34)* and 51% in 2009 *(36)*. Cases caused by strains of multiple antibiotic-resistant *Salmonella* spp. have also increased in recent years and illness is reported to be more serious than that caused by non-antibiotic resistant *Salmonella (18).*

Cross-contamination during poultry processing

Complex, high speed operations inside a poultry processing plant provide many opportunities for cross-contamination including that which may occur from the environment, plant equipment, poor worker hygiene, and/or among the carcasses. The typical unit operations in a poultry processing plant are presented in Appendix 5.

Live birds represent the primary entry point of pathogens into the processing plant, with a diverse microflora present on feathers, skin, feet, in the intestinal tract and respiratory system *(14, 42)*. According to Lillard *(87)*, USDA/FSIS indicates that 3 to 4% of broilers arriving to a poultry slaughterhouse are infected with *Salmonella* spp., while the rate of *Salmonella* contamination of broiler carcasses, either from processing plants or from retail markets, ranges from 2 to 100% with a mean value of 30% *(24, 45, 155)*. May *(92)* listed 57 points of possible cross-contamination in poultry processing plants that may have a potential

negative effect on the final microbiological safety of fresh eviscerated carcasses. These findings illustrate a correlation between cross-contamination, which occurs at various stages of poultry processing, and high percentage of *Salmonella* positive carcasses after slaughter in poultry plants. Based on a recent FSIS Nationwide Microbiological Baseline Data Collection Program for Young Chickens, the incidence of *Salmonella* on broilers decreased from 11.4% in 2006 to 7.2% in 2009 *(36, 148)*.

The number and incidence of salmonellae in raw poultry carcasses is greatly affected by the unit operations of killing, scalding, picking, evisceration, and chilling *(13, 94, 164)*. Although, killing is not considered a major source of contamination during poultry processing, a killing blade has been cited as a potential area of cross-contamination. Fifty percent of 20 analyzed swab samples from the blade of killing knife were found *Salmonella* positive in a Spanish processing plant *(28)*. Additionally, it was shown that an automatic killing knife can spread the organism, even to the 500^{th} bird passing through the machine *(95)*.

The process of scalding carcasses is used prior to picking. Both processes have an important effect on carcass microbial quality, and are often considered one of the major sites of cross-contamination with *Salmonella* spp. *(27)*. Moreover, the extent of cross-contamination during picking is governed by the hygiene of the scalding process *(68)*. More specifically, the bacteria that survive scalding may spread from carcass to carcasses, and/or to the rubber-fingers of the picking machine during the operation, subsequently resulting in a higher incidence of *Salmonella*-positive carcasses after picking *(69)*.

Most broilers in the U.S. are processed by immersion soft scalding *(102)*. The process is usually done in a scald tank at 53°C for up to 3 minutes *(124)*. Such scheme allows for easy feather removal during picking without causing appreciable damage to the stratum corneum, and leaves the skin with a desirable yellow appearance *(14, 93)*.

When the birds are immersed in the scald tank, significant numbers of microorganisms from feathers (7.7 $\log_{10}$ CFU/g), feet (6.2 $\log_{10}$ CFU/g), and skin (8.2 $\log_{10}$ CFU/g) are washed into scald water *(79)*. As a result, the scald water becomes heavily contaminated with microorganisms, and conditions are such that, after an initial build-up, the number of viable organisms remains relatively constant over the working period *(165)*. The incidence of *Salmonella* spp. on broiler chickens upon entering the scald tank ranges from 16.7 to 100% depending on the study *(2, 28, 79, 119)*. Breast skin of broiler chickens has a higher salmonellae incidence (45%) and bacterial count (6.3 $\log_{10}$ CFU/g) than the thigh (30%; 5.9 $\log_{10}$ CFU/g) and drum (27.5%; 5.8 $\log_{10}$ CFU/g) skins *(79)*.

Abu-Ruwaida et al. *(2)* showed that *Salmonella* counts of water samples at the scald tank entry (2.8 $\log_{10}$ CFU/ml), middle (2.2 $\log_{10}$ CFU/ml), and exit (2.3 $\log_{10}$ CFU/ml) remained relatively constant during the scalding period of 3 min at 51.5 to 53°C. At 52°C in scald water, *S.* Typhimurium and *S.* Newport have D values of 29.07 and 22.18 min, respectively *(108)*. Yang et al. *(164)* found that an increase in scalding temperature from 55 to 60°C reduced *S.* Typhimurium by 4 log CFU/ml; however, the thermal effects on bacterial survival were reduced if bacteria were attached to the skin, suggesting that microorganisms were protected by an oily layer on the chicken skin surface, as well as by residing within

clefts and crevices *(19)*. Additionally, Notermans and Kampelmacher *(104)* showed that salmonellae cells attached to chicken skin were more heat resistant than those in suspension (physiological saline solution (pH 7.2)). Conversely, Slavik et al. *(137)* indicated that scalding at 60°C allowed 1.1 to 1.3 higher *Salmonella* log counts compared to the skins scalded at 52 or 56°C. Temperature driven changes in poultry skin microtopography were reported to have some effect on *Salmonella* attachment. Thus, higher scalding temperatures can greatly reduce the bacterial survival in the scald water, but at the same time cause loss in the stratum corneum layer, making it easier for bacteria to adhere to chicken skin *(75)*. Scald water temperature may also have an impact on heat resistance of microorganisms and subsequently select for more heat resistant mesophiles *(67, 102)*.

Mulder et al. *(101)* demonstrated that after one carcass artificially contaminated with a marker organism was scalded at 52°C, the marker was detected on the 230th bird. Therefore, survival of salmonellae in the scald tank water or on the carcasses may lead to a substantial cross-contamination that may continue to spread throughout the plant, particularly during the picking process.

Feather removal during picking may contribute to carcass contamination by two means: air-borne dispersion of organisms in the vicinity of the picking machine and high bacterial load on the rubber fingers used to remove feathers *(7, 93)*. Mead et al. *(95)* demonstrated the potential of one contaminated carcass to cross-contaminate many others by using a marker organism that was detected up to the 200th carcass passing through the picking machine. Similarly, Clouser et al. *(39)* observed significant *Salmonella* cross-

contamination during a conventional defeathering operation in turkey processing. The number of *Salmonella* positive turkeys increased from 21% before defeathering to 71% after passing through the defeathering system *(39)*. Moreover, the relatively warm and moist conditions of the picking unit may favor bacterial survival and growth *(93)*, leading to an increase of *Salmonella* incidence on poultry carcasses during picking.

Gorskoy et al. *(57)* reported that the rate of *Salmonella* contaminated carcasses increased from 33.3 (after scalding) to 50% (after picking) in a turkey processing plant. Similar data was obtained by Carraminnana *(28)*, who found that 55% of broiler carcasses were *Salmonella* positive after feather removal in a Spanish poultry slaughterhouse, but Cason et al. *(30)* indicated that 23% of 30 sampled broiler carcasses were positive for salmonellae after picking. In another study, the incidence of *Salmonella* spp. on chicken carcasses increased to almost 100% during the picking process *(56)*. Such a high percentage of *Salmonella* positive carcasses may have been due to embedding of the pathogen in feather follicles after the feather was removed and before the follicles were reduced in size (57). In contrast, Morris and Wells *(98)* showed a 5.2% reduction in incidence of *Salmonella* spp. during picking process.

These studies imply that, even before evisceration, the majority of carcasses will already be contaminated with salmonellae. Intervention steps need to be introduced in the early stages of processing to reduce pathogen occurrence to a minimum prior to evisceration, to assist in the reduction of *Salmonella* prevalence in the final product.

The incidence of *Salmonella* spp. contamination during carcass evisceration usually tends to increase. Carraminana et al. *(28)* reported a 5% increase in *Salmonella* positive carcasses during evisceration, while Abu-Rawaida *(2)* found that the pathogen incidence did not change after vent opening (100%) or lung sucking (100%). In another study, handling and processing steps between feather removal and crop removal (prior to chilling) caused a 2.5-fold increase in *Salmonella* recovery from skin samples *(125)*. Hargis et al. *(60)* reported that crops were 85 times more likely to rupture than ceca during processing and *Salmonella* recovery from crops was 3.5 times more likely than from ceca. Thus, the increased number of *Salmonella* positive carcasses during eviscerating operations may result from occasionally ruptured ceca, and/or spillage of crop content as well as cross-contamination from contaminated equipment surfaces and employees hands *(24, 43)*.

The chill step is arguably the most recognized processing step contributing to cross-contamination of whole carcasses within a poultry processing plant. Though several types of chilling procedures are employed commercially, water-immersion chilling is the most common in the U.S. because of its effectiveness and low cost *(102)*. Eviscerated carcasses are typically chilled to a temperature of 4°C within 30 to 60 min depending on carcass weight *(14, 124)*. Some authors report no significant differences in the number of *Salmonella* positive carcasses between pre-chill and post-chill *(39, 125, 154)*. However, cross-contamination may take place due to removal of the pathogens from the skin of a contaminated carcass by the chill water and the subsequent transfer to other carcasses in the chill tank. James et al. *(71, 72)* found a 24% increase in prevalence of *Salmonella* spp. during

immersion chilling. Additionally, microtopographical changes in the skin surface are associated with uptake of water by skin tissue, opening and exposing channels and crevices to bacteria present in chill water which may contribute to carcasses contamination *(87, 144)*. Sarlin et al *(125)* showed that a flock with a low level of *Salmonella* contamination (4% *Salmonella* positive) was preceded in the immersion chiller by a highly contaminated flock (68% *Salmonella* positive), pathogen recovery increased to 68% at post-chill in the low level flock. Moreover, a recent study reported a 3.3% increase in the incidence of salmonellae from pre-chill to post-chill *(119)*. The rate of cross-contamination greatly depends on the level of salmonellae on eviscerated carcasses prior to entering the chilling tank *(88)*. These findings indicate that contamination and/or cross-contamination of broiler carcasses must be avoided or controlled throughout prior processes to prevent flocks from becoming contaminated with high levels of *Salmonella* during chilling operation.

With regard to the prevalence of *Salmonella* spp. in the poultry plant, there are many steps during processing where spread of pathogens may occur. The intensive nature of poultry slaughter, automation and an increase in production line speeds may contribute to the rate of cross-contamination during the normal course of processing *(16)*. Therefore, emphasis must be placed on the incorporation of various intervention strategies that would reduce the potential for *Salmonella* cross-contamination during a normal course of poultry slaughter.

Intervention sites for carcasses during poultry processing

Bacterial contamination during poultry processing is unavoidable. Thus, various interventions used to minimize the risk of broiler carcasses contamination during processing to subsequently reduce the prevalence of *Salmonella* spp. in the final product *(45, 145)*.

Processing operations such as scalding and immersion chilling have been considered as antimicrobial application sites to diminish cross-contamination. Additionally, pathogen reduction has been achieved by utilizing either antimicrobial sprays or dips while carcasses remain on-line or at post-chill (Appendix 5) *(16, 145).*

Scalding is one of the first steps in poultry processing where antimicrobials can be added to reduce cross-contamination at an early stage of processing and to potentially prevent the spread of salmonellae further down the processing scheme. Only 18% of broiler processing facilities in the U.S. employ antimicrobial applications at scalding operation *(120)*. Many scald water additives used as antimicrobials directly affect the pH of the scald water, since microbial survival is appreciably affected by pH. Tamblyn et al. *(142)* reported that acetic acid was the most effective in the scalder against firmly attached cells of *S.* Typhimurium; however, its practical use may be limited by the production of undesirable carcass characteristics. Bleaching and off odors were observed when skin samples were treated with ≥2% of acetic, citric, lactic, malic, mandelic, propionic, and tartaric acids *(142, 143)*.

Spray or immersion antimicrobial applications for broiler carcasses on-line are a common method to control pathogen levels during processing. Eighty-six percent of 100

broiler processing plants surveyed in the U.S. used on-line antimicrobial systems as a *Salmonella* intervention *(120)*. While some consider spray application of carcasses more effective compared to immersion treatments *(47)*, others prefer immersion in antimicrobial tanks to ensure a contact of the antimicrobial agent with all parts of carcass *(28)*. Additionally, a high pressure spray may force bacteria into the skin and subsequently promote cross-contamination *(28)*.

Chilling is often regarded as a common site for *Salmonella* cross-contamination due to the commingling a large number of carcasses in one communal tank. Expectedly, it is also the most commonly used site for antimicrobial interventions (93%) in broiler processing plants in the U.S. *(120)*. Although a longer contact time of antimicrobial to carcass is associated with chilling compared to contact times at other antimicrobial treatment locations, many researchers noted that antimicrobial activity of organic acids decreased at low temperatures *(22)*. However, Tamblyn et al. *(142)* found that 5% acetic acid was more effective against *S*. Typhimurium loosely attached cells in the chiller application (0°C/60min) compared to dipping (23°C/15sec) or scalding (50°C/2min). Although some organic acids showed effectiveness in reducing microbial contamination, they are not practical to use due to unfavorable changes in texture and sensorial characteristics of the carcasses. For example, lactic acid added to chill water resulted in the development of brown pigmentation, while acetic acid made poultry skin hard and leathery *(45)*.

Recently, establishment of antimicrobial immersion dip tanks at post-chill sites has gained popularity as a final line of defense against pathogens before entering further

processing or packing *(120)*. In 2006, 12% of 100 surveyed broiler processing plants were using post-chill interventions to control the *Salmonella* rate in the broilers *(120)*. Morris & Associates *(97)* manufactures finishing chill tanks that permit carcass immersion for 20 to 40 sec residence times allowing for a higher concentration of antimicrobial to be used in an effort to reduce pathogens to a minimum before further processing.

Determining which of the *Salmonella* interventions in the U.S. broiler industry are the most effective can be difficult for facilities because of the many variables that can affect performance. For instance, Byrd and McKee *(28)* emphasized that the effectiveness of various treatments depended on initial *Salmonella* load and its degree of attachment to the skin. Thus, production of a pathogen-free product cannot be guaranteed under current production conditions. However, incorporation of intervention steps during slaughter procedures can control microbial levels during processing and reduce the pathogen prevalence in the final product. Multiple interventions have been shown to be even more effective, but tremendous attention must be paid to the entire process. The advantage associated with antimicrobial interventions at scalding, chilling, and on-line immersion dipping is the ability to reduce bacterial contamination on the entire carcass surface during several processes throughout the operation *(31)*.

THE USE OF WEAK ORGANIC ACIDS IN POULTRY PROCESSING

Introduction

Weak organic acids, such as acetic, lactic, propionic, benzoic, and citric, have a long-standing history as effective food preservatives in ensuring food safety. The effectiveness of washes, sprays, scalders, and chillers containing organic acids have been extensively studied and evaluated as a means to reduce the levels of bacteria during poultry processing. Weak organic acids can reduce the numbers of pathogenic and spoilage microorganisms on meat by 1 to 3.5 log/g extending shelf life by 7 to 17 days, respectively *(70)*. However, not all weak organic acids are equally effective. Effectiveness depends upon method of application, concentration, time, temperature, menstruum, nature of target microorganism, and strength of microbial attachment to the meat surface prior to treatment *(21)*. This variation of factors may explain to some extent the numerous, seemingly contradictory, theories and publications concerning the antimicrobial actions of organic acids.

USDA/FSIS maintains a comprehensive listing of safe and suitable antimicrobials and their uses in Directive 7020.1 for meat, poultry, and egg products *(146)*. For example, weak organic acids, including lactic, citric, and acetic acid, are approved for use as a part of a carcass wash when applied at concentrations up to 2.5% *(146)*. Levels are generally dictated by four major considerations: minimum concentration to achieve desired effect, cost, antimicrobial activity, and the effect on organoleptic properties of the product.

Antimicrobial effect of weak organic acids

The effect of any weak organic acid depends on three factors: (i) the effect of pH, (ii) the extent of dissociation of the acid, and (iii) a specific effects related to a particular acid molecule *(70).*

The changes in the external pH have only a partial effect on the internal pH of microorganisms. Cells are able to maintain cytoplasmic pH near neutrality due to buffering capacity of the cytoplasm and limited influx of protons from the external medium into the microbial cell. However, lowering the external pH significantly is likely to cause some decrease in internal pH which leads to failure to maintain homeostasis and microbial cell death *(90)*. Sorrells et al. *(139)* reported that at the same pH values the antimicrobial activity of the following acids was: acetic > lactic > citric > malic.

Another factor in determining the antimicrobial effectiveness of weak organic acids is the concentration of the undissociated acid. Eklund *(51)* calculated that the inhibitory action of undissociated benzoic acid was 15 to 290 times greater than that of dissociated acid, when bacteria were grown in a medium acidified to pH 4.6 with benzoic acid. The extent of dissociation of weak acids depends on both pK_a and pH. If the external pH is lower than the pK_a, more than 50% of the acid will be in the undissociated form. The undissociated form of acids, due to lack of charge and hydrophobicity, are better able to diffuse through the cell membrane of bacteria *(90)*. Additionally, Casal et al. *(29)* showed that some acids, like acetic acid, can be transported across the cell membrane by inducible permeases. While inside of the cell, the protonated acid dissociates into anions, which accumulate in the cytoplasm and

cause acidification of the cytoplasm *(141)*. The magnitude of internal pH change determines the capability of cells to survive by metabolizing protons or ejecting them using proton pumps *(21)*. However, even a slight change in cytoplasmic pH may impact cellular metabolism by causing protein deformation and enzyme inhibition which could subsequently lead to a depletion of cellular ATP. Low internal pH may also inhibit metabolism by losing anionic metabolites from the cell, interrupting cellular signaling, neutralizing the electrochemical gradient which is essential for transport of many critical nutrients, and/or reducing the rate of synthesis of toxic molecules *(21)*.

The combined effect of pH and the level of undissociated molecules of the organic acids is sufficient to inhibit microbial growth or reduce the number of viable cells, but some acids may also exhibit an additional effect on cell activity *(21)*. For instance, citric acid is a good chelating agent for calcium and transition-metal ions *(58)*, while inhibition by lactate is partially attributed to chelation of ferric ions *(131)*. Organic acids could also act as uncouplers of oxidative phosphorylation, proton ionophores, or cause damage through increased membrane fluidity *(6)*. The antimicrobial efficacy of saturated fatty acids increases with increasing fatty acid chain length from C1 to C22 *(21)*.

However, not all weak organic acids exhibit comparable antimicrobial activity, including those that have similar pK_a values *(22)*. Warth *(156)* suggested that the degree of toxicity of acids may correlate with its transportation rate; for instance, benzoic acid is transported in 27 times faster than propionic acid. The transportation rate at which organic

acid enters the cell determines the energy requirement to maintain a lowered intracellular acid concentration in the cytoplasm. *(156).*

There is no single action that can account for inhibition by all acids. The antimicrobial action for weak organic acids may differ with environmental conditions or with the target microorganism. However, for all weak organic acids, undissociated molecules penetrate bacterial cell membranes and dissociate subsequently reducing intracellular pH, resulting in inhibition of growth or/and lethality.

Mechanisms of bacterial acid resistance

The ability of *Salmonella* to adapt and survive in high acid conditions (<4.0 pH) has been recognized and can be an important factor in food safety. Although much is known about the molecular responses of salmonellae to environmental stresses, the mechanisms involved in *Salmonella* survival upon exposure to some weak organic acids are addressed in this study by comparing wild type *S.* Typhimurium strains LT2 and UK1 versus their acid sensitive mutants during simulated poultry processing intervention treatments.

It is well established that *Salmonella* survival at harsh, acidic environment can be enhanced either by prior growth at mild or moderately low pH or by growth into the stationary phase *(21).* In general, logarithmically growing *Salmonella* cells are very sensitive to acid stress. However, logarithmic phase cells grown in E glucose medium (pH 7.7) and exposed to pH 5.8 prior to pH 3.3 acid challenge, adjusted with hydrochloric acid, survived the extreme acidic conditions in 5,000 times better than unadapted cells *(53).*

Acid survival of logarithmically grown salmonellae is attributed to the pH-dependent acid tolerant response (ATR). Log phase ATR is characterized as a two-stage system with some acid shock proteins (ASPs) induced at pH 5.8 (pre-acid shock) and others induced after a shift to pH 4.5 or below (post-acid shock). The pre-acid shock proteins induce a pH homeostasis which maintains the internal pH when housekeeping homeostasis systems fail *(90)*. The pH-inducible amino acid decarboxylases play an important role in surviving acid stress (pH 3.0) by decarboxylating amino acids available in a complex media (e.g. Luria-Bertani medium), forming a highly basic amine which is then secreted into the medium and causes pH to rise *(110)*. Additionally, several studies noted that arginine decarboxylase and glutamate decarboxylase may contribute to increased acid resistance of acid adapted cells *(74, 89)*. The ability of *S.* Typhimurium to survive at <pH 4.0 is dependent on the type of acid *(10)*. The order of acids in inducing the ATR in *S.* Typhimurium, grown in brain heart infusion broth acidified to pH 4.5, was citric > acetic > lactic > malic > hydrochloric > ascorbic *(10)*, which can be explained by the various abilities of organic acids to diffuse through the cell membrane *(59)*. This finding may have a significant impact on food safety because citric, acetic, and lactic acids are the most commonly used organic acids in carcass antimicrobial intervention treatments. Furthermore, a sustained ATR provides cross-resistance to other stresses such as heat *(82, 86)*, osmotic *(80, 82, 86, 145)*, and oxidative stress *(80, 82)*, as well as to ionizing radiation *(25)*. Several genes associated with the log phase ATR have been identified. Mutations in *atp* (Mg(II)-dependent proton-translocating ATPase) and *fur* (ferric uptake regulator) genes render the logarithmical cells very acid

sensitive because of defects in expression of ASPs in the pre- or post-acid shock stages of log phase ATR *(83)*.

Transition of *Salmonella* into stationary phase growth is associated with increased resistance to various stresses, including low pH conditions. *S.* Typhimurium (LT2) cells grown to stationary phase in minimal glucose medium were 1,000-fold more resistant to pH 3.0 than logarithmical cells after 1 hour of exposure *(83)*. This general stress resistance feature is dependent on the σ^s and does not require induction by low pH. Several researchers found that resistance of stationary phase cells upon exposure to low pH may relate to increased synthesis of saturated fatty acids and cyclopropane fatty acids *(10, 23)* which subsequently result in *S.* Typhimurium cells with decreased membrane fluidity *(11)*. Apparently, cells can regulate their lipid composition to achieve a degree of fluidity to cope with environmental conditions.

Furthermore, stationary phase cells also exhibit a *rpoS* independent ATR system which is functional when the cells are shifted to pH 4.5 or below *(55)*. The stationary phase ATR is characterized by the synthesis of 15 ASPs, which are distinct from the log phase proteins *(83)*. Foster *(54)* reported that acid shock at pH 4.3 for 20 to 30 min provides tolerance to pH 3.3; however, acid shock for 60 min or longer provides no protection. This phenomenon is called transiently induced ATR. In contrast to the log phase ATR, stationary phase ATR exhibited a sustained induction over the course of several hours at pH 4.3 *(160)*. The genetic difference responsible for a sustained versus transient ATR effect is traced to a mutation in the σ^s *(55)*.

Overall, the ability of *Salmonella* cells to withstand low pH environments depends on several complex systems and may be an important factor in permitting this foodborne pathogen to survive in foods and cause illness. The ATR is a complex inducible phenomenon in which exposure to moderately low pH produces a stress response capable of protecting the organism against more severe acidic challenges.

FUMARIC ACID

Introduction

Fumaric acid ($C_4H_4O_4$) is an unsaturated dicarboxylic acid, with two dissociation constants: pK_{a1} = 3.03, pK_{a2} = 4.54 (Appendix 6). Fumaric acid occurs in many plants, such as camellia flowers (*C. japonica* L.) *(76)*, *Fumariaceae* (*Fumaria officinalis* L.), *Boletaceae* (*Boletus scaber* Bull.), and *Polyporaceae* (*Fomes igniarius* Kickx.) *(161)*. Fumaric acid is commonly used in fruit drinks as an acidulant, in gelatin desserts as a thickening agent, in wines as an inhibitor of malo-lactic fermentation *(111)*, and in dairy-based products as antioxidant *(48)*.

Fumaric acid is a stronger weak organic acid than citric, malic, lactic, phosphoric, or tartaric acids *(15)* (Appendix 7), which makes it cost effective when compared to these other organic acids *(113)*. Isegen South Africa (PTY) Ltd. states that "fumaric acid cuts food acid cost as it can replace citric, malic, and tartaric acids in the ratio of 2:3" *(69)*. Unfortunately, the inhibitory effect of fumaric acid is limited by its low water solubility, 0.63% w/w at 25°C *(161)*. By using Lange and Sinks equation *(81)* (Appendix 8), the maximum calculated concentration that can be achieved at 53°C is 1.78% w/w and 0.26% w/w at 3°C.

Toxicology and regulatory status

Fumaric acid is designated as generally recognized as safe (GRAS) and it has been approved for human consumption under 21 CFR 172.350. The major food groups contributing to dietary intake of fumaric acid are desserts and sugar confectionery with the

maximum permitted level of 4,000 mg/kg being allowed in dry powdered dessert mixes, gel-like or fruit flavored desserts *(92)*. The acceptable daily intake (ADI) of fumaric acid for humans is up to 6 mg/kg body weight per day *(48)* and the proposed LD_{50} for fumaric acid is 8,000 mg/kg *(85)*.

Antimicrobial properties

Fumaric acid is effective in preserving foods by reducing microbiological spoilage and by increasing microbiological safety. Comes and Beelman *(40)* investigated the effect of fumaric acid against *E. coli* O157:H7 inoculated into unpreserved apple cider and found that fumaric acid was 2.3 log more bactericidal than malic, citric, or hydrochloric acid in pH 3.3 after a mild heat treatment (45°C, 20 min) and storage for 48 hrs at 4°C. More than a 5-log reduction of *E. coli* O157:H7 was obtained by treatment with 0.15% fumaric acid coupled with 0.05% sodium benzoate followed by holding for 6 hrs at 25ºC and 24 hrs at 4ºC. Additionally, total aerobic counts were reduced to levels below those typically achieved through pasteurization *(40)*.

Fumaric acid was proven more effective than lactic or acetic acid when used as an antimicrobial dip for meat products *(112-114)*. More specifically, when applied at concentrations of 1.0% for 30 sec at 55ºC to the surface of lean beef, levels of *L. monocytogenes, E. coli* O157:H7, and *S.* Typhimurium were reduced by 1.61, 1.38, and 1.51 log, respectively *(112)*. In another study, 0.5% fumaric acid treatment applied for 1 min decreased level of *S.* Typhimurium inoculated on turkey breast fillets by 2.5 log during 2 hrs

of refrigerated storage, without adversely affecting the color *(17)*. Fumaric acid was also shown to be more fungicidal against *Talaromyces flavus* and *Neosartorya fischeri* ascospors than acetic, citric, malic, or tartaric acid *(20, 41)*. However, Mountney and O'Malley *(100)* found that chill water acidified to pH 2.5 with acetic acid was a more effective antimicrobial treatment than fumaric acid. Additionally, Hiwaki et al. *(64)* demonstrated that soaking eviscerated carcasses in 1.37% sodium chlorite at pH 3.0 for 30 min had stronger bactericidal effect than 0.6% fumaric acid.

A relationship has been observed in growth medium between the dissociation constant of the acid used to control pH and the minimum pH at which enteric pathogens initiate growth *(123)*. Thus, the minimum pH for initiation of growth of *S.* Anatum, *S.* Tennessee, and *S.* Senftenberg in a medium acidified with fumaric acid (pK_{a1} 3.03, pK_{a2} 4.54) was pH 4.30, while for acetic acid (pK_a 4.75) it was 5.40 *(38)*. Acetic acid (pH 5.0, 0.012M) also exhibited the strongest antimicrobial activity toward *E. coli* O157:H7 followed by lactic (pH 4.5, 0.006M), fumaric (pH 4.5, 0.004M), and citric (pH 4.0, 0.004M) acids in tryptic soy broth at 37°C *(106)*. In another study, Skrivanova et al. *(136)* showed that neither fumaric, succinic, malic, nor lactic acids at a minimum inhibitory concentration of 5 mg/ml inhibited growth of *E. coli*, *C. perfringens*, or *Salmonella* spp. However, some studies noted a synergistic effect of fumaric acid with other weak organic acids *(17, 113, 132)*. For instance, greater mesophilic and psychotrophic bacteria reductions on turkey breasts were observed when 0.5% fumaric acid was combined with 1.0% lactic acid, which resulted in reductions of 1.8 and 1.1 log, respectively *(17)*. Also, the addition of 0.3% sodium propionate

to 0.2% fumaric acid to Arabic bread extended the shelf life of the bread to 16 days compared to 3 days shelf-life of bread containing single fumaric acid treatment *(1)*.

The effect of fumaric acid as an antimicrobial was also examined on lettuce *(78)*, cabbage *(107)*, and broccoli sprouts *(77)*. Fumaric acid was more effective than sodium hypochlorite in reducing native microflora of cut lettuce, and resulted in a 1.4-log reduction of the initial population. A dip application of 50 mM fumaric acid was also effective against attached *S. aureus*, *E. coli* O157:H7, and *S.* Typhimurium, but the treatment caused browning of the lettuce. The combination of mild heat and 5 mM fumaric acid was similar in effectiveness to the 50 mM fumaric acid treatment, although browning was apparent even at this low concentration *(78)*. A 2-log reduction of *L. monocytogenes*, *E. coli* O157:H7, and *S. aureus* were observed on cabbage within 2 days of refrigerated storage after treatment with 50 mM fumaric acid *(107)*, and a synergistic effect was shown by combination of 0.5% fumaric acid with 50 ppm chlorine dioxide, which resulted in a 2.74- and 2.65-log reduction of *S.* Typhimurium and *L. monocytogenes* on broccoli sprouts, respectively *(77)*.

The contradictions among fumaric acid studies may be due to use of different strains, experimental parameters and product composition. Overall, findings demonstrate that fumaric acid is an effective treatment for antimicrobial interventions and its effectiveness may be enhanced by combination with other known antimicrobials. Factors such as change in products organoleptic properties, cost, and labeling requirements should be evaluated to determine the practical applicability of fumaric acid as an antimicrobial agent in foods.

Unfortunately, there is no data available regarding the residual activity of fumaric acid or its impact on aroma, color, taste, and texture of poultry products.

OXALIC ACID

Introduction

Oxalic acid ($C_2H_2O_4$) is saturated short chain dicarboxylic acid, with two dissociation constants: pK_{a1} = 1.27 and pK_{a2} = 4.28 (Appendix 9). Among weak organic acids, oxalic acid is considered as one of the strongest and comparatively most cost-effective. The cost of 100 g 99+% pure oxalic acid is about $2.00 cheaper than the cost for the same weight and quality fumaric acid (Fisher Scientific, Pittsburg, PA). Oxalic acid occurs naturally in many fruits and vegetables *(3, 63, 65, 96, 103, 121, 122, 126, 158)*, meats *(149, 150)*, and fungi *(5)*. It may be present as insoluble calcium or magnesium oxalate crystals or as soluble sodium or potassium oxalate *(135, 140)*. Oxalic acid content varies widely within and between plant species, and is dependent on season, soil conditions, maturity, and location in the plant anatomy *(103, 135)*. Plants containing high concentrations of oxalic acid include spinach (5.60 to 7.64 mg/g) *(157, 158)*, tea (1.50 to 6.80 mg/g) *(37)*, sesame seeds (1.50 mg/g) *(96)*, amaranth (5.98 mg/g) *(96)*, rhubarb (5.36 to 6.02 mg/g) *(158)*, sweet potato (1.03 to 1.10 mg/g) *(99)*, almonds (2.61 mg/g) *(96)*, and soy based foods (0.02 to 2.06 mg/g) *(3)*.

The presence of oxalic acid in plants does not affect plant growth, suggesting that the synthesis of oxalates may have a significant function by serving as a possible defense mechanism against predation or other adverse environmental conditions *(109)*.

Toxicology and regulatory status

Oxalic acid is currently not registered as GRAS due to potential negative health effects associated with the intake of high concentrations of the acid. Soluble oxalate is absorbed in humans by passive diffusion in the colon *(66)* or in the small intestine *(117)*. The normal oxalate absorption is 2.3 to 4.5% in adults *(134)*, and varies depending on dietary conditions and source *(50)*. The mean daily intake of oxalate in English diets ranges between 70 and 150 mg *(166)*. In a small study (5 subjects) in the U.S., Holmes and Kennedy *(65)* found that the mean daily intake of oxalic acid is 152 mg, which corresponded to the mean daily oxalate intake in a Rajasthan hospital diet (139.4 mg) *(134)*. According to Noonan and Savage *(103)*, 10 to 15 g of oxalic acid is the usual amount required to cause fatalities, and the predicted LD_{50} for oxalic acid in rats is 375 mg/kg *(162)*.

The potential negative health effects of oxalic acid are due to its ability to form insoluble salts with calcium and magnesium, thus decreasing the minerals absorption in digestive tract *(62, 99, 103, 128, 134)*. One recent study showed that oxalic acid does not impact iron absorption nor does it contribute to the reported inhibitory effect of spinach on iron availability *(140)*. Additionally, Welch et al. *(159)* observed that zinc absorption was not affected by oxalic acid added to the diets of zinc-deficient rats. Other potential adverse effects associated with oxalic acid consumption may include mouth irritation and increase risk of kidney stone formation *(91)*.

It should be pointed out that possible adverse health effects of residual oxalic acid may diminish on treated products. Anang and et al. *(12)* estimated that if chicken breast

retained up to 5 ml of a 0.5% solution of oxalic acid in which it was dipped, then the amount of retained acid would be 1,000 fold less than the extrapolated LD_{50} for a 150 lbs individual. Several authors showed a significant loss of oxalates during soaking, cooking or drying of taro leaves *(32, 127)*, cocoyams *(130)*, beans, chickpeas, lentils *(118)*, oca *(4)*, beetroot, carrot, and spinach *(128)*. Sefa-Dedeh and Ahyir-Sackey *(130)* showed that drum drying of cocoams reduces the oxalic acid content by 50% due to collapse of the calcium oxalate-containing cells under high temperatures following the breakdown of oxalate structure.

Moreover, specific oxalate-degrading bacteria, including *Oxalobacter formigenes*, in the human gastrointestinal tract regulate oxalate homeostasis by preventing absorption and/or metabolizing oxalic acid by decarboxylase enzymes to formate and CO_2 *(8, 105)*. According to Singh et al. *(134)*, orthophosphates and high quality proteins like methionine *(152)* in the diet exert a protective effect and maintain a low level of urinary oxalate.

Antimicrobial properties

Despite recent attention to oxalic acid as an anti-browning agent *(9)* or antioxidant *(163)*, little work has been done to evaluate its antimicrobial potential against pathogenic microorganisms in foods. Currently, the only research on oxalic acid in poultry was described in 2005, which found it effective in reducing the initial bacterial population and controlling growth of spoilage bacteria naturally occurring on raw chicken. Raw chicken breasts were dipped in 0.5 to 2.0% oxalic acid solutions for 10, 20, and 30 min and stored at 4°C for up to 14 days. At 10 min, the 0.5% oxalic acid treatment resulted in a 4.0-log

reduction in total plate counts and a 3.02-log reduction in *Enterobacteriaceae* counts after 14 days of storage. It also was shown that treatment with oxalic acid caused a slight darkening in color, and increased yellowness and redness of treated samples *(12)*.

In an vitro study, 0.3% oxalic acid inhibited *S.* Typhimurium growth within 160 sec of contact, and the time for its lethality was reduced to 20 sec when oxalic acid was supplemented with 0.1 to 0.3% fumaric acid *(132)*. Oxalic acid was also reported to block growth and aflatoxin biosynthesis in *Aspergillus flavus (116).*

Oxalic acid use in the poultry industry should be evaluated by taking into account its bactericidal potential against pathogenic microorganisms, cost, labeling requirements, and sensorial changes of treated carcasses, as well as the possible risks involved with consumption of residual oxalic acid. The effect of certain poultry processing operations on oxalates, such as washing and cooking with water, can substantially reduce the amount of residual oxalic acid through diffusion into the washing or cooking water *(13)*. This knowledge, to some extent, may reduce concerns about extensive oxalate formation after consumption and any subsequent dietary problems *(128)*.

REFERENCES

1. Abu-Ghoush, M., T. J. Herald, F. Dowell, F. Xie, F. M. Aramouni, and R. Madl. 2008. Effect of preservatives addition on the shelf-life extensions and quality of flat bread as determined by near-infrared spectroscopy and texture analysis. *Int. J. Food Sci. Tech.* 43:357-364.

2. Abu-Rawaida, A. S., W. N. Sawaya, B. H. Dashti, M. Murad, and H. A. Al-Othman. 1994. Microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait. *J. Food Prot*. 57:887-892.

3. Al-Wahsh, I. A., H. T. Horner, R. G. Palmer, M. B. Reddy, and L. K. Massey. 2005. Oxalate and phytate of soy foods. *J. Agric. Food Chem*. 53:5670-5674.

4. Albihn, P. B. E., and G. P. Savage. 2000. The effect of cooking on the location and concentration of oxalate in three cultivars of New Zealand-grown oca (*Oxalis tuberosa* Mol). *J. Sci. Food Agric*. 81:1027-1033.

5. Aletor, V. A. 1995. Compositional studies on edible tropical species of mushrooms. *Food Chem*. 54:265-268.

6. Alexandre, H., B. Mathieu, and C. Charpentier. 1996. Alteration in membrane fluidity and lipid composition, and modulation of H^+-ATPase activity in *Saccharomyces cerevisiae* caused by decanoic acid. *Microb*. 142:469-475.

7. Allen, V. M., M. H. Hinton, D. B. Tinker, C. Gibson, G. C. Mead, and C. M. Wathes. 2003. Microbial cross-contamination by airborne dispersion and contagion during defeathering of poultry. *British Poultry Sci*. 44:567-576.

8. Allison, M. J., H. M. Cook, D. B. Milne, S. Gallagher, and R. V. Clayman. 1986. Oxalate degradation by gastrointestinal bacteria from humans. *J. Nutr*. 116:455-460.

9. Altunkaya, A., and V. Gokmen. 2009. Effect of various anti-browning agents on phenolic compounds profile of fresh lettuce (*L. sativa*). *Food Chem*. 117:122-126.

10. Alvarez-Ordonez, A., A. Fernandez, A. Bernardo, and M. Lopez. 2009. Comparison of acids on the induction of an acid tolerance response in *Salmonella* Typhimurium, consequences for food safety. *Meat Sci*. 81:65-70.

11. Alvarez-Ordonez, A., A. Fernandez, M. Lopez, R. Arenas, and A. Bernardo. 2008. Modifications in membrane fatty acid composition of *Salmonella typhimurium* in response to growth conditions and their effect on heat resistance. *Int. J. Food Microb*. 123:212-219.

12. Anang, D. M., G. Rusul, S. Radu, J. Bakar, and L. R. Beuchat. 2006. Inhibitory effect of oxalic acid on bacterial spoilage of raw chilled chicken. *J. Food Prot*. 69:1913-1919.

13. Bailey, J. S., J. E. Thomson, and N. A. Cox. 1987. Contamination of poultry during processing. p. 193-206. *In* F.E. Cunningham, and N.A. Cox (ed.), The microbiology of poultry meat products. Academic Press, Inc., Orlando, FL.

14. Barbut, S. 2002. Poultry products processing: an industry guide. CRC Press, Boca Raton.

15. Bartek Ingredients Inc. Available at: http://www.bartek.ca/fumaric_spec.html. Accessed 12 December 2010.

16. Bauermeister, L. J., J. W. J. Bower, J. C. Townsend, and S. R. McKee. 2008. The microbial and quality properties of poultry carcasses treated with peracetic acid as antimicrobial treatment. *Poultry Sci*. 87:2390-2398.

17. Baysal, A. H. D., and A. Unluturk. 2005. Effects of organic acid and phosphates on the microbiological stability and on survival of *Salmonella typhimurium* on turkey breast meat. XVII European Symposium on the Quality of Poultry Meat, Doorwerth, The Netherlands.

18. Bell, C., and A. Kyriakides. 2002. Salmonella: a practical approach to the organism and its control in foods. Blackwell Science, London.

19. Benedict, R. C., F. J. Schultz, and S. B. Jones. 1991. Attachment and removal of *Salmonella* spp. on meat and poultry tissues. *J. Food Safety*. 11:135-148.

20. Beuchat, L. R. 1988. Influence of organic acids on heat resistance characteristics of *Talaromyces flavus* ascospores. *Int. J. Food Microb.* 6:97-105.

21. Booth, I. R., and M. Stratford. 2003. Acidulants and low pH. p. 25-47. *In* N.J. Russell, and G.W. Gould (ed.), Food Preservatives. Kluwer Academic/Plenum Publishers, New York, N.Y.

22. Brenner, F. W., R. G. Villar, F. J. Angulo, R. Tauxe, and B. Swaminathan. 2000. *Salmonella* nomenclature. *J. Clin. Microb*. 38:2465-2467.

23. Brown, J. L., T. Ross, T. A. McMeekin, and P. D. Nichols. 1997. Acid habituation of *Escherichia coli* and the potential role of cyclopropane fatty acids in low pH tolerance. *Int. J. Food Microb*. 37:163-173.

24. Bryan, F. L., and M. P. Doyle. 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. *J. Food Prot*. 58:326-344.

25. Buchanan, R. L., S. G. Edelson-Mammel, G. Boyd, and B. S. Marmer. 2004. Influence of acidulant identity on the effects of pH and acid resistance on the radiation resistance of *Escherichia coli* O157:H7. *Food Microb*. 21:51-57.

