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Antimicrobial Efficacy of a Citric Acid/Hydrochloric Acid Blend, Peroxyacetic Acid, and Sulfuric Acid Against Salmonella on Inoculated Non-Conventional Raw Chicken Products

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ANTIMICROBIAL EFFICACY OF A CITRIC ACID/HYDROCHLORIC ACID BLEND, PEROXYACETIC ACID, AND SULFURIC ACID AGAINST *SALMONELLA* ON INOCULATED NON-CONVENTIONAL RAW CHICKEN PRODUCTS

By

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The utilization of non-conventional chicken parts as human food varies widely across countries. The high prevalence of *Salmonella*, especially in the giblets, poses a high risk to public health. Poultry processors have implemented multiple hurdle technology to reduce this gram-negative pathogen in chicken parts. This study was conducted to evaluate the antimicrobial efficacy of a commercial blend of citric acid/ hydrochloric acid (CP), peroxyacetic acid (PAA), and sulfuric acid (SA) in reducing *Salmonella* inoculated on two chicken giblets: livers and hearts. Chicken hearts and livers were inoculated (6 log CFU/ml of rinsate) by individually immersing them in a cocktail of five poultry-borne strains of *Salmonella* e*nterica* subsp. *Enterica* each for 30 s achieving initial mean *Salmonella* recovery of 4.75 ± 0.10 and 4.69 ± 0.10 log CFU/g for chicken hearts and livers respectively. Inoculated chicken hearts and livers were treated by immersing into solutions (4 °C) containing PAA (500 ppm, 90 s), 5% v/v CP (pH 0.66; 30 s), 2% v/v SA (pH 0.93; 30 s), or distilled water (control; 90 s) and analyzed for survivors immediately after treatment (0 h), after one (24 h), two (48 h) and three (72 h) days of aerobic storage at 4 °C. The effect of these treatments on the growth of aerobic mesophilic bacteria and their effect on the color of the chicken hearts and livers were also investigated at the same time points. Results for *Salmonella* log reductions and aerobic plate count (APC) showed that there was no interaction between the type of antimicrobial treatment and storage time. *Salmonella* survivors recovered in chicken hearts following

treatment (0 h) with PAA, SA, or CP were not significantly different ($p < 0.05$) from the control. However, SA-treated chicken hearts had significantly lower *Salmonella* counts than distilled water immediately after treatment. Unlike distilled water, all antimicrobials achieved greater than one-log reductions of *Salmonella* on both chicken hearts and livers, which indicated that immersing in antimicrobial solutions was more effective in reducing *Salmonella*. All treatments were effective in minimizing the growth of aerobic mesophilic microflora throughout the three days of storage with no significant differences ($p < 0.05$) in a* (redness), b*(yellowness) or L*(lightness) values on the third day of storage in both chicken parts. Hence the results of the study showed PAA, SA, and CP may be used in the poultry industry as part of a multiple hurdle system to reduce *Salmonella* in non-conventional chicken parts.

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DEDICATION

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CHAPTER 1. INTRODUCTION

1. Background

Salmonella is the second leading cause of foodborne illnesses in the United States, after norovirus (FDA, 2020). This pathogen is estimated to cause 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths annually (CDC, 2022). Not only is this pathogen a major public health concern in the US, but also worldwide. Majowicz et al. (2010) estimated 80.3 million foodborne illnesses and 155,000 deaths to be caused by non-typhoidal *Salmonella* annually at the global scale. While these numbers are good estimations, it is also likely that the actual burden is underestimated due to high cost of surveillance and lack of epidemiological infrastructure in many countries (Majowicz et al., 2010; Senior, 2009). Salmonellosis often causes gastroenteritis, with symptoms ranging from mild to severe, and these may include diarrhea, fever, nausea, vomiting, and stomach pains (FDA, 2020). Various food sources have been implicated in multiple salmonellosis illnesses. According to Ashrafudoulla et al. (2021), one third of salmonellosis cases worldwide are due to consumption of raw or undercooked contaminated food. Among the implicated food sources, animal products, particularly poultry meat, are largely represented. This is not surprising given that *Salmonella* is one of the two most common pathogens in raw poultry products, the other being *Campylobacter* (Henley et al., 2018). Over 2,600 serovars of *Salmonella* are known, and *S.* Enteritidis, *S.* Braenderup, *S.* Hadar, and *S.* Typhimurium, are some of the common poultry-borne strains implicated in foodborne illnesses (Popa & Papa, 2021; Procura et al., 2019).