26. Buzby, J., and H. Farah. Date, 2006, Chicken consumption continues long rise. Available at: http://www.ers.usda.gov/amberwaves/april06/findings/chicken.htm. Accessed 30 March 2010.

27. Byrd, J. A., and S. R. McKee. 2005. Improving slaughter and processing technologies. p. 310-332. *In* G.C. Mead (ed.), Food safety control in the poultry industry. CRC Press, Boca Raton, FL.

28. Carraminana, J. J., J. Yanguela, D. Blanco, C. Rota, A. I. Agustin, A. Arino, and A. Herrera. 1997. *Salmonella* incidence and distribution of serotypes throughout processing in a Spanish poultry slaughterhouse. *J. Food Prot*. 60:1312-1317.

29. Casal, M., H. Cardoso, and C. Leao. 1996. Mechanisms regulating the transport of acetic acid in *Saccharomyces cerevisiae. Microb*. 142:1385-1390.

30. Cason, J. A., J. S. Bailey, N. J. Stern, A. D. Whittemore, and N. A. Cox. 1997. Relationship between aerobic bacteria, salmonellae, and *Campylobacter* on broiler carcasses. *Poultry Sci*. 76:1037-1041.

31. Castillo, A., M. D. Hardin, G. R. Acuff, and J. S. Dickson. 2002. Reduction of microbial contamination on carcasses. p. 351-382. *In* V.K. Juneja, and J.N. Sofos (ed.), Control of foodborne microorganisms Marcel Dekker, Inc., New York, NY.

32. Catherwood, D. J., G. P. Savage, S. M. Mason, J. J. C. Scheffer, and J. A. Douglas. 2007. Oxalate content of cormels of Japanese taro (*Colocasia esculenta* (L.) Schott) and the effect of cooking. *J. Food Comp. Anal*. 20:147-151.

33. CDC. 2007. *Salmonella* surveillance: Annual summary, 2005. Atlanta, Georgia: U. S. Department of Health and Human Services. Available at: http://www.cdc.gov/ncidod/ dbmd/phlisdata/salmonella.htm. Accessed 25 December 2010.

34. CDC. 2009. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food -- 10 states, 2008. *MMWR*. 58:333-337.

35. CDC. 2009. Surveillance for foodborne disease outbreaks -- United States, 2006. *MMWR*. 58:609-615.

36. CDC. 2010. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food -- 10 states, 2009. *MMWR*. 59:418-422.

37. Charrier, M. J. S., and G. P. Savage. 2002. Oxalate content and calcium binding capacity of tea and herbal tea. *Asia Pacific J. Clin. Nutr.* 11:298-301.

38. Chung, K. C., and J. M. Goepfert. 1970. Growth of *Salmonella* at low pH. *J. Food Sci.* 35:326-328.

39. Clouser, C. S., S. Doores, M. G. Mast, and S. J. Knabel. 1995. The role of defeathering in the contamination of turkey skin by *Salmonella* species and *Listeria monocytogenes. Poultry Sci*. 74:723-731.

40. Comes, J. E., and R. B. Beelman. 2002. Addition of fumaric acid and sodium benzoate as an alternative method to achieve a 5-log reduction of *Escherichia coli* O157:H7 populations in apple cider. *J. Food Prot*. 65:476-483.

41. Conner, D. E., and L. R. Beuchat. 1987. Heat resistance of ascospores of *Neosartorya fischeri* as affected by sporulation and heating medium. *Int. J. Food Microb*. 4:303-312.

42. Conner, D. E., M. A. Davis, and L. Zhang. 2001. Poultry-borne pathogens: plant considerations. p. 137-159. *In* A.R. Sams (ed.), Poultry Meat Processing. CRC Press, Boca Raton, FL.

43. Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, and L. H. Stanker. 1999. Survival of *Salmonella* in the crop contents of market-age broilers during feed withdrawal. *Avian Diseas*. 43:453-460.

44. Cox, N. A., L. J. Richardson, J. S. Bailey, D. E. Cosby, J. A. Cason, and M. T. Musgrove. 2005. Bacterial contamination of poultry as a risk to human health. p. 21-35. *In* G.C. Mead (ed.), Food safety control in the poultry industry. CRC Press, Boca Raton, FL.

45. Cox, N. A., L. J. Richardson, J. S. Bailey, D. E. Cosby, J. A. Cason, M. T. Musgrove, Russell, Research, Center, and G. C. Mead. 2005. Bacterial contamination of poultry as a risk to human health. p. 21-43. *In* G.C. Mead (ed.), Food safety control in the poultry industry. CRC Press, Boca Raton, FL.

46. D'Aoust, J. 2000. Salmonella. p. 1233-1300. *In* B.M. Lund, T.C. Baird-Parker, and G.W. Gould (ed.), The microbiological safety and quality of food, vol. II. Aspen Publishers, Inc., Gaithersburg, MD.

47. D'Aoust, J., and J. Maurer. 2007. *Salmonella* species. p. 187-236. *In* M.P. Doyle, and L.R. Beuchat (ed.), Food microbiology: fundamentals and frontiers. ASM Press, Washington, DC.

48. Doores, S. 1993. Organic acids. p. 95-136. *In* P.M. Davidson, and A.L. Branen (ed.), Antimicrobials in foods Marcel Dekker, Inc., New Yourk, NY.

49. Duarte, D., A. Ribeiro, A. Vasconcelos, S. Santos, J. Silva, P. Andrade, and L. Falcao. 2009. Occurrence of *Salmonella* spp. in broiler chicken carcasses and their susceptibility to antimicrobial agents. *Brazil. J. Microb.* 40:569-573.

50. Earnest, D. L., H. E. Williams, and W. H. Admirand. 1975. A physico-chemical basis for treatment of enteric hyperoxaluria. *Trans. Assoc. Amer. Physicians*. 88:224-234.

51. Eklund, T. 1985. Inhibition of microbial growth at different pH levels by benzoic and propionic acids and esters of *p*-hydroxybenzoic acid. *Int. J. Food Microb*. 2:159-167.

52. Foley, S. L., A. M. Lynne, and R. Nayak. 2008. *Salmonella* challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. *J. Anim. Sci.* 86:E149-E162.

53. Foster, J. W. 1991. *Salmonella* acid shock proteins are required for the adaptive acid tolerance response. *J. Bact*. 173:6896-6902.

54. Foster, J. W. 1993. The acid tolerance of *Salmonella typhimurium* involves transient synthesis of key acid shock proteins. *J. Bact.* 175:1981-1987.

55. Foster, J. W. 1995. Low pH adaptation and the acid tolerance response of *Salmonella typhimurium. Critic. Rev. Microb*. 21:215-237.

56. Geornaras, I., A. E. Jesus, E. Zyl, and A. Holy. 1997. Bacterial populations of different sample types from carcasses in the dirty area of a South African poultry abattoir. *J. Food Prot.* 1997:551-554.

57. Goksoy, E. O., S. Kirkan, and F. Kok. 2004. Microbiological quality of broiler carcasses during processing in two slaughterhouses in Turkey. *Poultry Sci*. 83:1427-1432.

58. Graham, A. F., and B. M. Lund. 1986. The effect of citric acid on growth of proteolytic strains of *Clostridium botulinum. J. Appl. Bact*. 61:39-49.

59. Greenacre, E. J., T. F. Brocklehurst, C. R. Waspe, D. R. Wilson, and P. D. G. Wilson. 2003. *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* acid tolerance

response induced by organic acids at 20C: optimization and modeling. *Appl. Env. Microb*. 69:3945-3951.

60. Hargis, B. M., D. J. Caldwell, R. L. Brewer, D. E. Corrier, and J. R. DeLoach. 1995. Evaluation of the chicken crop as a source of *Salmonella* contamination of broiler carcasses. *Poultry Sci*. 74:1548-1552.

61. Hargis, B. M., D. J. Caldwell, and J. A. Byrd. 2001. Microbiological pathogens: live poultry considerations. p. 121-137. *In* A.R. Sams (ed.), Poultry meat processing. CRC Press, Boca Raton, FL.

62. Heaney, R. P., C. M. Weaver, and R. R. Recker. 1988. Calcium absorbability from spinach. *Amer. J. Clin. Nutr*. 47:707-709.

63. Hernandez Suarez, M., E. Rodriguez Rodriquez, and C. Diaz Romero. 2008. Analysis of organic acid content in cultivars of tomato harvested in Tenerife. *Eur Food Res Technol*. 226:423-435.

64. Hiwaki, H., M. Tsubakimoto, K. Kubokura, Y. Kurihara, and T. Oda. 1995. *Salmonella* contamination in livestock 'sashimi' (sliced raw meat and edible organs) and bacterial control of the 'sashimi' materials. p. 51-58. Annual report, vol. 20. Institute of Public Health, Fukuoka city.

65. Holmes, R. P., and M. Kennedy. 2000. Estimation of oxalate content of foods and daily oxalate intake. *Kidney Int*. 57:1662-1667.

66. Hughes, J., and R. W. Norman. 1992. Diet and calcium stones. *Can. Med. Assoc. J*. 146:137-143.

67. Humphrey, T. J., D. G. Lanning, and D. Beresford. 1981. The effect of pH adjustment on the microbiology of chicken scald-tank water with particular reference to the death rate of Salmonellas. *J. Appl. Bact*. 51:517-527.

68. Humphrey, T. J., G. C. Mead, and B. Rowe. 1988. Poultry meat as a source of human salmonellosis in England and Wales. *Epidemiol. Inf.* 100:175-184.

69. Isogen South Africa (PTY) LTD. Available at: http://www.isegen.co.za/Content/ products/fumaric.html. Accessed 12 December 2010.

70. James, C. 2002. New developments in decontaminating raw meat. p. 259-282. *In* J. Kerry, J. Kerry, and D. Ledward (ed.), Meat processing: improving quality. CRC Press, Boca Raton, FL.

71. James, W. O., R. L. Brewer, J. C. Prucha, W. O. Williams, and D. R. Parham. 1992. Effect of chlorination of chill water on the bacteriologic profile of raw chicken carcasses and giblets. *JAVMA*. 200:60-63.

72. James, W. O., W. O. Williams, J. C. Prucha, R. Johnson, and W. Christensen. 1992. Profile of selected bacterial counts and *Salmonella* prevalence on raw poultry in a poultry slaughter establishment. *JAVMA*. 200:57-59.

73. Janda, J. M., and S. L. Abbott. 2005. The enterobacteria. ASM Press, Washington, DC.

74. Kieboom, J., and T. Abee. 2006. Arginine-dependent acid resistance in *Salmonella enterica* serovar Typhimurium. *J. Bact*. 188:5650-5653.

75. Kim, J.-W., M. F. Slavik, C. L. Griffis, and J. T. Walker. 1993. Attachment of *Salmonella typhimurium* to skins of chicken scalded at various temperatures. *J. Food Prot*. 56:661-665.

76. Kim, K. Y., P. M. Davidson, and H. J. Chung. 2001. Antibacterial activity in extracts of *Camellia japonica* L. petals and its application to a model food system. *J. Food Prot*. 64:1255-1260.

77. Kim, Y. J., M. H. Kim, and K. B. Song. 2009. Efficacy of aqueous chlorine dioxide and fumaric acid for inactivating pre-existing microorganisms and *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on broccoli sprouts. *Food Control*. 20:1002-1005.

78. Kondo, N., M. Murata, and K. Isshiki. 2006. Efficiency of sodium hypochlorite, fumaric acid, and mild heat in killing native microflora and *Escherichia coli* O157:H7, *Salmonella* Typhimurium DT104, and *Staphylococcus aureus* attached to fresh-cut lettuce. *J. Food Prot*. 69:323-329.

79. Kotula, K. L., and Y. Pandya. 1995. Bacterial contamination of broiler chickens before scalding. *J. Food Prot*. 58:1326-1329.

80. Kwon, Y. M., and S. C. Ricke. 1998. Induction of acid resistance of *Salmonella typhimurium* by exposure to short-chain fatty acids. *Appl. Env. Microb*. 64:3458-3463.

81. Lange, N. A., and M. H. Sinks. 1930. The solubility, specific gravity and index of refraction of aqueous solutions of fumaric, maleic and *i*-malic acids *J. Am. Chem. Soc*. 52:2602-2604.

82. Lee, I. S., J. Lin, H. K. Hall, B. Bearson, and J. W. Foster. 1995. The stationary-phase sigma factor σ^s (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella typhimurium. Mol. Microb*. 17:155-167.

83. Lee, I. S., J. L. Slonczewski, and J. W. Foster. 1994. A low-pH-inducible, stationary-phase acid tolerance response in *Salmonella typhimurium. J. Bact*. 176:1422-1426.

84. Lestari, S. I., F. Han, F. Wang, and B. Ge. 2009. Prevalence and antimicrobial resistance of *Salmonella* serovars in conventional and organic chickens from Louisiana retail stores. *J. Food Prot*. 72:1165-1172.

85. Levey, S., A. G. Lasichak, R. Brimi, J. M. Orten, C. J. Smith, and A. H. Smith. 1946. A study to determine the toxicity of fumaric acid. *J. Am. Pharm. Assoc.* 35:298-304.

86. Leyer, G. J., and E. A. Johnson. 1993. Acid adaptation induces cross-protection against environmental stresses in *Salmonella* Typhimurium. *Appl. Env. Microb*. 59:1842-1847.

87. Lillard, H. S. 1989. Factors affecting the persistence of *Salmonella* during the processing of poultry. *J. Food Prot*. 52:829-832.

88. Lillard, H. S. 1989. Incidence and recovery of salmonellae and other bacteria from commercially processed poultry carcasses at selected pre- and post-evisceration steps. *J. Food Prot*. 52:88-91.

89. Lin, J., M. P. Smith, K. C. Chapin, H. S. Baik, G. N. Bennett, and J. W. Foster. 1996. Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli. Appl. Env. Microb*. 62:3094-3100.

90. Lund, B. M., and T. Eklund. 2000. Control of pH and use of organic acids. p. 175-199. *In* B.M. Lund, T.C. Baird-Parker, and G.W. Gould (ed.), The microbiological safety and quality of food, vol. I. Aspen Publishers, Inc, Gaithersburg, MD.

91. Massey, L. K., and R. A. L. Sutton. 1993. Modification of dietary oxalate and calcium reduces urinary oxalate inhyperoxaluric patients with kidney stones. *J. Am. Diet. Assoc*. 93:1305-1307.

92. May, K. N. 1974. Changes in microbial numbers during final washing and chilling of commercially slaughtered broilers. *Poultry Sci*. 53:1282-1285.

93. Mead, G. C. 2000. Fresh and further-processed poultry. p. 445-471. *In* B.M. Lund, T.C. Baird-Parker, and G.W. Gould (ed.), The microbiological safety and quality of food, vol. I. Aspen Publishers, Inc., Gaithersburg, MD.

94. Mead, G. C. 2004. Microbial hazards in production and processing. p. 232-257. *In* G.C. Mead (ed.), Poultry meat processing and quality. CRC Press, Boca Raton, FL.

95. Mead, G. C., W. R. Hudson, and M. H. Hinton. 1994. Use of a marker organism in poultry processing to identify sites of cross-contamination and evaluate possible control measures. *British Poultry Sci*. 35:345-354.

96. Meena, B. A., K. Padma, N. Pankaja, and J. Prakash. 1987. Soluble and insoluble oxalates in selected foods. *J. Food Sci. Technol*. 24:43-44.

97. Morris and Associates. Available at: http://www.morris-associates.com/page/home_alt. Accessed 5 April 2010.

98. Morris, G. K., and J. G. Wells. 1970. *Salmonella* contamination in poultry-processing plant. *J. Appl. Microb*. 19:795-799.

99. Mosha, T. C., H. E. Gaga, R. D. Pace, H. S. Laswai, and K. Mtebe. 1995. Effect of blanching on the content of antinutritional factors in selected vegetables. *Plant Foods Hum. Nutr*. 47:361-367.

100. Mountney, G. J., and J. O'Malley. 1965. Acids as poultry meat preservatives. *Poultry Sci.* 44:582.

101. Mulder, R. W. A. W., L. W. J. Dorresteijn, and J. Van Der Broek. 1978. Cross-contamination during the scalding and plucking of broilers. *British Poultry Sci*. 19:61-70.

102. National Advisory Committee on Microbiological Criteria for Foods. 1997. Generic HACCP application in broiler slaughter and processing. *J. Food Prot.* 60:579-604.

103. Noonan, S. C., and G. P. Savage. 1999. Oxalate content of foods and its effect on humans. *Asia Pacific J. Clin. Nutr*. 8:64-74.

104. Notermans, S., and E. H. Kampelmacher. 1975. Heat destruction of some bacterial strains attached to broiler skin. *British Poultry Sci*. 16:351-361.

105. Ogawa, Y., T. Miyazato, and T. Hatano. 2000. Oxalate and urinary stones. *World J. Surgery*. 24:1154-1159.

106. Oh, D.-H., J.-H. Park, and B.-K. Park. 2000. Effect of organic acids on the survival of *Escherichia coli* O157:H7. *J. Food Sci. Nutr.* 5:131-135.

107. Ohsone, M., K. Kaneko, H. Hayashidani, T. Takahashi, and M. Ogawa. 1999. Growth and inhibition by acids of five species of pathogenic bacteria inoculated in salad vegetables. *J. Food Hyg. Soc. Japan*. 40:297-303.

108. Okrend, A. J., R. W. Johnston, and A. B. Moran. 1986. Effect of acetic acid on the death rates at 52C of *Salmonella* Newport, *Salmonella* Typhimurium and *Campylobacter jejuni* in poultry scald water. *J Food Prot*. 49:500-503.

109. Palaniswamy, U. R., B. B. Bible, and R. J. McAvoy. 2004. Oxalic acid concentrations in Purslane (*Portulaca oleraceae* L.) is altered by the stage of harvest and the nitrate to ammonium ratios in hydroponics. *Scientia Horticulturae*. 102:267-275.

110. Park, Y.-K., B. Bearson, S. H. Bang, I. S. Bang, and J. W. Foster. 1996. Internal pH crisis, lysine decarboxylase and the acid tolerance response of *Salmonella typhimurium. Mol. Microb*. 20:605-611.

111. Pilone, G. J., B. C. Rankine, and D. A. Pilone. 1974. Inhibiting malo-lactic fermentation in Australian dry red wines by adding fumaric acid. *Amer. J. Enol. Viticult.* 25:99-107.

112. Podolak, R. K., J. F. Zayas, C. L. Kastner, and D. Y. C. Fung. 1995. Reduction of *Listeria monocytogenes, Escherichia coli O157:H7,* and *Salmonella typhimurium* during storage on beef sanitized with fumaric, acetic, and lactic acids. *J. Food Safety*. 15:283-290.

113. Podolak, R. K., J. F. Zayas, C. L. Kastner, and D. Y. C. Fung. 1996. Inhibition of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on beef by application of organic acids. *J. Food Prot*. 59:370-373.

114. Podolak, R. K., J. F. Zayas, C. L. Kastner, and D. Y. C. Fung. 1996. Reduction of bacterial populations on vacuum-packaged ground beef patties with fumaric and lactic acids. *J. Food Prot*. 59:1037-1040.

115. Popoff, M. Y., J. Bockemuhl, and F. W. Brenner. 2000. Supplement 1998 (no. 42) to the Dauffmann-White scheme. *Res. Microbiol*. 151:63-65.

116. Prasad, G., S. Sahay, and A. Masood. 1994. Inhibition in aflatoxin biosynthesis by hte extract of *Amorphophallus campanulatus* (OL) and calcium oxalate. *Lett. Appl. Microb*. 18:203-205.

117. Prenen, J. A. C., P. Boer, and M. J. D. Mees. 1984. Absorptin kinetics of oxalate from oxalate-rich food in man. *Amer. J. Clin. Nutr*. 40:1007-1010.

118. Quinteros, A., R. Farre, and M. J. Lagarda. 2003. Effect of cooking on oxalate content of pulses using an enzymatic procedure. *Int. J. Food Sci. Nutr*. 54:373-377.

119. Reiter, M. G., M. L. Fiorese, G. Moretto, M. C. Lopez, and R. Jordano. 2007. Prevalence of *Salmonella* in poultry slaughterhouse. *J. Food Prot*. 70:1723-1725.

120. Rice, J. T. Date, 2006, *Salmonella* interventions in the U. S. broiler industry. Available at: http://www.fsis.usda.gov/PDF/Slides_022406_EKrushinskie.pdf. Accessed 5 April 2010.

121. Ritter, M. M. C., and G. P. Savage. 2007. Soluble and insoluble oxalate content of nuts. *J. Food Comp. Anal*. 20:167-174.

122. Ross, A. B., G. P. Savage, R. J. Martin, and L. Vanhanen. 1999. Oxalates in Oca (New Zealand Yam) (*Oxalis tuberosa* Mol.). *J. Agric. Food Chem*. 47:5019-5022.

123. Rosso, L., E. Zuber, C. Pichat, and J. P. Flandrois. 1997. Simple relationship between acid dissociation constant and mimimal pH for microbial growth in laboratory medium. *Int. J. Food Microb.* 35:75-81.

124. Sams, A. R. 2001. First processing: slaughter through chilling. p. 19-35. *In* A.R. Sams (ed.), Poultry meat processing CRC Press, Boca Raton, FL.

125. Sarlin, L. L., E. T. Barnhart, D. J. Caldwell, R. W. Moore, J. A. Byrd, D. Y. Caldwell, D. E. Corrier, J. R. Deloach, and B. M. Hargis. 1998. Evaluation of alternative sampling methods for *Salmonella* critical control point determination at broiler processing. *Poultry Sci*. 77:1253-1257.

126. Savage, G. P. 2002. Oxalates in human foods. *Proceedings Nutr. Soc. New Zealand*. 27:4-24.

127. Savage, G. P., and M. Dubois. 2006. The effect of soaking and cooking on the oxalate content of taro leaves. *Int. J. Food Sci. Nutr*. 57:376-381.

128. Savage, G. P., L. Vanhanen, S. M. Mason, and A. B. Ross. 2000. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. *J. Food Comp. Anal* 13:201-206.

129. Scharff, R. L., J. McDowell, and L. Medeiros. 2009. Economic cost of foodborne illness in Ohio. *J. Food Prot*. 72:128-136.

130. Sefa-Dedeh, S., and E. K. Agyir-Sackey. 2004. Chemical composition and the effect of processing on oxalate contet of cocoyam *Xanthosoma sagittifolium* and *Colocasia esculenta* cormels. *Food Chem*. 84:479-487.

131. Shelef, L. A. 1994. Antimicrobial effects of lactates: a review. *J. Food Prot*. 57:445-450.

132. Shimizu, T., T. Takahata, and M. Kato. 1995. Antibacterial activity of several organic acids commonly used as food additives. *J. Food Hyg. Soc. Japan*. 36:50-54.

133. Shivaprasad, H. L. 2003. *Salmonella* infections: Pullorum disease and fowl typhoid. p. 567-615. *In* Y.M. Saif, et al. (ed.), Diseases of poultry Blackwell Publishing Professional., Ames, IA.

134. Singh, P. P., L. K. Kothari, D. C. Sharma, and S. N. Saxena. 1972. Nutritional value of foods in relation to their oxalic acid content. *Amer. J. Clin. Nutr.* 25:1147-1152.

135. Singh, P. P., and S. N. Saxena. 1972. Effect of maturity on the oxalate and cation contents of six leafy vegetables. *Indian J. Nutr. Diet*. 9:269-276.

136. Skrivanova, E., M. Marounek, V. Benda, and P. Brezina. 2006. Susceptibility of *Escherichia coli*, *Salmonella* sp. and *Clostridium perfringens* to organic acids and monolaurin. *Veterinarni Medicina*. 51:81-88.

137. Slavik, M. F., J.-W. Kim, and J. T. Walker. 1995. Reduction of *Salmonella* and *Campylobacter* on chicken carcasses by changing scalding temperature. *J. Food Prot*. 58:689-691.

138. Sockett, P. N., and E. C. D. Todd. 2000. The economic costs of foodborne disease. p. 1563-1584. *In* B.M. Lund, T.C. Baird-Parker, and G.W. Gould (ed.), The microbiological safety and quality of food, vol. II. Aspen Publishers, Inc., Gaithersburg, MD.

139. Sorrells, K. M., and D. C. Enigl. 1990. Effect of pH, acidulant, sodium chloride and temperature on the growth of *Listeria monocytogenes. J. Food Safety*. 11:31-37.

140. Storcksdieck gennant Bonsmann, S., T. Walczyk, S. Renggli, and R. F. Hurrell. 2008. Oxalic acid does not influence nonhaem iron absorption in humans: a comparison of kale and spinach meals. *Eur. J. Clin. Nutr.* 62:336-341.

141. Stratford, M., and T. Eklund. 2003. Organic acids and esters. p. 48-84. *In* N.J. Russell, and G.W. Gould (ed.), Food preservatives Kluwer Academic/Plenum Publishers, New York, N.Y.

142. Tamblyn, K. C., and D. E. Conner. 1997. Bactericidal activity of organic acids against *Salmonella typhimurium* attached to broiler chicken skin. *J. Food Prot*. 60:629-633.

143. Tamblyn, K. C., and D. E. Conner. 1997. Bactericidal activity of organic acids in combination with transdermal compounds against *Salmonella typhimurium* attached to broiler skin. *Food Microb*. 14:477-484.

144. Thomas, C. J., and T. A. McMeekin. 1980. Contamination of broiler carcass skin during commercial processing procedures: an electron microscopic study. *Appl. Env. Microb.* 40:133-143.

145. Tiganitas, A., N. Zeaki, A. S. Gounadaki, E. H. Drosinos, and P. N. Skandamis. 2009. Study of the effect of lethal and sublethal pH and a_w stresses on the inactivation or growth of *Listeria monocytogenes* and *Salmonella* Typhimurium *Int. J. Food Microb*. 134:104-112.

146. U. S. Department of Agriculture, Food Safety and Inspection Services. April 14 2010. Safe and suitable ingredients used in the production of meat, poultry, and egg products. Available at: http://origin-www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1Rev2.pdf. Accessed 5 May 2010.

147. U.S. Department of Agriculture, Food Safety and Inspection Services. 2008. Serotype profile of *Salmonella* isolates from meat and poultry products, 1998-2007. Available at: http:\\www.fsis.usda.gov/PDF/Serotypes_Profile_Salmonella_Tables_&_Figures. Accessed 14 September 2009.

148.U.S. Department of Agriculture, Food Safety and Inspection Services. 2010. New performance standards for *Salmonella* and *Campylobacter* in young chicken and turkey slaughter establishments; New compliance guides. Final Rule 75. Fed. Red 27288-27294 (May 14, 2010) (to be codified at 9 CFR 381.94).

149. Udoh, A. P., E. O. Akpanyung, and I. E. Igiran. 1995. Nutrients and anti-nutrients in small snails (*Limicolaria aurora*). *Food Chem*. 53:293-241.

150. Udoh, A. P., R. I. Effiong, and D. O. Edem. 1995. Nutrient composition of dogwhelk (*Thais cattifera*), a protein source for humans. *Trop. Sci*. 35:64-67.

151. Uzzau, S., D. J. Brown, T. Wallis, S. Rubino, G. Leori, S. Bernard, J. Casadesus, D. J. Platt, and J. E. Olsen. 2000. Host adapted serotypes of *Salmonella enterica. Epidemiol. Inf.* 125:229-255.

152. Van Reen, R., A. Valyasevi, and S. Dhanamitta. 1970. Studies of bladder stone disease in Thailand. *Amer. J. Clin Nutr.* 23:940-947.

153. Voetsch, A. C., T. J. Van Gilder, F. J. Angulo, M. M. Farley, S. Shallow, R. Marcus, P. R. Cieslak, V. C. Deneen, and T. R. V. 2004. FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. *Clin. Infect. Dis*. 38:S127-134.

154. Waldroup, A., B. Rathgeber, and N. Imel. 1993. Microbiological aspects of counter current scalding. *J. Appl. Poultry Res.* 2:203-207.

155. Waldroup, A. L., B. M. Rathgeber, and R. H. Forsythe. 1992. Effects of six modifications on the incidence and levels of spoilage and pathogenic organisms on commercially processed postchill broilers. *J. Appl. Poultry Res.* 1:226-234.

156. Warth, A. D. 1989. Transport of benzoic and propanoic acids by *Zygosaccharomyces bailii. J. Gen Microb.* 135:1383-1390.

157. Watanabe, Y., F. Uchiyama, and Y. Kiyoko. 1994. Compositional changes in spinach (*Spinacia oleracea* L.) grown in the summer and in the fall. *J. Japan. Soc. Hort. Sci.* 62:889-895.

158. Weaver, C. M., R. P. Heaney, K. P. Nickel, and P. I. Packard. 1997. Calcium bioavailability from high oxalate vegetables: Chinese vegetables, sweet potatoes, and rhubarb. *J. Food Sci*. 62:524-525.

159. Welch, R., W. House, and D. Van Campen. 1977. Effects of oxalic acid on availability of zinc from spinach leaves and zinc sulfate to rats. *J. Nutr*. 107:929-933.

160. Wilmes-Riesenberg, M. R., J. W. Foster, and R. Curtiss III. 1997. An altered *rpoS* allele contributes to the avirulence of *Salmonella typhimurium* LT2. *Infect. Immun.* 65:203-210.

161. Windholz, M. 1976. The Merck Index. p. 4137. *In* M. Windholz, et al. (ed.), An encyclopedia of chemicals and drugs. Merck & Co., Inc., Rahway, NJ.

162. Windholz, M. 1976. The Merck Index. p. 6744. *In* M. Windholz, et al. (ed.), An encyclopedia of chemicals and drugs. Merck & Co., Inc, Rahway, NJ.

163. Xiao-lin, Z., T. Shi-ping, Y. Hong, and X. Yong. 2007. Effect of oxalic acid treatment on mango fruit during storage. *Acta Horticulturae Sinica*. 34:579-584.

164. Yang, H., Y. Li, and M. C. Johnson. 2001. Survival and death of *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on chicken skin during poultry scalding and chilling. *J. Food Prot.* 64:770-776.

165. Young, L. L., and J. K. Northcutt. 2000. Poultry processing. p. 147-170. *In* S. Nakai, and H.W. Modler (ed.), Food Proteins. Wiley-VCH Publ., New York, NY.

166. Zarembski, P. M., and A. Hodgkinson. 1962. The oxalic acid content of English diets. *British J. Nutr*. 16:627-634.

167. Zou, W., J. G. Frye, C. W. Chang, J. Liu, C. E. Cerniglia, and R. Nayak. 2009. Microarray analysis of antimicrobial resistance genes in *Salmonella enterica* from preharvest poultry environment. *J. Appl. Microb*. 107:906-914.

EFFICACY OF OXALIC ACID TO REDUCE THE LEVELS OF *SALMONELLA* SPP. AT VARIOUS STAGES OF POULTRY PROCESSING

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ABSTRACT

The microbiological safety of fresh eviscerated poultry remains a major concern of the public and poultry industry due to foodborne illnesses associated with *Salmonella* spp. Oxalic acid was evaluated as an antimicrobial treatment at time and temperature combinations equivalent to dipping, chilling, and scalding for reduction of *Salmonella* spp. attached to raw chicken skin by using the skin attachment model. To mimic common commercial broiler processing practices, raw chicken skins were dipped in 1.0, 1.5, and 2.0% oxalic acid treatments at 22°C for 10, 20, 30, and 40 sec to simulate on-line or post-chill dipping and at 53°C for 1, 2, and 3 min to simulate soft scalding. To simulate water-immersion chilling 0.1, 0.25, and 0.5% oxalic acid was applied at 3°C for 30, 45, and 60 min. After treatment samples were stomached in buffered peptone water to neutralize any residual acid, serially diluted, and plated on XLD agar using the thin agar layer technique for acid injured cells. On-line or post-chill dipping with oxalic acid resulted in a ≤1.12-log reduction of *Salmonella* spp. for all tested combinations. The 0.5% oxalic acid treatment was found effective against *Salmonella* cells in the 60 min water-immersion chiller application by attaining a 2.25-log reduction. The greatest *Salmonella* reductions (2.43 log) were achieved when oxalic acid was applied in soft scalder applications at a concentration of 2.0% and a contact time of 3 min. Treatments with oxalic acid caused an initial darkening in color (decreased Hunter L value) and increase in yellowness (increased Hunter b value) of non-irradiated chicken skins at dipping, scalding, and chilling. There was no significant impact of refrigerated storage (4ºC) for 14 days on the color of the treated skins ($P > 0.05$). Treatments

with oxalic acid may have potential for use as an antimicrobial agent to reduce *Salmonella* spp. during poultry processing, but consumer perception of oxalic acid and toxicological studies should be done to evaluate its potential practical use.

INTRODUCTION

Salmonellosis is one of the most common enteric infections in the U.S. *(12)*. The reported incidence of *Salmonella* illness is about 15 cases per 100,000 people *(11)*, of those there are approximately 7,000 laboratory-confirmed cases of salmonellosis yearly *(9)*. Foods of animal origin, such as poultry, eggs, beef, and pork, are the major transmission vehicles of human salmonellosis *(7)*. Centers for Disease Control and Prevention (CDC) reports that among the outbreaks caused by a single food vehicle, poultry is recognized as the most common food commodity (21%) to which outbreak-related cases are attributed *(10)*.

Contamination of poultry with *Salmonella* may occur throughout the production chain *(17)*. Subsequently, there are continuous efforts to reduce the incidence of *Salmonella* positive carcasses during poultry processing *(14)*. The rapid evolution of slaughtering technologies *(41)* and passage of new referendums for the poultry industry *(50)* necessitate research on new methodologies to reduce prevalence of salmonellae during poultry processing. Additionally, the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA/FSIS) has proposed that all slaughter establishments apply at least one antimicrobial treatment or other approved intervention procedure to poultry carcasses to reduce levels of pathogenic microorganisms *(34)*.

The effects of various antimicrobial agents for carcasses, such as chlorine *(6, 20)*, trisodium phosphate (TSP) *(6, 19, 26, 27, 51)*, cetylpyridinium chloride (CPC) *(25, 26, 32)*, acidified sodium chlorite (ASC) *(24, 32)*, and peroxyacetic acid *(32)* have been extensively investigated for activity against *Salmonella* spp. and approved as antimicrobial agents for

broiler carcasses. Myhyar et al. *(32)* reported that TSP (10%), ASC (0.12%), CPC (0.5%), and peroxyacetic acid (0.2%) applied as a dip for 1 min reduced *Salmonella* inoculated onto unchilled drumettes by 1.56, 1.11, 1.36, and 0.04 log, respectively. Organic acids, including formic, citric, lactic, propionic, adipic, acetic, succinic, and peracetic *(5, 33)* have also been tested for application in poultry processing because they exhibit strong bactericidal potential against a wide range of bacteria *(6, 15, 16, 18, 19, 26, 31, 33, 35, 45)*, and are generally recognize as safe (GRAS) *(30)*. Acetic, citric, lactic, malic, and tartaric acids at concentration of 0.5% applied as a dip in scalder application (53°C/2 min) reduced *S.* Typhimurium attached to broiler skin by 1.48, 0.37, 0.86, 1.62, and 2.64 log, respectively *(45)*, but caused some alteration in skin color of carcasses *(8)*. The use of oxalic acid to reduce *Salmonella* population on raw poultry and its effect on chicken skin color has not been described.

Preliminary work demonstrated that 0.45% oxalic acid (pH 1.61) at 22ºC was able to reduce a four strain *Salmonella* cocktail by 3.71 log with a contact time of 40 sec in vitro *(44)*, which was the basis for evaluating the bactericidal efficacy against *Salmonella* spp. attached to chicken skin by using the modified skin-attachment model (mSAM) *(45)* under conditions mimicking those that found in poultry slaughter facilities at on-line dip or post-chill dip, chiller, and scalder processing steps. Using the mSAM, chicken skins were irradiated to eliminate background microflora and facilitate testing of the acid against salmonellae attached or embedded in poultry skin *(45)*.

Oxalic acid is not currently approved for use in the food industry due to potential negative effects (e.g. irritation in mouth and gastrointestinal tract, reduced calcium,

magnesium and zinc absorption, and increased risk of kidney stones formation) associated with its consumption in high levels *(42)*. However, based on calculations by Anang et al. *(2)* a 200 g chicken breast dipped into 0.5% oxalic acid solution would retain only 0.0025 g of the acid, which is 1,000-fold less than the extrapolated LD_{50} for a 150-lb person. If the oxalic acid concentration is increased to 2.0% then 0.1 g of the acid would remain on the chicken breast, which is 225 times lower than the extrapolated LD_{50} for a 150-lb person. The oxalic acid LD_{50} in rats is 375 mg/kg *(2)*, which is higher than LD_{50} values of some currently approved antimicrobials to treat the surface of raw poultry carcasses, such as CPC (21 CFR 173.375) (LD_{50}(rats) is 200 mg/kg) *(4)*. The effect of cooking taro and beans resulted in a 36% *(43)* and 60% *(38)* loss of oxalates, respectively, suggesting that rinsing and cooking of poultry with water would substantially reduce the amount of residual oxalic acid on treated carcasses, thereby decreasing potential negative effects associated with its consumption *(2)*.

Several factors should be considered when evaluating antimicrobial treatments, including efficacy in reducing pathogenic microflora at contact times appropriate for the particular processing operation, cost-effectiveness, and deleterious effects on product quality *(5)*. In this study various time temperature conditions, mimicking those found at various stages in poultry processing that can be used to apply antimicrobial treatments, were used to evaluate the effectiveness of oxalic acid in reducing *Salmonella* spp. during poultry slaughter operations. Additionally, oxalic acid effect on broiler skin color was also evaluated.

MATERIALS AND METHODS

Sample collection

To mimic natural variation that occurs in industry chicken breast skins were obtained from a local poultry processing plant (Case Farms Inc., Dudley, NC) in February, May, and August of 2009. Skins without visible damage on the surface were collected immediately after an automatic skinner, placed in plastic bags contained in an ice chest (≤4ºC) and transported to the Meat Pathogen Laboratory at North Carolina State University (NCSU), Raleigh, NC. Upon arrival, skins were trimmed into 62.2 cm² round pieces (on average, 2 mm thick) by placing the bottom portion of a petri dish over the skin area and tracing the perimeter using a sterile scalpel, similar to the method used by Conner and Bilgili *(13)*. Skin samples were individually vacuum packaged (UV225, Koch Industries Inc., Wichita, KS) in high oxygen barrier film (OTR 4.5 cc/100^2/24 hrs) vacuum pouches (Koch Equipment LLC, Kansas City, MO) at 7.6 mm Hg vacuum and then frozen at -20°C.

The vacuum-packaged samples were shipped overnight maintaining a temperature at or below 4°C to the U.S. Department of Agriculture-Agricultural Research Service Eastern Regional Research Center (USDA-ARS ERRC), Wyndmoor, PA to be irradiated at 12 to 25 kGy with a Co-60 source, similar to what have been used in previous studies using chicken skin samples *(45)*. After irradiation the skins were shipped frozen overnight to NCSU where they were immediately stored and maintained at -80°C until needed *(13, 45)*. The sterility of the skin pieces was confirmed after irradiation by bacterial enumeration on two randomly selected samples using 3M Petrifilm Aerobic Count Plates in accordance with Association of

Official Agricultural Chemists (AOAC) official method 990.12 for aerobic plate count in foods *(3)*.

Preparation of inoculum

A four-strain cocktail consisting of *Salmonella enterica* serovar Enteritidis (poultry isolate), *Salmonella enterica* serovar Heidelberg (environmental isolate) from turkey processing plant, *Salmonella enterica* serovar Typhimurium (poultry isolate) obtained from the Poultry Science department at NCSU, Raleigh, NC and *Salmonella enterica* serovar Newport (J1980 - tomato outbreak human isolate) obtained from the Food Science department at Virginia Polytechnic Institute and State University, Blacksburg, VA were used to inoculate chicken skins. The antibiotic susceptibility profile of *Salmonella* strains (Appendix 1) was performed by National Antimicrobial Resistance Monitoring System (NARMS), Atlanta, GA.

Salmonella strains were maintained at -80°C ± 1°C in brain heart infusion broth (BHIB; Fisher Scientific, Pittsburg, PA) containing 50% glycerol (Fisher Scientific, Pittsburg, PA) *(45)*. Isolates of each strain were activated by two successive transfers in BHIB at 37°C for 18 to 24 hrs *(13)*, streaked onto xylose lysine deoxycholate agar (XLD; Weber Scientific, Hamilton, NJ), examined for purity, and confirmed as *Salmonella* spp. using API 20E (bioMerieux Inc., Hazelwood, MO). Typical *Salmonella* colonies on XLD appeared black with clear and opaque zone *(52)*.

Inoculum was prepared by placing one colony into 9 ml of BHIB and incubating still at 37°C for 22 ± 1 hrs to achieve a level of *ca.* 9.0 $\log_{10}$ CFU/ml. Two ml aliquots from each of the four strains were combined in a sterile plastic tubes and used to inoculate samples *(19, 32)*. Exact inoculum concentration was determined on the day of skin inoculation by serial dilution in 0.1% w/v buffered peptone water (BPW; Fisher Scientific, Pittsburg, PA) and plating on XLD.

Inoculation of chicken skin samples

Irradiated frozen skins were thawed in a refrigerator at 4ºC ± 1°C for 24 hrs. The outside of each bag was sanitized with 70% ethyl alcohol, then cut with ethanol/flame sterilized scissors to allow the skin piece to be aseptically taken out with sterilized tweezers. Skins were placed on sterile 20 gauge stainless steel 9.5 x 9.5 cm racks (3.175 mm perforation) for inoculation (Appendix 10).

Chicken skin samples were drip inoculated on the de-feathered side of skin with a 0.1-ml cocktail of *Salmonella* inoculum using a sterile tuberculin syringe. The inoculum was evenly distributed with a disposable sterile plastic spreader (Weber Scientific, Hamilton, NJ). Drip inoculation method was preferred to dip inoculation to eliminate water uptake, uneven cell distribution in a suspending medium, and contamination of the cell suspension through repeated immersion *(13)*.

A 10-min contact time at 22ºC ± 1°C was allowed for cells to attach to the skin surface prior to application of treatments *(13, 45)*. To prevent contamination, the inoculated

samples and stainless racks were covered with the same foil that was used during autoclaving.

Chemical treatment preparation

Oxalic acid (CAS 114-62-7; Fisher Scientific, Pittsburg, PA) solutions were prepared at 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 % w/v by first weighing the acid using analytical scales (XS 64; Mettler Toledo, Northbrook, IL) and then transferring the acid to a sterile 100 ml beaker, where it was pre-dissolved with 50 ml of sterile distilled water. This minimized the effects of air flow and relative humidity on oxalic acid during weighing. After the chemical was completely dissolved the content was placed into a sterile 200 ml volumetric flask. The interior of the beaker was rinsed three times with additional sterile distilled water and the subsequent rinsate was added to the flask. The flask was appropriately filled to volume with sterile distilled water using the underside of the meniscus, and the pH of each chemical solution (Table 1) was measured using a calibrated digital pH meter (AB15 Accumet, Fisher Scientific, Pittsburgh, PA).

Chemical treatments were transferred into autoclaved plastic treatment containers with lids (18 x 10 x 5.4 cm) (Fisher Scientific, Pittsburg, PA) (Appendix 11), and left at room temperature (22°C ± 1°C) to simulate on-line or post-chill dipping, or placed in either low temperature incubator (3°C ± 1°C) to simulate water-immersion chilling or a warm temperature incubator (53°C ± 1°C) to simulate soft scalding prior to the skin treatments *(45)*. The temperature of treatment bathes was measured with a submerged sterile

thermocouple wire (TT-T-30-SLE-50, Omega, Stamford, CT) attached to a calibrated continuous digital thermometer (HH21, Omega, Stamford, CT). When chemical bathes reached the appropriate treatment temperatures the inoculated skins were submerged for various contact times according to oxalic acid treatment procedure.

Procedure for treating chicken skins with oxalic acid

The irradiated chicken skin samples were randomly divided into oxalic acid treatments and sterile distilled water (control) treatments. To mimic common commercial processing practices, samples in the first group (nine replicates per process simulation, concentration and time; total of 270 samples) were dipped into 200 ml of 1.0, 1.5, and 2.0% oxalic acid solutions for 10, 20, 30, and 40 sec to simulate on-line or post-chill dipping (22°C ± 1°C), into 200 ml of 1.0, 1.5, and 2.0% oxalic acid for 1, 2, and 3 min to simulate scalding (53°C ± 1°C), and into 200 ml of 0.1, 0.25, and 0.5% acid solution for 30, 45, and 60 min to simulate chilling (3°C ± 1°C) of broilers, roasters, and mature birds *(36, 45)*. The oxalic acid concentrations were predetermined by preliminary studies as levels that provided ≥1 log reductions without adversely effecting color of the treated chicken skin samples. Remaining samples in the control group (nine replicates per process simulation; total of 81 samples) were dipped into 200 ml of sterile distilled water at 22°C ± 1°C, 53°C ± 1°C, and 3°C ± 1°C. Each treatment was made fresh on the day of experiment, and only one skin sample was dipped into the acid treatment to limit the effect of organic matter on the potential of oxalic acid. After treatments, skins were drained for 1 min at 22°C ± 1°C to allow for removal of

excess water or acid solution. The drainage time for samples after antimicrobial treatments in previous studies have varied from 15 sec to 15 min *(32, 40)*.

Microbiological analysis

Each treated chicken skin sample was placed in a sterile stomacher bag containing 90 ml of sterile 0.1% w/v BPW and homogenized for 2 min at 230 rpm (400 Circulator, Seward Inc., Bohemia, NY) to remove remaining cells and neutralize the effects of treatment *(45, 47)*. One ml of solution was removed, serially diluted and plated in duplicate by using the thin agar layer (TAL) technique for acid-injured foodborne pathogens *(52)*. This method provides better recovery of surviving *Salmonella* cells by allowing the cells including those that are sublethally injured to resuscitate on the top nonselective agar (tryptic soy agar (TSA; Weber Scientific, Hamilton, NJ)) before selective ingredients (XLD) diffuses through to the top thin layer *(52)*. The plates were incubated at 37° ± 1°C for 24 hrs under aerobic conditions. *Salmonella* counts for each experiment were expressed as $\log_{10}$ CFU/skin.

On a periodic basis, two or three typical *Salmonella* colonies were picked from countable plates and confirmed by API 20E (bioMerieux Inc., Hazelwood, MO) as *Salmonella* spp. No further subtyping was performed for the confirmed *Salmonella* spp. colonies.

Expression of antimicrobial activity

Efficacy of oxalic acid as antimicrobial agent was assessed by determining the reduction in the viable population of attached *Salmonella* cells *(13)*. Reduction for each application was calculated as a difference between the population of cells recovered from the inoculated skin treated with water and the population of cells recovered from the inoculated skin treated with oxalic acid.

Color and pH measurement of treated chicken skins during refrigerated storage

To objectively assess the influence of oxalic acid on broiler skin color when used at poultry slaughter facility and to eliminate the effect of irradiation on the color change caused by oxalic acid treatment, the non-irradiated chicken skin samples were treated under dip, water-immersion chiller, and soft scalder applications according to the same procedure for treating inoculated chicken skins with oxalic acid. Samples were then individually vacuum packaged (UV225, Koch Industries Inc., Wichita, KS) in high oxygen barrier film (OTR 4.5 $cc/100^2/24$ hrs) vacuum pouches (Koch Equimpent LLC, Kansas City, MO) at 7.6 mm Hg vacuum, and stored for 14 days at 4ºC ± 1°C. The colorimetric changes in chicken skins treated with water or with oxalic acid were analyzed for Hunter L (dark to light), a (green to red), and b (blue to yellow) values with a colorimeter (CR-400, Konica Minolta, Ramsey, NJ) before treatment, immediately after treatment, and during subsequent storage for 14 days. The values were recorded by placing the hand-held colorimeter directly in contact with the vacuum packaged skin. During refrigerated storage, the measurements were randomly

taken at three different locations on each sample of chicken skin at day 0, 1, 3, 7, 10, and 14 in triplicate. The colorimeter was calibrated with a reflectance calibration plate supplied by the manufacturer (plate № 139333049).

To determine if treatment with oxalic acid would have a residual effect on broiler carcasses when used at poultry slaughter operation, the surface pH of chicken skins treated with 1.0. 1.5, and 2.0% oxalic acid solutions for 10 sec at 22ºC ± 1°C (on-line or post-chill dipping) was measured in triplicate. This treatment was chosen due to it being the shortest contact time among all tested applications; therefore speculation could be made on the acid's functional/technical properties at longer contact times. Skins' pH was measured at five locations with a pH meter (AB15 Accumet, Fisher Scientific, Pittsburg, PA) fitted with a poly flat surface electrode (Accumet, Fisher Scientific, Pittsburg, PA), and compared to the pH values of untreated or treated with water chicken skin samples before and after the treatments, and during subsequent storage at 4ºC ± 1°C for 14 days.

Statistical analysis

Effectiveness of antimicrobial agents depended on the method of application *(45)*. Thus, the data was analyzed separately for each application by using SAS software (version 9.1, SAS Institute, Cary, NC). Analysis of variance was carried out at a 95% significance level ($P < 0.05$), and a Tukey test was used for separation of means *(39)*.

RESULTS

Efficacy of oxalic acid to reduce salmonellae during on-line or post-chill dip application

The efficacy of oxalic acid when applied during a simulated on-line or post-chill dip application is presented in Table 2. Treatment with 1.0% oxalic acid at 22°C ± 1°C showed a 0.28-log reduction in recoverable *Salmonella* cells in 10 sec, which was not statistically different from the control ($P = 0.5870$). However, the number of recoverable cells decreased as treatment time increased. Thus, a 30-sec treatment with 1.0% oxalic acid resulted in a 0.55-log reduction, while a 40 sec treatment provided a 0.70-log reduction. The effect of oxalic acid concentration ($P < 0.0001$) was more pronounced on *Salmonella* reduction than the effects of time ($P = 0.0125$). Statistical significance within all contact times was observed between 1.0% and 2.0% oxalic acid treatments ($P < 0.0001$). Treatment with 1.5% oxalic acid showed a 0.70-log reduction at 10 sec and a 1.13-log reduction at 40 sec of exposure time. However, treatment with 2.0% oxalic acid did not result in further reduction of viable *Salmonella* cells with reductions of 0.79, 0.86, and 1.12 log at 10, 20, and 40 sec of contact time, respectively.

Efficacy of oxalic acid to reduce salmonellae during water-immersion chiller application

The bactericidal potential of oxalic acid against of *Salmonella* spp. during simulated water-immersion chilling is shown in Table 3. Treatment with 0.1% oxalic acid at 3°C ± 1°C showed a 0.52- and 0.85-log reduction in recoverable *Salmonella* cells within 30 and 60 min,

respectively. There was no significant difference between treatments for 30 and 45 min for all tested concentrations (P = 0.1389). Treatment with 0.25% oxalic acid showed a 1.09-log reduction after 45 min and a 1.32-log reduction after 60 min of exposure time. The most effective treatment was with 0.5% oxalic acid at a 60 min contact time, resulting in a 2.25-log reduction of *Salmonella.*

Efficacy of oxalic acid to reduce salmonellae during soft scalder application

The bactericidal potential of oxalic acid against of *Salmonella* spp. during a simulated soft scalding application is summarized in Table 4. Treatment with 1.0% oxalic acid at 53°C ± 1°C generated *Salmonella* reductions of 1.01 and 1.56 log at 1 and 3 min, respectively. A 1.0% oxalic acid treatment was not significantly different from the 1.5% treatment at all tested contact times (P = 0.5137), while it was found significantly different from 2.0% oxalic acid treatment (P < 0.0001). At a concentration of 2.0% and a contact time of 3 min, oxalic acid reduced the initial *Salmonella* levels by 2.43 log compared to the control. There was no significant difference between a contact times of 2 or 3 min for all tested concentrations (P = 0.1870). Treatment for 2 min with 1.0, 1.5, and 2.0% oxalic acid resulted in reductions of 1.31, 1.52, and 2.17 log, respectively.

Colorimetric and pH changes in chicken skin samples treated with oxalic acid

Hunter L, a, and b values of chicken skins treated with oxalic acid at simulated on-line or post-chill dip, soft scalder, and water-immersion chiller applications during

subsequent refrigerated storage at 4°C ± 1°C for 14 days were determined (Tables 5 through 7). These values were compared to the impact of refrigerated storage on the color of untreated chicken skin samples (Appendix 3).

Generally, on-line or post-chill dipping of skins resulted in significantly lower Hunter L and a values and significantly higher b values compared to the control (Table 5). Chicken skins treated with 1.0% oxalic acid had higher Hunter L values than samples treated with 1.5 or 2.0%, and were not statistically different from the control at contact time of 10 sec ($P > 0.05$). Contact time of 10 sec was also not significantly different from a contact time of 20, 30, and 40 sec for Hunter L and b values ($P > 0.05$), and there was no significant difference among Hunter b values of skin control samples and samples treated with 1.0, 1.5, or 2.0% oxalic acid ($P > 0.05$). Additionally, refrigerated storage at 4°C for 14 days did not impact Hunter b values of treated samples ($P = 1.0000$), and Hunter L values of chicken skins at day 0 were not significantly different from day 1 or day 14 ($P > 0.05$).

Treatment with oxalic acid under simulated chiller conditions affected the color of chicken skins by reducing Hunter L and a values and increasing b values of treated samples compared to the control (Table 6). A 30-min oxalic acid treatment was not significantly different from 45 or 60 min treatments for Hunter L values ($P > 0.05$), but there was a significant difference between chicken skins treated with oxalic acid at concentrations of 0.1 to 0.5% and control on Hunter L values ($P < 0.0001$), but not on b values ($P > 0.05$) at 30 to 60 min of chilling time. Although, treated samples appeared lighter at the end of refrigerated storage, there was no statistical difference between day 0 versus day 1 or day 14 ($P > 0.05$).

Significant oxalic acid effect on Hunter L, a, and b values was observed during simulated scalder application (Table 7). Skins had lower Hunter L and a values and higher b values compared to the control ($P < 0.0001$). No significant difference was observed among treatments with 1.0, 1.5, and 2.0% oxalic acid on Hunter L values ($P > 0.05$), but a contact time of 1 min was significantly different from 2 min and 3 min for Hunter L and b values ($P < 0.05$). During refrigerated storage there was no significant difference between color of oxalic acid treated samples at day 0 versus day 1 ($P > 0.05$).

A post-chill dip for 10 sec with 1.0 and 2.0% oxalic acid reduced the surface pH of chicken skins from 5.57 to 3.68 and 3.49, respectively. There was significant difference between the pH values of oxalic acid treated samples and pH values of water treated or untreated samples ($P < 0.05$). The effect of storage at 4ºC for 14 days had no significant impact on the pH values of oxalic acid treated chicken skins at 1.0, 1.5, and 2.0% ($P > 0.05$) (Table 9).

DISCUSSION

There are many factors that must be considered when determining if an antimicrobial is appropriate for use in the poultry industry. The first factor is its antimicrobial efficacy. Oxalic acid treatments were considered effective if they were able to reduce *Salmonella* counts by 2 log, because carcasses are known to carry from 10 to 100 salmonellae cells on average following processing *(21)*. Simulated on-line or post-chill dipping (22°C ± 1°C) with oxalic acid showed the least quantitative reductions compared to chiller and scalder applications. Although the number of recoverable cells decreased with increased contact time or oxalic acid concentration, the mean *Salmonella* log reduction remained ≤2 log. Similarly, Tamblyn and Conner *(46)* found that neither acetic, citric, lactic, malic, mandelic, propionic or tartaric acid treatments were effective in a dip application against *S.* Typhimurium cells attached to broiler skin. Short contact time, associated with on-line or post-chill dipping, may be a limiting factor of oxalic acid efficacy against salmonellae that have become embedded in or firmly attached to the skin. Li et al. *(26)* demonstrated that more than 30 sec of spraying time was required to achieve a ≥2 log *Salmonella* reduction on chicken carcasses. Taking into account the speed of a processing shackle line, the longest contact time that can be achieved during on-line or post-chill dip application in a poultry slaughter operation is approximately 40 sec. Thus, the use of oxalic acid at higher concentrations (2.5 and 3.0%, w/v) and exposure time of 40 sec was evaluated against of *Salmonella* attached to raw chicken skin during simulated on-line or post-chill dip application. Treatment with 2.5 and 3.0% oxalic acid solutions did not result in significant reductions of attached *Salmonella* cells

compared to the treatment with 2.0% oxalic acid (P = 0.0503) (Table 8). Additionally, the use of higher oxalic acid concentrations may increase the potential risks associated with the consumption of residual oxalic acid, produce undesirable carcass characteristics, and/or increase production cost.

Lower oxalic acid concentrations (0.1 to 0.5%, w/v) were tested during water-immersion chilling due to the longer contact times associated with this application. Oxalic acid at 0.5% was effective against *Salmonella* cells in a water-immersion chiller application (3°C ± 1°C), achieving more than a 2-log reduction in 60 min. Tamblyn and Conner *(45)* reported that ≥4.0% of malic, mandelic, or propionic acids were needed to achieve similar reductions (≥2 log) of firmly attached *S.* Typhimurium cells in a 0°C chiller, while 25 ppm of chlorine had no effect on *Salmonella* prevalence during immersion chilling *(20)*. Anang et al. *(2)* showed that treatment of chicken breasts with 0.5% oxalic acid for 10 min at room temperature reduced *Enterobacteriaceae* count by >3 log compared to the control, while 1.0% oxalic acid was sufficient to maintain a count of <2 log throughout 14 days of refrigerated storage, perhaps due to presence of residual oxalic acid on the chicken breasts.

The greatest *Salmonella* reductions (up to 2.43 log CFU/skin) were achieved when oxalic acid was applied as a simulated soft scalder application (53°C ± 1°C). These results are consistent with the Tamblyn et al. study *(47)*, which demonstrated a higher efficacy of acetic acid against *S.* Typhimurium during scalder application than during dip or chiller applications. A 2.41-log reduction was achieved using 2.0% acetic acid or 1.0% lactic acid applied during a simulated scalder application *(45)*. Higher microbial reductions during soft

scalding compared to other applications indicate a synergistic effect between organic acids and heat. Alvarez-Ordonez et al. *(1)* showed that the membrane fatty acid profile of *S.* Typhimurium cells grown at 45°C did not change significantly upon acidification of the growth medium, and cyclopropane fatty acids provided only limited protection to the membrane at elevated temperatures. Possibly, damage to the membrane structure and function may to some extent explain the synergistic effect between heat and acids, and the higher reductions observed during scalding. Tsuchido et al. *(48)* found that release of LPS and lipids from the outer membrane of *E. coli* upon heat treatment brings disorganization to the membrane structure and results in the partial disruption of the permeability barrier function. Also, loss of enzymes may occur as a result of blebbing and vesiculation of the outer membrane in *E. coli (22).*

Particularly in the scalder application, Tamblyn and Conner *(46)* noted a greater bactericidal activity of acetic and lactic acids at higher concentrations. However, Lillard et al. *(28)* found no significant reduction in the incidence of salmonellae on unpicked carcasses sampled after scalding in 0.5% acetic acid-treated scald water. In another study, a decrease of acetic acid concentration in scald water from 0.2 to 0.1% resulted in a 1.36-min higher LD_{52} value for *S.* Newport *(35).* Similarly, we determined that bactericidal activity of oxalic acid was proportional to increasing the acid concentration and exposure time of scalder application. However, the reductions achieved at 2 min contact time were not significantly different from 3 min. Mehyar et al. *(32)* indicated that treatments with commercial antimicrobials (e.g. TSP, Sanova, Inspexx 100, and Cecure) for 20 min were more effective

than for 1 min in reducing *Salmonella* on chicken skin, but no further reductions were observed when treatment time exceeded 20 min.

Martin and Maris *(31)* calculated that the minimal bactericidal concentration of oxalic acid on *Salmonella* is 0.08%, where 6.33% of the acid is in undissociated form and 93.67% is in dissociated form. A small study conducted in our laboratory *(44)* indicated that at pH 1.03 hydrochloric acid (strong acid) was 0.35 and 0.51 log less effective in reducing *Salmonella* spp. during simulated on-line or post-chill dipping and soft scalding, respectively, than 2.0% oxalic acid (pH 1.03) (weak acid) applied at the same treatment parameters (40 sec/22 ± 1ºC) (Appendix 2). No significant difference was observed between treatments of 0.5% oxalic acid (pH 1.48) and hydrochloric acid (pH 1.48) when applied during 60-min water-immersion chilling. This reveals that bactericidal effect of oxalic acid mostly depends upon the number of free hydrogen ions present in the solution (effect of low pH).

The second factor that impacts potential use of antimicrobial agents in the poultry industry is its effect on color of broiler carcasses. At the concentrations previously discussed organic acids alter the appearance of poultry carcasses by either bleaching or darkening them. Bautista et al. *(6)* observed discoloration of turkey skin tissues after treatment with 1.24% lactic acid for 15 sec, and chicken skin bleaching and softening was reported by Mehyar *(32)* after a 30-sec lactic acid treatment. Chilling of broiler carcasses for 30 min with hydrogen peroxide made poultry skin bloated, rubbery, and bleached *(29)*. Dickens and Whittemore *(15)* found that a pre-chill treatment with 0.3% acetic acid for 10 min made chicken carcasses appear darker and more yellow in color, and Anang et al. *(2)* showed that oxalic acid (0.5 to

2.0%) caused color darkening and an increase in yellowness of chicken breasts. Similarly, our study showed that chicken skins treated with oxalic acid appeared darker (lower Hunter L values) and more yellow (higher Hunter b values) compared to the controls at all tested applications. Both the treatment time and concentration of oxalic acid were determining factors in the skin color change. The reduction in the lightness of skin samples during on-line or post-chill dipping with oxalic acid was lower than those obtained after scalding or chilling. Results of the refrigerated storage study indicated insignificant color change of untreated, treated with water (control) or oxalic acid chicken skin samples.

One of the other factors that influence implementation of antimicrobials in industry is financial burden *(5)*. The wholesale price for oxalic acid is about $450 to $650 per metric ton which is almost half the cost of lactic acid *(37)*. Therefore, oxalic acid is a cheaper alternative for use in poultry industry.

Another aspect that has to be taken to in account is current labeling requirements and consumer perception of those ingredients. Organic acids are permitted to be applied at pre-chilling without labeling if they have no technical and/or function effect on poultry carcasses *(49)*. Oxalic acid applied at concentrations of 1.0, 1.5, and 2.0% during simulated on-line or post-chill dip for a contact time of 10 sec at 22ºC reduced the pH of chicken skins from 5.61 to 3.68, 3.73, and 3.49, respectively (Table 9). The pH values of treated samples were conveyed through 14 days of refrigerated storage (4°C) ($P > 0.05$), suggesting the pH effect of oxalic acid on poultry skin may still be functioning, and therefore if used must be listed on the label as an ingredient.

The next factor to consider is the effect of organic matter on the antimicrobial potential of oxalic acid during dip applications, since its presence increases pH of the solution which will reduce the bactericidal efficacy of oxalic acid against salmonellae. The maximum effectiveness of oxalic acid occurs at pH close to pH 1.0 for on-line or post-chill dipping and scalding, and a pH 1.5 for chiller application. Such low pH's would be corrosive to stainless steel equipment *(23),* and long term use of oxalic acid may potentially lead to equipment damage.

The results of this study establish that antimicrobial effectiveness of oxalic acid depends on the point of application in the processing plant. The greatest *Salmonella* reductions were achieved during simulated water-immersion chilling and soft scalding, suggesting a potential for oxalic acid to reduce or prevent cross-contamination at these processing steps. The issue of discoloration *(6)* and residual pH effect carried by oxalic acid may be minimized by an immediate water rinse. However, improvements are needed to overcome quality obstacles, toxicity concerns, and corrosive pH levels associated with oxalic acid's application at high concentrations.

REFERENCES

1. Alvarez-Ordonez, A., A. Fernandez, M. Lopez, R. Arenas, and A. Bernardo. 2008. Modifications in membrane fatty acid composition of *Salmonella typhimurium* in response to growth conditions and their effect on heat resistance. *Int. J. Food Microb*. 123:212-219.

2. Anang, D. M., G. Rusul, S. Radu, J. Bakar, and L. R. Beuchat. 2006. Inhibitory effect of oxalic acid on bacterial spoilage of raw chilled chicken. *J. Food Prot*. 69:1913-1919.

3. AOAC. 2005. Official method 990.12. *In* Official methods of analysis of AOAC International. AOAC International, Gaithersburg, MD.

4. Ash, M., and I. Ash. 2004. Handbook of preservatives. Synapse Information Resources, Inc., Endicott, NY.

5. Bauermeister, L. J., J. W. J. Bower, J. C. Townsend, and S. R. McKee. 2008. The microbial and quality properties of poultry carcasses treated with peracetic acid as antimicrobial treatment. *Poultry Sci*. 87:2390-2398.

6. Bautista, D. A., N. Sylvester, S. Barbut, and M. W. Griffiths. 1997. The determination of efficacy of antimicrobial rinses on turkey carcasses using response surface designs. *Int. J. Food Microb*. 34:279-292.

7. Bell, C., and A. Kyriakides. 2002. Salmonella: a practical approach to the organism and its control in foods. Blackwell Science, London.

8. Bilgili, S. F., D. E. Conner, J. L. Pinion, and K. C. Tamblyn. 1998. Broiler skin color as affected by organic acids: influence of concentration and method of application. *Poultry Sci.* 77:752-757.

9. CDC. 2008. Foodborne Disease Active Surveillance Network (FoodNet). FoodNet Surveillance Final Report for 2005. U.S. Department of Health and Human Services, Atlanta, GA. Available at: http://www.cdc.gov/foodnet/reports.htm. Accessed 5 October 2010.

10. CDC. 2009. Surveillance for foodborne disease outbreaks -- United States, 2006. *MMWR*. 58:609-615.

11. CDC. 2010. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food -- 10 states, 2009. *MMWR*. 59:418-422.

12. Clark, M. *Salmonella*. Available at: http://www.about-salmonella.com. Accessed 18 December 2010.

13. Conner, D. E., and S. F. Bilgili. 1994. Skin attachment model for improved laboratory evaluation of potential carcass disinfectants for their efficacy against *Salmonella* attached to broiler skin. *J. Food Prot.* 57:684-688.

14. Conner, D. E., M. A. Davis, and L. Zhang. 2001. Poultry-borne pathogens: plant considerations. p. 137-159. *In* A.R. Sams (ed.), Poultry Meat Processing. CRC Press, Boca Raton, FL.

15. Dickens, J. A., and A. D. Whittemore. 1994. The effect of acetic acid and air injection on appearance, moisture pick-up, microbiological quality, and *Salmonella* incidence on processed poultry carcasses. *Poultry Sci*. 73:582-586.

16. Dickens, J. A., and A. D. Whittemore. 1997. Effects of acetic acid and hydrogen peroxide application during defeathering on the microbiological quality of broiler carcasses prior to evisceration. *Poultry Sci*. 76:657-660.

17. Heyndrickx, M., D. Vandekerchove, L. Herman, I. Rollier, K. Grijspeerdt, and L. De Zutter. 2002. Routes for *Salmonella* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. *Epidemiol. Inf.* 129:253-265.

18. Humphrey, T. J., D. G. Lanning, and D. Beresford. 1981. The effect of pH adjustment on the microbiology of chicken scald-tank water with particular reference to the death rate of Salmonellas. *J. Appl. Bact*. 51:517-527.

19. Hwang, C., and L. R. Beuchat. 1995. Efficacy of selected chemicals for killing pathogenic and spoilage microorganisms on chicken skin. *J. Food Prot*. 58:19-23.

20. James, W. O., R. L. Brewer, J. C. Prucha, W. O. Williams, and D. R. Parham. 1992. Effect of chlorination of chill water on the bacteriologic profile of raw chicken carcasses and giblets. *JAVMA*. 200:60-63.

21. Jetton, J. P., S. F. Bilgili, D. E. Conner, J. S. Kotrola, and M. A. Reiber. 1992. Recovery of salmonellae from chilled broiler carcasses as affected by rinse media and enumeration method. *J. Food Prot*. 55:329-332.

22. Katsui, N., T. Tsuchido, R. Hiramatsu, S. Fujikawa, M. Takano, and I. Shibasaki. 1982. Heat-induced blebbing and vesiculation of the outer membrane of *Escherichia coli. J. Bact*. 151:1523-1531.

23. Katsuyama, A. M. 1993. Principles of food processing sanitation. The Food Processors Institute, Washington, DC.

24. Kemp, G. K., M. L. Aldrich, M. L. Guerra, and K. R. Schneider. 2001. Continuous online processing of fecal- and ingesta- contaminated poultry carcasses using and acidified sodium chlorite antimicrobial intervention. *J. Food Prot*. 64:807-812.

25. Kim, J.-W., and M. F. Slavik. 1995. Cetylpyridinium Chloride (CPC) treatment on poultry skin to reduce attached *Salmonella. J. Food Prot*. 59:322-326.

26. Li, Y., M. F. Slavik, J. T. Walker, and H. Xiong. 1997. Pre-chill spray of chicken carcasses to reduce *Salmonella typhimurium. J. Food Sci*. 62:605-607.

27. Lillard, H. S. 1994. Effect of Trisodium Phosphate on salmonellae attached to chicken skin. *J. Food Prot*. 57:465-469.

28. Lillard, H. S., L. C. Blankenship, J. A. Dickens, S. E. Craven, and A. D. Shackelford. 1987. Effect of acetic acid on the microbiological quality of scalded picked and unpicked broiler carcasses. *J. Food Prot*. 50:112-114.

29. Lillard, H. S., and J. E. Thomson. 1983. Efficacy of hydrogen peroxide as a bactericide in poultry chiller water. *J. Food Sci*. 48:125-126.

30. Lund, B. M., and T. Eklund. 2000. Control of pH and use of organic acids. p. 175-199. *In* B.M. Lund, T.C. Baird-Parker, and G.W. Gould (ed.), The microbiological safety and quality of food, vol. I. Aspen Publishers, Inc, Gaithersburg, MD.

31. Martin, H., and P. Maris. 2005. An assessment of the bactericidal and fungicidal efficacy of seventeen mineral and organic acids on bacterial and fungal food industry contaminants. *Sci. Aliments*. 25:105-127.

32. Mehyar, G., G. Blank, J. H. Han, A. Hydamaka, and R. A. Holley. 2005. Effectiveness of trisodium phosphate, lactic acid and commercial antimicrobials against pathogenic bacteria on chicken skin. *J. Food Prot*. 25:351-362.

33. Mountney, G. J., and J. O'Malley. 1965. Acids as poultry meat preservatives. *Poultry Sci.* 44:582.

34. Nissen, H., T. Maugesten, and P. Lea. 2001. Survival and growth of *Escherichia coli* O157:H7, *Yersinia enterocolitica* and *Salmonella enteritidis* on decontaminated and untreated meat. *Meat Sci*. 57:291-298.

35. Okrend, A. J., R. W. Johnston, and A. B. Moran. 1986. Effect of acetic acid on the death rates at 52C of *Salmonella* Newport, *Salmonella* Typhimurium and *Campylobacter jejuni* in poultry scald water. *J. Food Prot*. 49:500-503.

36. Oyarzabal, O. A. 2005. Reduction of *Campylobacter* spp. by commercial antimicrobials applied during the processing of broiler chickens: a review from the United States perspective. *J.Food Prot*. 68:1752-1760.

37. Price search. 2010. Available at: http://www.aaabbbcccddd.com/. Accessed 18 December 2010.

38. Quinteros, A., R. Farre, and M. J. Lagarda. 2003. Effect of cooking on oxalate content of pulses using an enzymatic procedure. *Int. J. Food Sci. Nutr.* 54:373-377.

39. Rao, P. V. 1997. Statistical research methods in life sciences. Thomson Wadsworth, Mason, OH.

40.Rio, E., M. Panizo-Moran, M. Prieto, C. Alonso-Calleja, and R. Capita. 2007. Effect of various chemical decontamination treatments on natural microflora and sensory characteristics of poultry. *Int. J. Food Microb*. 115:268-280.

41. Salvat, G., P. Coppen, J. C. Allo, S. Fenner, M. J. Laisney, M. T. Toquin, F. Humbert, and P. Colin. 1997. Effects of AvGard^{TM} treatment on the microbiological flora of poultry carcasses. *British Poultry Sci*. 38:489-498.

42. Savage, G. P. 2002. Oxalates in human foods. *Proceedings Nutr. Soc. New Zealand*. 27:4-24.

43. Savage, G. P., L. Vanhanen, S. M. Mason, and A. B. Ross. 2000. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. *J. Food Comp. Anal*. 13:201-206.

44. Sybirtseva, I. 2009. Unpublished data. North Carolina State University, Raleigh, NC.

45. Tamblyn, K. C., and D. E. Conner. 1997. Bactericidal activity of organic acids against *Salmonella typhimurium* attached to broiler chicken skin. *J. Food Prot*. 60:629-633.

46. Tamblyn, K. C., and D. E. Conner. 1997. Bactericidal activity of organic acids in combination with transdermal compounds against *Salmonella typhimurium* attached to broiler skin. *Food Microb*. 14:477-484.

47. Tamblyn, K. C., D. E. Conner, and S. F. Bilgili. 1997. Utilization of the skin attachment model to determine the antibacterial efficacy of potential carcass treatments. *Poultry Sci*. 76:1318-1323.

48. Tsuchido, T., N. Katsui, A. Takeuchi, M. Takano, and I. Shibasaki. 1985. Destruction of the outer membrane permeability barrier of *Escherichia coli* by heat treatment. *Appl. Env. Microb*. 50:298-303.

49. U. S. Department of Agriculture, Food Safety and Inspection Service. 2010. Safe and suitable ingredients used in the production of meat, poultry, and egg products. Available at: http://origin-www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1Rev2.pdf. Accessed 5 May 2010.

50. U.S. Department of Agriculture, Food Safety and Inspection Service. 2010. New performance standards for *Salmonella* and *Campylobacter* in young chicken and turkey slaughter establishments; New compliance guides. Final Rule 75 Fed. Red. 27288-27294 (May 14, 2010) (to be codified at 9 CFR 381.94).

51. Whyte, P., J. D. Collins, K. McGill, C. Monahan, and H. O'Mahony. 2001. Quantitative investigation of the effects of chemical decontamination procedures on the microbiological status of broiler carcasses during processing. *J. Food Prot*. 64:179-183.

52. Wu, V. C. H., D. Y. C. Fung, D. H. Kang, and L. K. Thompson. 2001. Evaluation of thin agar layer method for recovery of acid-injured foodborne pathogens. *J. Food Prot*. 64:1067-1071.

Table 1. pH values of oxalic acid solutions at 25°C.

Oxalic acid (% w/v)	pH ± std. error[1]
0.1	2.07 ± 0.01
0.25	1.72 ± 0.00
0.5	1.48 ± 0.02
1.0	1.29 ± 0.02
1.5	1.13 ± 0.00
2.0	1.03 ± 0.01
2.5	0.98 ± 0.01
3.0	0.92 ± 0.01

[1] n = 3.

Table 2. Efficacy of oxalic acid to reduce *Salmonella* spp. on raw chicken skins during simulated on-line or post-chill dipping (22°C ± 1°C).

Oxalic acid (% w/v)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]			
	10 sec dipping	20 sec dipping	30 sec dipping	40 sec dipping
1.0	0.28 ± 0.06^{aA}	0.37 ± 0.11^{aA}	0.55 ± 0.12^{aA}	0.70 ± 0.13^{aA}
1.5	0.70 ± 0.09^{bA}	0.73 ± 0.11^{bA}	0.88 ± 0.10^{abA}	1.13 ± 0.09^{bA}
2.0	0.79 ± 0.05^{bA}	0.86 ± 0.08^{bA}	0.99 ± 0.08^{bA}	1.12 ± 0.07^{bA}

[1] n = 9.

[2] Within the same column, mean values with different lowercase superscripts are significantly different ($P < 0.05$). Within the same row, mean values with different uppercase superscripts are significantly different ($P < 0.05$).

Table 3. Efficacy of oxalic acid to reduce *Salmonella* spp. on raw chicken skins during simulated water-immersion chilling (3°C ± 1°C).

Oxalic acid (% w/v)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]		
	30 min chilling	45 min chilling	60 min chilling
0.1	0.52 ± 0.10^{aA}	0.69 ± 0.08^{aA}	0.85 ± 0.15^{aA}
0.25	1.01 ± 0.19^{bA}	1.09 ± 0.17^{abA}	1.32 ± 0.24^{abA}
0.5	1.29 ± 0.20^{bA}	1.58 ± 0.14^{bAB}	2.25 ± 0.31^{bB}

[1, 2] See Table 2 footnotes.

Table 4. Efficacy of oxalic acid to reduce *Salmonella* spp. on raw chicken skins during simulated soft scalding (53°C ± 1°C).

Oxalic acid (% w/v)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]		
	1 min scalding	2 min scalding	3 min scalding
1.0	1.01 ± 0.05^{aA}	1.31 ± 0.17^{aA}	1.56 ± 0.19^{aA}
1.5	1.07 ± 0.09^{aA}	1.52 ± 0.18^{abAB}	1.91 ± 0.20^{abB}
2.0	1.97 ± 0.26^{bA}	2.17 ± 0.27^{bA}	2.43 ± 0.31^{bA}

[1, 2] See Table 2 footnotes.

Table 5. Hunter values of raw chicken skins treated with oxalic acid during simulated on-line or post-chill dipping (22°C ± 1°C) and storage at 4°C for 14 days.