According to the USDA Foreign Agricultural Service (FAS, 2022), the U.S. is the leading producer of chicken meat globally, producing approximately 20.8 million metric tons. The production is expected to increase given the high demand and consumption of broiler meat products. Poultry meat is the second most consumed meat type globally, the first being pork (Bux & Amicarelli, 2022). The USDA Livestock and Poultry sector reported the global chicken

meat consumption to be approximately 98 million metric tons (excluding chicken paws), with 17 million metric tons (17.3%) in the U.S. alone. The per capita consumption of chicken meat is 99.3 pounds in the US (USDA National Chicken Council, 2022) and is expected to grow by 16.7% in 2030 (USDA Agriculture and Livestock, 2022). This high consumption can be attributed to a variety of reasons, including that chicken meat is easy to prepare and has no cultural limitations in many countries (Toldrá et al., 2012). Besides domestic consumption, a significant amount of the poultry meat, amounting to $4.25 billion (11.5 million metric tons), is exported annually from the U.S. (USDA-FAS, 2019). With the high domestic and global consumption of poultry products, the risk of foodborne salmonellosis remains a big threat to public health and a heavy burden to the economic sector, representing $4.1 billion annually in the U.S. alone (USDA Economic Research Service, 2021).

The large volume of production of broiler meat in the U.S. implies that a lot of non-conventional chicken products are inevitably produced from the chicken slaughterhouses. Non-conventional chicken products, also known as “variety meats” (Jayathilakan et al., 2012) include all the chicken parts aside from legs, wings, and breasts, with the edible parts being livers, hearts, gizzards, necks, and paws (USDA FSIS Notice, 2021). These non-conventional chicken products are utilized in various ways. Chicken by-products such as chicken feet are at times considered “excess parts” and ground into pet food, contributing significantly to that market (Richburg, 2011). The global pet food production was 34.17 million metric tons in 2021, with Europe being the highest producer by volume (11.59 million metric tons) and the U.S. producing 9.80 million metric tons (Pet Food Processing site, 2022). With the current global pet food market valued at $115.5 billion and the 5.11% projected growth by 2029 (Animal Nutrition Market Research Report, 2022), the demand for non-conventional chicken products in the pet food industry (particularly among the Raw Meat-Based Diets - RMBDs) is expected to increase further. According to the American Pet Products Associations (APPA),

the sale of pet food and treats in the U.S increased by 9.7% in 2020. Pet food is comprised of various ingredient sources which supply different nutrients. According to the 2020 Pet Food production and ingredient analysis report, chicken by-products are the third most significant source of animal protein contributing over 360 tons, the lead ingredients being chicken (584 tons) and meat plus bone (533 tons). Chicken livers (CL) are the predominant organ meat (Procura et al., 2019), primarily because of the size and high nutrient composition. They contain high amounts of protein (17.7%), iron, potassium, and vitamins (Seong et al., 2015). Chicken gizzards and hearts are also significantly represented in pet food.

Currently, pet owners are increasingly utilizing meat and poultry by-products to prepare raw meat-based diets (RMBDs) for their pets as they desire to provide a more natural healthy diet, but also partly to respect the ancestral carnivorous nature of cats and dogs (Morelli et al., 2019). Aside from the pet food industry, significant amounts of the non-conventional chicken parts are exported to many Asian countries - mostly to China, Japan, Korea, and the Philippines; countries in the Middle East like Iran and Iraq and some in Africa (USDA Agricultural Research Service, 2021; The Poultry Site, 2022). In 2011, Richburg reported over 370,000 metric tons of chicken paws, worth $278 million, were exported to China annually, and this was expected to increase significantly given the rapidly increasing population in the country which skyrockets the demand. In addition, according to the latest data from the Observatory of Economic Complex-OEC (2022), the U.S. is the world's largest exporter of chicken gizzards and ranks third for chicken livers, with Brazil being the first and Australia second one.

The utilization of non-conventional chicken parts as human food varies widely across countries due to differences imposed by certain traditions, cultures, and religions. Regulatory requirements on utilization of these variety meats in products may restrict the margin of their applications (Jayathilakan et al., 2012). The U.S. Department of Agriculture requires for variety

meats to be specifically identified as ingredients on product labels (USDA Food Standards and Labeling Policy Book, 2005). The domestic consumption market value is not documented, however, the cultural diversity in the country, especially with people originating from different countries where these products are a delicacy indicates their active consumption. Moreover, these products are sold in significant amounts in many grocery stores across the U.S.

Various researchers have reported high prevalence levels of *Salmonella* in some non-conventional chicken products, especially giblets. El-Aziz (2013) detected *S.* Typhimurium in 48% (12/25) and 40% (10/25) of the samples of chicken hearts and livers tested, respectively. In another study on chicken gizzards by Abd-Elghany et al. (2014), 30 out of 50 samples (60%) tested positive for *Salmonella.* More recently, *Salmonella* prevalence rates of 54.7% (52/95) and 35.7% (10/28) were reported for chicken livers and hearts, respectively (Mohammed et al., 2022). Some non-conventional chicken products, especially livers, have been implicated in various *Salmonella* outbreaks (CDC, 2019; Hanson et al., 2014; Lanier et al., 2018), and this is no surprise considering the high prevalence rates of the pathogen in these products. More so, some people prefer to consume chicken livers undercooked for its distinct texture and taste (Little et al., 2010).