Time	Oxalic acid (% w/v)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
10 sec dipping	0	0	80.48 ± 0.49^{aA}a	-1.68 ± 0.18^{aA}a	5.68 ± 2.49^{aA}a
		1	81.28 ± 0.74^{aA}a	-1.82 ± 0.15^{aA}a	6.07 ± 2.29^{aA}a
		3	80.63 ± 0.64^{aA}a	-1.94 ± 0.17^{aA}a	5.84 ± 2.51^{aA}a
		7	81.46 ± 0.74^{aA}a	-1.85 ± 0.16^{aA}a	6.12 ± 2.20^{aA}a
		10	80.55 ± 0.56^{aA}a	-1.76 ± 0.22^{aA}a	5.81 ± 2.43^{aA}a
		14	80.91 ± 0.80^{aA}a	-1.84 ± 0.23^{aA}a	6.17 ± 2.30^{aA}a
	1.0	0	73.92 ± 0.99^{aB}a	-2.50 ± 0.35^{aA}a	9.20 ± 2.42^{aA}a
		1	74.22 ± 0.94^{aB}a	-2.60 ± 0.36^{aA}a	9.72 ± 1.69^{aA}a
		3	74.29 ± 1.01^{aB}a	-2.85 ± 0.30^{aA}a	10.16 ± 1.47^{aA}a
		7	74.46 ± 0.75^{aB}a	-2.71 ± 0.34^{aA}a	9.70 ± 1.74^{aA}a
		10	74.38 ± 0.76^{aB}a	-2.63 ± 0.36^{aA}a	9.56 ± 1.60^{aA}a
		14	74.93 ± 0.96^{aB}a	-2.75 ± 0.40^{aA}a	9.54 ± 1.65^{aA}a
	1.5	0	73.37 ± 0.34^{aB}a	-2.58 ± 0.25^{aA}a	12.20 ± 0.38^{aA}a
		1	73.78 ± 0.46^{aB}a	-2.94 ± 0.21^{aA}a	11.98 ± 0.45^{aA}a
		3	74.39 ± 0.68^{aB}a	-3.09 ± 0.21^{aA}a	11.67 ± 0.67^{aA}a
		7	74.67 ± 0.28^{aB}a	-3.04 ± 0.22^{aA}a	11.80 ± 0.33^{aA}a
		10	75.10 ± 0.24^{aB}a	-3.01 ± 0.24^{aA}a	11.72 ± 0.46^{aA}a
		14	75.12 ± 0.23^{aB}a	-3.19 ± 0.16^{aA}a	12.08 ± 0.34^{aA}a
	2.0	0	73.22 ± 0.14^{aB}a	-2.36 ± 0.12^{aA}a	12.31 ± 0.10^{aA}a
		1	72.60 ± 0.44^{aB}a	-2.64 ± 0.03^{aA}a	11.72 ± 0.07^{aA}a
		3	73.98 ± 0.46^{aB}a	-2.99 ± 0.11^{aA}a	11.88 ± 0.09^{aA}a
		7	73.30 ± 0.24^{aB}a	-2.86 ± 0.07^{aA}a	11.74 ± 0.18^{aA}a
		10	74.58 ± 0.50^{aB}a	-2.86 ± 0.10^{aA}a	11.62 ± 0.25^{aA}a
		14	73.80 ± 0.46^{aB}a	-3.05 ± 0.13^{aA}a	12.08 ± 0.16^{aA}a
20 sec dipping	0	0	79.21 ± 0.42^{aA}a	-1.49 ± 0.32^{aA}a	8.08 ± 0.38^{aA}a
		1	80.02 ± 0.04^{aA}a	-1.68 ± 0.39^{aA}a	8.77 ± 0.38^{aA}a
		3	79.11 ± 0.39^{aA}a	-1.78 ± 0.36^{aA}a	8.77 ± 0.66^{aA}a
		7	79.75 ± 0.08^{aA}a	-1.72 ± 0.39^{aA}a	8.67 ± 0.63^{aA}a
		10	79.35 ± 0.30^{aA}a	-1.79 ± 0.34^{aA}a	9.13 ± 0.69^{aA}a
		14	79.69 ± 0.18^{aA}a	-1.64 ± 0.36^{aA}a	9.08 ± 0.70^{aA}a
	1.0	0	73.62 ± 0.89^{aB}a	-3.04 ± 0.14^{aA}a	9.49 ± 0.47^{aA}a
		1	74.63 ± 0.85^{aB}a	-3.11 ± 0.15^{aA}a	11.92 ± 0.52^{aA}a
		3	74.47 ± 1.04^{aAB}a	-3.31 ± 0.23^{aA}a	11.75 ± 0.71^{aA}a
		7	75.40 ± 1.13^{aAB}a	-3.28 ± 0.10^{aA}a	11.69 ± 1.00^{aA}a
		10	74.46 ± 1.02^{aAB}a	-3.23 ± 0.16^{aA}a	11.91 ± 0.80^{aA}a
		14	75.19 ± 1.00^{aAB}a	-3.31 ± 0.10^{aA}a	11.75 ± 0.89^{aA}a

Table 5. Continued

Time	Oxalic acid (% w/v)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
20 sec dipping	1.5	0	71.34 ± 1.72^{aB}a	-2.40 ± 0.34^{aA}a	11.98 ± 0.86^{aA}a
		1	72.55 ± 1.24^{aB}a	-2.64 ± 0.29^{aA}a	11.88 ± 0.96^{aA}a
		3	72.74 ± 1.65^{aB}a	-2.93 ± 0.33^{aA}a	12.07 ± 0.94^{aA}a
		7	72.76 ± 1.34^{aB}a	-2.93 ± 0.31^{aA}a	12.00 ± 1.01^{aA}a
		10	73.27 ± 1.30^{aB}a	-2.86 ± 0.29^{aA}a	11.67 ± 1.12^{aA}a
		14	72.91 ± 1.16^{aB}a	-2.93 ± 0.34^{aA}a	11.63 ± 1.21^{aA}a
	2.0	0	72.92 ± 0.88^{aB}a	-3.12 ± 0.10^{aA}a	11.91 ± 0.71^{aA}a
		1	73.15 ± 0.90^{aB}a	-3.15 ± 0.19^{aA}a	11.37 ± 0.76^{aA}a
		3	74.55 ± 0.90^{aAB}a	-3.53 ± 0.25^{aA}a	11.92 ± 1.01^{aA}a
		7	73.77 ± 1.02^{aB}a	-3.40 ± 0.23^{aA}a	11.72 ± 0.85^{aA}a
		10	74.61 ± 1.18^{aAB}a	-3.32 ± 0.14^{aA}a	11.72 ± 0.91^{aA}a
		14	73.58 ± 1.12^{aB}a	-3.32 ± 0.22^{aA}a	11.46 ± 0.83^{aA}a
30 sec dipping	0	0	79.09 ± 0.51^{aA}a	-1.70 ± 0.53^{aA}a	8.24 ± 2.58^{aA}a
		1	79.51 ± 0.33^{aA}a	-1.78 ± 0.53^{aA}a	8.09 ± 2.55^{aA}a
		3	79.36 ± 0.46^{aA}a	-2.01 ± 0.55^{aA}a	8.24 ± 2.53^{aA}a
		7	79.53 ± 0.49^{aA}a	-1.99 ± 0.51^{aA}a	8.15 ± 2.51^{aA}a
		10	79.95 ± 0.24^{aA}a	-1.91 ± 0.50^{aA}a	7.89 ± 2.42^{aA}a
		14	79.66 ± 0.47^{aA}a	-1.87 ± 0.52^{aA}a	8.37 ± 2.40^{aA}a
	1.0	0	72.91 ± 0.29^{aB}a	-3.15 ± 0.05^{aA}a	13.57 ± 0.12^{aA}a
		1	73.59 ± 0.45^{aB}a	-3.27 ± 0.06^{aA}a	13.15 ± 0.14^{aA}a
		3	74.21 ± 0.75^{aAB}a	-3.57 ± 0.04^{aA}a	13.24 ± 0.09^{aA}a
		7	73.99 ± 0.20^{aB}a	-3.59 ± 0.02^{aA}a	12.72 ± 0.27^{aA}a
		10	74.75 ± 0.65^{aAB}a	-3.49 ± 0.02^{aA}a	12.90 ± 0.15^{aA}a
		14	75.59 ± 0.31^{aAB}a	-3.40 ± 0.08^{aA}a	12.73 ± 0.15^{aA}a
	1.5	0	71.05 ± 2.04^{aB}a	-2.26 ± 0.31^{aA}a	11.03 ± 0.33^{aA}a
		1	71.52 ± 2.05^{aB}a	-2.52 ± 0.15^{aA}a	10.89 ± 0.39^{aA}a
		3	72.00 ± 1.70^{aB}a	-2.92 ± 0.07^{aA}a	11.28 ± 0.54^{aA}a
		7	71.86 ± 2.11^{aB}a	-2.75 ± 0.07^{aA}a	10.68 ± 0.52^{aA}a
		10	72.62 ± 1.46^{aB}a	-2.67 ± 0.10^{aA}a	10.39 ± 0.81^{aA}a
		14	72.96 ± 1.71^{aB}a	-2.70 ± 0.11^{aA}a	10.66 ± 0.39^{aA}a
	2.0	0	72.16 ± 1.21^{aB}a	-2.63 ± 0.58^{aA}a	13.04 ± 1.39^{aA}a
		1	71.49 ± 1.15^{aB}a	-2.95 ± 0.62^{aA}a	12.65 ± 1.59^{aA}a
		3	73.47 ± 1.18^{aB}a	-3.25 ± 0.55^{aA}a	12.96 ± 1.58^{aA}a
		7	72.26 ± 0.73^{aB}a	-3.10 ± 0.56^{aA}a	12.22 ± 1.55^{aA}a
		10	73.77 ± 1.29^{aB}a	-3.08 ± 0.50^{aA}a	12.21 ± 1.51^{aA}a
		14	72.62 ± 1.02^{aB}a	-3.04 ± 0.52^{aA}a	12.05 ± 1.54^{aA}a

Table 5. Continued

Time	Oxalic acid (% w/v)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
40 sec dipping	0	0	79.49 ± 0.22^{aA}a	-2.09 ± 0.28^{aA}a	8.27 ± 0.94^{aA}a
		1	80.05 ± 0.55^{aA}a	-2.26 ± 0.31^{aA}a	8.87 ± 1.19^{aA}a
		3	79.97 ± 0.31^{aA}a	-2.32 ± 0.25^{aA}a	9.34 ± 0.94^{aA}a
		7	80.37 ± 0.66^{aA}a	-2.32 ± 0.25^{aA}a	9.44 ± 0.83^{aA}a
		10	80.30 ± 0.34^{aA}a	-2.28 ± 0.25^{aA}a	9.47 ± 0.69^{aA}a
		14	80.72 ± 0.23^{aA}a	-2.26 ± 0.31^{aA}a	10.14 ± 0.80^{aA}a
	1.0	0	73.12 ± 0.54^{aB}a	-2.69 ± 0.17^{aA}a	10.33 ± 1.02^{aA}a
		1	73.68 ± 0.51^{aB}a	-2.92 ± 0.08^{aA}a	10.17 ± 0.93^{aA}a
		3	74.02 ± 0.64^{aB}a	-2.99 ± 0.20^{aA}a	9.87 ± 1.03^{aA}a
		7	74.44 ± 0.30^{aB}a	-2.98 ± 0.25^{aA}a	9.68 ± 1.09^{aA}a
		10	75.06 ± 0.61^{aAB}a	-2.87 ± 0.19^{aA}a	9.65 ± 0.83^{aA}a
		14	74.06 ± 0.55^{aB}a	-2.84 ± 0.24^{aA}a	9.77 ± 1.04^{aA}a
	1.5	0	72.09 ± 0.71^{aB}a	-3.09 ± 0.32^{aA}a	11.52 ± 0.48^{aA}a
		1	73.19 ± 0.73^{aB}a	-3.27 ± 0.21^{aA}a	11.43 ± 0.33^{aA}a
		3	73.02 ± 1.00^{aB}a	-3.33 ± 0.17^{aA}a	11.36 ± 0.37^{aA}a
		7	74.10 ± 0.88^{aB}a	-3.38 ± 0.19^{aA}a	11.59 ± 0.37^{aA}a
		10	73.71 ± 0.73^{aB}a	-3.15 ± 0.23^{aA}a	11.22 ± 0.45^{aA}a
		14	73.31 ± 0.93^{aB}a	-3.16 ± 0.16^{aA}a	11.24 ± 0.38^{aA}a
	2.0	0	71.48 ± 0.52^{aB}a	-2.52 ± 0.08^{aA}a	11.93 ± 0.83^{aA}a
		1	72.10 ± 0.33^{a}a	-2.79 ± 0.08^{aA}a	11.66 ± 0.76^{aA}a
		3	72.41 ± 0.69^{aB}a	-3.05 ± 0.11^{aA}a	11.54 ± 0.84^{aA}a
		7	72.94 ± 0.50^{aB}a	-3.10 ± 0.11^{aA}a	11.74 ± 0.93^{aA}a
		10	72.14 ± 0.45^{aB}a	-3.00 ± 0.13^{aA}a	11.55 ± 0.93^{aA}a
		14	72.60 ± 0.62^{aB}a	-2.95 ± 0.12^{aA}a	11.64 ± 0.87^{aA}a

[1] n = 3.

[2] Within the same time and concentration, mean values with different lowercase superscript are significantly different (P <0.05). Within the same time and day, mean values with different uppercase superscript are significantly different (P < 0.05). Within the same day and concentration, means values followed different letters are significantly different (P < 0.05).

Table 6. Hunter values of raw chicken skins treated with oxalic acid during simulated water-immersion chilling (3°C ± 1°C) and storage at 4°C for 14 days.

Time	Oxalic acid (% w/v)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
30 min chilling	0	0	79.96 ± 0.78^{aA}a	-1.54 ± 0.27^{aA}a	6.55 ± 2.31^{aA}a
		1	79.98 ± 0.77^{aA}a	-1.74 ± 0.25^{aA}a	6.93 ± 2.21^{aA}a
		3	80.20 ± 0.69^{aA}a	-1.68 ± 0.21^{aA}a	7.34 ± 2.12^{aA}a
		7	79.82 ± 0.76^{aA}a	-1.80 ± 0.23^{aA}a	7.57 ± 2.30^{aA}a
		10	80.46 ± 0.71^{aA}a	-1.77 ± 0.19^{aA}a	7.69 ± 2.14^{aA}a
		14	80.14 ± 0.71^{aA}a	-1.82 ± 0.24^{aA}a	8.01 ± 2.05^{aA}a
	0.1	0	74.64 ± 0.86^{aB}a	-2.76 ± 0.28^{aB}a	9.58 ± 0.12^{aA}a
		1	76.17 ± 0.78^{aAB}a	-3.26 ± 0.23^{aB}a	10.19 ± 0.26^{aA}a
		3	76.01 ± 0.97^{aB}a	-3.17 ± 0.14^{aB}a	10.07 ± 0.38^{aA}a
		7	75.01 ± 0.39^{aB}a	-3.36 ± 0.19^{aB}a	10.43 ± 0.15^{aA}a
		10	76.37 ± 0.76^{aB}a	-3.32 ± 0.14^{aB}a	10.11 ± 0.33^{aA}a
		14	75.78 ± 0.91^{aB}a	-3.33 ± 0.12^{aB}a	10.18 ± 0.16^{aA}a
	0.25	0	73.00 ± 0.72^{aB}a	-2.56 ± 0.14^{aAB}a	10.29 ± 0.52^{aA}a
		1	74.10 ± 0.86^{aBC}a	-3.03 ± 0.01^{aB}a	10.23 ± 0.42^{aA}a
		3	74.12 ± 0.53^{aBC}a	-303 ± 0.05^{aB}a	9.83 ± 0.02^{aA}a
		7	73.48 ± 0.46^{aB}a	-3.13 ± 0.04^{aB}a	9.99 ± 0.07^{aA}a
		10	73.55 ± 0.35^{aBC}a	-3.23 ± 0.01^{aB}a	10.10 ± 0.14^{aA}a
		14	74.90 ± 0.61^{aB}a	-3.15 ± 0.05^{aB}a	10.06 ± 0.31^{aA}a
	0.5	0	70.86 ± 1.43^{aB}a	-2.50 ± 0.13^{aAB}a	10.63 ± 0.17^{aA}a
		1	72.12 ± 1.55^{aC}a	-3.03 ± 0.14^{aB}a	10.92 ± 0.27^{aA}a
		3	70.25 ± 1.43^{aC}a	-2.83 ± 0.17^{aB}a	10.10 ± 0.28^{aA}a
		7	71.09 ± 0.88^{aB}a	-2.90 ± 0.15^{aAB}a	10.32 ± 0.27^{aA}a
		10	70.18 ± 0.83^{aC}a	-3.08 ± 0.14^{aB}a	10.36 ± 0.19^{aA}a
		14	72.04 ± 1.11^{aB}a	-3.03 ± 0.07^{aB}a	10.28 ± 0.03^{aA}a
45 min chilling	0	0	80.45 ± 0.56^{aA}a	-1.48 ± 0.11^{aA}a	5.95 ± 0.62^{aA}a
		1	80.60 ± 0.43^{aA}a	-1.52 ± 0.10^{aA}a	6.15 ± 0.23^{aA}a
		3	81.01 ± 0.25^{aA}a	-1.74 ± 0.09^{aA}a	6.39 ± 0.32^{aA}a
		7	81.32 ± 0.20^{aA}a	-1.73 ± 0.06^{aA}a	6.64 ± 0.38^{aA}a
		10	80.92 ± 0.26^{aA}a	-1.75 ± 0.11^{aA}a	7.41 ± 0.26^{aA}a
		14	80.67 ± 0.35^{aA}a	-1.74 ± 0.07^{aA}a	7.17 ± 0.31^{aA}a
	0.1	0	73.00 ± 0.34^{aB}a	-2.59 ± 0.13^{aB}a	9.48 ± 0.55^{aA}a
		1	73.49 ± 0.61^{aB}a	-2.99 ± 0.16^{aB}a	9.94 ± 0.56^{aA}a
		3	73.80 ± 0.46^{aB}a	-3.04 ± 0.13^{aB}a	9.66 ± 0.64^{aA}a
		7	72.92 ± 0.37^{aB}a	-3.23 ± 0.23^{aB}a	9.76 ± 0.85^{aA}a
		10	73.70 ± 0.49^{aB}a	-3.22 ± 0.19^{aB}a	9.85 ± 0.88^{aA}a
		14	72.92 ± 0.52^{aB}a	-3.23 ± 0.27^{aB}a	9.27 ± 0.94^{aA}a

Table 6. Continued

Time	Oxalic acid (% w/v)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
45 min chilling	0.25	0	71.39 ± 0.65^{aB}a	-2.67 ± 0.06^{aB}a	9.94 ± 0.33^{aA}a
		1	71.46 ± 0.68^{aB}a	-2.84 ± 0.07^{aB}a	9.67 ± 0.17^{aA}a
		3	72.24 ± 0.57^{aB}a	-2.86 ± 0.09^{aB}a	9.72 ± 0.19^{aA}a
		7	70.76 ± 0.96^{aB}a	-2.90 ± 0.04^{aB}a	9.38 ± 0.28^{aA}a
		10	71.86 ± 0.14^{aB}a	-2.98 ± 0.06^{aB}a	9.61 ± 0.23^{aA}a
		14	71.63 ± 0.67^{aB}a	-2.97 ± 0.02^{aB}a	9.42 ± 0.29^{aA}a
	0.5	0	69.65 ± 0.49^{aB}a	-2.48 ± 0.16^{aAB}a	9.74 ± 0.52^{aA}a
		1	69.68 ± 0.52^{aB}a	-2.66 ± 0.23^{aB}a	9.92 ± 0.48^{aA}a
		3	70.86 ± 0.63^{aB}a	-2.70 ± 0.23^{aAB}a	10.03 ± 0.59^{aA}a
		7	69.43 ± 0.53^{aB}a	-2.76 ± 0.21^{aAB}a	9.52 ± 0.45^{aA}a
		10	70.05 ± 0.65^{aB}a	-2.79 ± 0.19^{aAB}a	9.55 ± 0.41^{aA}a
		14	70.26 ± 0.59^{aB}a	-3.11 ± 0.19^{aB}a	9.96 ± 0.34^{aA}a
60 min chilling	0	0	81.26 ± 0.82^{aA}a	-1.57 ± 0.25^{aA}a	6.30 ± 1.06^{aA}a
		1	81.53 ± 0.60^{aA}a	-1.85 ± 0.26^{aA}a	7.01 ± 1.18^{aA}a
		3	81.60 ± 0.84^{aA}a	-1.94 ± 0.23^{aA}a	7.32 ± 1.18^{aA}a
		7	81.05 ± 0.74^{aA}a	-2.03 ± 0.24^{aA}a	7.28 ± 1.07^{aA}a
		10	81.44 ± 0.74^{aA}a	-1.97 ± 0.23^{aA}a	7.68 ± 1.12^{aA}a
		14	81.09 ± 0.81^{aA}a	-2.10 ± 0.27^{aA}a	7.74 ± 0.88^{aA}a
	0.1	0	72.83 ± 0.34^{aB}a	-3.00 ± 0.04^{aB}a	10.02 ± 0.25^{aA}a
		1	73.50 ± 0.72^{aB}a	-3.66 ± 0.05^{aB}a	11.14 ± 0.15^{aA}a
		3	73.80 ± 0.32^{aB}a	-3.84 ± 0.07^{aB}a	11.44 ± 0.05^{aA}a
		7	72.89 ± 0.10^{aB}a	-3.85 ± 0.07^{aB}a	10.97 ± 0.11^{aA}a
		10	74.06 ± 0.25^{aB}a	-3.84 ± 0.11^{aB}a	11.26 ± 0.18^{aA}a
		14	73.67 ± 0.30^{aB}a	-3.88 ± 0.04^{aB}a	10.81 ± 0.41^{aA}a
	0.25	0	69.93 ± 0.44^{aB}a	-2.21 ± 0.16^{aAB}a	9.42 ± 0.86^{aA}a
		1	71.07 ± 0.53^{aB}a	-2.75 ± 0.23^{aAB}a	9.72 ± 0.81^{aA}a
		3	71.04 ± 0.08^{aB}a	-2.73 ± 0.19^{aA}a	8.99 ± 0.72^{aA}a
		7	70.75 ± 0.48^{aB}a	-2.95 ± 0.25^{aAB}a	9.47 ± 0.86^{aA}a
		10	71.11 ± 0.20^{aB}a	-2.99 ± 0.24^{aAB}a	9.58 ± 0.65^{aA}a
		14	70.80 ± 0.42^{aB}a	-3.06 ± 0.26^{aAB}a	9.57 ± 0.69^{aA}a
	0.5	0	71.18 ± 0.43^{aB}a	-2.87 ± 0.28^{aB}a	11.01 ± 1.26^{aA}a
		1	71.80 ± 0.57^{aB}a	-3.14 ± 0.24^{aB}a	11.33 ± 1.44^{aA}a
		3	71.08 ± 0.66^{aB}a	-3.34 ± 0.30^{aB}a	11.27 ± 1.42^{aA}a
		7	71.63 ± 0.33^{aB}a	-3.33 ± 0.34^{aB}a	11.21 ± 1.57^{aA}a
		10	71.58 ± 0.70^{aB}a	-3.39 ± 0.30^{aB}a	11.11 ± 1.42^{aA}a
		14	72.05 ± 0.21^{aB}a	-3.41 ± 0.35^{aB}a	11.05 ± 1.39^{aA}a

[1, 2] See Table 5 footnotes.

Table 7. Hunter values of raw chicken skins treated with oxalic acid during simulated soft scalding (53°C ± 1°C) and storage at 4°C for 14 days.

Time	Oxalic acid (% w/v)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
1 min scalding	0	0	79.04 ± 0.22^{aA}a	-1.49 ± 0.10^{aA}a	8.27 ± 0.77^{aA}a
		1	79.46 ± 0.49^{aA}a	-1.49 ± 0.10^{aA}a	8.44 ± 0.76^{aA}a
		3	79.29 ± 0.37^{aA}a	-1.54 ± 0.11^{aA}a	8.23 ± 1.15^{aA}a
		7	79.48 ± 0.24^{aA}a	-1.76 ± 0.12^{aA}a	8.28 ± 0.94^{aA}a
		10	80.33 ± 0.23^{aA}a	-1.79 ± 0.12^{aA}a	8.60 ± 0.83^{aA}a
		14	80.06 ± 0.22^{aA}a	-1.93 ± 0.13^{aA}a	8.68 ± 0.79^{aA}a
	1.0	0	69.00 ± 0.80^{aB}a	-1.93 ± 0.18^{aA}a	12.29 ± 0.50^{aAB}a
		1	69.97 ± 0.92^{aB}a	-2.27 ± 0.19^{aA}a	12.33 ± 0.45^{aAB}a
		3	70.24 ± 0.53^{aB}a	-2.44 ± 0.21^{aA}a	12.03 ± 0.64^{aAB}a
		7	71.68 ± 0.98^{aB}a	-2.67 ± 0.17^{aA}a	12.23 ± 0.54^{aAB}a
		10	71.61 ± 0.46^{aB}a	-2.82 ± 0.22^{aA}a	11.83 ± 0.43^{aA}a
		14	72.43 ± 1.02^{aB}a	-3.01 ± 0.23^{aA}a	12.05 ± 0.47^{aA}a
	1.5	0	69.80 ± 0.57^{aB}a	-2.35 ± 0.36^{aA}a	14.65 ± 0.69^{aB}a
		1	69.71 ± 0.69^{aB}a	-2.87 ± 0.30^{aA}a	14.22 ± 0.63^{aB}a
		3	69.96 ± 0.79^{aB}a	-3.02 ± 0.34^{aA}a	13.94 ± 0.57^{aB}a
		7	71.13 ± 0.69^{aB}a	-3.22 ± 0.28^{aA}a	13.72 ± 0.65^{aB}a
		10	71.30 ± 0.56^{aB}a	-3.39 ± 0.26^{aA}a	13.68 ± 0.58^{aA}a
		14	71.72 ± 0.58^{aB}a	-3.56 ± 0.28^{aA}a	13.83 ± 0.61^{aA}a
	2.0	0	70.01 ±1.40^{aB}a	-2.46 ± 0.27^{aA}a	11.58 ± 0.76^{aAB}a
		1	70.11 ± 0.85^{aB}a	-2.57 ± 0.23^{aA}a	11.15 ± 0.83^{aAB}a
		3	69.91 ± 0.83^{aB}a	-2.72 ± 0.24^{aA}a	10.75 ± 0.82^{aAB}a
		7	71.32 ± 0.60^{aB}a	-2.88 ± 0.14^{aA}a	10.81 ± 0.95^{aAB}a
		10	71.52 ± 0.92^{aB}a	-2.89 ± 0.15^{aA}a	10.67 ± 0.84^{aA}a
		14	71.87 ± 0.55^{aB}a	-2.98 ± 0.17^{aA}a	10.68 ± 0.84^{aA}a
2 min scalding	0	0	77.33 ± 0.22^{aA}a	-0.57 ± 0.22^{aA}a	8.89 ± 0.81^{aA}a
		1	77.69 ± 0.20^{aA}a	-0.69 ± 0.27^{aA}a	9.47 ± 0.80^{aA}a
		3	77.08 ± 0.59^{aA}a	-0.70 ± 0.32^{aA}a	9.46 ± 0.82^{aA}a
		7	77.60 ± 0.30^{aA}a	-0.75 ± 0.23^{aA}a	9.70 ± 0.79^{aA}a
		10	77.51 ± 0.20^{aA}a	-0.75 ± 0.24^{aA}a	9.51 ± 0.89^{aA}a
		14	78.14 ± 0.39^{aA}a	-0.90 ± 0.26^{aA}a	9.95 ± 0.85^{aA}a
	1.0	0	68.55 ± 0.59^{aB}a	-1.78 ± 0.27^{aA}a	13.65 ± 0.85^{aA}a
		1	69.80 ± 0.39^{aB}a	-2.38 ± 0.22^{aA}a	13.24 ± 0.52^{aA}a
		3	70.41 ± 0.78^{aB}a	-2.49 ± 0.20^{aA}a	13.19 ± 0.42^{aA}a
		7	70.69 ± 0.43^{aB}a	-2.89 ± 0.24^{aA}a	13.07 ± 0.43^{aA}a
		10	70.77 ± 0.24^{aB}a	-3.00 ± 0.19^{aA}a	13.28 ± 0.51^{aA}a
		14	72.38 ± 0.27^{aB}a	-3.06 ± 0.13^{aAB}a	13.15 ± 0.52^{aA}a

Table 7. Continued

Time	Oxalic acid (% w/v)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
2 min scalding	1.5	0	69.69 ± 0.68^{aB}a	-2.33 ± 0.47^{aA}a	14.22 ± 0.87^{aA}a
		1	70.85 ± 0.67^{aB}a	-2.82 ± 0.34^{aA}a	13.62 ± 0.83^{aA}a
		3	71.21 ± 0.48^{aB}a	-2.92 ± 0.33^{aA}a	13.61 ± 0.82^{aA}a
		7	71.20 ± 0.55^{aB}a	-3.06 ± 0.31^{aA}a	13.61 ± 0.83^{aA}a
		10	71.66 ± 0.53^{aB}a	-3.18 ± 0.30^{aA}a	13.65 ± 0.72^{aA}a
		14	71.64 ± 0.53^{aB}a	-3.30 ± 0.33^{aAB}a	13.65 ± 0.88^{aA}a
	2.0	0	67.99 ± 0.94^{aB}a	-2.14 ± 0.26^{aA}a	13.79 ± 0.68^{aA}a
		1	68.41 ± 0.84^{aB}a	-2.56 ± 0.10^{aA}a	13.39 ± 0.76^{aA}a
		3	69.66 ± 0.63^{aB}a	-2.68 ± 0.09^{aA}a	13.23 ± 0.91^{aA}a
		7	68.66 ± 1.20^{aB}a	-2.73 ± 0.09^{aA}a	13.18 ± 0.82^{aA}a
		10	70.28 ± 0.98^{aB}a	-2.85 ± 0.09^{aA}a	13.28 ± 0.90^{aA}a
		14	68.51 ± 0.92^{aB}a	-2.83 ± 0.08^{aAB}a	12.89 ± 0.96^{aA}a
3 min scalding	0	0	77.10 ± 1.60^{aA}a	-0.59 ± 0.74^{aA}a	9.52 ± 0.30^{aA}a
		1	76.93 ± 1.53^{aA}a	-0.69 ± 0.79^{aA}a	9.06 ± 0.26^{aA}a
		3	77.30 ± 1.55^{aA}a	-0.74 ± 0.82^{aA}a	9.26 ± 0.27^{aA}a
		7	76.91 ± 1.57^{aA}a	-0.70 ± 0.76^{aA}a	9.54 ± 0.07^{aA}a
		10	77.57 ± 1.30^{aA}a	-0.61 ± 0.83^{aA}a	9.52 ± 0.17^{aA}a
		14	77.06 ± 1.36^{aA}a	-0.65 ± 0.82^{aA}a	9.29 ± 0.14^{aA}a
	1.0	0	69.61 ± 1.54^{aB}a	-2.31 ± 0.20^{aA}a	12.54 ± 0.84^{aA}a
		1	70.72 ± 1.44^{aB}a	-2.73 ± 0.24^{aA}a	12.56 ± 0.90^{aA}a
		3	70.03 ± 1.20^{aB}a	-2.69 ± 0.24^{aA}a	12.01 ± 1.12^{aA}a
		7	71.51 ± 1.06^{aB}a	-2.79 ± 0.26^{aA}a	12.11 ± 1.07^{aA}a
		10	70.69 ± 1.00^{aB}a	-3.00 ± 0.27^{aB}a	12.11 ± 0.91^{aA}a
		14	71.78 ± 0.90^{aB}a	-2.98 ± 0.23^{aA}a	12.20 ± 0.87^{aA}a
	1.5	0	68.65 ± 1.14^{aB}a	-2.10 ± 0.71^{aA}a	13.67 ± 1.97^{aA}a
		1	68.96 ± 1.32^{aB}a	-2.50 ± 0.57^{aA}a	13.37 ± 1.74^{aA}a
		3	69.96 ± 0.94^{aB}a	-2.58 ± 0.50^{aA}a	13.26 ± 1.42^{aA}a
		7	70.21 ± 0.85^{aB}a	-2.74 ± 0.46^{aA}a	13.51 ± 1.34^{aA}a
		10	70.31 ± 0.80^{aB}a	-2.97 ± 0.44^{aB}a	13.52 ± 1.15^{aA}a
		14	69.63 ± 1.04^{aB}a	-2.97 ± 0.41^{aA}a	13.29 ± 1.24^{aA}a
	2.0	0	69.78 ± 1.56^{a}a	-2.47 ± 0.69^{aA}a	13.92 ± 1.25^{aA}a
		1	71.18 ±1.71^{aB}a	-2.85 ± 0.68^{aA}a	14.29 ± 1.63^{aA}a
		3	70.76 ± 1.07^{aB}a	-2.89 ± 0.64^{aA}a	13.91 ± 1.27^{aA}a
		7	71.66 ± 1.31^{aB}a	-2.88 ± 0.61^{aA}a	14.07 ± 1.29^{aA}a
		10	70.74 ± 1.01^{aB}a	-3.04 ± 0.64^{aB}a	14.21 ± 1.34^{aA}a
		14	71.57 ± 1.53^{aB}a	-2.95 ± 0.58^{aA}a	14.07 ± 1.22^{aA}a

[1, 2] See Table 5 footnotes

Table 8. Efficacy of 2.5% and 3.0% oxalic acid to reduce *Salmonella* spp. on raw chicken skins during 40 sec of simulated on-line or post-chill dipping (22°C ± 1°C).

Oxalic acid (% w/v)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]
2.0	1.12 ± 0.06^{a}
2.5	1.30 ± 0.08^{a}
3.0	1.32 ± 0.09^{a}

[1] n = 3.
[2] Within the same column, mean values with different superscripts are significantly different ($P < 0.05$).

Table 9. pH values of untreated raw chicken skins or skins treated with water or oxalic acid during simulated on-line or post-chill dipping (22ºC ± 1ºC) for 10 sec and subsequent storage at 4ºC for 14 days.

Time (day)	Untreated	Treated with water (control)	Treated with oxalic acid (% w/v)		
			1.0	1.5	2.0
	pH ± std. error[1]				
0	5.57 ± 0.07^{aA}	5.46 ± 0.12^{aA}	3.68 ± 0.18^{aB}	3.73 ± 0.25^{aB}	3.49 ± 0.26^{aB}
1	5.54 ± 0.08^{aA}	5.47 ± 0.13^{aA}	3.89 ± 0.14^{aB}	3.82 ± 0.11^{aB}	3.77 ± 0.22^{aB}
3	5.61 ± 0.19^{aA}	5.52 ± 0.14^{aA}	3.85 ± 0.04^{aB}	3.79 ± 0.19^{aB}	3.56 ± 0.09^{aB}
7	5.66 ± 0.11^{aA}	5.56 ± 0.14^{aA}	3.92 ± 0.02^{aB}	3.84 ± 0.27^{aB}	3.50 ± 0.09^{aB}
10	5.72 ± 0.06^{aA}	5.55 ± 0.11^{aA}	3.89 ± 0.06^{aB}	3.65 ± 0.11^{aB}	3.50 ± 0.14^{aB}
14	5.77 ± 0.09^{aA}	5.58 ± 0.15^{aA}	3.91 ± 0.08^{aB}	3.59 ± 0.04^{aB}	3.25 ± 0.26^{aB}

[1] n =3.
[2] Within the same column, mean values with different lowercase superscripts are significantly different ($P < 0.05$). Within the same row, mean values with different uppercase superscripts are significantly different ($P < 0.05$).

EFFICACY OF FUMARIC ACID TO REDUCE LEVELS OF *SALMONELLA* SPP. AT VARIOUS STAGES OF POULTRY PROCESSING

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ABSTRACT

Salmonella remains one of the most common causes of foodborne illness in the U.S., and poultry has been identified as a major transmission vehicle for salmonellosis. Mandated *Salmonella* testing and consumer demand for "natural" products have created a need for alternative interventions to reduce pathogens in poultry processing operations. Fumaric acid was evaluated as an antimicrobial treatment applied at simulated post-chill dipping (22ºC), scalding (53ºC), and chilling (3ºC) steps for reduction of *Salmonella* spp. attached to raw chicken skin. Irradiated chicken skin samples were inoculated with a four-strain cocktail of *Salmonella* spp. Fumaric acid treatments at concentrations of 0.25 and 0.5% were applied for 40 and 20 sec during simulated on-line or post-chill dipping, 0.05, 0.1, and 0.25% for 60, 45, and 30 min during simulated water-immersion chilling, and 0.5, 1.0, and 1.5% for 1, 2, and 3 min during simulated soft scalding of broiler carcasses. Among all tested applications, the most significant *Salmonella* reductions were obtained when 1.5% fumaric acid was applied during the soft scalding, resulting in reductions of 1.47 and 1.53 log at 2 and 3 min, respectively. The number of recoverable cells decreased as scald treatment time increased, thus a 0.72-log reduction was observed at 60 min contact time and fumaric acid concentration of 1.5%. On-line or post-chill dipping with fumaric acid (≤0.5%) showed a ≤0.31-log reduction in *Salmonella* spp. at ≤40 sec exposure time. A simulated chill step acidified with 0.25% fumaric acid for 45 min resulted in *Salmonella* reductions of 0.63 log. At all tested applications, the color of chicken skins treated with fumaric acid appeared darker and more yellow compared to the control samples, and the skins appeared lighter at

the end of refrigerated storage, but there was no significant difference between Hunter L and b values of samples at day 0 versus day 14. Fumaric acid at the tested concentrations and processing parameters reduced *Salmonella* counts on average by <2 log, and caused alterations in chicken skin color. Therefore, fumaric acid under tested conditions may not be an appropriate antimicrobial agent for use in poultry industry.

INTRODUCTION

Salmonella remains a significant food safety concern for the poultry industry, consumer, and impacts the economy *(4)*. Frequent occurrence of salmonellae on carcasses and equipment in poultry processing plants has been well documented by Rasschaert et el. *(37)*, Reiter et al. *(38)*, and Rose et al *(40)*. Based on the Nationwide Young Chicken Microbiological Baseline Data Collection Program, the prevalence of *Salmonella* on young chicken carcasses reduced from 20.0% in (1994 to 1995) *(48)* to 7.5% (2007 to 2008) *(49)*. However, a reported *Salmonella* incidence of 33.9% *(42)* in whole chicken carcasses at retail is substantially higher than the one reported by the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA/FSIS) baseline studies. The higher incidence of salmonellae in market studies compared to the USDA/FSIS surveys may be caused by differences in sampling locations and methods, possibility of post-processing contamination, variation in sampling frequencies of retail and in-plant carcasses, as well as presence of giblets in retail carcasses, versus giblet free post-chill carcasses *(42)*.

The effort to reduce prevalence of salmonellae in poultry is ongoing, and various steps are taken throughout the production chain to improve microbiological safety of poultry products. USDA/FSIS expects 90% of broiler processing plants to meet the *Salmonella* performance standard of 7.5%, which is based on 2007 to 2008 baseline study, by the end of 2010 *(50)*. To assist the industry with compliance and to provide a microbiologically safer product to the consumer, new interventions to reduce prevalence of *Salmonella* positive carcasses at post-chill should be investigated.

Organic acids are used as efficient antimicrobial treatments to decrease pathogen levels and incidences, inhibit the growth of spoilage bacteria, and to extend the shelf life of fresh eviscerated poultry carcasses *(6)*. Acetic *(8, 16, 17, 26, 44-46)*, lactic *(5, 17, 25, 44, 45, 52)*, citric *(44, 45)*, malic *(44, 45)*, mandelic *(44)*, propionic *(44)*, tartaric *(44, 45)*, and peracetic *(4)* acids have been applied as antimicrobial sprays and dips, as well as chiller and scalder additives to effectively reduce levels of salmonellae on carcasses. Little data has been published on the bactericidal potential of fumaric acid against *Salmonella* spp. in poultry processing.

Fumaric acid is a cost-effective, generally recognized as safe (GRAS) food-grade acidulant *(24)*. It has been used as an antimicrobial agent in meat products *(6, 33-35)*, produce *(22-24, 30)*, and apple cider *(12)*. Treatment with 1.5% of fumaric acid for 15 sec at 55ºC caused a 1.1- and 1.94-log reduction in population of *S.* Typhimurium and *E. coli* O157:H7, respectively, on lean beef muscles *(33)*. Turkey breast meat treated with 0.5% fumaric acid solution for 1 min, and then stored for 2 hrs (4ºC) had a 2.5-log lower *S.* Typhimurium count compared to the control *(6)*.

In this study, fumaric acid was evaluated to determine its bactericidal efficacy against *Salmonella* spp. attached to broiler skin by using the modified skin-attachment model (mSAM) in conditions mimicking those found in three common sites for application of antimicrobial agents in poultry slaughter facilities: on-line dip or post-chill dip, chiller and scalder. Chicken skins were irradiated to eliminate background microflora and permit testing of the acid against salmonellae attached or embedded in poultry skin *(44)*. Since fumaric acid

has limited water solubility (0.63% w/v at 25ºC), which varies depending on the temperature of the solution in which it is dissolved, the maximum tested concentrations for each application step were determined by the temperature associated with this processing operation.

MATERIALS AND METHODS

Sample collection

To account for the natural variation that occurs in poultry industry among broiler carcasses, chicken breast skins were collected from post-chill carcasses after automatic skinning in May and August of 2009 at a local poultry processing plant (Case Farms Inc., Dudley, NC). Skins were transported at 4°C to the Meat Pathogen Laboratory at North Carolina State University (NCSU), where they were cut into round pieces (*ca*. 62.2cm^2), individually vacuum-packaged, and then irradiated (12 to 25 kGy with Co-60) at the USDA-ARS ERRC, Wyndmoor, PA according to the procedure described previously by Sybirtseva et al. (Sybirtseva et al., unpublished, "Efficacy of oxalic acid to reduce the levels of *Salmonella* spp. at various stages of poultry processing").

The sterility of the irradiated skin samples was confirmed on two randomly selected skins by using 3M Petrifilm Aerobic Count Plates in accordance with Association of Official Agricultural Chemists (AOAC) official method 990.12 for aerobic plate count in foods *(3)*.

Culture preparation and inoculation of chicken skin samples

Salmonella enterica serovar Enteritidis (poultry isolate), *Salmonella enterica* serovar Heidelberg (environmental isolate from turkey processing plant), *Salmonella enterica* serovar Typhimurium (poultry isolate) obtained from the Poultry Science department at NCSU, Raleigh, NC and *Salmonella enterica* serovar Newport (J1980) (human isolate from tomato outbreak) obtained from the Food Science department at Virginia Polytechnic Institute and

State University, Blacksburg, VA (Appendix 1) were used to create a cocktail of four *Salmonella* strains. Cultures were maintained at -80°C ± 1°C in brain heart infusion broth (BHIB; Fisher Scientific, Pittsburg, PA) containing 50% glycerol (Fisher Scientific, Pittsburg, PA) *(44)*. *Salmonella* strains were activated via two successive transfers in BHIB at 37°C for 18 to 24 hours *(14)*, streaked onto xylose lysine deoxycholate agar (XLD; Weber Scientific, Hamilton, NJ), and confirmed by API 20E (bioMerieux Inc., Hazelwood, MO) as *Salmonella* spp. Each strain was loop-transferred from XLD plate to 9 ml of BHIB, and incubated at 37°C for 22 ± 1 hrs to achieve populations of *ca.* 9.0 $\log_{10}$ CFU/ml. Inoculum for chicken skin samples was prepared by combining equal volumes (2 ml) of each *Salmonella* strain in a sterile plastic tube *(19, 28)*. Exact inoculum concentration was determined prior to sample inoculation by serial dilution in 0.1% w/v buffered peptone water (BPW; Fisher Scientific, Pittsburg, PA) and plating on XLD.

The inoculation procedure described previously in Material and Methods for "Efficacy of oxalic acid to reduce the levels of *Salmonella* spp. at various stages of poultry processing" was utilized. Briefly, irradiated chicken skin samples were inoculated on sterile 20 gauge stainless steel racks (9.5 x 9.5 cm) (Appendix 10) with a four-strain *Salmonella* cocktail (0.1 ml) using a drip inoculation method. Drip inoculation was chosen over a dip inoculation method to eliminate water uptake, uneven cell distribution within the suspension medium, and potential contamination of the cell suspension through repeated immersion *(14)*. Bacterial cells were allowed to attach to the skin surface for 10 min at 22ºC ± 1°C prior to application of treatments *(14, 44)*.

Chemical treatment preparation

Fumaric acid (CAS 110-17-8; Fisher Scientific, Pittsburg, PA) treatments were prepared at 0.05, 0.1, 0.25, 0.5, 1.0, and 1.5% w/w by dissolving the acid with sterile distilled water in a sterile 250 ml beaker on a stirring hotplate (SP131325, Barnstead/Thermolyne, Dubuque, IA). Since fumaric acid levels used to prepare 1.0 and 1.5% solutions are exceeded the maximum fumaric acid solubility concentration at room temperature, the chemical was dissolved by heating the solution to approximately 53ºC on stirring hotplate.

Fumaric acid treatments were transferred into autoclaved treatment containers (18 x 10 x 5.4 cm) (Fisher Scientific, Pittsburg, PA) (Appendix 11) held at room temperature (22°C ± 1°C) to simulate on-line or post-chill dipping, or placed in either low temperature incubator (3°C ± 1°C) to simulate water-immersion chilling or a warm temperature incubator (53°C ± 1°C) to simulate soft scalding. The pH of solutions were measured using a calibrated digital pH meter with temperature compensation (AB15, Fisher Scientific, Pittsburgh, PA) at temperatures associated with the applications (Table 10) The temperature of fumaric acid solutions was monitored with a submerged sterile thermocouple wire (TT-T-30-SLE-50, Omega, Stamford, CT) attached to a calibrated continuous digital thermometer (HH21, Omega, Stamford, CT).

Procedure for treating chicken skins with fumaric acid

To mimic common commercial processing practices, inoculated chicken skin samples were submersed into: fumaric acid treatments (six replicates per temperature, concentration

and time; total of 162 samples) and sterile distilled water (control) treatments (six replicates per temperature and time; total of 54 samples). Fumaric acid treatments (200 ml) included: 0.25 and 0.5% solutions to simulate on-line or post-chill dipping (22°C ± 1°C), 0.05, 0.1, and 0.25% to simulate chilling (3°C ± 1°C), and 0.5, 1.0, and 1.5% acid solutions to simulate scalding (53°C ± 1°C). Temperature and time combinations associated with each application determined the maximum fumaric acid concentration tested at that application. Control samples were submersed into 200 ml of sterile distilled water at 22°C ± 1°C, 53°C ± 1°C, and 3°C ± 1°C. After treatments, skins were allowed to drain for 1 min at 22°C ± 1°C for removal of excess water or fumaric acid solution. In previous studies, the time of drainage varied from 15 sec to 15 min *(28, 39)*.