According to the USDA-FSIS guideline for controlling *Salmonella* in raw chicken products, the maximum acceptable percent positive for chicken parts is 15.4% (USDA-FSIS, 2021). Various procedures are being employed in the food industry to mitigate the risk of *Salmonella* such as the use of antimicrobials. Antimicrobials must be approved for use in the food industry and need to achieve a pathogen reduction of at least one logarithmic cycle to be considered of practical significance (Brashears, & Chaves, 2017). This is in addition to having documented efficacy, cost effectiveness, minimal alterations to product quality and lastly, the concentration and contact time need to be appropriate for the processing step (Bauermeister et al., 2008).

According to the Code of Federal Regulations, (21 CFR 101.100 (a)), any processing aid needs to comply with one of the following: be removed in some manner from the food before it is packaged in its finished form; be converted into constituents normally present in the food and does not significantly increase the amount of the constituents naturally present in that food; or be present in the finished product at insignificant levels and does not have any technical or functional effect in that food.

Peroxyacetic acid (PAA) is one of the popular antimicrobials used by most establishments to decontaminate both chicken carcasses and parts (Cano et al., 2021). PAA is a combination of peracetic acid, hydrogen peroxide (Bauermeister et al., 2008) and 1-hydroxyethylidene1,1-diphosphonic acid (HEDP) and it exists in various combinations of the components. This antimicrobial has no potential health concern if used within acceptable limits for conditions (FAO/WHO Expert Committee on Food Additives, 2006; European Food Safety Authority-EFSA, 2014). According to USDA Food Contact Substance Notification (FCN No. 2036), up to 2000 ppm of PAA, 1474 ppm of hydrogen peroxide, and 136 ppm of 1-hydroxyethylidene1,1-diphosphonic acid (HEDP) is permitted to be used for inactivating pathogens like *Salmonella* in poultry process water for spraying, washing, rinsing, dipping, chill water, low temperature (less than 40 degrees F) immersion baths, or scald water on poultry parts, and organs (USDA FSIS Directive 7120.1).

Other blends of antimicrobials exist on the market including Citrilow Plus™ (Safe Foods Corporation, North Little Rock, AR, USA), a citric and hydrochloric acid blend and Assist™ (Safe Foods Corporation, North Little Rock, AR, USA), a sulfuric acid product. Some studies have shown efficacy of the citric and lactic acid blend in reducing *Escherichia coli* in beef and chicken carcasses (Laury et al., 2009). In another study, the effectiveness of sulfuric acid and sodium sulfate (Scott et al., 2015) was investigated together with PAA, and the blend was able to achieve a one logarithmic reduction of *Salmonella* inoculated on the chicken wings.

Information on the efficacy of various acid blends on the more popular chicken parts has been documented, however, much less is available about the non-conventional chicken parts. This study focused on evaluating the effectiveness of PAA, Citrilow Plus™, and Assist™, in reducing *Salmonella* artificially inoculated onto non-conventional raw chicken products. We hypothesized that Citrilow Plus™ and Assist™ would be at least as effective as PAA at reducing *Salmonella* in artificially inoculated non-conventional poultry products. Additionally, the effect of the three chemicals on meat color and aerobic (mesophilic) bacteria populations was evaluated over a three-day period.

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CHAPTER 2. LITERATURE REVIEW

2.1. Introduction

Microorganisms are ubiquitous in foods and the food processing environment (Wali & Abed, 2019). Spoilage microorganisms cause deterioration in food products, reducing the quality and product shelf life, however, those of major concern are the pathogens, as these cause foodborne illnesses. The U.S. Centers for Disease Control and Prevention (CDC) estimate that, in the US alone, 48 million people get sick, 128,000 are hospitalized and 3,000 die annually due to foodborne illnesses (2021). There are 31 major pathogens (Scallan et al., 2011) of which *Salmonella, Clostridium perfringens*, and *Campylobacter* are the ones most commonly found on raw poultry products (Henley et al., 2018).

Salmonella can be found everywhere in the environment, gastrointestinal tract of animals, and in raw foods, especially poultry and meat products. *Salmonella* bacteria have an optimal growth temperature of 37 ˚C (Mohammed et al., 2022), however, they are capable of surviving conditions of stress such as heat and low water activity for long periods (Podolak et al., 2010). In addition, *Salmonella* spp. are capable of forming biofilms (Popa & Papa, 2021) on food contact surfaces and this way they are partially protected from external physical and chemical stressors. This protection enables these pathogens to survive and thus the risk of cross-contamination during processing increases (Ashrafudoulla et al., 2021).