Microbiological analysis

Following treatments, chicken skin samples were homogenized for 2 min at 230 rpm (400 Circulator, Seward Inc., Bohemia, NY) in sterile stomacher bags containing 90 ml of sterile 0.1% w/v BPW to remove remaining *Salmonella* cells and neutralize the effect of treatments *(44, 46)*. The thin agar plating (TAL) method was chosen as a plating technique and is accomplished by overlaying the selective media (XLD) with non-selective media, such tryptic soy agar (TSA; Weber Scientific, Hamilton, NJ), which permits *Salmonella* cells to recover from acid injury before the selective ingredients migrate through the top layer. The plates were incubated at 37° ± 1°C for 24 hrs under aerobic conditions, and *Salmonella* counts for each treatment were expressed as $\log_{10}$ CFU/skin. On a periodic basis, two or three

typical *Salmonella* colonies were picked from countable plates and confirmed by API 20E (bioMerieux Inc., Hazelwood, MO) as *Salmonella* spp. Typical *Salmonella* colonies appear black with clear and opaque zones *(51)*.

Expression of antimicrobial activity

Fumaric acid efficacy for each treatment was expressed as a reduction in the viable population of *Salmonella* cells *(14)* between the population of cells recovered from the inoculated skin treated with water and the population of cells recovered from the inoculated skin treated with fumaric acid.

Color measurement of treated chicken skins after fumaric acid treatments and during refrigerated storage

The impact of fumaric acid treatments on the color of non-irradiated chicken skins after on-line or post-chill dipping, water-immersion chilling, and soft scalding as well as during refrigerated storage was evaluated based on Hunter L (dark to light), a (green to red), and b (blue to yellow) values. Non-irradiated chicken skin samples were also treated with antimicrobial the same as described previously. The samples were then individually vacuum packaged (UV225, Koch Industries Inc., Wichita, KS) at 7.6 mm Hg in high oxygen barrier vacuum pouches (Koch Equipment LLC, Kansas City, MO) and stored at 4ºC ± 1°C for up to 14 days. The Hunter values were measured by placing a calibrated hand-held colorimeter (CR-400, Konica Minolta, Ramsey, NJ) directly in contact with the vacuum packaged skin

before and after fumaric acid treatment and during refrigerated storage at day 1, 3, 7, 10, and 14. Measurements were performed in triplicate and average values are being reported.

Statistical analysis

The data were analyzed separately for each application by using SAS software (version 9.1, SAS Institute, Cary, NC). Analysis of variance was performed at a 95% significance level ($P < 0.05$), and the difference in means was separated by using a Tukey test *(36)*.

RESULTS

Efficacy of fumaric acid to reduce salmonellae during on-line or post-chill dipping

Treatment of skins with 0.25% fumaric acid at 22°C ± 1°C resulted in a 0.06- and 0.18-log reduction of *Salmonella* in 20 and 40 sec, respectively, compared to the control. Treatment with 0.5% fumaric acid showed reductions of 0.27 and 0.31 log at 20 and 40 sec exposure time, respectively. Main effect of concentration appeared significant ($P < 0.0001$), while main effect of treatment time was not significant for *Salmonella* reduction ($P = 0.9649$) (Table 11).

Efficacy of fumaric acid to reduce salmonellae during water-immersion chilling

Chicken skins treated with 0.05% fumaric acid at 3°C ± 1°C resulted in a 0.20-log reduction in recoverable cells in 30 min, and there was no significant difference between 0.05% and 0.1% fumaric acid treatments for all tested contact times ($P = 1.0000$). Simulated chilling with 0.25% fumaric acid for 45 and 60 min resulted in a 0.63- and 0.72-log reduction of *Salmonella*, respectively (Table 12).

Efficacy of fumaric acid to reduce salmonellae during soft scalding

Fumaric acid applied at a concentration of 0.5% and a scalding temperature of 53°C ± 1°C reduced *Salmonella* counts by 0.73 and 1.14 log at 1 and 3 min, respectively. There was no significant difference in log reductions between a contact time of 1 and 2 min for all tested concentrations ($P = 0.1138$). At a concentration of 1.5% and a contact time of 3 min,

fumaric acid reduced the initial *Salmonella* levels by 1.53 log compared to the control. Statistically there was no difference in fumaric acid treatment applied at 0.5, 1.0, or 1.5% for 1, 2, and 3 min of scalding ($P > 0.05$) (Table 13).

Colorimetric changes in chicken skin samples treated with fumaric acid

On-line or post-chill dipping resulted in lower Hunter L and a values, and higher b values compared to the control (Table 14). However, no statistical significant difference was observed between treatments with 0.25 or 0.5% fumaric acid and control ($P > 0.05$), and a contact time of 20 sec was not significantly different from 40 sec for Hunter L and b values ($P > 0.05$). Although, treated samples appeared lighter at the end of refrigerated storage, there was no statistical difference between day 0 and day 1, or day 14 at tested fumaric acid concentrations of 0.25 and 0.5% ($P > 0.05$).

Treatment with fumaric acid under simulated chiller conditions affected the color of chicken skins by reducing Hunter L and a values and increasing b values of samples (Table 15). No statistical significant difference was observed between the contact times for Hunter L values ($P = 0.1248$), and the 0.05 and 0.1% fumaric acid treatments were not statistically different from the control at 30, 45, and 60 min ($P > 0.05$). Additionally, refrigerated storage at 4°C for 14 days did not impact Hunter L or b values of any treated samples ($P > 0.05$).

Significant fumaric acid effects on Hunter L, a, and b values were observed during simulated scalder applications (Table 16). Skins had lower Hunter L and a values and higher b values compared to the control ($P < 0.0001$). No significant difference, however, was

observed among Hunter L values of chicken skins treated with 0.5, 1.0, and 1.5% fumaric acid ($P > 0.05$). A contact time of 1 min was significantly different from 2 and 3 min for Hunter L and b values ($P > 0.05$), and there was no significant impact of refrigerated storage on the color of chicken skins treated with fumaric acid at concentrations of 0.5, 1.0, and 1.5% ($P > 0.05$).

DISCUSSION

Among all tested applications, fumaric acid treatments were least effective during simulated on-line or post-chill dipping (22°C ± 1°C), resulting in ≤0.31-log reduction in *Salmonella* spp. attached to raw chicken skin. Similarly, no reduction in *S.* Typhimurium was reported by Tamblyn and Corner *(44, 45)* when acetic, citric, or lactic acid at a concentration of 0.5% was applied at 22ºC for 15 sec, but when acid concentrations were increased to 6.0% reductions of 0.27, 0.13, and 0.73 log, respectively, were observed. Also, no significant difference was found between *S.* Typhimurium counts on turkey breast fillets after treatment with 1.5% lactic (pH 2.72) and 0.5% fumaric acid (pH 2.70) applied for 1 min and stored for 2 hrs at 4ºC *(6)*. The short contact time (≤40 sec) associated with dip application and limited solubility of fumaric acid at room temperature may have limited antimicrobial efficacy of fumaric acid against *Salmonella* cells that had become embedded in or firmly attached to the poultry skin. Hiwaki et al *(18)* found that when eviscerated carcasses were soaked in 0.6% fumaric acid for 30 min, the population of *Salmonella* decreased by 5 log. In another study, 0.5% fumaric acid treatment for 5 min reduced the population of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on broccoli sprouts by 2.39, 2.74, and 2.63 log CFU/g, respectively *(23)*. Several studies indicated higher antimicrobial activity of fumaric acid in comparison with other weak organic acids *(30, 33-35, 41)*. For example, a 0.3% fumaric acid exhibited stronger growth-inhibiting action on *S.* Typhi and *S.* Typhimurium than 1.0% malic, tartaric, citric, and ascorbic acids *(41)*, and was more lethal to *L. monocytogenes* and *E. coli* O157: H7 than acetic and lactic acids on lean beef *(33)*. Additionally, Beuchat *(9)*

reported that 0.5% fumaric acid was more lethal than 0.5% acetic, malic, citric, and tartaric acids in inactivating *T. flavus* ascospores in vitro. In contrast, Oh et al. *(29)* found that a population of *E. coli* O157:H7 grown in the tryptic soy broth at 37ºC acidified with acetic acid (pH 5.0) was *ca.* 1.5 log lower than in broth acidified with fumaric acid (pH 4.5), presumably due to the higher undissociated concentration of acetic acid (0.012M) compared to fumaric acid (0.004M).

Fumaric acid concentrations of 0.05 to 0.25% were tested during water-immersion chilling (3°C ± 1°C) due to decreased solubility of the acid at low temperatures. All tested combinations of concentrations and contact times resulted in a <1.0 log *Salmonella* reduction. Tamblyn and Conner *(44)* reported that ≥4.0% of malic, mandelic, or propionic acids was needed to achieve a 2-log reduction of firmly attached *S.* Typhimurium cells on broiler chicken skin in a 0°C chiller. Reductions of <1 log observed during water-immersion chilling in our study may have resulted from low fumaric acid concentrations and the ability of *Salmonella* to induce protective systems at low temperature and acidic pH *(10)*. For example, Leitch and Stewart *(27)* found that efficacy of lactate against *E. coli* O157 and non-O157 isolates was reduced at lower temperatures, perhaps due to the reduction in the proton gradient and intracellular lactate anion concentration. Additionally, bacteria have the ability to retain membrane fluidity by increasing the concentration of unsaturated fatty acids and shortening the fatty acid chain length when temperature decreases *(7)*. Alvarez-Ordonez et al. *(1)* found that at 10°C the proportion of unsaturated fatty acids in *S.* Typhimurium, mainly C18:1, increased, modifying the physical properties of the bacterial membrane. A significant

proportion of unsaturated fatty acids is converted to cyclopropane fatty acids during acid adaptation of *Salmonella,* which improves the structural stability and dynamic properties of membrane *(1, 2, 21).* Other responses to an abrupt reduction in temperature may involve induction of cold shock proteins *(7)* and this may also explain the reduced antimicrobial potential of fumaric acid when used at chilling step.

The most significant *Salmonella* reductions were obtained when fumaric acid was applied during soft scalding (53°C ± 1°C). Treatment with 1.5% fumaric acid resulted in reductions of 1.47 and 1.53 log at 2 and 3 min, respectively. These results are consistent with other studies, which demonstrated a higher efficacy of fumaric acid at elevated temperatures *(11, 13, 33),* and indicated a synergistic effect between the acid and heat. Kondo et al. *(24)* showed that treatment of 5 mM fumaric at 50ºC for 1 min had similar effect on the native microflora of cut lettuce as treatment of 50 mM fumaric acid for 10 min at room temperature. Significant reductions in *S.* Typhimurium were reported on lean beef samples treated with fumaric acid at 55°C, accounting for 1.51 and 2.54 log in 15 and 30 sec, respectively *(33).* Alvarez-Ordonez et al. *(1)* noted that acidification of the growth medium from pH 6.4 to 4.5 with acetic, citric, lactic, and hydrochloric acids did not significantly affect the membrane fatty acid profile of *S.* Typhimurium cells grown at 45°C, suggesting that the effect of acid adaptation on the membrane fatty acid composition was disguised by the fatty acid profile changes associated with high growth temperature. Tsuchido et al. *(47)* found that the release of LPS and lipids from the outer membrane of *E. coli* upon heat treatment (55ºC) causes disorganization of the membrane structure and results in the partial disruption of the

permeability barrier function. Also, a loss of enzymes may occur as a result of blebbing and vesiculation of the outer membrane in *E. coli* cells when heated to 55ºC for 30 min in Tris-hydrochloride buffer *(20)*. In general, bactericidal efficacy of fumaric acid against *Salmonella* spp. increased with increased concentration, even though there was no statistical significance among the tested concentrations at the scalder application. Other researchers also noted an increase in bactericidal effect of fumaric acid with increased concentrations *(24, 31, 35)*. Fumaric acid greatly increased the rate of heat inactivation of *Neosartorya fisheri (13)* and *Talaromyces flavus (9)* ascospores, and the rate of inactivation increased as the pH of fumaric acid solution decreased. In another study, addition of fumaric acid to wine provided almost a 200-fold decrease in *Leuconostoc oenos* viability when pH was reduced from 4.1 to 3.5 *(31)*. Failure to detect significance among concentrations in scalding to some extent may be due to higher variation in log reductions and the use of more conservative Tukey test *(36)* for separation of means.

It is known that the antimicrobial effect of weak organic acids is the result of a rapid decrease in external pH to below the pathogen growth limit *(10)*, as well as inhibition of bacterial activity by the undissociated form of the acid that can freely penetrate inside of the cell and imbalance intracellular pH leading to the cell death *(29)*. Comes and Beelman *(12)* found a linear correlation between concentration of undissociated fumaric acid and log reduction of *E. coli* O157:H7 in apple cider. A comparison study of bactericidal potential of fumaric acid versus hydrochloric acid (strong acid) mimicking three common sites for application of antimicrobial agents was performed in our laboratory to determine the

mechanism of action *(43)*. Fumaric acid at concentrations of 0.5 (pH 2.30), 0.25 (pH 2.25), and 1.5% (2.03) applied during simulated on-line or post-chill dipping, water-immersion chilling, and soft-scalding, respectively, provided statistically higher reductions in *Salmonella* attached to raw chicken skins than hydrochloric acid at the same pH values and treatment parameters (Appendix 4). The bactericidal effect of fumaric acid against salmonellae is produced by the combined actions of free hydrogen ions and undissociated molecules present in the solution.

Visual alteration of the chicken skin samples when treated with fumaric acid at levels of 0.05 to 1.5% during on-line or post-chill dipping, water-immersion chilling, and soft scalding was observed. Fumaric acid treated skins appeared darker (lower Hunter L values) and more yellow (higher b values) compared to the controls, and intensity of color alteration increased with the increased acid concentration. .Bautista et al. *(5)* detected a discoloration of turkey skins after treatment with 1.24% lactic acid for 15 sec, while 0.3% acetic acid applied at pre-chill for 10 min made chicken carcasses appear darker and more yellow *(15)*. Browning was also promoted on fresh-cut lettuce samples treated for 1 min at 50ºC with 50 mM fumaric acid *(24)*. Additionally, Podolak et al. *(32)* reported more noticeable yellowish discoloration of ground beef patties when 3.0 or 5.0% of fumaric acid was added to patties formulations. During refrigerated storage for 14 days fumaric acid treated and control samples appeared slightly lighter and more yellow compared to day 0, but no statistical significance was observed.

Effectiveness of fumaric acid as an antimicrobial agent is limited by its low water solubility, resulting in <2 log reduction of *Salmonella* attached to raw chicken skins during simulated on-line or post-chill dipping, water-immersion chilling, or soft scalding. Additionally, the color alterations of broiler chicken skins associated with application of fumaric acid need to be overcome. Future work on fumaric acid antimicrobial activity should focus on increasing the concentration of dissolved acid and any potential synergistic effects with other antimicrobials, e.g. organic acids, which may subsequently increase bactericidal potential of the acid.

REFERENCES

1. Alvarez-Ordonez, A., A. Fernandez, M. Lopez, R. Arenas, and A. Bernardo. 2008. Modifications in membrane fatty acid composition of *Salmonella typhimurium* in response to growth conditions and their effect on heat resistance. *Int. J. Food Microb*. 123:212-219.

2. Alvarez-Ordonez, A., A. Fernandez, M. Lopez, and A. Bernardo. 2009. Relationship between membrane fatty acid composition and heat resistance of acid and cold stressed *Salmonella senftenberg* CECT 4384. *Food Microb*. 26:347-353.

3. AOAC. 2005. Official method 990.12. *In* Official methods of analysis of AOAC. International AOAC International, Gaithersburg, MD.

4. Bauermeister, L. J., J. W. J. Bower, J. C. Townsend, and S. R. McKee. 2008. The microbial and quality properties of poultry carcasses treated with peracetic acid as antimicrobial treatment. *Poultry Sci*. 87:2390-2398.

5. Bautista, D. A., N. Sylvester, S. Barbut, and M. W. Griffiths. 1997. The determination of efficacy of antimicrobial rinses on turkey carcasses using response surface designs. *Int. J. Food Microb*. 34:279-292.

6. Baysal, A. H. D., and A. Unluturk. 2005. Effects of organic acid and phosphates on the microbiological stability and on survival of *Salmonella typhimurium* on turkey breast meat. XVII European Symposium on the Quality of Poultry Meat, Doorwerth, The Netherlands.

7. Beales, N. 2004. Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. *Compr. Rev. Food Sci. Food Safety*. 3:1-20.

8. Bell, C., C. N. Cutter, and S. S. Sumner. 1997. Reduction of foodborne micro-organisms on beef carcasses tissue using acetic acid, sodium bicarbonate, and hydrogen peroxide spray washes. *Food Microb*. 14:439-448.

9. Beuchat, L. R. 1988. Influence of organic acids on heat resistance characteristics of *Talaromyces flavus* ascospores. *Int. J. Food Microb.* 6:97-105.

10. Booth, I. R., and M. Stratford. 2003. Acidulants and low pH. p. 25-47. *In* N.J. Russell, and G.W. Gould (ed.), Food Preservatives. Kluwer Academic/Plenum Publishers, New York, N.Y.

11. Chung, K. C., and J. M. Goepfert. 1970. Growth of *Salmonella* at low pH. *J. Food Sci.* 35:326-328.

12. Comes, J. E., and R. B. Beelman. 2002. Addition of fumaric acid and sodium benzoate as an alternative method to achieve a 5-log reduction of *Escherichia coli* O157:H7 populations in apple cider. *J. Food Prot*. 65:476-483.

13. Conner, D. E., and L. R. Beuchat. 1987. Heat resistance of ascospores of *Neosartorya fischeri* as affected by sporulation and heating medium. *Int. J. Food Microb*. 4:303-312.

14. Conner, D. E., and S. F. Bilgili. 1994. Skin attachment model for improved laboratory evaluation of potential carcass disinfectants for their efficacy against *Salmonella* attached to broiler skin. *J. Food Prot*. 57:684-688.

15. Dickens, J. A., and A. D. Whittemore. 1994. The effect of acetic acid and air injection on appearance, moisture pick-up, microbiological quality, and *Salmonella* incidence on processed poultry carcasses. *Poultry Sci*. 73:582-586.

16. Dickens, J. A., and A. D. Whittemore. 1995. The effects of extended chilling times with acetic acid on the temperature and microbiological quality of processes poultry carcasses. *Poultry Sci*. 74:1044-1048.

17. Dorsa, W. J., C. N. Cutter, and G. R. Siragusa. 1998. Bacterial profile of ground beef made from carcasses tissue experimentally contaminated with pathogenic and spoilage bacteria before being washed with hot water, alkaline solution, or organic acid and then stored at 4 or 12C. *J. Food Prot*. 61:1109-1118.

18. Hiwaki, H., M. Tsubakimoto, K. Kubokura, Y. Kurihara, and T. Oda. 1995. *Salmonella* contamination in livestock 'sashimi' (sliced raw meat and edible organs) and bacterial control of the 'sashimi' materials. p. 51-58. Annual report, vol. 20. Institute of Public Health, Fukuoka city.

19. Hwang, C., and L. R. Beuchat. 1995. Efficacy of selected chemicals for killing pathogenic and spoilage microorganisms on chicken skin. *J. Food Prot*. 58:19-23.

20. Katsui, N., T. Tsuchido, R. Hiramatsu, S. Fujikawa, M. Takano, and I. Shibasaki. 1982. Heat-induced blebbing and vesiculation of the outer membrane of *Escherichia coli. J. Bact*. 151:1523-1531.

21. Katsui, N., T. Tsuchido, M. Takano, and I. Shibasaki. 1981. Effect of preincubation temperature on the heat resistance of *Escherichia coli* having different fatty acid compositions. *J. Gen. Microb.* 122:357-361.

22. Kim, J. Y., J. W. Kim, G. O. Lim, S. A. Jang, and K. B. Song. 2010. The effects of aqueous chlorine dioxide of fumaric acid treatment combined with UV-C on postharvest quality of 'Maehyang' strawberries. *Postharv. Biol. Technol*. 56:254-256.

23. Kim, Y. J., M. H. Kim, and K. B. Song. 2009. Efficacy of aqueous chlorine dioxide and fumaric acid for inactivating pre-existing microorganisms and *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on broccoli sprouts. *Food Control*. 20:1002-1005.

24. Kondo, N., M. Murata, and K. Isshiki. 2006. Efficiency of sodium hypochlorite, fumaric acid, and mild heat in killing native microflora and *Escherichia coli* O157:H7, *Salmonella* Typhimurium DT104, and *Staphylococcus aureus* attached to fresh-cut lettuce. *J. Food Prot*. 69:323-329.

25. Li, Y., M. F. Slavik, J. T. Walker, and H. Xiong. 1997. Pre-chill spray of chicken carcasses to reduce *Salmonella typhimurium. J. Food Sci*. 62:605-607.

26. Lillard, H. S., L. C. Blankenship, J. A. Dickens, S. E. Craven, and A. D. Shackelford. 1987. Effect of acetic acid on the microbiological quality of scalded picked and unpicked broiler carcasses. *J. Food Prot*. 50:112-114.

27. McWilliam Leitch, E. C. M., and C. S. Stewart. 2002. Susceptibility of *Escherichia coli* O157 and non-O157 isolates to lactate. *Lett. Appl. Microb*. 35:176-180.

28. Mehyar, G., G. Blank, J. H. Han, A. Hydamaka, and R. A. Holley. 2005. Effectiveness of trisodium phosphate, lactic acid and commercial antimicrobials against pathogenic bacteria on chicken skin. *J. Food Prot*. 25:351-362.

29. Oh, D.-H., J.-H. Park, and B.-K. Park. 2000. Effect of organic acids on the survival of *Escherichia coli* O157:H7. *J. Food Sci. Nutr.* 5:131-135.

30. Ohsone, M., K. Kaneko, H. Hayashidani, T. Takahashi, and M. Ogawa. 1999. Growth and inhibition by acids of five species of pathogenic bacteria inoculated in salad vegetables. *J. Food Hyg. Soc. Japan*. 40:297-303.

31. Pilone, G. J., B. C. Rankine, and D. A. Pilone. 1974. Inhibiting malo-lactic fermentation in Australian dry red wines by adding fumaric acid. *Amer. J. Enol. Viticult.* 25:99-107.

32. Podolak, R. K., C. S. Setser, C. L. Kastner, and J. F. Zayas. 1997. Aroma, color, and texture of ground beef patties treated with fumaric and lactic acids. *J. Food Quality*. 20:513-524.

33. Podolak, R. K., J. F. Zayas, C. L. Kastner, and D. Y. C. Fung. 1995. Reduction of *Listeria monocytogenes, Escherichia coli O157:H7,* and *Salmonella typhimurium* during storage on beef sanitized with fumaric, acetic, and lactic acids. *J. Food Safety*. 15:283-290.

34. Podolak, R. K., J. F. Zayas, C. L. Kastner, and D. Y. C. Fung. 1996. Inhibition of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on beef by application of organic acids. *J. Food Prot*. 59:370-373.

35. Podolak, R. K., J. F. Zayas, C. L. Kastner, and D. Y. C. Fung. 1996. Reduction of bacterial populations on vacuum-packaged ground beef patteis with fumaric and lactic acids. *J. Food Prot*. 59:1037-1040.

36. Rao, P. V. 1997. Statistical research methods in life sciences. Thomson Wadsworth, Mason, OH.

37. Rasschaert, G., K. Houf, C. Godard, C. Wildemauwe, M. Pastuszczak-Frak, and L. De Zutter. 2008. Contamination of carcasses with *Salmonella* during poultry slaughter. *J. Food Prot.* 71:146-152.

38. Reiter, M. G., M. L. Fiorese, G. Moretto, M.C. Lopez, and R. Jardano. 2007. Prevelance of *Salmoenlla* in poultry slaughterhouse. *J. Food Prot.* 70:1723-1725.

39. Rio, E., M. Panizo-Moran, M. Prieto, C. Alonso-Calleja, and R. Capita. 2007. Effect of various chemical decontamination treatments on natural microflora and sensory characteristics of poultry. *Int. J. Food Microb*. 115:268-280.

40. Rose, B. E., W. E. Hill, R. Umholtz, G. M. Ransom, and W. O. James. 2002. Testing for *Salmonella* in raw meat and poultry products collected at federally inspected establishments in the United States, 1998 through 2000. *J. Food Prot.* 65:937-947.

41. Shimizu, T., T. Takahata, and M. Kato. 1995. Antibacterial activity of several organic acids commonly used as food additives. *J. Food Hyg. Soc. Japan*. 36:50-54.

42. Simmons, M. D., L. Fletcher, J. A. Cason, and M. E. Berrang. 2003. Recovery of *Salmonella* from retail broilers by whole-carcass enrichment procedure. *J. Food Prot.* 66:446-450.

43. Sybirtseva, I. 2009. Unpublished data. North Carolina State University, Raleigh, NC.

44. Tamblyn, K. C., and D. E. Conner. 1997. Bactericidal activity of organic acids against *Salmonella typhimurium* attached to broiler chicken skin. *J. Food Prot*. 60:629-633.

45. Tamblyn, K. C., and D. E. Conner. 1997. Bactericidal activity of organic acids in combination with transdermal compounds against *Salmonella typhimurium* attached to broiler skin. *Food Microb*. 14:477-484.

46. Tamblyn, K. C., D. E. Conner, and S. F. Bilgili. 1997. Utilization of the skin attachment model to determine the antibacterial efficacy of potential carcass treatments. *Poultry Sci*. 76:1318-1323.

47. Tsuchido, T., N. Katsui, A. Takeuchi, M. Takano, and I. Shibasaki. 1985. Destruction of the outer membrane permeability barrier of *Escherichia coli* by heat treatment. *Appl. Env. Microb*. 50:298-303.

48. U.S. Department of Agriculture, Food Safety and Inspection Services. 2006. Nationwide broiler chicken microbiological baseline data collection program, July 1994 - June 1995. Available at: http://www. fsis.usda.gov/Science/Baseline_Data/index.asp. Accessed 18 December 2010.

49. U.S. Department of Agriculture, Food Safety and Inspection Service. 2010. Nationwide broiler chicken microbiological baseline data collection program, July 2007 - June 2008. Available at: http://www. fsis.usda.gov/Science/Baseline_Data/index.asp. Accessed 18 December 2010.

50. U.S. Department of Agriculture, Food Safety and Inspection Service. 2010. New performance standards for *Salmonella* and *Campylobacter* in young chicken and turkey slaughter establishments; New compliance guides, Final Rule, 75 Fed. Red. 27288-27294 (May 14, 2010) (to be codified at 9 CFR 381.94).

51. Wu, V. C. H., D. Y. C. Fung, D. H. Kang, and L. K. Thompson. 2001. Evaluation of thin agar layer method for recovery of acid-injured foodborne pathogens. *J. Food Prot*. 64:1067-1071.

52. Zeitoun, A. A. M., and J. M. Debever. 1990. The effect of treatment with buffered lactic acid on microbial decontamination and on shelf life of poultry. *Int. J. Food Microb*. 11:305-312.

Table 10. pH values of fumaric acid solutions.

Temperature (°C)	Fumaric acid (% w/w)	pH ± std. error[1]
3 ± 1	0.05	2.69 ± 0.10
	0.1	2.59 ± 0.04
	0.25	2.25 ± 0.06
22 ± 1	0.25	2.46 ± 0.01
	0.5	2.30 ± 0.01
53 ± 1	0.5	2.30 ± 0.01
	1.0	2.15 ± 0.01
	1.5	2.03 ± 0.01

[1] n = 3.

Table 11. Efficacy of fumaric acid to reduce *Salmonella* spp. on raw chicken skins during simulated on-line or post-chill dipping (22°C ± 1°C).

Fumaric acid (% w/w)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]	
	20 sec dipping	40 sec dipping
0.25	0.06 ± 0.03^{aA}	0.18 ± 0.02^{aA}
0.5	0.27 ± 0.03^{bA}	0.31 ± 0.02^{bA}

[1] n = 6.

[2] Within the same column, mean values with different lowercase superscripts are significantly different ($P < 0.05$). Within the same row, mean values with different uppercase superscripts are significantly different ($P < 0.05$).

Table 12. Efficacy of fumaric acid to reduce *Salmonella* spp. on raw chicken skins during simulated water-immersion chilling (3°C ± 1°C).

Fumaric acid (% w/w)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]		
	30 min chilling	45 min chilling	60 min chilling
0.05	0.20 ± 0.03^{aA}	0.24 ± 0.03^{aA}	0.26 ± 0.04^{aA}
0.1	0.23 ± 0.02^{aA}	0.26 ± 0.04^{aA}	0.29 ± 0.01^{aA}
0.25	0.46 ± 0.07^{bA}	0.63 ± 0.03^{bB}	0.72 ± 0.05^{bB}

[1, 2] See Table 11 footnotes.

Table 13. Efficacy of fumaric acid to reduce *Salmonella* spp. on raw chicken skins during simulated soft scalding (53°C ± 1°C).

Fumaric acid (% w/w)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]		
	1 min scalding	2 min scalding	3 min scalding
0.5	0.73 ± 0.08^{aA}	1.04 ± 0.12^{aA}	1.14 ± 0.06^{aA}
1.0	0.89 ± 0.16^{aA}	1.13 ± 0.11^{aA}	1.24 ± 0.09^{aA}
1.5	0.97 ± 0.14^{aA}	1.47 ± 0.22^{aA}	1.53 ± 0.11^{aA}

[1, 2] See Table 11 footnotes.

Table 14. Hunter values of chicken skins treated with fumaric acid during simulated on-line or post-chill dipping (22°C ± 1°C) and storage at 4°C for 14 days.

Time	Fumaric acid (% w/w)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
20 sec dipping	0	0	$78.85 \pm 0.73^{aA}a$	$-1.16 \pm 0.35^{aA}a$	$6.51 \pm 0.79^{aA}a$
		1	$79.49 \pm 0.77^{aA}a$	$-1.35 \pm 0.38^{aA}a$	$6.88 \pm 0.72^{aA}a$
		3	$79.48 \pm 0.53^{aA}a$	$-1.43 \pm 0.32^{aA}a$	$6.92 \pm 0.66^{aA}a$
		7	$79.68 \pm 0.62^{aA}a$	$-1.76 \pm 0.29^{aA}a$	$7.62 \pm 0.73^{aA}a$
		10	$79.32 \pm 0.71^{aA}a$	$-1.68 \pm 0.33^{aA}a$	$7.83 \pm 0.67^{aA}a$
		14	$79.80 \pm 0.78^{aA}a$	$-1.72 \pm 0.39^{aA}a$	$8.07 \pm 0.61^{aA}a$

Table 14. Continued

Time	Fumaric acid (% w/w)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
20 sec dipping	0.25	0	78.65 ± 0.17^{aA}a	-1.57 ± 0.12^{aA}a	6.40 ± 0.60^{aA}a
		1	79.30 ± 0.25^{aA}a	-2.00 ± 0.09^{aA}a	7.09 ± 0.49^{aA}a
		3	79.59 ± 0.26^{aA}a	-2.17 ± 0.07^{aA}a	7.31 ± 0.50^{aA}a
		7	79.87 ± 0.38^{aA}a	-2.43 ± 0.06^{aA}a	7.44 ± 0.45^{aA}a
		10	78.88 ± 1.26^{aA}a	-2.40 ± 0.05^{aA}a	7.53 ± 0.52^{aA}a
		14	79.92 ± 0.^{45aA}a	-2.46 ± 0.04^{aA}a	7.73 ± 0.51^{aA}a
	0.5	0	74.79 ± 1.02^{aA}a	-1.64 ± 0.30^{aA}a	7.43 ± 0.57^{aA}a
		1	74.42 ± 0.26^{aA}a	-2.33 ± 0.32^{aA}a	8.15 ± 0.38^{aA}a
		3	76.05 ± 1.02^{aA}a	-2.56 ± 0.31^{aA}a	8.39 ± 0.41^{aA}a
		7	75.69 ± 1.05^{aA}	-2.80 ± 0.37^{aA}a	8.59 ± 0.37^{aA}a
		10	76.45 ± 1.13^{a}a	-2.84 ± 0.34^{aA}a	8.86 ± 0.33^{aA}a
		14	76.70 ± 0.77^{A}a	-2.92 ± 0.32^{aA}a	8.77 ± 0.36^{aA}a
40 sec dipping	0	0	78.50 ± 0.29^{aA}a	-1.46 ± 0.01^{aA}a	7.42 ± 0.30^{aA}a
		1	78.64 ± 0.26^{aA}a	-1.53 ± 0.02^{aA}a	7.30 ± 0.13^{aA}a
		3	78.68 ± 0.23^{aA}a	-1.58 ± 0.01^{aA}a	7.22 ± 0.14^{aA}a
		7	78.91 ± 0.20^{aA}a	-1.69 ± 0.01^{aA}a	7.53 ± 0.19^{aA}a
		10	79.10 ± 0.25^{aA}a	-1.88 ± 0.03^{aA}a	7.74 ± 0.20^{aA}a
		14	79.04 ± 0.24^{aA}a	-1.85 ± 0.01^{aA}a	8.12 ± 0.08^{aA}a
	0.25	0	76.07 ± 0.99^{aA}a	-1.86 ± 0.19^{aA}a	8.62 ± 1.19^{aA}a
		1	76.91 ± 1.06^{aA}a	-2.28 ± 0.06^{aA}a	8.80 ± 1.11^{aA}a
		3	77.30 ± 1.04^{aA}a	-2.36 ± 0.00^{aA}a	8.80 ± 1.18^{aA}a
		7	77.21 ± 0.96^{aA}a	-2.41 ± 0.02^{aA}a	8.88 ± 1.06^{aA}a
		10	77.35 ± 1.02^{aA}a	-2.61 ± 0.05^{aA}a	9.02 ± 1.22^{aA}a
		14	77.39 ± 1.03^{aA}a	-2.61 ± 0.03^{aA}a	9.28 ± 1.23^{aA}a
	0.5	0	74.42 ± 1.85^{aA}a	-1.88 ± 0.34^{aA}a	8.85 ± 0.29^{aA}a
		1	75.18 ± 1.95^{aA}a	-2.23 ± 0.40^{aA}a	9.07 ± 0.43^{aA}a
		3	75.37 ± 1.61^{aA}a	-2.36 ± 0.32^{aA}a	9.01 ± 0.36^{aA}a
		7	75.56 ± 1.50^{aA}a	-2.40 ± 0.31^{aA}a	8.93 ± 0.50^{aA}a
		10	75.92 ± 1.40^{aA}a	-2.56 ± 0.28^{aA}a	8.98 ± 0.55^{aA}a
		14	75.88 ± 1.36^{aA}a	-2.58 ± 0.28^{aA}a	9.08 ± 0.58^{aA}a

[1] n = 3.

[2] Within the same time and concentration, mean values with different lowercase superscript are significantly different (P <0.05). Within the same time and day, mean values with different uppercase superscript are significantly different (P < 0.05). Within the same day and concentration, means values followed different letters are significantly different (P < 0.05).

Table 15. Hunter values of chicken skins treated with fumaric acid during simulated water-immersion chilling (3°C ± 1°C) and storage at 4°C for 14 days.

Time	Fumaric acid (% w/w)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
30 min chilling	0	0	78.31 ± 0.58^{aA}a	-1.11 ± 0.20^{Aa}a	8.43 ± 1.09^{aA}a
		1	78.41 ± 0.66^{aA}a	-1.23 ± 0.17^{aA}a	9.01 ± 1.03^{aA}a
		3	78.96 ± 0.62^{aA}a	-1.27 ± 0.23^{aA}a	9.22 ± 1.05^{aA}a
		7	78.46 ± 0.46^{aA}a	-1.42 ± 0.23^{aA}a	9.70 ± 1.17^{aA}a
		10	78.59 ± 0.43^{aA}a	-1.40 ± 0.22^{aA}a	9.93 ± 1.08^{aA}a
		14	78.10 ± 0.66^{aA}a	-1.49 ± 0.19^{aA}	10.43 ± 1.08^{aA}a
	0.05	0	75.89 ± 0.85^{aAB}a	-1.84 ± 0.42^{aA}a	11.78 ± 1.16^{aA}a
		1	76.49 ± 0.85^{aAB}a	-2.52 ± 0.34^{aA}a	12.69 ± 1.50^{aA}a
		3	76.21 ± 0.92^{aAB}a	-2.78 ± 0.31^{aA}a	12.80 ± 1.41^{aA}a
		7	76.72 ± 0.78^{aAB}a	-3.04 ± 0.28^{aB}a	12.95 ± 1.01^{aA}a
		10	76.64 ± 0.72^{aAB}a	-3.15 ± 0.24^{aB}a	12.64 ± 0.89^{aA}a
		14	77.30 ± 0.57^{aAB}a	-3.08 ± 0.39^{aA}a	13.00 ± 0.74^{aA}a
	0.1	0	73.63 ± 0.85^{aAB}a	-1.38 ± 0.10^{aA}a	10.70 ± 0.56^{aA}a
		1	74.02 ± 1.20^{aAB}a	-1.99 ± 0.24^{aA}a	11.02 ± 0.93^{aA}a
		3	73.90 ± 0.94^{aB}a	-2.28 ± 0.21^{aA}a	11.18 ± 0.88^{aA}a
		7	74.37 ± 1.23^{aAB}a	-2.58 ± 0.28^{aAB}a	11.28 ± 1.03^{aA}a
		10	74.33 ± 1.03^{aAB}a	-2.68 ± 0.27^{aAB}a	11.03 ± 1.10^{aA}a
		14	75.14 ± 1.30^{aAB}a	-2.85 ± 0.31^{aA}a	11.19 ± 1.18^{aA}a
	0.25	0	71.58 ± 0.90^{aB}a	-1.48 ± 0.15^{aA}a	9.00 ± 0.67^{aA}a
		1	72.36 ± 0.96^{aB}a	-2.04 ± 0.16^{aA}a	9.17 ± 0.64^{aA}a
		3	72.52 ± 0.52^{aB}a	-2.10 ± 0.18^{aA}a	9.03 ± 0.71^{aA}a
		7	72.24 ± 0.70^{aB}a	-2.27 ± 0.19^{aAB}a	9.22 ± 0.73^{aA}a
		10	72.46 ± 0.61^{aB}a	-2.34 ± 0.22^{aAB}a	9.30 ± 0.64^{aA}a
		14	73.05 ± 0.81^{aB}a	-2.51 ± 0.22^{aA}a	9.42 ± 0.52^{aA}a
45 min chilling	0	0	79.13 ± 0.82^{aA}a	-1.39 ± 0.38^{aA}a	7.41 ± 1.40^{aA}a
		1	79.16 ± 0.80^{aA}a	-1.42 ± 0.36^{aA}a	7.71 ± 1.40^{aA}a
		3	79.31 ± 0.91^{aA}a	-1.54 ± 0.34^{aA}	8.18 ± 1.13^{aA}a
		7	79.43 ± 0.67^{aA}a	-1.67 ± 0.29^{aA}a	8.39 ± 1.26^{aA}a
		10	79.18 ± 0.70^{aA}a	-1.49 ± 0.40^{aA}a	8.90 ± 1.13^{aA}a
		14	79.14 ± 0.77^{aA}a	-1.74 ± 0.34^{aA}a	9.23 ± 1.03^{aA}a
	0.05	0	73.85 ± 1.84^{aB}a	-1.76 ± 0.34^{aA}a	8.65 ± 0.78^{aA}a
		1	74.49 ± 1.86^{aAB}a	-1.98 ± 0.18^{aA}a	8.94 ± 0.46^{aA}a
		3	74.95 ± 1.72^{aAB}a	-2.33 ± 0.12^{aA}a	9.46 ± 0.44^{aA}a
		7	75.21 ± 1.62^{aAB}a	-2.46 ± 0.16^{aA}a	9.74 ± 0.51^{aA}a
		10	75.39 ± 1.54^{aAB}a	-2.58 ± 0.23^{aA}a	9.71 ± 0.50^{aA}a
		14	75.36 ± 1.49^{aAB}a	-2.73 ± 0.15^{aA}a	9.93 ± 0.53^{aA}a

Table 15. Continued

Time	Fumaric acid (% w/w)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
45 min chilling	0.1	0	75.13 ± 0.51^{aAB}a	-1.75 ± 0.42^{aA}a	8.65 ± 0.78^{aA}a
		1	75.54 ± 0.40^{aAB}a	-2.13 ± 0.28^{aA}a	8.94 ± 0.46^{aA}a
		3	76.19 ± 0.52^{aAB}a	-2.42 ± 0.22^{aA}a	9.46 ± 0.44^{aA}a
		7	76.32 ± 0.38^{aAB}a	-2.50 ± 0.23^{aA}a	9.74 ± 0.51^{aA}a
		10	76.37 ± 0.48^{aAB}a	-2.48 ± 0.21^{aA}a	9.71 ± 0.50^{aA}a
		14	76.31 ± 0.28^{aAB}a	-2.70 ± 0.23^{aA}a	9.93 ± 0.53^{aA}a
	0.25	0	71.99 ± 0.42^{aB}a	-1.85 ± 0.33^{aA}a	7.30 ± 1.14^{aA}a
		1	72.74 ± 0.07^{aB}a	-2.09 ± 0.30^{aA}a	7.69 ± 0.67^{aA}a
		3	72.47 ± 0.55^{aB}a	-2.10 ± 0.30^{aA}a	7.70 ± 0.68^{aA}a
		7	72.56 ± 0.49^{aB}a	-2.11 ± 0.29^{aA}a	7.80 ± 0.45^{aA}a
		10	72.37 ± 0.60^{aB}a	-2.06 ± 0.28^{aA}a	8.05 ± 0.39^{aA}a
		14	72.62 ± 0.39^{aB}a	-2.29 ± 0.27^{aA}a	8.34 ± 0.33^{aA}a
60 min chilling	0	0	77.97 ± 0.61^{aA}a	-0.78 ± 0.34^{aA}a	7.97 ± 0.92^{aA}a
		1	77.90 ± 0.52^{aA}a	-0.87 ± 0.34^{aA}a	8.52 ± 0.92^{aA}a
		3	78.20 ± 0.45^{aA}a	-1.06 ± 0.29^{aA}a	8.51 ± 0.99^{aA}a
		7	78.45 ± 0.51^{aA}a	-1.36 ± 0.20^{aA}a	9.08 ± 1.05^{aA}a
		10	78.07 ± 0.60^{aA}a	-1.25 ± 0.28^{aA}a	9.35 ± 0.95^{aA}a
		14	78.14 ± 0.52^{aA}a	-1.36 ± 0.27^{aA}a	9.58 ± 1.21^{aA}a
	0.05	0	75.70 ± 1.05^{aAB}a	-1.85 ± 0.54^{aA}a	7.82 ± 0.59^{aA}a
		1	75.89 ± 1.07^{aAB}a	-2.26 ± 0.30^{aA}a	8.47 ± 0.34^{aA}a
		3	76.25 ± 0.79^{aAB}a	-2.44 ± 0.21^{aA}a	8.80 ± 0.37^{aA}a
		7	76.45 ± 0.79^{aAB}a	-2.59 ± 0.18^{aA}a	8.89 ± 0.35^{aA}a
		10	76.36 ± 0.69^{aA}a	-2.52 ± 0.17^{aA}a	8.83 ± 0.41^{aA}a
		14	76.42 ± 0.72^{aAB}a	-2.68 ± 0.23^{aA}a	8.97 ± 0.53^{aA}a
	0.1	0	73.09 ± 0.01^{aAB}a	-1.90 ± 0.10^{aA}a	8.51 ± 0.92^{aA}a
		1	73.19 ± 0.10^{aAB}a	-2.11 ± 0.07^{aA}a	8.82 ± 1.25^{aA}a
		3	73.55 ± 0.20^{aAB}a	-2.20 ± 0.06^{aA}a	8.83 ± 1.42^{aA}a
		7	73.99 ± 0.06^{aAB}a	-2.34 ± 0.06^{aA}a	8.76 ± 1.53^{aA}a
		10	73.82 ± 0.13^{aA}a	-2.32 ± 0.05^{aA}a	9.17 ± 1.56^{aA}a
		14	73.76 ± 0.05^{aAB}a	-2.55 ± 0.06^{aA}a	9.35 ± 1.49^{aA}a
	0.25	0	71.73 ± 0.72^{aB}a	-2.07 ± 0.18^{aA}a	9.07 ± 1.26^{aA}a
		1	72.15 ± 0.57^{aB}a	-2.43 ± 0.30^{aA}a	9.34 ± 1.32^{aA}a
		3	73.15 ± 0.69^{aB}a	-2.54 ± 0.34^{aA}a	9.55 ± 1.53^{aA}a
		7	72.90 ± 0.49^{aB}a	-2.68 ± 0.36^{aA}a	9.70 ± 1.46^{aA}a
		10	73.38 ± 0.67^{aA}a	-2.56 ± 0.30^{aA}a	9.63 ± 1.21^{aA}a
		14	72.93 ± 0.45^{aB}a	-2.76 ± 0.31^{aA}a	9.66 ± 1.28^{aA}a

[1, 2] See Table 14 footnotes.

Table 16. Hunter values of chicken skins treated with fumaric acid during simulated soft scalding (53°C ± 1°C) and storage at 4°C for 14 days.

Time	Fumaric acid (% w/w)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
1 min scalding	0	0	78.49 ± 0.55^{aA}a	-1.39 ± 0.19^{aA}a	8.51 ± 0.55^{aA}a
		1	78.76 ± 0.33^{aA}a	-1.55 ± 0.19^{aA}a	8.99 ± 0.54^{aA}a
		3	78.92 ± 0.19^{aA}a	-1.51 ± 0.14^{aA}a	8.94 ± 0.58^{aA}a
		7	79.02 ± 0.30^{aA}a	-1.66 ± 0.14^{aA}a	9.20 ± 0.59^{aA}a
		10	78.74 ± 0.31^{aA}a	-1.71 ± 0.14^{aA}a	9.49 ± 0.61^{aA}a
		14	78.67 ± 0.35^{aA}a	-1.67 ± 0.17^{aA}a	9.74 ± 0.58^{aA}a
	0.5	0	74.23 ± 0.38^{aAB}a	-2.22 ± 0.39^{aB}a	13.05 ± 0.35^{aA}a
		1	74.93 ± 0.43^{aAB}a	-2.85 ± 0.22^{aB}a	13.73 ± 0.30^{aA}a
		3	75.24 ± 0.56^{aAB}a	-3.00 ± 0.13^{aB}a	13.90 ± 0.17^{aB}a
		7	75.85 ± 0.50^{aAB}a	-3.17 ± 0.13^{aB}a	13.76 ± 0.25^{aA}a
		10	75.59 ± 0.27^{aAB}a	-3.25 ± 0.13^{aB}a	13.85 ± 0.12^{aA}a
		14	75.73 ± 0.18^{aAB}a	-3.30 ± 0.13^{aB}a	13.90 ± 0.25^{aA}a
	1.0	0	72.32 ± 0.47^{aB}a	-2.01 ± 0.18^{aAB}a	11.10 ± 0.90^{aA}a
		1	72.98 ± 0.30^{aB}a	-2.37 ± 0.19^{aAB}a	10.99 ± 0.81^{aA}a
		3	73.08 ± 0.29^{aB}a	-2.45 ± 0.15^{aAB}a	11.01 ± 0.72^{aAB}a
		7	74.14 ± 0.20^{aAB}a	-2.60 ± 0.18^{aAB}a	11.18 ± 0.80^{aA}a
		10	74.00 ± 0.38^{aAB}a	-2.63 ± 0.14^{aAB}a	11.06 ± 0.67^{aA}a
		14	73.89 ± 0.21^{aAB}a	-2.65 ± 0.16^{aAB}a	11.20 ± 0.76^{aA}a
	1.5	0	70.41 ± 1.67^{aB}a	-1.97 ± 0.41^{aAB}a	12.22 ± 0.55^{aA}a
		1	71.36 ± 1.46^{aB}a	-2.38 ± 0.30^{aAB}a	12.14 ± 0.76^{aA}a
		3	72.59 ± 1.46^{aB}a	-2.43 ± 0.28^{aAB}a	12.30 ± 0.76^{aAB}a
		7	72.51 ± 1.41^{aB}a	-2.56 ± 0.27^{aAB}a	12.04 ± 0.83^{aA}a
		10	73.36 ± 1.60^{aB}a	-2.66 ± 0.29^{aAB}a	12.25 ± 0.85^{aA}a
		14	72.68 ± 1.63^{aB}a	-2.64 ± 0.29^{aAB}a	12.17 ± 0.81^{aA}a
2 min scalding	0	0	79.37 ± 0.35^{Aa}a	-1.31 ± 0.07^{aA}a	5.94 ± 0.51^{aA}a
		1	79.60 ± 0.30^{aA}a	-1.61 ± 0.09^{aA}a	6.01 ± 0.58^{aA}a
		3	79.77 ± 0.25^{aA}a	-1.66 ± 0.11^{aA}a	6.45 ± 0.56^{aA}a
		7	79.77 ± 0.24^{aA}a	-1.83 ± 0.07^{aA}a	6.59 ± 0.55^{aA}a
		10	79.76 ± 0.30^{aA}a	-1.83 ± 0.07^{aA}a	6.87 ± 0.62^{aA}a
		14	79.83 ± 0.23^{aA}a	-1.88 ± 0.03^{aA}a	6.69 ± 0.73^{aA}a
	0.5	0	73.28 ± 0.51^{aA}a	-2.09 ± 0.04^{aA}a	12.22 ± 0.61^{aB}a
		1	73.29 ± 0.53^{aB}a	-2.49 ± 0.03^{aA}a	12.85 ± 1.06^{aB}a
		3	73.74 ± 0.38^{aB}a	-2.58 ± 0.07^{aA}a	12.95 ± 1.00^{aB}a
		7	73.76 ± 0.67^{aB}a	-2.80 ± 0.02^{aA}a	12.89 ± 0.95^{aB}a
		10	74.13 ± 0.64^{aB}a	-2.74 ± 0.09^{aA}a	13.01 ± 0.94^{aB}a
		14	74.15 ± 0.92^{aB}a	-2.81 ± 0.11^{aA}a	12.92 ± 0.87^{aB}a

Table 16. Continued

Time	Fumaric acid (% w/v)	Day	Hunter value ± std. error[1, 2]		
			L	a	b
2 min scalding	1.0	0	72.20 ± 1.35^{aB}a	-2.30 ± 0.11^{aA}a	11.21 ± 0.51^{aB}a
		1	72.93 ± 1.10^{aB}a	-2.65 ± 0.10^{aA}a	11.47 ± 0.59^{aB}a
		3	72.88 ± 0.88^{aB}a	-2.72 ± 0.11^{aA}a	11.51 ± 0.54^{aB}a
		7	73.38 ± 1.01^{aB}a	-2.86 ± 0.11^{aA}a	11.45 ± 0.49^{aB}a
		10	73.49 ± 0.90^{aB}a	-2.85 ± 0.13^{aA}a	11.40 ± 0.50^{aAB}a
		14	74.02 ± 1.15^{aB}a	-2.91 ± 0.12^{aA}a	11.55 ± 0.39^{aB}a
	1.5	0	70.73 ± 1.10^{aB}a	-2.08 ± 0.20^{aA}a	12.34 ± 1.35^{aB}a
		1	71.48 ± 1.28^{aB}a	-2.38 ± 0.20^{aA}a	12.33 ± 1.27^{aB}a
		3	71.85 ± 1.19^{aB}a	-2.43 ± 0.20^{aA}a	12.39 ± 1.33^{aB}a
		7	72.33 ± 1.47^{aB}a	-2.63 ± 0.19^{aA}a	12.51 ± 1.28^{aB}a
		10	72.72 ± 0.95^{aB}a	-2.63 ± 0.24^{aA}a	12.49 ± 1.26^{aB}a
		14	72.69 ± 1.22^{aB}a	-2.69 ± 0.19^{aA}a	12.73 ± 1.20^{aB}a
3 min scalding	0	0	79.26 ± 0.93^{aA}a	-1.18 ± 0.05^{aA}a	5.84 ± 1.38^{aA}a
		1	79.74 ± 0.58^{aA}a	-1.47 ± 0.07^{aA}a	6.15 ± 1.57^{aA}a
		3	79.75 ± 0.55^{aA}a	-1.49 ± 0.09^{aA}a	6.40 ± 1.56^{aA}a
		7	79.79 ± 0.52^{aA}a	-1.63 ± 0.09^{aA}a	6.72 ± 1.60^{aA}a
		10	79.86 ± 0.55^{aA}a	-1.87 ± 0.10^{aA}a	6.90 ± 1.55^{aA}a
		14	79.86 ± 0.51^{aA}a	-1.84 ± 0.08^{aA}a	7.27 ± 1.60^{aA}a
	0.5	0	72.48 ± 0.70^{aB}a	-2.03 ± 0.12^{aA}a	11.76 ± 0.49^{aB}a
		1	72.62 ± 0.81^{aB}a	-2.24 ± 0.17^{aA}a	12.20 ± 0.35^{aB}a
		3	73.04 ± 0.41^{aB}a	-2.44 ± 0.10^{aA}a	12.06 ± 0.31^{aB}a
		7	73.71 ± 0.70^{aB}a	-2.61 ± 0.04^{aA}a	10.66 ± 0.87^{aAB}a
		10	74.08 ± 0.21^{aB}a	-2.81 ± 0.02^{aA}a	11.90 ± 0.25^{aB}a
		14	74.20 ± 0.50^{aB}a	-2.85 ± 0.02^{aA}a	11.83 ± 0.21^{aAB}a
	1.0	0	71.45 ± 0.85^{aB}a	-2.16 ± 0.37^{aA}a	12.93 ± 0.55^{aB}a
		1	71.94 ± 1.00^{aB}a	-2.40 ± 0.41^{aA}a	12.59 ± 0.32^{aB}a
		3	72.34 ± 0.68^{aB}a	-2.50 ± 0.40^{aA}a	12.86 ± 0.28^{aB}a
		7	73.09 ± 0.78^{aB}a	-2.69 ± 0.37^{aA}a	12.85 ± 0.32^{aB}a
		10	73.22 ± 0.87^{aB}a	-2.84 ± 0.35^{aA}a	13.06 ± 0.19^{aB}a
		14	73.29 ± 0.71^{aB}a	-2.84 ± 0.34^{aA}a	13.25 ± 0.17^{aB}a
	1.5	0	70.28 ± 1.18^{aB}a	-2.24 ± 0.51^{aA}a	13.84 ± 0.92^{aB}a
		1	70.98 ± 1.20^{aB}a	-2.32 ± 0.55^{aA}a	13.70 ± 0.58^{aB}a
		3	71.45 ± 1.24^{aB}a	-2.47 ± 0.56^{aA}a	13.78 ± 0.56^{aB}a
		7	71.97 ± 1.30^{aB}a	-2.65 ± 0.49^{aA}a	14.02 ± 0.46^{aB}a
		10	72.59 ± 1.30^{aB}a	-2.83 ± 0.47^{aA}a	14.15 ± 0.47^{aB}a
		14	72.58 ± 1.19^{aB}a	-2.84 ± 0.47^{aA}a	14.40 ± 0.38^{aB}a

[1, 2] See Table 14 footnotes.

SYNERGISTIC EFFECT OF FUMARIC ACID WITH OTHER ORGANIC ACIDS IN REDUCING *SALMONELLA* SPP. IN VITRO

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ABSTRACT

Salmonella is a major public health concern, influencing research, policy making, and surveillance by government agencies and the food industry. Various antimicrobial agents are implemented at different steps of food production to prevent and reduce the incidence of product contamination with the pathogen, and subsequently decrease the burden of foodborne disease. The synergistic effects of fumaric acid coupled with other organic acids were evaluated for the reduction of *Salmonella* spp. in vitro. Test tubes containing 9 ml of 0.45% fumaric acid alone or in combination with 0.45 or 1.35% of acetic, citric, lactic, oxalic, and propionic acids, and with 0.45% of ascorbic, malic, and tartaric acids, were inoculated with a four-strain cocktail of *Salmonella* spp. After a contact time of 40 sec at 22°C, treatments were serially diluted in buffered BPW and plated onto XLD agar using the thin agar layer (TAL) method. Each treatment was performed in triplicate and log reductions were determined by comparison to a sterile distilled water control. Treatment with 0.45% fumaric acid alone showed a 0.94-log reduction in recoverable cells. No significant differences were observed between control and fumaric acid coupled with 0.45% ascorbic, malic, or tartaric acids. At an initial concentration of 0.45%, with the exception of oxalic acid, combining fumaric acid with selected organic acids (0.45%) made fumaric acid less effective in reducing *Salmonella* spp. The synergistic effectiveness was only improved by the addition of 1.35% acetic, oxalic, and lactic acids. The greatest synergistic effect was observed when 0.45% fumaric acid (pH 2.74) was coupled with 0.45% (pH 1.93) or 1.35% (pH 1.50) oxalic acid resulting in *Salmonella* reductions of 2.09 and ≥6 log. However, oxalic acid treatments

of 0.45% (pH 1.61) and 1.35% (pH 1.22) alone achieved a 3.41- and ≥6-log reductions, suggesting that oxalic acid is more lethal to salmonellae than fumaric acid, and improved bactericidal potential of fumaric acid resulted from a pH drop upon addition of oxalic acid. Synergistic effects of fumaric acid with other weak organic acids to reduce salmonellae in vitro depended on the type of supplemented acid and its concentration.

INTRODUCTION

Salmonellae are a major cause of foodborne illness in humans worldwide, and in the U.S. foodborne outbreaks of salmonellosis are routinely observed and reported *(19)*. On average, *Salmonella* causes an estimated 1.4 million illnesses, resulting in approximately 16,000 hospitalizations and 580 deaths yearly *(17)*. In an attempt to reduce the burden of foodborne disease, numerous improvements in food `production, food technology, and the monitoring of foodborne salmonellosis and the pathogen itself have been successfully implemented *(19)*. The incidence of *Salmonella* infection per 100,000 population declined slightly in 2009 (15.19) compared to 2008 (16.20) *(5)*, accounting for 7,039 laboratory-confirmed cases of salmonellosis in the U.S. *(7)*. Poultry and poultry products are often implicated in sporadic cases and in outbreaks of human salmonellosis *(3)*. In surveillance for foodborne disease outbreaks in the U.S., poultry was associated with 17% out of 235 outbreaks caused by a single food commodity in 2007 *(6)*. A comparison of human cases caused by salmonellae *(4)* with prevalence of serotypes found in poultry *(10)*, indicates Enteritidis, Typhimurium, Newport, and Heidelberg are the most common strains that belong to both groups. These four *Salmonella* serotypes accounted for 51% of all *Salmonella* infections in 2009 *(7)*. Despite the efforts of scientists, government agencies, and industry *Salmonella* spp. continues to be a major public health problem. The U.S. Department of Agriculture, Economic Research Service (USDA/ERS) estimated that *Salmonella* infections' cost was about $2.65 billion in 2009, based on medical cost due to illness, the value of time lost from work due to nonfatal illness, and value of premature death *(27)*. Extrapolating from

the above, the cost of salmonellosis due to the consumption of contaminated poultry and poultry production is about 450 million in the U.S.

Fumaric acid is a generally recognized as safe (GRAS) food-grade acidulant *(16)* and has been found effective in increasing the microbiological safety of foods by decreasing the levels of enteric pathogens, including salmonellae *(1, 15, 16, 20)*. It has been used as an antimicrobial agent in meat products *(1, 20-22)*, produce *(14-16)*, and apple cider *(8)*. Fumaric acid has limited water solubility, which hinders its bactericidal potential. The maximum concentration soluble in water at 25°C is 0.63% *(28)*. Several researchers have noted that the antimicrobial activity of fumaric acid increases when it is used in combination with other chemical or physical interventions. For instance, the combined treatment of fumaric acid (0.5%) and ultraviolet-C irradiation (UV-C) (5kJ m^{-2}) was more effective at reducing the initial population of total aerobic bacteria, yeasts and molds on strawberries than fumaric acid or UV-C alone *(14)*. Use of 0.5% fumaric acid with 50 ppm chlorine dioxide reduced populations of *S.* Typhimurium and *L. monocytogenes* inoculated on broccoli sprouts by 0.74 and 0.39 log CFU/g, respectively, compared to fumaric acid or chlorine dioxide alone *(15)*. In another study, a combination of 0.15% fumaric acid and 0.05% sodium benzoate reduced *E. coli* O157:H7 levels by 5 log in apple cider *(8)*.

Fumaric acid in combination with other weak organic acids may be an effective intervention against salmonellae and subsequently enhance microbiological safety of food.

MATERIALS AND METHODS

Preparation of inoculum

S. Enteritidis (poultry isolate), *S.* Heidelberg (environmental isolate from turkey processing plant), and *S.* Typhimurium (poultry isolate) were obtained from the Poultry Science department at NCSU, Raleigh, NC, and *S.* Newport (J1980) (human isolate from tomato outbreak) was acquired from the Food Science department at Virginia Polytechnic Institute and State University, Blacksburg, VA. The antibiotic susceptibility profile of the four *Salmonella* strains was performed by NARMS, Atlanta, GA (Appendix 1). The strains were stored at -80°C ± 1°C in brain heart infusion broth (BHIB; Fisher Scientific, Fair Lawn, NJ) containing 50% glycerol (Fisher Scientific, Fair Lawn, NJ) *(26)*, and then activated by two successive transfers in BHIB *(9)*. A cocktail of all four *Salmonella* strains was prepared by combining 2 ml of aliquots from each culture grown to a level of *ca.* 9.0 $\log_{10}$ CFU/ml. (37°C) in a sterile plastic tube *(13, 18)*. The cocktail concentration was determined by serial dilution in 0.1% w/v buffered peptone water (BPW; Weber Scientific, Hamilton, NJ) and plating on xylose lysine deoxycholate agar (XLD; Weber Scientific, Hamilton, NJ).

Chemical treatment preparation

Fumaric acid (CAS 110-17-8) treatments, at concentrations of 0.5% w/w, were prepared by dissolving the chemical in sterile distilled water in a sterile 100 ml beaker on a stirring plate at room temperature (SP131325, Barnstead/Thermolyne, Dubuque, IA). Ascorbic (CAS 50-81-7), tartaric (CAS 133-37-9), and malic (CAS 6915-15-7) acids at

concentrations of 0.5% w/v, and citric (CAS 77-92-9) and oxalic (CAS 114-62-7) acids, at concentrations of 0.5 and 1.5% w/v, were prepared by separately pre-dissolving each chemical in sterile distilled water in a sterile 100 ml beaker. After the chemicals were completely dissolved, the contents were placed into sterile 100 ml volumetric flasks and volume adjusted with additional sterile distilled water until the bottom of the meniscus reached the volumetric line. Acetic (CAS 64-19-7), propionic (CAS 79-09-4), and lactic (CAS 598-8231) acids, at concentrations of 0.5 and 1.5% v/v, were prepared by dissolving the chemical in sterile distilled water in a sterile 100 ml volumetric flask. The flask was filled until the bottom of the meniscus reached the volumetric line with sterile distilled water. All chemicals tested were purchased from Fisher Scientific (Fair Lawn, NJ), and made fresh on the day of experimentation.

Procedure for testing synergistic effect of fumaric acid with other organic acids

The test tubes containing 9 ml of sterile distilled water (control), 0.5% fumaric acid alone or 1:1 combination of 0.5% fumaric and 0.5% of one of the following: acetic, ascorbic, citric, lactic, malic, oxalic, propionic, or tartaric acid were inoculated with 1 ml of *Salmonella* cocktail. The contact time for all treatments was 40 sec at 22°C ± 1°C. These treatment parameters would allow simulating an application of fumaric acid with other organic acids as they have been applied as antimicrobial treatment during on-line or post-chill dipping of broiler chicken carcasses. The pH of each solution (Table 17) was measured using a calibrated digital pH meter (AB15, Fisher Scientific, Pittsburgh, PA).

To determine if a synergistic effect of fumaric acid with other organic acids depended on the concentration of the supplemented organic acid, the combinations that provided the best reductions were tested according to an additional scheme of 0.5% fumaric acid + 1.5% supplemented organic acid. The contact time for all treatments was 40 sec at 22°C ± 1°C. The pH of each solution (Table 18) was measured using a calibrated digital pH meter (AB15, Fisher Scientific, Pittsburgh, PA).

Microbiological analysis

One ml of solution was removed, serially diluted in BPW and plated in duplicate on XLD by using the thin agar layer (TAL) plating technique for acid-injured foodborne pathogens *(29)*. This method allows for maximum recovery of surviving *Salmonella* cells including those that are sublethally injured *(12)*. The plates were incubated at 37° ± 1°C for 24 hours under aerobic conditions and the subsequent *Salmonella* count for each experiment was expressed as $\log_{10}$ CFU/ml. Typical *Salmonella* colonies on XLD appeared black with clear and opaque zone *(29)*. On a periodic basis, two or three putative *Salmonella* colonies were picked from the countable plates and confirmed by API 20E (bioMerieux Inc., Hazelwood, MO) as *Salmonella* spp.

Expression of antimicrobial activity

Efficacy of fumaric acid treatments was assessed by determining the reduction in viable population of *Salmonella* cells *(9)*. Reduction for each application was calculated as

the difference between the population of cells recovered from the control and the population of cells recovered from the organic acid treatment.

Statistical analysis

The data were analyzed by using SAS software (version 9.1, SAS Institute, Cary, NC). Analysis of variance was carried out at a 95% significance level ($P < 0.05$), and a Tukey test was used for separation of means *(23)*.

RESULTS AND DISCUSSION

Based on the procedure described previously, the concentrations of the acids in the solutions would change from 0.5 to 0.45% and from 1.5 to 1.35% as a result of addition of 1 ml inoculum to 9 ml of acid treatments. Treatment with 0.45% fumaric acid alone showed a 0.94-log reduction in recoverable *Salmonella* cells after 40 sec. At a concentration of 0.45%, with the exception of oxalic acid, combinations of fumaric acid with 0.45% acetic, ascorbic, citric, lactic, malic, propionic, or tartaric acids resulted in *Salmonella* reductions of ≤0.38 log in vitro (Table 17). No significant differences were observed between control and fumaric acid coupled with 0.45% ascorbic, malic, and tartaric acids ($P > 0.05$). Shimizu et al. *(25)* indicated that 0.3% fumaric acid inhibited *S.* Typhimurium growth after 160 sec, and supplementation with 0.3% ascorbic, malic, citric, and tartaric acids decreased growth inhibition time to 20, 80, 80, and 40 sec, respectively. In another study, 1.0% fumaric acid combined with 1.0% acetic or lactic acid reduced *E. coli* O157:H7 counts on lean beef muscles by 0.32 and 0.40 log compared to treatment with 1.0% fumaric acid alone; however, after 90 days of refrigerated storage *E. coli* O157:H7 counts on fumaric acid treated samples were lower compared to otherwise similar samples treated with fumaric and lactic acids *(21)*. The antagonistic effect observed in our study between fumaric acid and 0.45% lactic, acetic, propionic, citric, ascorbic, tartaric, and malic acids was in contrast to the synergy between the acids reported in the literature. This may have resulted from the differences in bacterial strains and design of the experiment, e.g. matrix and treatment time. Thus, at levels of 0.45%, the bactericidal potential of fumaric acid was only improved by the addition of oxalic acid,

which generated a 2.09-log reduction of *Salmonella*. Similarly, oxalic acid at concentrations of 0.05 to 0.3% was also the most effective in combination with 0.3% fumaric acid in the Shimizu et al. study, inhibiting salmonellae growth after 20 sec *(25)*.

The synergistic effect of fumaric acid combined with weak organic acids improved when the concentration of the supplement acid increased (Table 18). Baysal and Unluturk *(1)* found that a combination of 0.5% fumaric acid with 1.0% lactic acid showed greater immediate inhibitory effect on mesophilic and psychrotrophic bacteria on turkey breasts compared to single treatments of 0.5% fumaric acid or 1.0% lactic acid. Shimizu et al. *(25)* reported that increasing tartaric acid (supplemented acid) concentration from 0.1 to 0.3% in solution with 0.3% fumaric acid decreased growth inhibition time of *S.* Typhimurium by 120 sec. In our study, an increase of the supplemented organic acid concentration to 1.35% improved fumaric acid effectiveness against *Salmonella* only in case of oxalic, acetic, and lactic acids, resulting in ≥6, 1.48, and 1.27 log reductions, respectively. There was no significant difference in log reductions between 0.45% fumaric acid coupled with 1.35% citric acid and 0.45% fumaric acid coupled with 1.35% propionic acid ($P > 0.05$).

It worth note that treatments of 0.45% fumaric acid with 0.45% (pH 1.93) or 1.35% (pH 1.50) oxalic acid had lower pH values compared to the treatments of fumaric acid with lactic, acetic, propionic, citric, ascorbic, tartaric, and malic acids. Oxalic acid alone at concentrations of 0.45% (pH 1.61) and 1.35% (pH 1.22) was more effective than 0.45% fumaric acid (pH 2.49), and reduced *Salmonalla* counts by 3.71 and ≥6 log, respectively, compared to control. These research findings indicate the strong bactericidal potential of

oxalic acid which may at least in part be explained by the rapid decrease in the external pH below the pathogen survival limit *(2)*. Additionally, weak organic acids may also exhibit a specific inhibitory effect on cell activity *(2)*, such as transition-metal ions chelation *(11, 24)*.

The synergistic effect of fumaric acid with other weak organic acids to reduce salmonellae in vitro depends on the type and concentration of supplemented acid. Future work should focus on evaluation of the bactericidal potential of fumaric acid with 1.35% acetic or lactic acids and with 0.45 or 1.35% oxalic acid when applied as antimicrobial surface treatments to decrease *Salmonella* in food matrixes.

REFERENCES

1. Baysal, A. H. D., and A. Unluturk. 2005. Effects of organic acid and phosphates on the microbiological stability and on survival of *Salmonella typhimurium* on turkey breast meat. XVII European Symposium on the Quality of Poultry Meat, Doorwerth, The Netherlands.

2. Booth, I. R., and M. Stratford. 2003. Acidulants and low pH. p. 25-47. *In* N.J. Russell, and G.W. Gould (ed.), Food Preservatives. Kluwer Academic/Plenum Publishers, New York, N.Y.

3. Bryan, F. L., and M. P. Doyle. 1995. Health risks and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. *J. Food Prot*. 58:326-344.

4. CDC. 2007. *Salmonella* surveillance: annual summary, 2005. U.S. Department of Health and Human Services, Atlanta, GA.

5. CDC. 2009. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food -- 10 states, 2008. *MMWR*. 58:333-337.

6. CDC. 2010. Surveillance for foodborne disease outbreaks -- United States, 2007. *MMWR*. 59:973-979.

7. CDC. 2010. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food -- 10 states, 2009. *MMWR*. 59:418-422.

8. Comes, J. E., and R. B. Beelman. 2002. Addition of fumaric acid and sodium benzoate as an alternative method to achieve a 5-log reduction of *Escherichia coli* O157:H7 populations in apple cider. *J. Food Prot*. 65:476-483.

9. Conner, D. E., and S. F. Bilgili. 1994. Skin attachment model for improved laboratory evaluation of potential carcass disinfectants for their efficacy against *Salmonella* attached to broiler skin. *J. Food Prot*. 57:684-688.

10. Foley, S. L., A. M. Lynne, and R. Nayak. 2008. *Salmonella* challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. *J. Anim. Sci*. 86:E149-E162.

11. Graham, A. F., and B. M. Lund. 1986. The effect of citric acid on growth of proteolytic strains of *Clostridium botulinum. J. Appl. Bact*. 61:39-49.

12. Humphrey, T. J. 1981. The effects of pH and levels of organic matter on the death rates of Salmonellas in chicken scald-tank water. *J. Appl. Bact*. 51:27-39.

13. Hwang, C., and L. R. Beuchat. 1995. Efficacy of selected chemicals for killing pathogenic and spoilage microorganisms on chicken skin. *J. Food Prot*. 58:19-23.

14. Kim, J. Y., J. W. Kim, G. O. Lim, S. A. Jang, and K. B. Song. 2010. The effects of aqueous chlorine dioxide of fumaric acid treatment combined with UV-C on postharvest quality of 'Maehyang' strawberries. *Postharv. Biol. Technol*. 56:254-256.

15. Kim, Y. J., M. H. Kim, and K. B. Song. 2009. Efficacy of aqueous chlorine dioxide and fumaric acid for inactivating pre-existing microorganisms and *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on broccoli sprouts. *Food Control*. 20:1002-1005.

16. Kondo, N., M. Murata, and K. Isshiki. 2006. Efficiency of sodium hypochlorite, fumaric acid, and mild heat in killing native microflora and *Escherichia coli* O157:H7, *Salmonella* Typhimurium DT104, and *Staphylococcus aureus* attached to fresh-cut lettuce. *J. Food Prot*. 69:323-329.

17. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis*. 5:607-625.

18. Mehyar, G., G. Blank, J. H. Han, A. Hydamaka, and R. A. Holley. 2005. Effectiveness of trisodium phosphate, lactic acid and commercial antimicrobials against pathogenic bacteria on chicken skin. *J. Food Prot*. 25:351-362.

19. Newell, D. G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, H. Sprong, M. Opsteegh, M. Langelaar, J. Threfall, F. Scheutz, J. Giessen, and H. Kruse. 2010. Food-borne disease -- The challenge of 20 years ago still persist while new ones continue to emerge. *Int. J. Food Microb*. 139:3-15.

20. Podolak, R. K., J. F. Zayas, C. L. Kastner, and D. Y. C. Fung. 1995. Reduction of *Listeria monocytogenes, Escherichia coli O157:H7,* and *Salmonella typhimurium* during storage on beef sanitized with fumaric, acetic, and lactic acids. *J. Food Safety*. 15:283-290.

21. Podolak, R. K., J. F. Zayas, C. L. Kastner, and D. Y. C. Fung. 1996. Inhibition of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on beef by application of organic acids. *J. Food Prot*. 59:370-373.

22. Podolak, R. K., J. F. Zayas, C. L. Kastner, and D. Y. C. Fung. 1996. Reduction of bacterial populations on vacuum-packaged ground beef patties with fumaric and lactic acids. *J. Food Prot*. 59:1037-1040.

23. Rao, P. V. 1997. Statistical research methods in life sciences. Thomson Wadsworth, Mason, OH.

24. Shelef, L. A. 1994. Antimicrobial effects of lactates: a review. *J. Food Prot*. 57:445-450.

25. Shimizu, T., T. Takahata, and M. Kato. 1995. Antibacterial activity of several organic acids commonly used as food additives. *J. Food Hyg. Soc. Japan*. 36:50-54.

26. Tamblyn, K. C., and D. E. Conner. 1997. Bactericidal activity of organic acids against *Salmonella typhimurium* attached to broiler chicken skin. *J. Food Prot*. 60:629-633.

27. U.S. Department of Agriculture, Economic Research Service. 2010. Foodborne illness cost calculator: *Salmonella.* Available at: http://www.www.ers.usda.gov/Data/Foodborne Illness/salm_Intro.asp. Accessed 11 March 2010.

28. Windholz, M. 1976. The Merck Index. p. 4137. *In* M. Windholz, et al. (ed.), An encyclopedia of chemicals and drugs. Merck & Co., Inc., Rahway, NJ.

29. Wu, V. C. H., D. Y. C. Fung, D. H. Kang, and L. K. Thompson. 2001. Evaluation of thin agar layer method for recovery of acid-injured foodborne pathogens. *J. Food Prot*. 64:1067-1071.

Table 17. Synergistic effect of 0.45% fumaric acid with 0.45% weak organic acid to reduce *Salmonella* spp. in vitro.

Treatment[1]	pH	Mean *Salmonella* log reduction ± std. error (CFU/ml)[2, 3]
0.45% fumaric acid	2.49	0.94 ± 0.01^{d}
0.45% fumaric acid + 0.45% oxalic acid	1.93	2.06 ± 0.06^{e}
0.45% fumaric acid + 0.45% lactic acid	2.69	0.38 ± 0.06^{c}
0.45% fumaric acid + 0.45% acetic acid	2.77	0.33 ± 0.04^{c}
0.45% fumaric acid + 0.45% propionic acid	2.80	0.29 ± 0.04^{bc}
0.45% fumaric acid + 0.45% citric acid	2.53	0.26 ± 0.01^{abc}
0.45% fumaric acid + 0.45% ascorbic acid	2.70	0.07 ± 0.05^{ab}
0.45% fumaric acid + 0.45% tartaric acid	2.44	0.06 ± 0.01^{ab}
0.45% fumaric acid + 0.45% malic acid	2.56	0.04 ± 0.06^{a}

[1] Treatments of fumaric acid alone and fumaric acid coupled with weak organic acids in a ratio 1:1 are applied for 40 sec at 22 ± 1°C.
[2] n = 3.
[3] Within the same column, mean values with different superscripts are significantly different ($P < 0.05$).

Table 18. Synergistic effect of 0.45% fumaric with 1.35% weak organic acid to reduce *Salmonella* spp. in vitro.

Treatment[1]	pH	Mean *Salmonella* log reduction ± std. error (CFU/ml)[2, 3]
0.45% fumaric acid + 1.35% oxalic acid	1.50	$\geq 6^{a}$
0.45% fumaric acid + 1.35% acetic acid	2.75	1.48 ± 0.03^{b}
0.45% fumaric acid + 1.35% lactic acid	2.59	1.27 ± 0.08^{b}
0.45% fumaric acid + 1.35% citric acid	2.38	0.70 ± 0.06^{c}
0.45% fumaric acid + 1.35% propionic acid	2.75	0.53 ± 0.05^{c}

[1] Treatments of fumaric acid alone and fumaric acid coupled with weak organic acids in a ratio 1:1 are applied for 40 sec at 22 ± 1°C.
[2] n = 3.
[3] Within the same column, mean values with different superscripts are significantly different ($P < 0.05$).

VARIATION IN THE EFFICACY OF OXALIC ACID TO REDUCE *SALMONELLA* SPP. AT VARIOUS STAGES OF POULTRY PROCESSING

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ABSTRACT

Salmonellosis is the most frequently reported foodborne illness in the U.S., and poultry is recognized as a major source of *Salmonella* infections. Numerous antimicrobial agents have been studied to determine their efficacy against pathogenic bacteria in model systems. The bactericidal potential of weak organic acids is well documented; however, inconsistencies exist in the efficacy of antimicrobials among the studies. Previous studies on bactericidal efficacy of oxalic and fumaric acids against salmonellae in poultry processing (Sybirtseva et al., 2011, unpublished) detected a disparity in the potential of the acids to reduce the levels of *Salmonella* spp. attached to chicken skins collected several months apart. Each of the three sets of skins collected was considered a separate batch. Skins from all three batches were analyzed with the modified skin attachment model (mSAM) for determination of antimicrobial effectiveness. More significant variations in mean *Salmonella* log reduction was observed among the three batches treated with oxalic acid than the variation between each of the three replications within each batch ($P < 0.05$). Treatment with 0.5% oxalic acid applied at 53°C for 3 min resulted in reductions of 3.46, 1.65, and 2.16 log for batch 1, 2, and 3, respectively. The mean reduction in recoverable cells at chilling application at a 60 min contact time was 3.33 log for batch 1, 1.22 log for batch 2, and 2.18 log for batch 3. When 2.0% oxalic acid applied at simulated 40 sec post-chill dipping, *Salmonella* mean reduction for batch 1, batch 2, and batch 3 was 1.30, 0.87, and 1.19 log, respectively. The effect of contact time and oxalic acid concentration on *Salmonella* reduction was also dependent on the batch ($P < 0.05$).

Treatment with fumaric acid at concentrations of 0.5, 1.0, and 1.5% applied during soft scalding yielded greater *Salmonella* reductions with batch 3 than with batch 2. A 1.0% fumaric acid treatment applied at 53ºC for 3 min resulted in *Salmonella* reductions of 0.92 log with batch 2, and 1.34 log with batch 3. No significant difference was detected between *Salmonella* reductions achieved with batch 2 versus batch 3 when fumaric acid applied during on-line or post-chill dipping at concentrations of 0.25 and 0.5% and during water-immersion chilling at concentrations of 0.05, 0.1, and 0.25% ($P > 0.05$), possibly due to use of smaller fumaric acid concentrations compared to treatments applied at the scalder step (0.5 to 1.5%).

Various hypotheses were considered to explain the variation in mean *Salmonella* reductions among the three batches, including the effect of scalder water temperature, variation in initial pH, fat and moisture content of collected samples, as well as presence of channels and crevices on skins. Due to lack of samples from batch 1, scanning electron microscopy and skin histology were used to evaluate microtopographical differences between batch 2 and batch 3. No obvious difference was observed among the skin batches that could explain the variation in *Salmonella* reduction. Variation in antimicrobial log reduction among the different chicken skin batches emphasizes the importance of collecting samples from different flocks, days and seasons for testing antimicrobial agents. This would best simulate the natural variation that occurs within industry and more accurately assess the potential reduction range of antimicrobials while decreasing inconsistency among the reported results.

INTRODUCTION

Salmonellae are one of the leading causes of gastroenteritis in the world, and are responsible for 1.4 million illnesses, 16,000 hospitalizations, and 580 deaths in the U.S. each year *(24)*. Poultry is commonly associated with foodborne salmonellosis *(8, 11)*, and is recognized by CDC as the most frequent single food commodity (21%) contributing to human cases of *Salmonella* infections in 2006 *(6)*. In spite of continuous regulatory oversight, media attention, and industry attempts to prevent or reduce broiler carcasses contamination with salmonellae *(27)*, approximately 34% of whole broiler carcasses are found *Salmonella* positive in retail market stores *(30)*.

Chemical interventions such as antimicrobial agents are known as one of the most practical and effective means to improve the microbiological safety of poultry *(3)*. The immediate effect of various carcass antimicrobial agents, such as chlorine *(1, 14)*, trisodium phosphate (TSP) *(1, 13, 20, 21, 34)*, cetylpyridinium chloride (CPC) *(17, 20, 25)*, hydrogen peroxide *(10, 22)*, and organic acids *(1, 9, 10, 12, 13, 20, 23, 26, 28)* have been investigated for their activity against *Salmonella* spp; but inconsistencies exist in the efficacy of antimicrobials among studies.

Previous work in our laboratory (Sybirtseva et al., unpublished; "Efficacy of oxalic acid to reduce the levels of *Salmonella* spp. at various stages of poultry processing" and "Efficacy of fumaric acid to reduce the levels of *Salmonella* spp. at various stages of poultry processing") has demonstrated the antimicrobial potential of oxalic and fumaric acids with the modified skin attachment model (mSAM) to reduce a cocktail of four *Salmonella* strains,

Enteritidis, Heidelberg, Typhimurium, and Newport (J1980), from the surface of broiler chicken skins collected during three different seasons: February, May, and August of 2009. Each season a batch of skins and antimicrobial potential of acids was evaluated by averaging the mean reductions of three batches for oxalic acid and two batches for fumaric acid. Prior to inoculation, chicken skins from all batches were irradiated at 12 to 25 kGy with Co-60 to eliminate background microflora, and permitting testing of organic acids against salmonellae in a pure culture situation. Mean *Salmonella* log reductions of 1.91 (three batches, total of 9 replications) and 1.53 log (two batches, total of 6 replications) were achieved with 1.5% oxalic and fumaric acid applied at soft scalder application (53°C/3 min), respectively. The disparity in the efficacy of oxalic and fumaric acid against *Salmonella* was noted among the batches under simulated on-line or post-chill dipping, water-immersion chilling, and soft scalding

MATERIALS AND METHODS

Chicken skin analysis

The surface pH of the chicken skins was measured at five locations with a pH meter (AB15, Fisher Scientific, Pittsburgh, PA) fitted with a poly flat surface electrode (flat surface pH combination, Ag/AgCl reference, Fisher Scientific, Pittsburgh, PA). The measurements from each skin were taken at 25°C and then averaged for analysis.