2.2.*Salmonella* Survival and Prevalence

Salmonella remains a major public health problem. This pathogen is the second leading cause of bacterial foodborne illnesses in the U.S, following *Campylobacter spp.* (Byomi et al., 2019; USDA FSIS Revised Appendix A, 2021). *Salmonella* alone is responsible for an estimated 1 million illnesses, 26000 hospitalizations and 400 deaths annually in the United States (CDC, 2022). According to a survey conducted by the United States Department of Agriculture's (USDA) Economic Research Service (2010), the annual cost of salmonellosis is

over $2.6 billion USD (Taskila, et al., 2012), and this is expected to have increased over the past decade due to the increased cases of illnesses. *Salmonella* is not only a concern in the US, but also globally in both developed and developing countries (Byomi et al., 2019). Researchers have also reported that *Salmonella* is responsible for approximately 39.2% of the foodborne illnesses in the European Union (European Food Safety Authority (EFSA), 2009) and 80-90% of those are in China (Jun, et al., 2007). Most salmonellosis foodborne outbreaks are related to consumption of raw or undercooked meat and poultry products (Benshaban et al., 2014). While salmonellosis infections are rarely fatal, they may cause severe sequalae such as reactive arthritis and irritable bowel syndrome. In addition, outbreaks due to salmonellosis are quite common and often lead to large product recalls with large economic losses to companies, aside the vast numbers of illnesses. A single salmonellosis outbreak due to contaminated ground beef in 2018 caused a recall of about 11 million pounds of ground beef across 30 US states that were affected; 403 illnesses were reported, with 117 hospitalizations (CDC, 2019). Recently in 2021, a multistate salmonellosis outbreak in salami sticks products caused the company to issue a recall of 119,091 pounds across 10 US states (USDA FSIS, 2022). According to Hoffmann (2015), *Salmonella* ranks first in terms of the economic burden caused by the major pathogens in the US. Although the numbers of deaths due to salmonellosis might seem low, 90% of the $3.7 billion imposed by the illness annually is due to the deaths (Hoffman, 2015). Overall, this high economic burden, public health risk, and the high prevalence rates of the *Salmonella* pathogen in raw poultry and poultry products are the reason for concern. It should be noted, however, that most of the research on *Salmonella* in poultry has focused on the more popular parts like wings, drumsticks, thighs, and, breasts putting less emphasis on non-conventional chicken parts, which are also referred to as “other poultry products”.

2.3.Non-conventional poultry products

USDA FSIS created the "Other poultry products" group in 2015, and this was meant to encompass all poultry parts that were not legs, breasts, and wings. The eligible products in this group included livers, gizzards, hearts, necks, and feet (USDA FSIS Notice, 2021). Livers, gizzards, necks, and hearts are collectively termed as 'giblets' (Procura et al., 2019). Contamination of chicken carcasses and parts can occur at any point during post-harvest/ slaughter of the chicken. The slaughter process starts at live receiving and hanging; stunning and bleeding; scalding; picking; evisceration and finally chilling of carcasses and parts (Dookeran et al., 2014). Evisceration is the step at which the internal organs of the chicken, collectively referred to as the viscera, are removed (Perez-Arnedo et al., 2021). The viscera include the gastro-intestinal tract (GIT) as well as the edible organs like livers, and hearts. Attention needs to be taken during this process to avoid rupture of the GIT and minimize cross-contamination of microorganisms, which inevitably include *Salmonella*, not only to the carcass but also the internal edible organs (Dias et al., 2016).

2.4. *Salmonella* prevalence in non-conventional chicken parts

2.4.1. Chicken livers

Chicken livers contain proteins, vitamins, and minerals, particularly iron, zinc, and B-vitamins in considerable amounts and this makes them very nutritious for humans and animals alike (Jung et al., 2019). The high nutrition composition also makes the livers highly prone to pathogen growth especially *Salmonella and Campylobacter spp.* The two pathogens are frequently recovered in chicken parts, especially livers. Some researchers have also reported internal contamination of chicken organs including livers with these pathogens even when they are aseptically dressed (Chaloner et al., 2014; Gast et al., 2013; He et al., 2010). From one survey conducted on chicken liver samples by the USDA FSIS in 2016, it was reported that *Salmonella* was recovered at a rate of 67.1% (57/85) (Lanier et al., 2018). USDA-FSIS in 2018

conducted another survey in which they estimated the incidence of *Salmonella* in raw chicken necks, hearts, livers, and gizzards (Jung et al., 2019) combined at 53.9% (172 of 319 samples). The incidence of *Salmonella* in chicken livers varies widely from 1 to 67% in both fresh and frozen products (Lanier et al., 2018). Procura et al. (2019) reported, of the 666 frozen chicken livers in their study, 32 samples were found to be positive for *Salmonella*, hence a 4.8% incidence rate as shown in Table 2.1. A more recent study about *Salmonella* in chicken livers by Khan et al., (2021) reported a prevalence of 24% (12 out of 50 samples).

2.4.2. Chicken hearts

Salmonella continues to be a major concern even in the would-be minor chicken parts consumed by both human beings and pets. Mohammed et al. (2022) noted that the pathogen can survive for long periods at low temperatures common in poultry processing. The researchers also reported a prevalence of *Salmonella* of 35.7% (10/28) in hearts compared to 54.7% (52 of 95 samples) in livers. Mohammed's results were not different from those reported by El-Aziz (2013) where 48% (12 of 25 samples) in hearts, and 40% (10 of 25 samples) in livers tested positive for *Salmonella*. Most researchers agree that sanitation of the processing environment and hygiene of the employees especially during the evisceration process (Jackson et al., 2001; Nychas et al., 2007) needs to be improved to minimize incidences of pathogens in chicken parts. All equipment, personal protective equipment (PPEs) and the entire chicken slaughter environment need to be adequately cleaned and sanitized before and after the harvest process; microbiological sampling and testing need to be conducted to ensure that the environment is free of pathogens.

2.4.3. Chicken gizzards

Chicken gizzards are another part of the edible viscera that is widely consumed across the globe. The prevalence of *Salmonella* in gizzards has also been reported and for some researchers this is higher than that in livers. In a study conducted in Morocco, Abdellah et al.

(2008) reported *Salmonella* to be more prevalent in gizzards amongst the chicken parts tested. The prevalence was 13.88% (20/144), 11.11% (16/144), 8.33% (12/144) and 6.25% (9/144) in gizzards, livers, legs, and breasts respectively. Abd-Elghany et al., (2014) also reported a higher

Table 2. 1. Prevalence of *Salmonella* isolated from non-conventional chicken parts in different countries

Product	Number of Samples	Number of positives	Percent positive	Sample location	Reference
Livers	144	16	11.11	Morocco	Abdellah et al., 2008
Livers	25	10	40	Egypt	El-Aziz, 2013
Livers	50	16	32	Egypt	Abd-Elghany et al., 2014
Livers	120	26	21.6	Iran	Sodagari et al., 2015
Livers	666	32	4.8	Argentina	Procura et al., 2018
Livers	52	37	71.15	Egypt	Byomi et al., 2019
Livers	50	12	24	Pakistan	Khan et al., 2021
Livers	95	52	54.7	Iraq	Mohammed et al., 2022
Hearts	25	12	48	Egypt	El-Aziz, 2013
Hearts	120	17	14.1	Iran	Sodagari et al., 2015
Hearts	30	18	60	Egypt	Byomi et al., 2019
Hearts	28	10	35.7	Iraq	Mohammed et al., 2022
Gizzards	144	20	13.88	Morocco	Abdellah et al., 2008
Gizzards	50	30	60	Egypt	Abd-Elghany et al., 2014
Gizzards	120	10	8.3	Iran	Sodagari et al., 2015
Gizzards	32	19	59.4	Egypt	Byomi et al., 2019
Feet	144	12	8.33	Morocco	Abdellah et al., 2008

prevalence of *Salmonella* in gizzards (60%; 30/50) than in livers (32%; 16/50) in their study. Sodagari et al. (2015) reported a value of 8.3% (10/120) in chicken gizzards which had a reduced prevalence than in chicken liver (21.6%; 26/120) and heart (14.1%; 17/120). However, later in 2019, Byomi et al. reported a higher prevalence for *Salm onella* in gizzards, 59.4% (19/32), in samples collected from different outlets. Most researchers have recommended adequate sanitation and inclusion of *Salmonella* as a pathogen highly likely to occur in the HACCP system for slaughter and processing of chicken parts.

2.4.4 Chicken feet

Chicken feet though not consumed widely by humans in many countries including the United States, are exported to eastern countries like China in considerable amounts. China, the largest importer of chicken feet, developed microbiological criteria that require absence of *Salmonella* and *Escherichia coli* O157:H7 in 25-g samples of all imported chicken meat. This requirement is the same for all chicken meat imported into China (Santos et al., 2011). In addition to the standards, it is no surprise that chicken feet are one of the chicken products for which less information is available about its sanitary quality given that chicken are hang by their feet during the evisceration process, which minimizes cross-contamination, and their preparation before consumption involves long cooking times which in addition reduces the risk of salmonellosis. Kotula & Pandya (1995) conducted a study about the microbiological quality of chicken feet before scalding and reported a *Salmonella* prevalence of 55% (22/40). Santos who later in 2011 conducted a similar study got an incidence of 68% (13/19), however, this reduced considerably after scalding to 5.26% (1/19). It was concluded by both researchers that even minimal sanitation during processing can minimize pathogens like *Salmonella* in chicken feet to achieve the standards required. Brizio et al. (2013) analyzed a total of 98 frozen chicken feet in Brazil for various pathogens including *Salmonella* and found out that 99% (97 of 98

samples) of the results were within the acceptable standards for chicken feet set by China and the Brazilian legislation for raw meat.