Water activity of chicken skin samples was measured by using a water activity meter (AQUALAB 4TE, Decagon Devices, Inc., Pullman, WA) following sample preparation and measurements in accordance with the manufacturer's guidelines.

Moisture and fat analysis of chicken skins was carried out by a private testing laboratory using a SMART Trac (NMR system, CEM Corp., Metthews, NC).

All measurements were performed in triplicates for the skins from batch 2 and batch 3. No measurements could be made from batch 1 due to lack of samples.

Microscopic examination of chicken skins

Scanning electron microscopy (SEM) of chicken skin samples was performed at the Department of Microbiology at NCSU, Raleigh, NC. The procedure described by Kim and Doores *(16)* was followed with some modifications. Briefly, irradiated chicken skins (1 cm^2) with or without inoculated *Salmonella* cocktail (three pieces per skin, three replicates per batch) were fixed in 3% glutaraldehyde buffered with 0.1M sodium cacodylate (pH 7.2), held for one week at 4°C, rinsed three times (30 min each) with 0.1M sodium cacodylate buffer

(pH 7.2), and dehydrated with graded ethanol series (30, 50, 70, 95, and 100%) for 30 min per bath. Then, skin samples were critical point dried in liquid carbon dioxide for 10 min to equilibrium using a Tousimis Samdri -795 (Tousimis Research Corporation, Rockville, MD). Chicken skins were mounted, coated with gold/palladium using a Hummer 6.2 sputtering system (Anatech U.S.A., Union City, CA), and viewed by a JEOL JSM-5900LV SEM (JEOL USA, Peabody, MA) at 15kV.

Histology of irradiated chicken skins (three replicates per batch) was carried out at the Department of Population Health and Pathobiology at NCSU, Raleigh, NC by using the standard histological procedure with hematoxylin and eosin staining (H&E). The samples were viewed at 10X with a Leica microscope (DM LB2, Vashaw Scientific Inc., Raleigh, NC). Photographs were made with Flash Bus program and measurements were made with image J (NIH software, Bethesda, MD).

Statistical analysis

The data were analyzed by using SAS software (version 9.1, SAS Institute, Cary, NC), and analysis of variance was carried out at a 95% significance level ($P < 0.05$) and a Tukey test was used for separation of means *(29)*.

RESULTS AND DISCUSSION

Although significant research has been conducted on the bactericidal activity of potential antimicrobial compounds, inconsistencies exist in the published efficacy of these chemicals. Reasons for this disparity in the potential of oxalic acid to reduce *Salmonella* spp. attached to raw chicken skins could include the collection of skins at different seasons. The variation in a mean *Salmonella* log reduction among the three batches of chicken skin treated with oxalic and fumaric acid during on-line or post-chill dipping, water-immersion chilling and soft scalding is presented in Tables 19 through 24.

The variation in salmonellae reduction observed among the chicken skin batches treated with oxalic acid was more significant than the variation between each of three replications within each batch ($P < 0.05$). Significant batch main effect and interactions were detected in simulated on-line or post-chill dipping, water-immersion chilling and soft scalding with oxalic acid ($P < 0.05$). In general, oxalic acid was more effective against salmonellae when tested with batch 1 compared to batch 2, and batch 3 revealed intermediate reductions. Treatments of 2.0% oxalic acid applied at 22°C ± 1°C for 20 sec resulted in 1.16-, 0.63-, and 0.81-log reduction in batch 1, batch 2, and batch 3, respectively. When contact time was increased to 40 sec, mean *Salmonella* log reduction for batch 1, batch 2, and batch 3 decreased to 1.30, 0.87, and 1.19 log compared to the control, respectively. Even though a mean *Salmonella* log reduction varied among the batches, statistically no significant difference was observed until the dipping time increased to 30 sec ($P > 0.05$).

More significant variations in salmonellae reduction among the batches were observed during water-immersion chiller application (3°C ± 1°C). The mean reduction in recoverable *Salmonella* cells using 0.5% oxalic acid and a 60-min chilling time was 3.33 log for batch 1, 1.22 log for batch 2, and 2.18 log for batch 3.

When 2.0% oxalic acid was applied at simulated 1 min soft scalding (53°C ± 1°C) *Salmonella* mean reduction for batch 1, batch 2, and batch 3 was 2.95, 1.36, and 1.47 log, respectively. At an exposure time of 3 min, 2.0% oxalic acid reduced salmonellae counts by a 3.46-, 1.65-, and 2.16-log reduction in batch 1, batch 2, and batch 3, respectively. No significant difference in *Salmonella* reduction was observed among the batches treated with 1.0 and 1.5% oxalic acid at 1 min scalding time ($P = 1.0000$).

No significant variation in *Salmonella* reductions was observed among batch 2 and batch 3 when treated with fumaric acid under simulated on-line or post-chill dipping and water-immersion chilling ($P > 0.05$). Fumaric acid (0.25%) applied during a 40-sec on-line or post-chill dip step reduced *Salmonella* spp. attached to irradiated raw chicken skins by 0.31 log in both batch 2 and batch 3. Similarly, no significant variation in *Salmonella*'s reduction was observed when 0.25% fumaric acid applied during 60 min water-immersion chilling, resulting in 0.65- and 0.78-log reduction in batch 2 and batch 3, respectively. Possibly, fumaric acid concentrations of 0.05 and 0.25% tested during dipping and chilling were too low to detect the disparity among the batches. This is evidenced by the variation in *Salmonella* reduction was observed among batch 2 and batch 3 when fumaric acid applied during soft scalding at concentrations of 0.5 to 1.5%, and the variation between two batches

was more significant than the variation within each batch ($P < 0.05$). Treatment with 1.5% fumaric acid applied at 53ºC for 3 min reduced *Salmonella* spp. by 1.25 log in batch 2 and 1.79 log in batch 3, respectively. Although, the main effect of the batch was significant during soft scalding with fumaric acid ($P < 0.05$), no significant difference was observed between batch 2 and batch 3 at all tested combinations of times and concentrations ($P > 0.05$).

Various hypotheses were considered to explain the variation in *Salmonella* reduction among the batches, including initial variations in pH, water activity, fat, and moisture content of collected chicken skin samples, as well as the effect of various water temperatures during conventional scalding operations and the presence of channels and crevices on the surface of chicken skin samples. Since all the chicken skins were collected from the same poultry processing plant and no changes occurred in the processing procedures between the collection time periods, the effect of breed of broilers and method of scalding or chilling were excluded from a list of potential factors that could alter the skin structure and subsequently oxalic acid effectiveness among the batches. Additionally, Suderman and Cunningham *(31)* stated that age of broilers did not affect the physical nature of poultry skin ultrastructure noticeably. Due to the lack of skin samples from batch 1, all following results and discussion are related only to batch 2 and batch 3.

Several investigators indicated that surface fat characteristics of meat is important for the effectiveness of antimicrobial interventions, since fat, being hydrophobic, may interfere with the effectiveness of water washes to remove bacterial contamination *(5)*. For instance,

Kim and Doores *(16)* observed a fatty liquid film on the surface of skins which could protect the bacterial cells. Additionally, we suspected a possibility of residual plant antimicrobial treatments (acidified sodium chlorite and peroxyacetic acid), remaining on the surface of chicken skins, to interfere with oxalic acid action. However, no significant difference in surface pH was observed between batch 2 and batch 3 samples ($P > 0.05$) (Table 25). Also, the samples from batch 2 and batch 3 were not statistically different in water activity, moisture, or fat content ($P > 0.05$) (Table 25).

SEM was used to examine differences in skin surface morphology between the skin batches. High variation was observed in skins microtopography among and within skin batches, but no obvious visual difference was found between the skins from batch 2 (Figure 1) and batch 3 (Figure 2) that could impact oxalic acid effectiveness and subsequently cause variation in *Salmonella* reduction. Both batches appeared to have numerous crevices and channels, but they seemed to be insufficient in size to entrap inoculated *Salmonella* cells (Figure 3). Taking into account that skins were collected from processed broiler carcasses, water temperature variation during a normal plant scalding operation could impact the thickness of epidermal layer and subsequently influence the strength of *Salmonella* cell attachment to the skin. Previously, it was reported that variation in water temperature during conventional scalding impacts the thickness of lost epidermis and influences the bacterial attachment, as well as causes entrapment of the cells in channels and crevices of the revealed skin layer where bacteria would be protected from antimicrobial agent action *(2, 18, 31, 33)*.

On other hand, Kim et al. *(18)* found no significant differences in the number of salmonellae attached to the skins processed at different temperatures.

Histology of chicken skins revealed a lack of the epidermal layer in both batches (Figure 4) and partial presence of epithelial fragments on the surface of chicken skins (Figure 5). Also, there was no significant difference in the collagen thickness of skins between batches ($P > 0.05$) (Table 26). The presence of partial epithelial fragments may impact the strength of *Salmonella* attachment. Although a physical entrapment of cells within the delaminated layers is also expected and would result in withholding bacteria from being releasing by stomaching *(16)*. Kim et al. *(18)* pointed out that salmonellae attached more readily to the dermis than to epidermis layer of chicken skins, possibly due to formation of attachment fibrils on the surface of dermis. However, large craters and small irregularities were noted in both skin batches (Figure 6). Similarly, Thomas and McMeekin *(32)* observed that conventionally scalded chicken skins were missing the outer skin layer and exposed dermal skin tissue was covered with capillary-sized channels, crevices and epidermal fragments. Possibly, these skin surface characteristics caused variation in *Salmonella* reductions among the batches. Butler et al. *(4)* supports this by indicating that "inoculum may trap in capillary crevices or troughs" where it will be protected from antimicrobial agent action *(32)*. Some of the large craters were of a sufficient size (0.356 mm wide and 1.166 mm long (Figure 7)) to entrap *Salmonella* cells which range from 0.7 to 1.5 µm in diameter and from 2 to 5 µm in length *(7)*. Kim et al. *(19)* observed that inoculated *Salmonella* were mostly located in the cervices and feather follicles of broiler skins.

Surface morphologies of chicken skins varied significantly within and between the batches, although the skins had the same structure based on the histological results. Variation in *Salmonella* log reduction among the batches cannot be simply explained by the microtopography and physicochemical properties of chicken skins, strength of bacterial attachment must be also considered. Lillard indicated that the mechanism of salmonellae attachment to poultry skin involves more than changes in tissue morphology mediated by processing procedures *(19).* Bacterial attachment is a complex two-step process of reversible adhesion and irreversible exopolymer formation involving many disparate factors including hydrophilic and hydrophobic mechanisms, entrapment, electrostatic repulsion, and formation of extracellular polysaccharides or attachment fibrils *(2, 15).*

Magnitude of *Salmonella* reduction varied across batches due to concentration, time and possibly various skin surface conditions. Variation in antimicrobial log reduction among different chicken skin batches emphasizes the importance of collecting samples from different flocks, days, and seasons when testing antimicrobial agents and averaging the results. This sampling scheme would best simulate the natural variation that occurs within industry and more accurately assess the potential reduction range of antimicrobials while decreasing reported inconsistencies.

REFERENCES

1. Bautista, D. A., N. Sylvester, S. Barbut, and M. W. Griffiths. 1997. The determination of efficacy of antimicrobial rinses on turkey carcasses using response surface designs. *Int. J. Food Microb*. 34:279-292.

2. Benedict, R. C., F. J. Schultz, and S. B. Jones. 1991. Attachment and removal of *Salmonella* spp. on meat and poultry tissues. *J. Food Safety*. 11:135-148.

3. Bilgili, S. F., D. E. Conner, J. L. Pinion, and K. C. Tamblyn. 1998. Broiler skin color as affected by organic acids: influence of concentration and method of application. *Poultry Sci.* 77:752-757.

4. Butler, J. L., C. Vanderzant, Z. L. Carpenter, G. C. Smith, R. E. Lewis, and T. R. Dutson. 1980. Influence of certain processing steps on attachment of microorganisms to pork skin. *J. Food Prot*. 43:699-705.

5. Castillo, A., M. D. Hardin, G. R. Acuff, and J. S. Dickson. 2002. Reduction of microbial contamination on carcasses. p. 351-382. *In* V.K. Juneja, and J.N. Sofos (ed.), Control of foodborne microorganisms Marcel Dekker, Inc., New York, NY.

6. CDC. 2009. Surveillance for foodborne disease outbreaks -- United States, 2006. *MMWR*. 58:609-615.

7. Cox, J. 2000. *Salmonella.* p. 1928-1937. *In* R.K. Robinson, C.A. Batt, and P. Patel (ed.), Encyclopedia of food microbiology, vol. 2. Academic Press, Inc., San Diego, CA.

8. Cox, N. A., L. J. Richardson, J. S. Bailey, D. E. Cosby, J. A. Cason, M. T. Musgrove, Russell, Research, Center, and G. C. Mead. 2005. Bacterial contamination of poultry as a risk to human health. p. 21-43. *In* G.C. Mead (ed.), Food safety control in the poultry industry. CRC Press, Boca Raton, FL.

9. Dickens, J. A., and A. D. Whittemore. 1994. The effect of acetic acid and air injection on appearance, moisture pick-up, microbiological quality, and *Salmonella* incidence on processed poultry carcasses. *Poultry Sci*. 73:582-586.

10. Dickens, J. A., and A. D. Whittemore. 1997. Effects of acetic acid and hydrogen peroxide application during defeathering on the microbiological quality of broiler carcasses prior to evisceration. *Poultry Sci*. 76:657-660.

11. Hargis, B. M., D. J. Caldwell, R. L. Brewer, D. E. Corrier, and J. R. DeLoach. 1995. Evaluation of the chicken crop as a source of *Salmonella* contamination of broiler carcasses. *Poultry Sci*. 74:1548-1552.

12. Humphrey, T. J., D. G. Lanning, and D. Beresford. 1981. The effect of pH adjustment on the microbiology of chicken scald-tank water with particular reference to the death rate of Salmonellas. *J. Appl. Bact*. 51:517-527.

13. Hwang, C., and L. R. Beuchat. 1995. Efficacy of selected chemicals for killing pathogenic and spoilage microorganisms on chicken skin. *J. Food Prot*. 58:19-23.

14. James, W. O., R. L. Brewer, J. C. Prucha, W. O. Williams, and D. R. Parham. 1992. Effect of chlorination of chill water on the bacteriologic profile of raw chicken carcasses and giblets. *JAVMA*. 200:60-63.

15. Kim, J.-W., and S. Doores. 1993. Attachment of *Salmonella typhimurium* to skin of turkey that had been defeathered through three different systems: scanning electron microscopic examination. *J. Food Prot*. 56:395-400.

16. Kim, J.-W., and S. Doores. 1993. Influence of three defeathering systems on microtopography of turkey skin and adhesion of *Salmonella typhimurium. J. Food Prot*. 56:286-291, 305.

17. Kim, J.-W., and M. F. Slavik. 1995. Cetylpyridinium Chloride (CPC) treatment on poultry skin to reduce attached *Salmonella. J. Food Prot*. 59:322-326.

18. Kim, J.-W., M. F. Slavik, C. L. Griffis, and J. T. Walker. 1993. Attachment of *Salmonella typhimurium* to skins of chicken scalded at various temperatures. *J. Food Prot*. 56:661-665.

19. Kim, K., J. F. Frank, and S. E. Craven. 1996. Three-dimensional visualization of *Salmonella* attachment to poultry skin using confocal scanning laser microscopy. *Lett. Appl. Microb*. 22:280-282.

20. Li, Y., M. F. Slavik, J. T. Walker, and H. Xiong. 1997. Pre-chill spray of chicken carcasses to reduce *Salmonella typhimurium. J. Food Sci*. 62:605-607.

21. Lillard, H. S. 1994. Effect of Trisodium Phosphate on salmonellae attached to chicken skin. *J. Food Prot*. 57:465-469.

22. Lillard, H. S., and J. E. Thomson. 1983. Efficacy of hydrogen peroxide as a bactericide in poultry chiller water. *J. Food Sci*. 48:125-126.

23. Martin, H., and P. Maris. 2005. An assessment of the bactericidal and fungicidal efficacy of seventeen mineral and organic acids on bacterial and fungal food industry contaminants. *Sci. Aliments*. 25:105-127.

24. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis*. 5:607-625.

25. Mehyar, G., G. Blank, J. H. Han, A. Hydamaka, and R. A. Holley. 2005. Effectiveness of trisodium phosphate, lactic acid and commercial antimicrobials against pathogenic bacteria on chicken skin. *J. Food Prot*. 25:351-362.

26. Mountney, G. J., and J. O'Malley. 1965. Acids as poultry meat preservatives. *Poultry Sci.* 44:582.

27. Newell, D. G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, H. Sprong, M. Opsteegh, M. Langelaar, J. Threfall, F. Scheutz, J. Giessen, and H. Kruse. 2010. Food-borne disease -- The challenge of 20 years ago still persist while new ones continue to emerge. *Int. J. Food Microb*. 139:3-15.

28. Okrend, A. J., R. W. Johnston, and A. B. Moran. 1986. Effect of acetic acid on the death rates at 52C of *Salmonella* Newport, *Salmonella* Typhimurium and *Campylobacter jejuni* in poultry scald water. *J Food Prot*. 49:500-503.

29. Rao, P. V. 1997. Statistical research methods in life sciences. Thomson Wadsworth, Mason, OH.

30. Simmons, M., D. L. Fletcher, J. A. Cason, and M. E. Berrang. 2003. Recovery of *Salmonella* from retail broilers by whole-carcass enrichment procedure. *J. Food Prot*. 66:446-450.

31. Suderman, D. R., and F. E. Cunningham. 1980. Factors affecting adhesion of coating to poultry skin. Effect of age, method of chilling and scald temperature on poultry skin ultrastructure. *J. Food Sci*. 45:444-449.

32. Thomas, C. J., and T. A. McMeekin. 1980. Contamination of broiler carcass skin during commercial processing procedures: an electron microscopic study. *Appl. Env. Microb.* 40:133-143.

33. Thomas, C. J., and T. A. McMeekin. 1981. Attachment of *Salmonella* spp. to chicken muscle surfaces. *Appl. Env. Microb*. 42:130-134.

34. Whyte, P., J. D. Collins, K. McGill, C. Monahan, and H. O'Mahony. 2001. Quantitative investigation of the effects of chemical decontamination procedures on the microbiological status of broiler carcasses during processing. *J. Food Prot*. 64:179-183.

Table 19. Variation in mean *Salmonella* log reduction among poultry skin batches during simulated on-line or post-chill dipping with oxalic acid (22°C ± 1°C).

Time (sec)	Oxalic acid (% w/v)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]		
		Batch 1	Batch 2	Batch 3
10	1.0	0.45 ± 0.07[a]	0.17 ± 0.11[a]	0.21 ± 0.09[a]
	1.5	0.86 ± 0.07[a]	0.47 ± 0.18[a]	0.78 ± 0.12[a]
	2.0	0.82 ± 0.05[a]	0.70 ± 0.06[a]	0.86 ± 0.12[a]
20	1.0	0.78 ± 0.09[a]	0.13 ± 0.14[a]	0.21 ± 0.05[a]
	1.5	1.13 ± 0.04[a]	0.54 ± 0.17[a]	0.53 ± 0.08[a]
	2.0	1.16 ± 0.09[a]	0.63 ± 0.06[a]	0.81 ± 0.12[a]
30	1.0	0.95 ± 0.11[a]	0.18 ± 0.14[b]	0.52 ± 0.10[ab]
	1.5	1.13 ± 0.10[a]	0.56 ± 0.18[a]	0.94 ± 0.15[a]
	2.0	1.20 ± 0.03[a]	0.65 ± 0.06[b]	1.12 ± 0.12[a]
40	1.0	1.02 ± 0.20[a]	0.30 ± 0.09[b]	0.78 ± 0.16[ab]
	1.5	1.34 ± 0.02[a]	0.81 ± 0.09[b]	1.24 ± 0.08[ab]
	2.0	1.30 ± 0.01[a]	0.87 ± 0.04[b]	1.19 ± 0.02[a]

[1] n =3.
[2] Within the same row, mean values with different superscripts are significantly different ($P < 0.05$).

Table 20. Variation in mean *Salmonella* log reduction among poultry skin batches during simulated on-line or post-chill dipping with fumaric acid (22°C ± 1°C).

Time (min)	Fumaric acid (% w/w)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]	
		Batch 2	Batch 3
20	0.25	0.03 ± 0.02[a]	0.08 ± 0.02[b]
	0.5	0.25 ± 0.02[a]	0.33 ± 0.02[a]
40	0.25	0.17 ± 0.03[a]	0.18 ± 0.02[a]
	0.5	0.31 ± 0.02[a]	0.31 ± 0.03[a]

[1, 2] See Table 19 footnotes.

Table 21. Variation in mean *Salmonella* log reduction among poultry skin batches during simulated water-immersion chilling with oxalic acid (3°C ± 1°C).

Time (min)	Oxalic acid (% w/v)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]		
		Batch 1	Batch 2	Batch 3
30	0.1	0.92 ± 0.15[a]	0.16 ± 0.05[b]	0.48 ± 0.04[b]
	0.25	1.80 ± 0.12[a]	0.39 ± 0.13[b]	0.82 ± 0.17[b]
	0.5	2.16 ± 0.02[a]	0.61 ± 0.06[b]	1.10 ± 0.06[ab]
45	0.1	1.15 ± 0.02[a]	0.39 ± 0.05[b]	0.52 ± 0.07[b]
	0.25	1.84 ± 0.03[a]	0.46 ± 0.11[b]	0.95 ± 0.05[a]
	0.5	2.19 ± 0.04[a]	1.03 ± 0.09[b]	1.51 ± 0.05[a]
60	0.1	1.41 ± 0.10[a]	0.43 ± 0.09[b]	0.71 ± 0.02[ab]
	0.25	2.08 ± 0.18[a]	0.50 ± 0.10[b]	1.37 ± 0.15[c]
	0.5	3.33 ± 0.09[a]	1.22 ± 0.25[b]	2.18 ± 0.02[c]

[1, 2] See Table 19 footnotes.

Table 22. Variation in mean *Salmonella* log reduction among poultry skin batches during simulated water-immersion chilling with fumaric acid (3°C ± 1°C).

Time (sec)	Fumaric acid (% w/w)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]	
		Batch 2	Batch 3
30	0.05	0.2 ± 0.05[a]	0.22 ± 0.0[a]
	0.1	0.19 ± 0.03[a]	0.28 ± 0.02[a]
	0.25	0.45 ± 0.06[a]	0.49 ± 0.14[a]
45	0.05	0.22 ± 0.04[a]	0.26 ± 0.06[a]
	0.1	0.23 ± 0.08[a]	0.29 ± 0.05[a]
	0.25	0.61 ± 0.06[a]	0.64 ± 0.04[a]
60	0.05	0.22 ± 0.07[a]	0.29 ± 0.07[a]
	0.1	0.24 ± 0.03[a]	0.32 ± 0.01[a]
	0.25	0.65 ± 0.09[a]	0.78 ± 0.06[a]

[1, 2] See Table 19 footnotes.

Table 23. Variation in mean *Salmonella* log reduction among poultry skin batches during simulated soft scalding with oxalic acid (53°C ± 1°C).

Time (min)	Oxalic acid (% w/v)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]		
		Batch 1	Batch 2	Batch 3
1	1.0	1.16 ± 0.05[a]	0.86 ± 0.10[a]	1.03 ± 0.11[a]
	1.5	1.29 ± 0.13[a]	0.94 ± 0.22[a]	0.98 ± 0.12[a]
	2.0	2.95 ± 0.24[a]	1.36 ± 0.01[ab]	1.47 ± 0.19[b]
2	1.0	1.68 ± 0.27[a]	1.08 ± 0.12[b]	1.14 ± 0.12[ab]
	1.5	1.91 ± 0.17[a]	1.04 ± 0.06[b]	1.58 ± 0.19[a]
	2.0	2.92 ± 0.14[a]	1.44 ± 0.11[b]	2.12 ± 0.23[a]
3	1.0	2.16 ± 0.15[a]	1.12 ± 0.06[b]	1.40 ± 0.16[ab]
	1.5	2.58 ± 0.14[a]	1.47 ± 0.06[b]	1.69 ± 0.12[b]
	2.0	3.46 ± 0.29[a]	1.65 ± 0.03[b]	2.16 ± 0.21[a]

[1, 2] See Table 19 footnotes.

Table 24. Variation in mean *Salmonella* log reduction among poultry skin batches during simulated soft scalding with fumaric acid (53°C ± 1°C).

Time (min)	Fumaric acid (% w/w)	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]	
		Batch 2	Batch 3
1	0.5	0.54 ± 0.01[a]	0.91 ± 0.09[a]
	1.0	0.59 ± 0.01[a]	1.18 ± 0.22[a]
	1.5	0.78 ± 0.07[a]	1.15 ± 0.26[a]
2	0.5	0.83 ± 0.04[a]	1.25 ± 0.14[a]
	1.0	0.92 ± 0.03[a]	1.34 ± 0.05[a]
	1.5	1.16 ± 0.27[a]	1.78 ± 0.24[a]
3	0.5	0.94 ± 0.03[a]	1.32 ± 0.08[a]
	1.0	1.10 ± 0.20[a]	1.37 ± 0.05[a]
	1.5	1.25 ± 0.17[a]	1.79 ± 0.06[a]

[1, 2] See Table 19 footnotes.

Table 25. Analysis of chicken skin samples.

Batch	Physicochemical properties[1, 2]			
	Water activity ± std. error	**Moisture ± std. error (%)**	**Fat ± std. error (%)**	**pH ± std. error**
1	No data	No data	No data	No data
2	0.991 ± 0.003[a]	39.33 ± 2.64[a]	50.14 ± 3.47[a]	5.57 ± 0.07[a]
3	0.989 ± 0.001[a]	40.80 ± 3.69[a]	47.74 ± 4.77[a]	5.39 ± 0.15[a]

[1] n =3.
[2] Within the same column, mean values with different superscripts are significantly different ($P < 0.05$).

Table 26. Thickness of collagen layer of chicken skin samples.

Batch	Thickness ± std. error (mm)[1, 2]
1	No data
2	0.048 ± 0.003[a]
3	0.051 ± 0.005[a]

[1] n = 3.
[2] Within the same column, mean values with different superscripts are significantly different ($P < 0.05$).

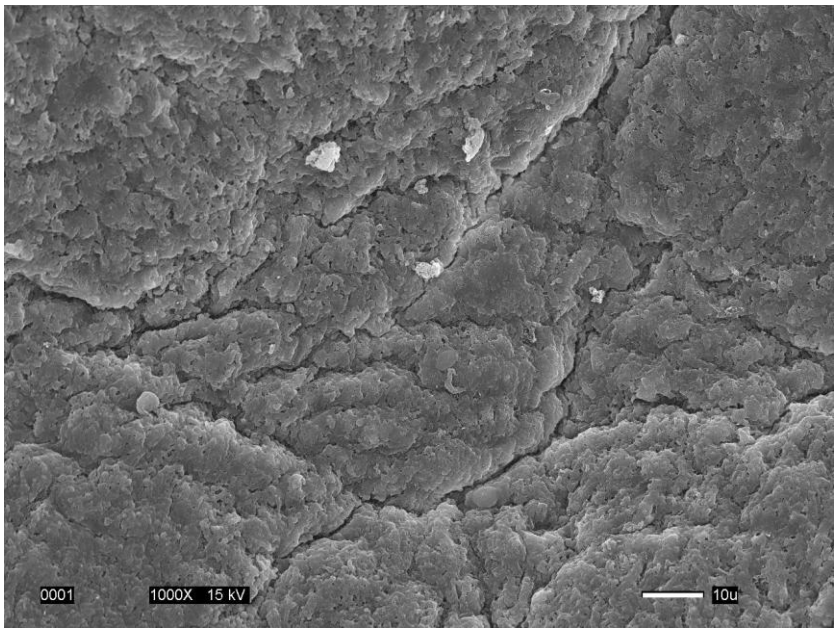

Figure 1. Scanning electron micrograph of raw chicken skin from batch 2.

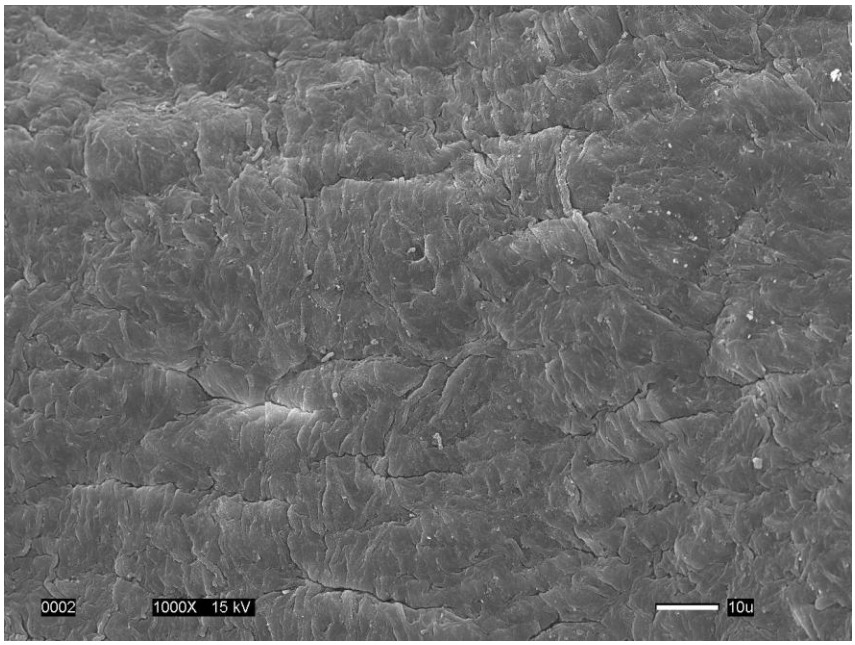

Figure 2. Scanning electron micrograph of raw chicken skin from batch 3.

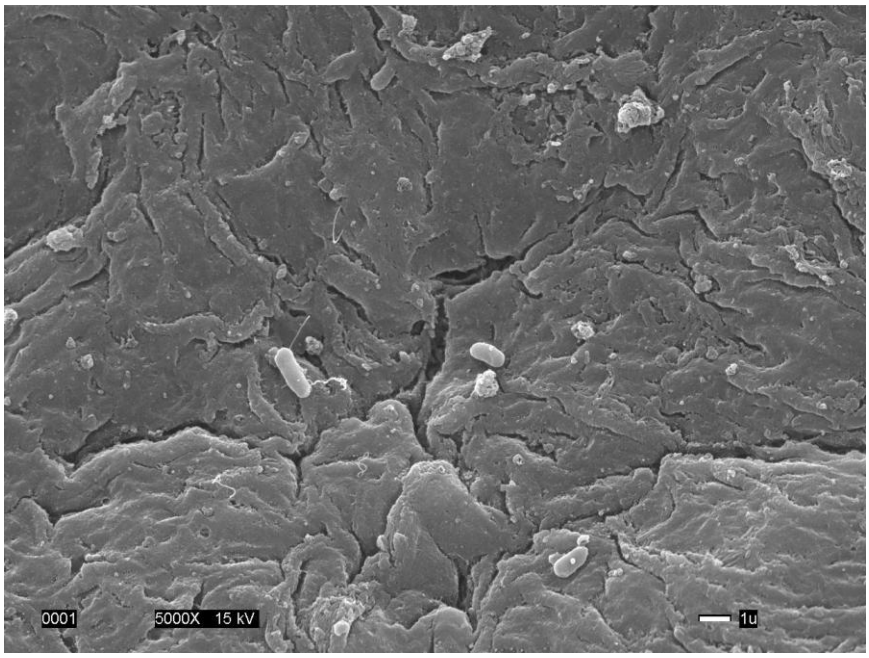

Figure 3. Scanning electron micrograph showing *Salmonella* cells on the surface of raw chicken skin.

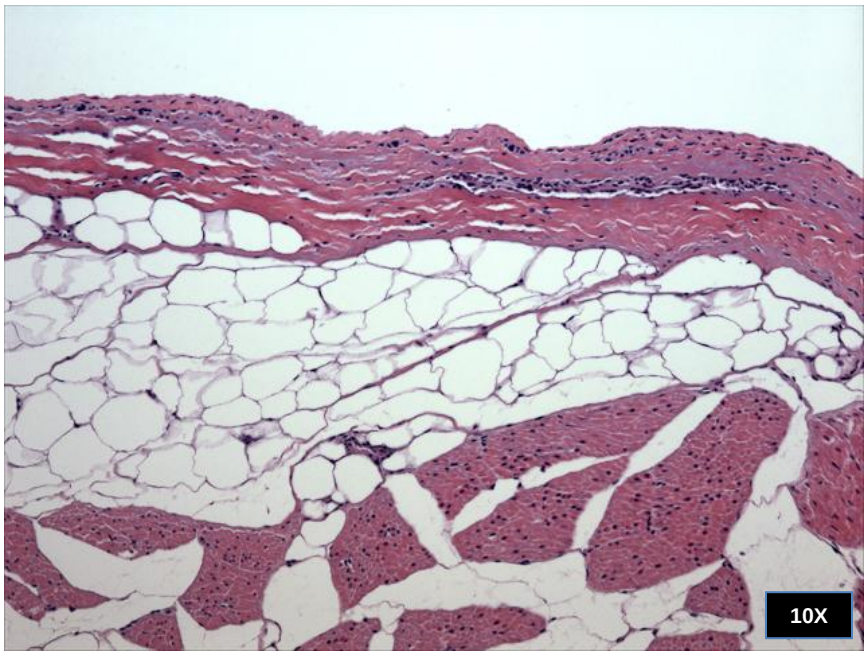

Figure 4. Histology of skin samples showing absence of epidermal layer.

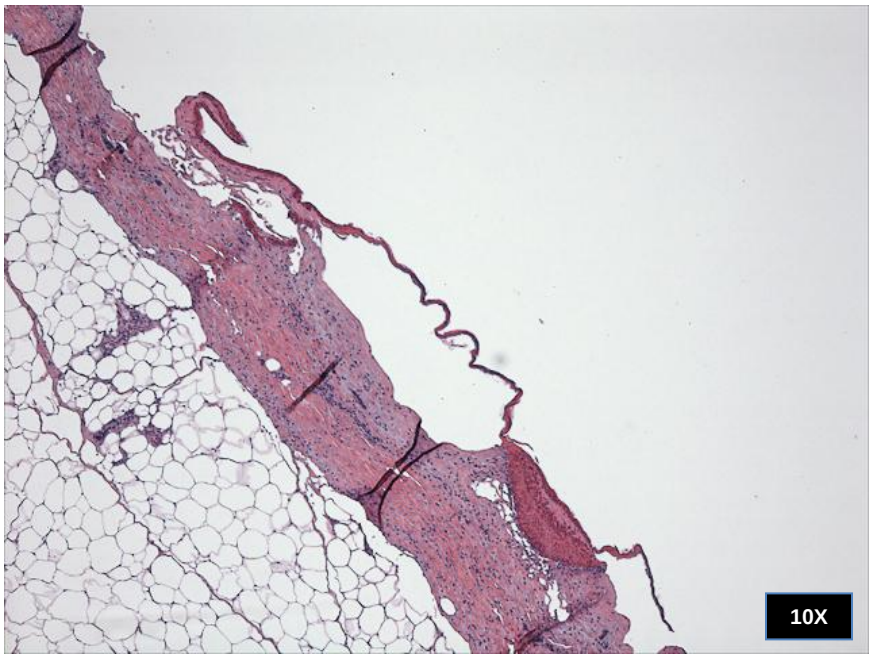

Figure 5. Histology of skin samples showing presence of an epithelial fragment.

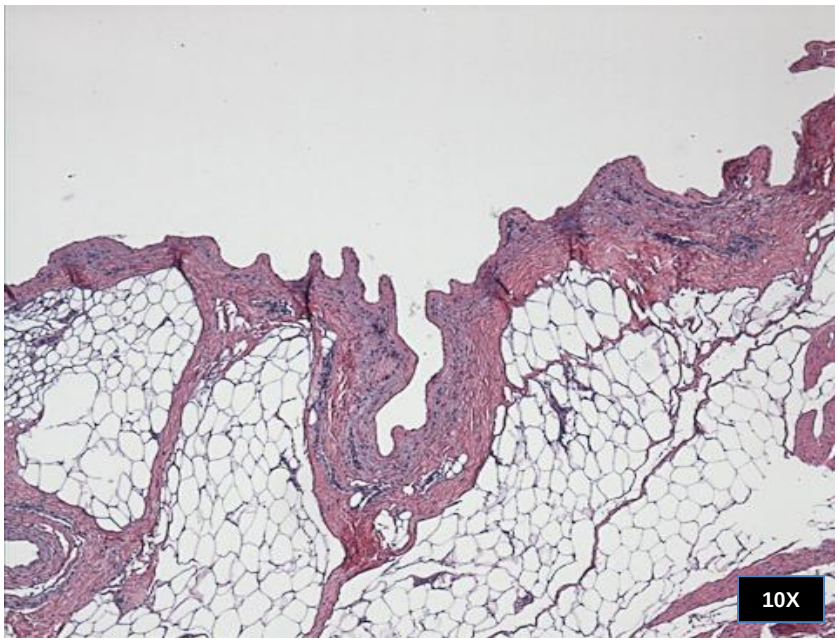

Figure 6. Histology of skin samples showing small irregularities.

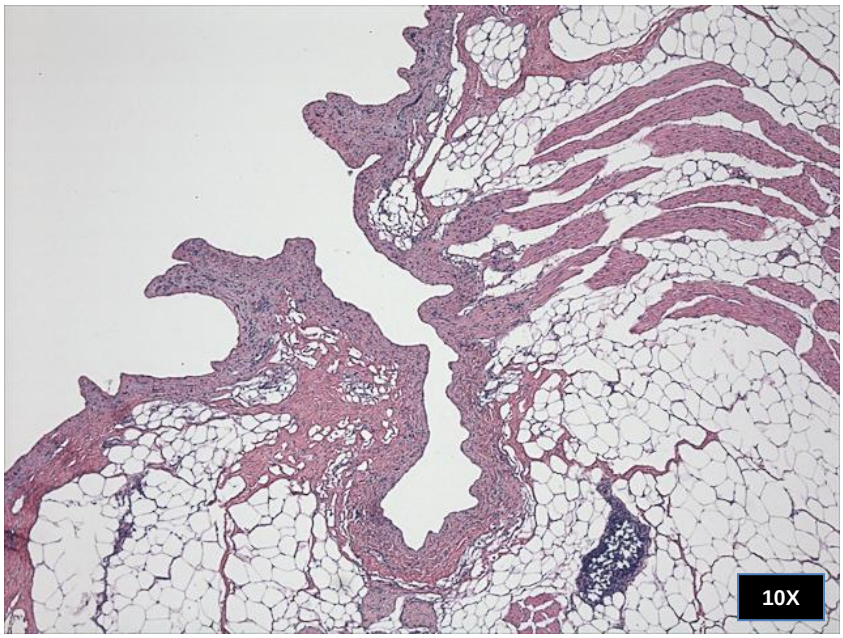

Figure 7. Histology of skin samples showing a large crater of sufficient size to entrap *Salmonella* cell.

EFFECT OF MUTATIONS IN *ATP, FUR,* AND *RPOS* ON SURVIVAL OF *SALMONELLA* TYPHIMURIUM UPON TREATMENT WITH OXALIC ACID DURING POULTRY PROCESSING

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ABSTRACT

The role of *atp, fur,* and *rpoS* in survival of *S.* Typhimurium upon treatment with 2.0% oxalic acid applied during poultry processing was studied by using strains with directed mutation in these loci. To mimic common processing practices, oxalic acid treatments were applied at 22°C for 40 sec to simulate on-line or post-chill dipping and at 53°C for 3 min to simulate soft scalding of broiler carcasses. After treatment samples were stomached in buffered peptone water to neutralize the effect of treatment, serially diluted and plated on XLD agar using the thin agar layer (TAL) technique for acid-injured cells. Treatment of logarithmic phase *S.* Typhimurium LT2 cells attached to raw chicken skins resulted in reductions of 1.72 and 3.57 log during dipping and scalding treatments, respectively, compared to the controls. There was no significant difference in reductions between wild type LT2 and its *atp* or *fur* mutants treated during dipping or scalding ($P > 0.05$). A higher tolerance to 2.0% oxalic acid treatment was noted in stationary phase cells compared to logarithmic phase cells in both wild type and mutants; however no statistical significance was observed ($P > 0.05$). A 1.47 log and 1.71 log reduction was observed in LT2 during dipping and scalding with 2.0% oxalic acid, while a 1.67- and 2.4- log reduction was achieved in strain UK1 at the same applications compared to the controls. Although the *rpoS* mutants were more sensitive to oxalic acid, differences were not significant when compared to wild strains ($P > 0.05$). In summary, mutations in *fur, atp* or *rpoS* did not impact survival of *S.* Typhimurium cells attached to broiler skins when treated with oxalic acid during dipping and scalding compared to control treatments. Further work may focus on testing

other genes involved in acid resistance to understand and determine the mechanism of *Salmonella* survival upon treatment with oxalic acid.