2.4.5. Chicken necks

Chicken necks are also another edible part of the chicken for which there is little information published about microbial quality. This is partly because most times the necks are not considered for human consumption and are often prepared with the rest of chicken meat following the required temperature/time combination if they are to be consumed by humans. Chicken necks are, however, an important constituent in dog and cat food, especially in form rendered products (Thompson, 2008). Regardless of the consumption pattern, researchers have used the neck skin to evaluate the microbial quality of chicken's carcasses in general (Cox & Pavic, 2010; Perez-Arnedo et al., 2021). There is a high possibility of contamination of the chicken necks when the head is being removed from the chicken and during the picking step (Dookeran et al., 2014; Russell, 2012). During these steps, pathogens in the feathers can be passed onto the chicken carcass including the necks. Scalding is another step at which pathogens from the feathers which are passed into the scald water contaminate the chicken carcass (Carson et al., 2007). Contamination occurs when scalding is done under unsanitary conditions such as stagnant water, excessive excreta, or non-bactericidal temperatures are used (Cox & Pavic, 2010). Overall, the microbiological quality of chicken necks can be controlled through managing the quality of the entire chicken carcass.

2.5.Salmonellosis outbreaks in non-conventional chicken parts

Multiple outbreaks in non-conventional chicken parts have been associated with chicken livers, particularly, consumption of undercooked products (Hanson et al., 2014). This is of little surprise considering the widely varying incidence rates of *Salmonella* in the product

and the fact that in some dishes like pâté, the livers are intentionally undercooked to achieve the texture and appearance desired by the consumers (Little et al., 2010). In almost all the forms of preparation of chicken livers, which include skewing, broiling and pan frying, the cooking temperature is rarely monitored, and results in an undercooked product. Undercooking poses a high risk given that CL, even when aseptically dressed, are already internally contaminated (Chaloner et al., 2014), in that only surface cooking alone will not kill all the pathogens (Gast et al., 2013; He et al., 2010). A total of 28 outbreaks were reported between 2000 – 2016 in the US concerning chicken livers, 10.7% of these were salmonellosis, 82.1% campylobacteriosis and 7.1% were caused by both pathogens (Lanier et al., 2018). Of the 361 illnesses reported, 190 were from a multistate salmonellosis outbreak occurring across seven states implicating Kosher broiled chicken livers in 2011 (CDC, 2019). Little et al. (2010), in his "A recipe for disaster" study noted that cooking the chicken livers to an internal temperature of 165 °F, as recommended by USDA FSIS, would reduce the multiple outbreaks of illnesses associated with foodborne pathogens like *Campylobacter* and *Salmonella* which are the most common ones associated with undercooked livers. Generally, *Salmonella*, and other microorganisms in poultry may survive well and multiply in internal organs, especially the livers and hearts, as these sites enable them to multiply without interruption from the host defense mechanisms (El-Aziz, 2013).

Even when the prevalence rates are high, salmonellosis outbreaks resulting from consumption of the rest of the non-conventional chicken parts are not very evident in the literature reviewed. This can be partly attributed to the difference in cooking methods for these parts and because they take a relatively longer period to cook which inactivates the pathogen in the process.

2.6.Food industry guidelines on control of *Salmonella*

Due to the worldwide public health concern and economic burden of the pathogen, USDA FSIS has a guideline of controlling *Salmonella* in raw poultry products (USDA-FSIS, 2022). This guideline was designed to help raw poultry establishments including small and very small processors and emphasizes control of the pathogen through pre- and post-harvest interventions included in the Hazard Analysis and Critical Control Point (HACCP) system. The guideline further recommends microbial testing as a means of monitoring the pathogen during processing operations (USDA FSIS, 2021) and this would aid process control and inform important decision making in these raw poultry processing establishments. The maximum acceptable percent positive for chicken parts, according to the FSIS performance standard for raw chicken products is 15.4%. The performance standards are 9.8% for broiler carcasses and 25% for comminuted chicken (USDA FSIS, 2015). As noted above, much of research on *Salmonella* in poultry has focused on the more popular parts like wings, drumsticks, thighs, and breasts, and less emphasis has been accorded to non-conventional chicken parts. However, with the high prevalence rates of *Salmonella* observed, it is worthwhile to conduct research into the non-conventional chicken parts, especially the chicken livers and hearts which overall present a relatively higher risk.

2.7. Antimicrobial interventions

While meat and poultry products are very good vehicles for foodborne pathogens (Little et al., 2010), cross-contamination starts right away at slaughter (Perez-Arnedo et al., 2021). The handling of carcass meat after slaughter heavily impacts the microbial quality of the meat and this will ultimately affect the products that move into commerce (Mead, et al., 2005). Nevertheless, contamination of food products can occur at any point along the food chain (Perez-Arnedo et al., 2021), and due to this, food processing plants face multiple challenges regarding food safety and sustainability to achieve compliance with HACCP regulations and

performance standards required by USDA FSIS. This body suggests adequate sanitation in the processing environment, antimicrobial interventions and microbiological sampling and testing to control *Salmonella* in raw poultry products during post-harvest activities such as slaughter and processing (USDA-FSIS, 2021). Some researchers have recommended similar strategies, coupled with employee hygiene especially during the evisceration process (Nychas et al., 2007) to minimize incidences of pathogens especially *Salmonella* in chicken parts. Periodic employee training and other good house-keeping practices can also be used to control other pathogens like *Salmonella*, not only in poultry but also in meat products (Kotula & Pandya, 1995). Overall, *Salmonella* needs to be included as a pathogen highly likely to occur in the HACCP system (Lanier et al., 2018) for slaughter and processing of chicken parts.

Generally, acceptable antimicrobials used in the food industry need to be approved for industry use, have documented efficacy at an appropriate concentration and contact time for a particular processing step, have minimal adverse effect on the quality of food product, must attain at least one logarithmic reduction on tested pathogen(s), and be cost-effective (Bauermeister et al., 2008). Antimicrobials can be added to processing water used in food facilities and, particularly in poultry processing, they can be added to scalder and pre-chilling tanks, IOBW (inside-outside bird washers), and post-chill applications (Wideman et al., 2016). At times, antimicrobials used in food products are considered as processing aids if they comply with one of the following: the antimicrobials are removed in some manner from the food before it is packaged in its finished form; they are converted into constituents normally present in the food and do not significantly increase the amount of the constituents naturally present in that food; and, lastly, they are present in the finished product at insignificant levels and do not have any technical or functional effect in that food (Code of Federal Regulation, 21 CFR 101.100(a)). When used as processing aids, antimicrobials do not need to be included on the product label. Antimicrobial agents can be categorized as either inorganic or organic in nature.

Inorganic antimicrobials may include chlorine dioxide (ClO_2), and hydrogen peroxide (H_2O_2). The two inorganic antimicrobials mentioned above, together with peroxyacetic acid – PAA (organic) and ozone are collectively referred to as oxidizing antimicrobial agents as they share chemical oxidation as a basic mode of action (Finnegan et al., 2010). These oxidizing agents remove electrons from susceptible chemical groups of the cell membrane and the cellular components of the pathogen. Their effect can be reversible at low concentrations of antimicrobial but irreversible when high concentrations are used because high concentrations lead to severe damage of cell structure, cell wall and intracellular components (Maillard, 2002). These oxidizing agents, due to their low molecular weight, diffuse easily through the cell membrane and may either react with the cellular components leading to apoptosis and necrosis or may severely damage the microbial structure, leading to release of the cellular components which are then oxidized (Denyer & Stewart, 1998). Among all antimicrobials, PAA is the most popularly used antimicrobial in the food industry (Ebel et al., 2019), especially in poultry processing as it decomposes quickly into carbon dioxide, oxygen, and water, and unlike chlorine, leaves no residues in the product (Cano et al., 2021). The effectiveness of PAA has been proven (Cano et al., 2021) in traditional raw chicken products. However, it should be noted that this chemical is highly corrosive, and this poses occupational and storage concerns.

Organic acids (OAs) are natural compounds present in various food products and are produced by some microorganisms (Kim & Rhee, 2013). OAs are increasingly used in the food industry (Lingham et al., 2012) to inhibit pathogen growth as their effectiveness is known and because they are relatively affordable. They are generally recognized as safe (GRAS) by the United States Food and Drug Authority (US FDA) and are approved for use as additives in food by the European Commission, the World Health Organization (WHO), (Surekha & Reddy, 2014). Organic acids include citric acid, acetic acid (commonly referred to as vinegar), lactic acid, and PAA and some can also be added in their salt form, such as sodium lactate, or

potassium lactate. The mode of action of weak acids against bacteria has been widely investigated. OAs diffuse across the bacterial cell membrane into the cytoplasm where they dissociate into charged protons and anions, which alters the hydrogen ion equilibrium inside the cell and raises the pH (Brul & Coote, 1999; Davidson et al., 2005). These antimicrobial actions upset intracellular pH homeostasis, inhibit essential metabolic reactions, and cause accumulation of toxic anions in the bacterial cells, eventually causing cell death (Kim et al., 2013).