INTRODUCTION

The presence of salmonellae on poultry and poultry products causes continuous public health concerns *(4, 13)*, and reduction of *Salmonella* levels and cross-contamination during poultry processing is one of the major goals of poultry processors and food safety researchers *(15)*. Numerous intervention methods have been incorporated into poultry processing to reduce pathogen prevalence and cross-contamination, including the use of organic acids as antimicrobials *(4, 6)*. A previous study conducted in our laboratory (I. Sybirtseva et al., unpublished) indicated that 2.0% oxalic acid applied during soft scalding for 3 min at 53°C reduced the level of *Salmonella* attached to raw chicken skin by 2.43 log compared to the control. Although oxalic acid was effective, the pathogen still had the ability to survive at highly acidic conditions (pH 1.03). *Salmonella* Typhimurium survives in moderate acid conditions, down to pH 4.5, due to its constitutive pH homeostasis system that maintains internal pH at neutral *(10)*. However, when the external pH drops <pH 4.0, internal hydrogen ion concentration rises to lethal levels and protein synthesis capacity is severely compromised *(11)*, pH homeostasis system fails, and cells rapidly lose viability *(10)*. Acid shock proteins (ASPs) are not synthesized when the external pH drops directly from slightly alkaline pH 7.7 to ≤pH 4.0 *(11)*, presumably because the internal pH is too acidic for ribosome function *(21)*.

Several laboratories have initiated studies to examine the mechanisms that could protect *S.* Typhimurium from acid pH *(10, 26)* and identified mutations which alter the bacterium's ability to mount an acid tolerance response (ATR) in which exposure to slight or

moderate acid stress results in the synthesis of proteins that protect the bacterium from severe acid challenge *(16, 26)*. Mutations in *atp* (Mg (II) - dependent proton-translocating ATPase) *(8)*, *fur* (Fe (II) - binding regulatory protein), and *rpoS* (alternate sigma factor (σ^s)) confer an acid-sensitive phenotype on *S.* Typhimurium *(9, 10)*.

The *atp* operon codes for the F_0F_1 proton-transolocating ATPase (Figure 11) required for oxidative synthesis of ATP and for the generation of proton motive force under anaerobic conditions *(12)*. The F_0 subunits are intrinsic membrane proteins that form pores through which protons can pass. The F_1 sector (ATPase) binds to F_0 and protrudes into the cytoplasm. Protons passing into the cell through F_0 generate ATP via F_1, whereas exiting protons expend ATP *(12)*. Thus, F_0F_1 can pump protons out of the cell and conceivably raise the cell internal pH *(12)*. Mutation in *atp* renders the logarithmic phase cells acid sensitive because of defects in the pre- or post-acid shock stages of log phase ATR *(18)*.

Fur protein is the major regulator of iron metabolism in *S*. Typhimurium (Figure 12), but it has broad impact on cellular physiology *(11)*, including sensing changes in internal pH of the cell and regulating a set of low pH-inducible genes *(8, 12)*. The acid sensitivity of *fur* mutants has been attributed to reduced expression of several acid-regulated genes *(12)* and cancelled induction of six transiently induced ASPs *(9, 10)*.

The transition of bacterial cells into stationary phase growth is associated with increased resistance to a variety of environmental stresses, including high acid pH *(10)*. This general stress-resistance feature is dependent on σ^s *(10)*, encoded by the *rpoS* locus, which

mediates the transcription of specific genes during starvation and selectively expressed at the stationary phase of bacterial growth *(7)*.

The purpose of this study was to examine a mechanism of *Salmonella*'s survival upon exposure to oxalic acid under simulated on-line or post-chill dip and soft scalding application by using the modified skin attachment model (mSAM). We chose *S.* Typhimurium as a tested bacterium because of its well-defined genetics and its ability to endure a variety of acidic non-host and host conditions *(10)*. Screening of wild type *S.* Typhimurium versus *atp*, *fur*, and *rpoS* mutants would allow us to determine whether these loci or their products are essential for *Salmonella* survival when exposed to oxalic acid during on-line or post-chill dipping or/and soft scalding.

MATERIALS AND METHODS

Preparation of inoculum

Salmonella strains used in this study were generously provided by J. W. Foster (Department of Microbiology and Immunology, University of South Alabama) are listed in Table 27. Strains were maintained at -80°C ± 1°C in brain heart infusion broth (BHIB; Fisher Scientific, Pittsburg, PA) containing 50% glycerol (Fisher Scientific, Pittsburg, PA) *(24)*. Each strain was activated by two successive transfers in BHIB at 37°C for 18 to 24 hours *(3)*, and examined for purity on xylose lysine deoxycholate agar (XLD; Weber Scientific, Hamilton, NJ). Typical *Salmonella* colonies on XLD appear black with clear and opaque zone *(28)*.

Growth patterns of *Salmonella* strains and their acid-sensitive mutants were determined by monitoring the optical density at 600 nm (OD_{600}) over 24 hrs incubation period in BHIB at 37°C ± 1°C using a spectrophotometer (SmartSpec^{TM}300, Bio-Rad, Hercules, CA). The growth curves of *Salmonella* strains and their acid-sensitive mutants are presented in Figure 8.

Inoculum was prepared by placing one colony into 9 ml of BHIB and incubating at 37°C ± 1°C to an OD_{600} of 0.8 ± 0.1 nm for logarithmic phase cells or for 22 ± 1 hrs for stationary phase cells.

Chemical treatment preparation

Oxalic acid (CAS 114-62-7; Fisher Scientific, Pittsburg, PA) treatments were prepared at 2.0% w/v using sterile distilled water. All treatments were placed in autoclave sterilized containers (18 x 10 x 5.4 cm) (Fisher Scientific, Pittsburg, PA), and held at room temperature (22°C ± 1°C, on-line or post-chill dipping) or in a warm temperature incubator (53°C ± 1°C, soft scalding) prior to application. The temperature of treatment solutions was measured with a submerged sterile thermocouple wire (TT-T-30-SLE-50, Omega, Stamford, CT) attached to a calibrated continuous digital thermometer (HH21, Omega, Stamford, CT).

Sample collection and mSAM experiments

The procedure of Sybirtseva et al. (Sybirtseva et al., unpublished) was used, and the general method was as follows. Chicken breast skins were collected from a local poultry processing plant (Case Farms Inc., Dudley, NC) immediately after an automatic skinner, cut into 62.2 cm^2 round pieces using a sterile scalpel, individually vacuum packaged, irradiated at 25 kGy with Co-60 source to eliminate background microflora, and stored at -80°C until they were used. Skin samples were thawed at 4°C ± 1°C for 24 hrs and inoculated with a 0.1 ml of *Salmonella* strain. A 10 minutes contact time at 22ºC ± 1°C was allowed for bacterial cells to attach to the skin surface prior to application of treatments *(3, 24)*. Chicken skin samples (three replicates per strain and two applications; total of 72 samples) were dipped into 200 ml of 2.0% oxalic acid solutions (pH 1.03) or sterile distilled water (control) to simulate on-line or post-chill dipping (22°C ± 1°C for 40 sec) or soft scalding (53°C ± 1°C

for 3 min). This would allow determining the impact of mutation when conditions change to more severe ones. The oxalic acid concentration and contact time were predetermined by previous study conducted in our laboratory as one that provided the highest log reductions in a cocktail of four *Salmonella* strains attached to irradiated raw broiler skins during soft scalding and on-line or post-chill dipping. Following treatments, skins were drained for 1 min at 22°C ± 1°C to allow removal of the excess water or acid solution, placed in 90 ml of sterile 0.1% w/v buffered peptone water (BPW; Fisher Scientific, Pittsburg, PA) and homogenized for 2 min at 230 rpm (400 Circulator, Seward Inc., Bohemia, NY) to remove remaining cells and neutralize the effect of treatment *(24, 25)*. All samples were plated on XLD using the thin agar layer (TAL) plating technique for acid-injured foodborne pathogens *(28)*, which allows maximum recovery of surviving *Salmonella* cells including those that are sublethally injured *(14)*. The plates were incubated at 37° ± 1°C for 24 hrs under aerobic conditions. On a periodical basis, two or three putative *Salmonella* colonies were picked from countable plates and confirmed by API 20E (bioMerieux Inc., Hazelwood, MO) as *Salmonella* spp.

Mechanism of oxalic acid bactericidal action

Determination of the bactericidal mechanism of oxalic acid is based on the comparison of wild type *Salmonella* strains versus their acid-sensitive mutants. Reduction for each implicated mechanism was calculated as the difference between the population of cells

recovered from LT2 or UK1-inoculated skins treated with 2.0% oxalic acid and the population of cells of the corresponding mutants, similarly treated with 2.0% oxalic acid.

Statistical analysis

Data for each experiment, expressed as $\log_{10}$ CFU/skin, were analyzed by using SAS software (version 9.1, SAS Institute, Cary, NC). Analysis of variance was carried out at a 95% significance level ($P < 0.05$) and a Tukey test was used for separation of means *(23)*.

RESULTS AND DISCUSSION

The survival of *S.* Typhimurium strain LT2 wild type and *S.* Typhimurium strains LT2 *atp* (JF1923) and *fur* (JF2023) mutants during logarithmic and stationary phase of growth at on-line and post-chill dipping and soft scalding with 2.0% oxalic acid is presented in Figure 9. Treatment of logarithmic phase LT2 cells attached to raw chicken skins resulted in a 1.72- and 3.57-log reduction during dipping and scalding, respectively, compared to the controls. There was no significant difference in reductions observed between LT2 and JF1923 or LT2 and JF2023 in both applications ($P > 0.05$). These results suggest that neither *fur* nor *atp* alone played an important role in survival of wild type *S.* Typhimurium attached to broiler skin when treated with 2.0% oxalic acid during on-line or post-chill dipping or soft scalding. Foster showed that both *atp* and *fur* mutants of LT2 were tolerant to pH 4.3 created by hydrochloric acid *(8)* and no significant loss in viability was observed in either mutant when exposed to pH 3.3 for 1 hr *(22)*.

Although higher tolerance to 2.0% oxalic acid treatment was noted in stationary phase LT2, JF1923, and JF2023 cells compared to the logarithmic phase cells in both applications; the statistical significance was only detected between LT2 logarithmic and stationary cells at 53ºC ($P = 0.0002$). This general stress resistance associated with stationary phase is dependent on σ^s and does not require induction by low pH *(18)*. Additionally, the stationary phase cells exhibit *rpoS*-independent ATR system which is apparent when the cells are shifted to ≤pH 4.5 *(10)*.

We also tested if mutations in *rpoS* would impact wild type *S.* Typhimurium survival upon treatment with oxalic acid at simulated on-line or post-chill dip and soft scalder application. Two genetically distinct wild strains of *S.* Typhimurium, LT2 and UK1, and their *rpoS* mutants, strains JF 2560 and JF 2960, respectively, were used to test this hypothesis. *Salmonella* Typhimurium strain LT2 was isolated in 1940s and used in phage-mediated transduction studies *(19, 20)*; while strain UK1 is extensively used in vaccine developing studies *(5).* This was desired in order to assess the impact of *rpoS* mutations in *Salmonella* Typhimurium strains of different genetic background. Lee et al. *(17)* showed that UK1 exhibited a sustained ATR over a period of 60 min, while LT2 could only mount an ATR after 20 min of pH 4.3 acid shock, but not after 60 min or longer in minimal E glucose medium at room temperature. The genetic difference responsible for a sustained versus transient ATR was traced to a mutation in the σ^s *(10)*. The *rpoS* gene of LT2 harbors an extremely rare start codon (UUG) that limited the production of σ^s, while alleles of UK1 *rpoS* contain a typical AUG start codon *(10)*. We used two wild type strains of *S.* Typhimurium to account for the different acid tolerance phenotypes previously observed *(17)*.

During soft scalding oxalic acid (2.0%) treatment of strains JF2560 and JF2960 attached to raw chicken skin resulted in reductions of 0.45 and 0.19 log compared to strains LT2 and UK1, respectively (Figure 10). Although the *rpoS* mutants were more sensitive to oxalic acid, no significant statistical difference was observed when compared to wild strains ($P > 0.05$). Fang et al. *(7)* found that an *rpoS* mutant was 10-fold more susceptible to low pH

after 60 min and more sensitive to acetic acid *(2)* than its parental strain. In contrast, Kwon and Ricke *(16)* found that mutations in *rpoS* did not change the acid resistance after adaptation to propionate compared to the wild type. We speculated that pH inducible ATR is independent of *rpoS* and may be enough to protect stationary phase *Salmonella* cells from oxalic acid acidic pH.

Strain LT2 is severely attenuated in terms of mouse virulence when compared with strain UK1 *(17, 27)*. Portillo et al. *(22)* reported that strain UK1 was more acid resistant than strain LT2, which may account, at least in part, for its virulence *(1)*. Our observations are in contrast with other researchers' findings. A 1.47- and 1.71-log reductions were observed for strain LT2 attached to raw chicken skin and treated with 2.0% oxalic acid during on-line or post-chill dipping and soft scalding, while reductions of 1.67 and 2.40 log were achieved for strain UK1 using the same treatment conditions, compared to the controls.

The results presented in this chapter show that *atp*, *fur*, or r*poS* do not directly impact the ability of *Salmonella* to withstand severe oxalic acid stress during on-line or post-chill dipping or soft scalding. *S.* Typhimurium may have more than one pathway functioning to protect itself from acid damage. Further work should focus on testing other genes involved in acid resistance to understand and determine the mechanism of *Salmonella* survival upon treatment with oxalic acid.

REFERENCES

1. Bang, I. S., J. P. Audia, Y. K. Park, and J. W. Foster. 2002. Autoinduction of the *ompR* response regulator by acid shock and control of the *Salmonella enterica* acid tolerance response. *Mol. Microb*. 44:1235-1250.

2. Bearson, B. L., L. Wilson, and J. W. Foster. 1998. A low pH-inducible, PhoPQ-dependent acid tolerance response protects *Salmonella typhimurium* against inorganic acid stress. *J. Bact*. 180:2409-2417.

3. Conner, D. E., and S. F. Bilgili. 1994. Skin attachment model for improved laboratory evaluation of potential carcass disinfectants for their efficacy against *Salmonella* attached to broiler skin. *J. Food Prot*. 57:684-688.

4. Cox, N. A., L. J. Richardson, J. S. Bailey, D. E. Cosby, J. A. Cason, M. T. Musgrove, Russell, Research, Center, and G. C. Mead. 2005. Bacterial contamination of poultry as a risk to human health. p. 21-43. *In* G.C. Mead (ed.), Food safety control in the poultry industry. CRC Press, Boca Raton, FL.

5. Curtiss III, R., S. B. Porter, M. Munson, S. A. Tinge, J. O. Hassan, C. Gentry-Weeks, and S. M. Kelly. 1991. Nonrecombinant and recombinant avirulent *Salmonella* live vaccines for poultry. p. 169-198. *In* J.S. Bailey, et al. (ed.), Colonization control of human bacterial enteropathogens in poultry Academic Press, New York, N.Y.

6. Doores, S. 2005. Organic acids. p. 91-143. *In* P.M. Davidson, J.N. Sofos, and A.L. Branen (ed.), Antimicrobials in foods. CRC Press, Boca Raton, FL.

7. Fang, F. C., S. J. Libby, N. A. Buchmeier, P. C. Loewen, J. Switala, J. Harwood, and D. G. Guiney. 1992. The alternate σ factor KatF (RpoS) regulates *Salmonella* virulence *Proc. Natl. Acad. Sci. USA*. 89:11978-11982.

8. Foster, J. W. 1991. *Salmonella* acid shock proteins are required for the adaptive acid tolerance response. *J. Bact*. 173:6896-6902.

9. Foster, J. W. 1993. The acid tolerance of *Salmonella typhimurium* involves transient synthesis of key acid shock proteins. *J. Bact*. 175:1981-1987.

10. Foster, J. W. 1995. Low pH adaptation and the acid tolerance response of *Salmonella typhimurium. Critic. Rev. Microb*. 21:215-237.

11. Foster, J. W., and B. Bearson. 1994. Acid-sensitive mutants of *Salmonella typhimurium* identified through a dinitrophenol lethal screening strategy. *J. Bact*. 176:2596-2602.

12. Foster, J. W., and H. K. Hall. 1991. Inducible pH homeostasis and the acid tolerance response of *Salmonella typhimurium. J. Bact*. 173:5129-5135.

13. Hargis, B. M., D. J. Caldwell, and J. A. Byrd. 2001. Microbiological pathogens: live poultry considerations. p. 121-137. *In* A.R. Sams (ed.), Poultry meat processing. CRC Press, Boca Raton, FL.

14. Humphrey, T. J. 1981. The effects of pH and levels of organic matter on the death rates of Salmonellas in chicken scald-tank water. *J. Appl. Bact*. 51:27-39.

15. Kim, J.-W., M. F. Slavik, C. L. Griffis, and J. T. Walker. 1993. Attachment of *Salmonella typhimurium* to skins of chicken scalded at various temperatures. *J. Food Prot*. 56:661-665.

16. Kwon, Y. M., and S. C. Ricke. 1998. Induction of acid resistance of *Salmonella typhimurium* by exposure to short-chain fatty acids. *Appl. Env. Microb*. 64:3458-3463.

17. Lee, I. S., J. Lin, H. K. Hall, B. Bearson, and J. W. Foster. 1995. The stationary-phase sigma factor σ^S (RpoS) is required for a sustained acid tolerance response in virulent *Salmonella typhimurium. Mol. Microb*. 17:155-167.

18. Lee, I. S., J. L. Slonczewski, and J. W. Foster. 1994. A low-pH-inducible, stationary-phase acid tolerance response in *Salmonella typhimurium. J. Bact*. 176:1422-1426.

19. McClelland, M., K. E. Sanderson, J. Spieth, S. W. Clifton, P. Latreille, L. Courtney, S. Porwollik, J. Ali, M. Dante, F. Du, S. Hou, D. Layman, S. Leonard, C. Nguyen, K. Scott, A. Holmes, N. Grewal, E. Mulvaney, E. Ryan, H. Sun, L. Florea, W. Miller, T. Stoneking, M. Nhan, R. Waterston, and R. K. Wilson. 2001. Complete genome sequence of *Salmonella* enterica serovar Typhimurium LT2. *Nature*. 413:852-856.

20. Neidhardt, F. C. 1996. *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology. ASM Press, Washington DC.

21. Park, Y.-K., B. Bearson, S. H. Bang, I. S. Bang, and J. W. Foster. 1996. Internal pH crisis, lysine decarboxylase and the acid tolerance response of *Salmonella typhimurium. Mol. Microb*. 20:605-611.

22. Portillo, F. G., J. W. Foster, and B. B. Finlay. 1993. Role of acid tolerance response genes in *Salmonella typhimurium* virulence. *Infect. Immun.* 61:4489-4492.

23. Rao, P. V. 1997. Statistical research methods in life sciences. Thomson Wadsworth, Mason, OH.

24. Tamblyn, K. C., and D. E. Conner. 1997. Bactericidal activity of organic acids against *Salmonella typhimurium* attached to broiler chicken skin. *J. Food Prot*. 60:629-633.

25. Tamblyn, K. C., D. E. Conner, and S. F. Bilgili. 1997. Utilization of the skin attachment model to determine the antibacterial efficacy of potential carcass treatments. *Poultry Sci*. 76:1318-1323.

26. Wilmes-Riesenberg, M. R., B. Bearson, J. W. Foster, and R. Curtiss III. 1996. Role of the acid tolerance response in virulence of *Salmonella typhimurium. Infect. Immun.* 64:1085-1092.

27. Wilmes-Riesenberg, M. R., J. W. Foster, and R. Curtiss III. 1997. An altered *rpoS* allele contributes to the avirulence of *Salmonella typhimurium* LT2. *Infect. Immun.* 65:203-210.

28. Wu, V. C. H., D. Y. C. Fung, D. H. Kang, and L. K. Thompson. 2001. Evaluation of thin agar layer method for recovery of acid-injured foodborne pathogens. *J. Food Prot*. 64:1067-1071.

Table 27. *Salmonella* Typhimurium strains used in this study.

Strain	Relevant genotype	Reference/Source
UK1	Wild type	*(5)*
LT2	Wild type	*(19, 20)*
JF1923	LT2 *atp*::Tn*10*	*(18)*
JF2023	LT2 *fur-1 zfi*::Tn*10*	*(18)*
JF2560	LT2 *rpoS*::*Ap*^{r}	*(17)*
JF2690	UK1 *rpoS*::*Ap*^{r}	*(17)*

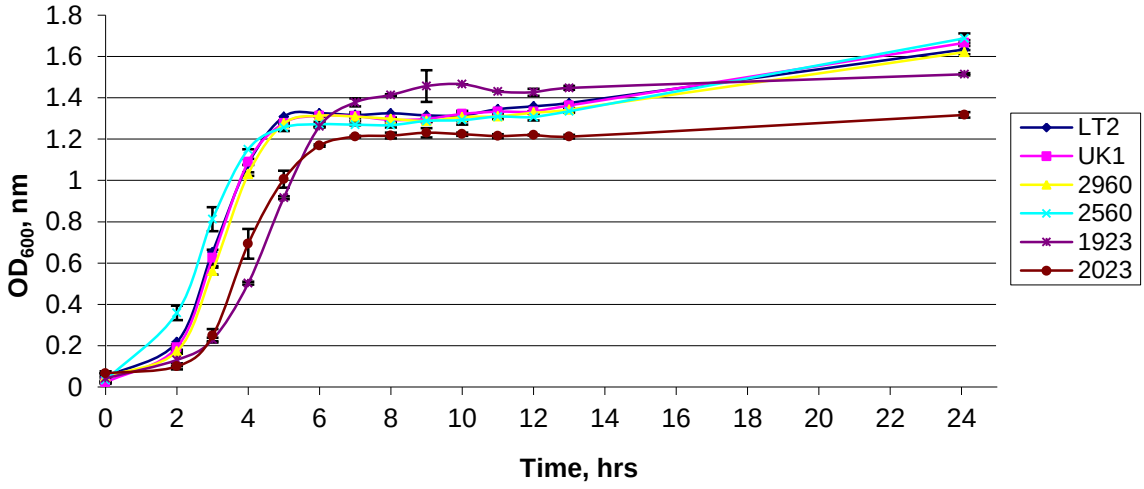


Figure 8. Growth curves of two wild *Salmonella* strains (LT2 and UK1) and their acid-resistant mutants[1].

[1] n = 2.

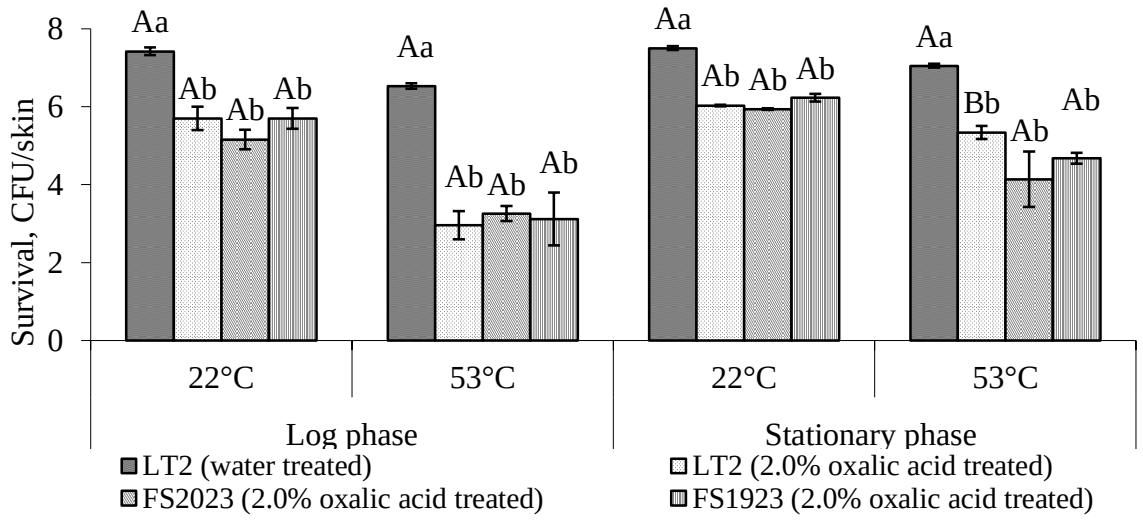


Figure 9. Survival of wild type *S.* Typhimurium LT2 and *atp* and *fur* logarithmic phase and stationary phase mutants and upon treatment with 2.0% oxalic acid during 40 sec on-line or post-chill dipping (22°C ± 1°C) and 3 min soft scalding (53°C ± 1°C)[1, 2].

[1] n = 3.

[2] Within the same application and growth phase, mean values with different lowercase letters are significantly different ($P < 0.05$). Within the same application and strain, mean values with different uppercase letters are significantly different ($P < 0.05$).

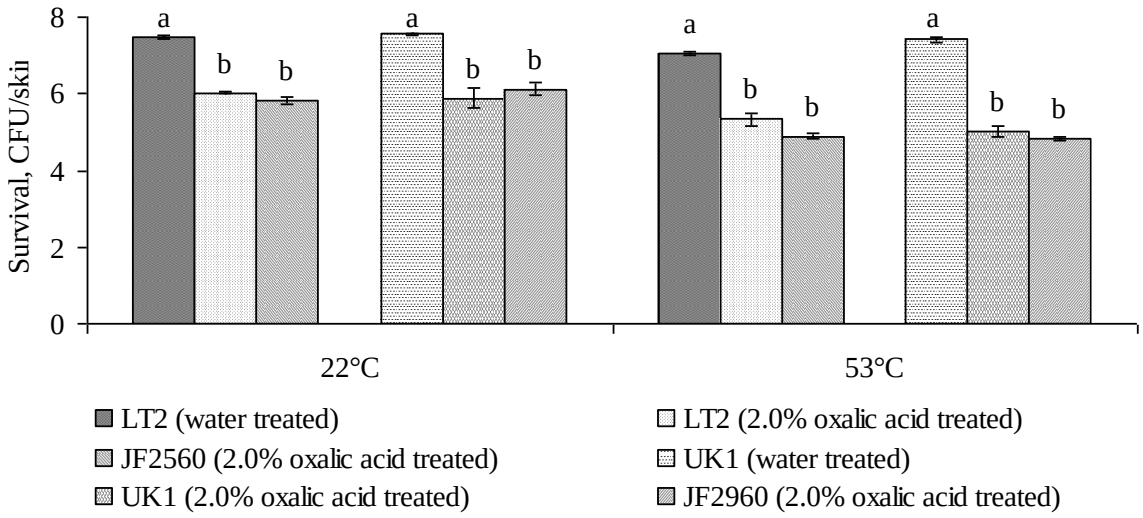


Figure 10. Survival of wild type *S.* Typhimurium LT2 and UK1 and their *rpoS* stationary phase mutants during 40 sec on-line or post-chill dipping (22°C ± 1°C) and 3 min soft scalding (53°C ± 1°C) with 2.0% oxalic acid[1, 2].

[1] n = 3.
[2] Within the same application and growth phase, mean values with different lowercase letters are significantly different ($P < 0.05$).

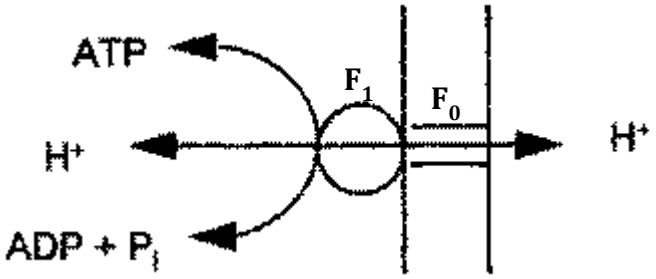


Figure 11. Schematic representation of F_0F_1 proton-translocating ATPase mechanism[1].

[1] Modified from Montville, T. J., and K. R. Matthews. 2007. Growth, survival, and death of microbes in foods. P. 3-23. *In* Doyle, M. P., and L. R. Beuchat (ed.), Food Microbiology: Fundamentals and Frontiers. ASM Press, Washington DC.

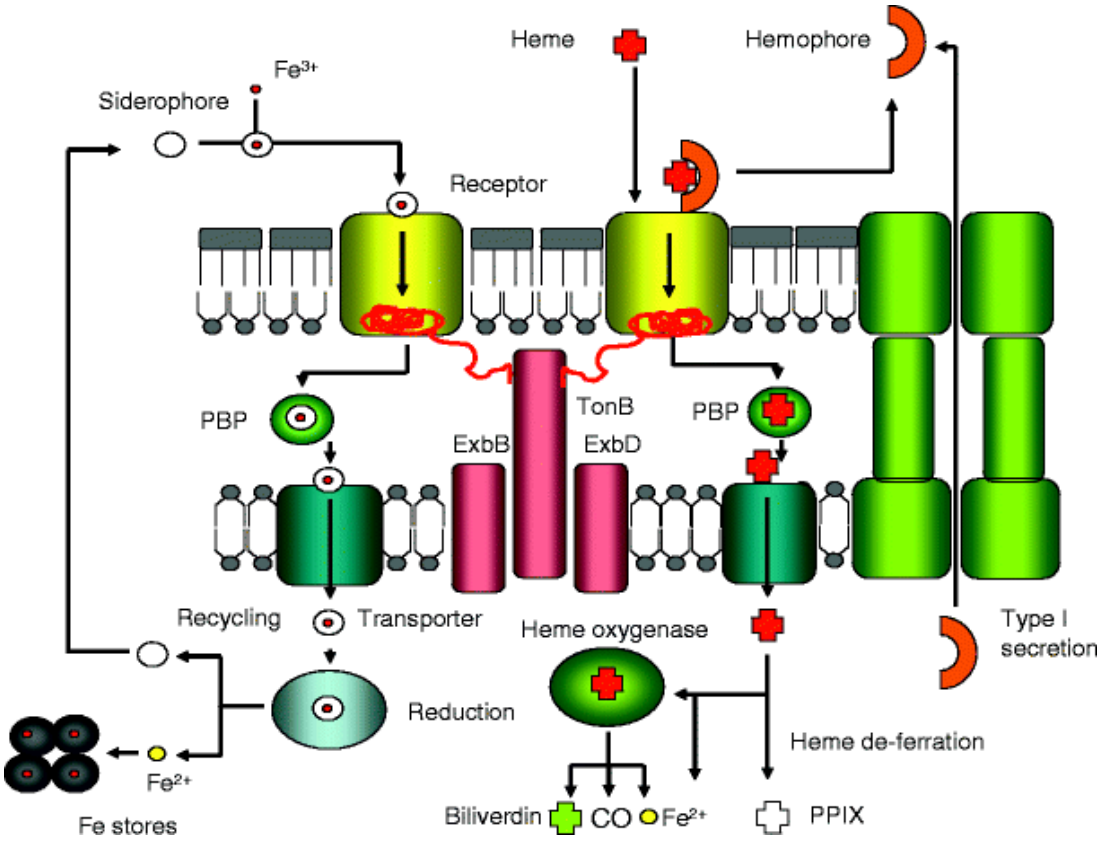


Figure 12. Schematic representation of iron uptake via siderophores in Gram-negative bacteria[1].

[1] Adapted from Cornelis, P. 2010. Iron uptake and metabolism in pseudomonads. *Appl. Microb. Biotechnol.* 86:1637-1645. The siderophores produced by Fur gene bind Fe^{3+} and form the ferrisiderophore complex recognized by an outer membrane TonB-dependent receptor. TonB together with ExbB and ExbD transport the ferrisiderophore to the periplasm where it is bound by a periplasmic binding protein (*PBP*). The PBP donates the ferrisiderophore to a transporter and inside the cytoplasm a reductase releases iron under the Fe^{2+} form where it can be incorporated into Fe-containing proteins.

APPENDICES

APPENDIX 1. *Salmonella* spp. antibiotic resistance profile.

Antibiotic	*Salmonella* spp. clearance zone			
	Newport[1]	**Typhimurium[2]**	**Enteritidis[3]**	**Heidelberg[4]**
Amikacin (AMI)	=1	=2	=2	=4
Amixicillin/Clavulanic acid (AUG)	≤1	≤1	≤1	=16
Ampicillin (AMP)	≤1	≤1	≤1	>32
Cefoxitin (FOX)	=2	=2	=2	=2
Ceftiofur (TIO)	=1	=1	=1	=1
Ceftriaxone (AXO)	≤0.25	≤0.25	≤0.25	≤0.25
Chloramphenicol (CHL)	>32	>32	>32	=4
Ciprofloxacin (CIP)	≤0.015	≤0.015	≤0.015	≤0.015
Gentamicin (GEN)	=0.5	=0.5	=1	=0.5
Kanamycin (KAN)	>64	>64	>64	≤8
Nalidixic acid (NAL)	=4	=4	=4	>32
Streptomycin (STR)	>64	>64	>64	>64
Sulfisoxazole (FIS)	>256	>256	>256	>256
Tetracycline (TET)	>32	>32	>32	>32
Trimethoprim/Sulfamethoxazole (COT)	≤0.12	≤0.12	≤0.12	≤0.12

[1] *S.* Newport is resistant to CHL, KAN, STR, FIS, TET.
[2] *S.* Typhimurium is resistant to CHL, KAN, STR, FIS, TET.
[3] *S.* Enteritidis is resistant to CHL, KAN, STR, FIS, TET.
[4] *S.* Heidelberg is resistant to AMP, NAL, STR, FIS, TET.

APPENDIX 2. Efficacy of hydrochloric and oxalic acid to reduce Salmonella spp. on raw chicken skins during simulated on-line or post-chill dipping (22°C ± 1°C), water-immersion chilling (3°C ± 1°C) and soft scalding (53°C ± 1°C).

Acid	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]		
	40 sec dipping (pH 1.03)	60 min chilling (pH 1.48)	3 min scalding (pH 1.03)
Oxalic acid	0.87 ± 0.04^{a}	1.22 ± 0.25^{a}	1.65 ± 0.03^{a}
Hydrochloric acid	0.52 ± 0.01^{b}	1.28 ± 0.29^{a}	1.14 ± 0.05^{b}

[1] n = 3.
[2] Within the same column, mean values with different superscripts are significantly different ($P < 0.05$).

APPENDIX 3. Hunter values of untreated raw chicken skins during refrigerated storage at 4°C for 14 days.

Day	Hunter value ± std. error[1, 2]		
	L	a	b
0	77.71 ± 1.58[a]	-2.06 ± 0.10[a]	6.77 ± 1.17[a]
1	79.82 ± 0.57[a]	-2.25 ± 0.09[a]	8.75 ± 1.68[a]
3	79.83 ± 0.74[a]	-2.40 ± 0.12[a]	8.59 ± 1.91[a]
7	72.62 ± 0.85[a]	-2.37 ± 0.10[a]	8.70 ± 1.98[a]
10	80.09 ± 0.52[a]	-2.51 ± 0.21[a]	9.21 ± 2.00[a]
14	80.34 ± 0.59[a]	-2.52 ± 0.12[a]	9.47 ± 2.06[a]

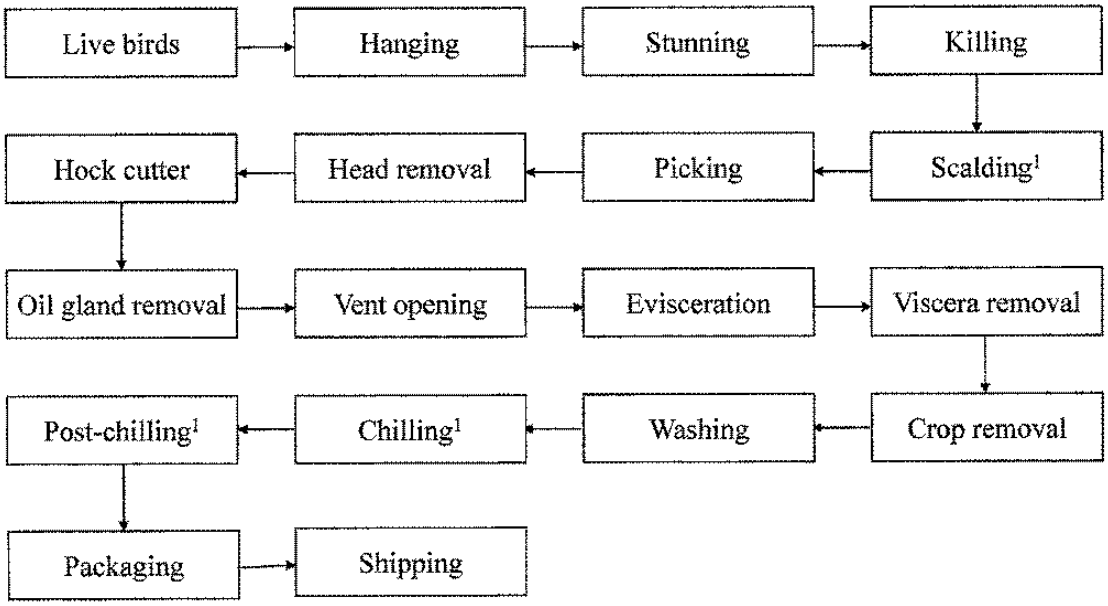
[1] n = 3.
[2] Within the same column, mean values with different superscriptsr are significantly different ($P < 0.05$).

APPENDIX 4. Efficacy of hydrochloric and fumaric acid to reduce *Salmonella* spp. on raw chicken skins during simulated on-line or post-chill dipping (22°C ± 1°C), water-immersion chilling (3°C ± 1°C) and soft scalding (53°C ± 1°C).

Acid	Mean *Salmonella* log reduction ± std. error (CFU/skin)[1, 2]		
	40 sec dipping (pH 2.30)	60 min chilling (pH 2.25)	3 min scalding (pH 2.03)
Fumaric acid	0.31 ± 0.02^{a}	0.65 ± 0.09^{a}	1.25 ± 0.17^{a}
Hydrochloric acid	0.08 ± 0.04^{b}	0.13 ± 0.08^{b}	0.98 ± 0.23^{b}

[1] n = 3.
[2] Within the same column, mean values with different superscripts are significantly different ($P < 0.05$).

APPENDIX 5. Typical unit operations in poultry processing plant.



[1] Poultry slaughter unit operations that are approached as sites for application of antimicrobial agents to diminish cross-contamination during poultry processing.

APPENDIX 6. Physical and chemical properties of fumaric acid.

Chemical name	*(E)-butenedioic acid*
Molecular formula	$C_4H_4O_4$
Appearance	White crystals
Odor	None
Taste	Tart
Molecular weight	116.07
Density (g/cm^3)	1.635
Melting point (ºC)	187 to 302
Solubility at 25ºC (g/100ml)	0.63

APPENDIX 7. Fumaric acid strength in comparison with other weak organic acids[1].

Acid	pH						
	2.5	**3.0**	**3.5**	**4.0**	**4.5**	**5.0**	**5.5**
Fumaric	0.372	0.128	0.070	0.048	0.037	0.032	0.030
Citric	0.717	0.239	0.128	0.090	0.69	0.055	0.047
Lactic	1.884	0.484	0.177	0.093	0.068	0.060	0.057
Malic	0.953	0.273	0.121	0.076	0.057	0.045	0.038
Tartaric	0.469	0.161	0.086	0.059	0.046	0.041	0.039

[1] Adapted from www.Bartek.ca.

APPENDIX 8. Lange and Sinks equation for solubility of fumaric acid[1].

$$\log(C) = 0.01672(T) - 0.604,$$

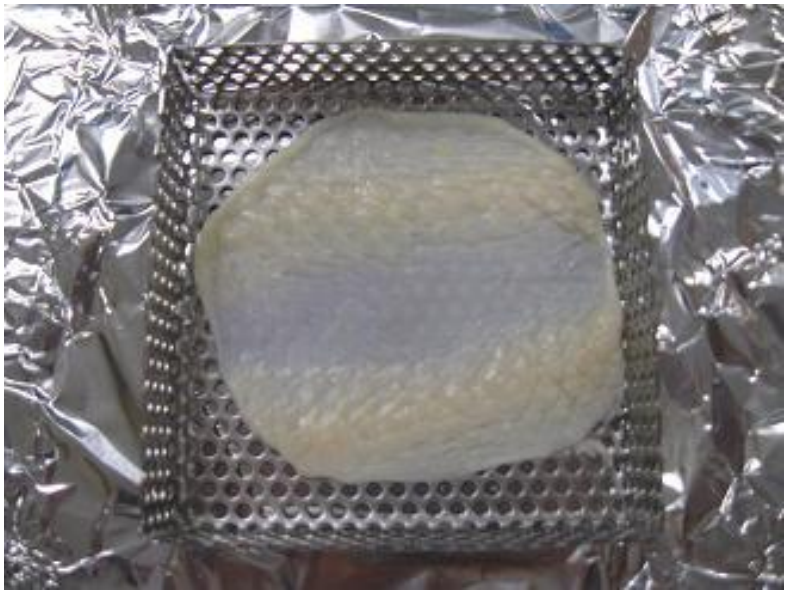
where C is % w/w fumaric acid concentration and T is temperature (ºC).

[1] Adapted from Lange, N. A., and M. H. Sinks. 1930. The solubility, specific gravity and index of refraction of aqueous solutions of fumaric, maleic and *i*-malic acids *J. Am. Chem. Soc.* 52:2602-2604.

APPENDIX 9. Physical and chemical properties of oxalic acid.

Chemical name	*Ethanedioic acid*
Molecular formula	$C_2H_2O_4$
Appearance	Transparent, colorless crystals
Odor	None
Taste	N/A
Molecular weight	90.04
Density (g/cm^3)	1.90
Melting point (ºC)	149 to 160
Solubility (g/100ml)	14.3

APPENDIX 10. A stainless steel perforated rack with a sample of chicken skin prepared for inoculation.



APPENDIX 11. Plastic containers used for simulated on-line or post-chill dipping, water-immersion chilling, and soft scalding of chicken skin samples.