Certain fatty acids (FAs) and their derivatives have also proven to be good antimicrobials and have potential to replace antibiotic use in the food industry (Marounek et al., 2003). This is partly due to their broad spectrum microbicidal activity against pathogens both *in vitro* and *in vivo,* and because the bacteria are also unlikely to acquire resistance against these FA antimicrobials (Borrelli et al., 2021; Jackman et al., 2020; Schlievert & Peterson, 2012). The mode of action of FAs against spoilage and pathogenic bacteria is considered broad spectrum and non-specific which makes them attractive to use in diverse applications, both in the food industry, clinical medicine, cosmetic formulations, and nutraceuticals (Desbois & Smith, 2010). Even when the specific mechanism used by FAs against the bacteria is not well understood, studies show that the key mechanism is related to disruption of membrane functionality. FAs and their derivatives interfere with the electron transport chain and disrupt oxidative phosphorylation, which affects production of energy needed for metabolic activities (Wieckowski & Wojtczak, 1998). Once the cell metabolism is disrupted, the pathogen growth is inhibited, and its survival is put at risk. However, it is also discussed that other processes like impairment of nutrient uptake, inhibition of enzyme activity (Zheng et al., 2005), cell lysis, generation of toxic peroxidation and auto oxidation products are possible ways in which FAs cause growth inhibition and death of pathogens (Schönfeld & Wojtczak, 2008; Shin et al., 2007; Yoon et al., 2018). Much as they have a similar mode of action, FAs differ in

effectiveness against controlling microorganism growth, and this can be explained by the difference in structure and shape, particularly, the length of the carbon chain, presence, number, position and even the orientation of the double bond in the chain affect the potency and spectrum (Desbois & Smith, 2010; Zheng et al., 2005). Generally, pathogens are more susceptible to unsaturated FAs than saturated ones (Nieman, 1994). According to Feldlaufer et al. (1993), FAs with a *cis* orientation are more effective than those with a *trans* orientation, and this is probably because the *trans*-bonded FAs resemble saturated FAs. Among, the FAs that have been researched widely are acetic, lauric, formic, propionic, capric and caprylic acid among others. Acetic acid has been documented in literature as a good preservative in beef, poultry, and pork products. Jeong & Ha, (2019) reported a synergistic relationship between irradiation and acetic acid in microbial inhibition of *S.* Typhimurium.

More natural antimicrobials, also referred to as 'clean label' options are being utilized in the food industry especially in whole food products. According to USDA-FSIS labeling standards defined in 21 CFR 101.22, "natural" products are not permitted to contain any artificial flavoring, coloring ingredient or chemical preservative (USDA Food Safety and Inspection Service, 2021). Clean-label products are not defined by FSIS; however, these have been defined to contain simple ingredients easily recognized by consumers (McDonnell et al., 2013). The 'definition' of clean label limits the antimicrobials that can be utilized in such products, but the most common ones include fermentates (cultured sugars, dextroses, milks) and buffered vinegar. Buffered vinegar is acetic acid buffered using an alkali such as potassium or sodium hydroxide to increase its pH and minimize the acid's effect on the functional properties of the proteins in processed meats (Badvela et al., 2016). Cultured sugars like dextrose fermentate (CDF) are comprised of active compounds such as diacetyl, lactic, propionic, and acetic acids, which are products obtained from fermentation of milk or dextrose by propionic bacteria or specific *Lactococci* (von Staszewski & Jagus, 2008*)*.

Most of the research have focused on antimicrobials used singly in the food industry and the effectiveness of blending antimicrobials has been explored. However, studies on antimicrobial interventions against pathogens in poultry parts have mostly been conducted on the more popular chicken parts and little emphasis has been placed on their use on non-conventional chicken parts. This study therefore focused on the use of some novel antimicrobials and their blends in the food industry; Citrilow Plus™ (Safe Foods Corporation, North Little Rock, AR, USA), a citric and hydrochloric acid blend and Assist™ (Safe Foods Corporation, North Little Rock, AR, USA), a sulfuric acid product. The effectiveness of these antimicrobials against *Salmonella* artificially inoculated onto chicken livers and hearts, was compared to that of PAA, whose effectiveness has been proven. The study was also conducted on non-conventional parts, particularly the livers and hearts, given the high prevalence rates reported in these by various researchers.

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CHAPTER 3. MATERIALS AND METHODS

3.1 Inoculum preparation and sample inoculation

Five poultry-borne strains of *Salmonella enterica* subsp. *Enterica* were individually streaked onto tryptic soy agar (TSA) plates and incubated at 37 °C for 24 h. The strains were Hadar (JE 322 2013 MI), Enteritidis (IV/NVSL 94-13062), Braenderup (NVSL 96 - 12528), Typhimurium (NVSL 96 - 6904), and Enteritidis (JE 605 2013 MI). Each strain was re-streaked on Xylose Lysine Deoxycholate agar (XLD) using the same incubation time and temperature to revive the colonies and isolate them. From XLD agar, 1 large distinct colony was picked and individually transferred into 9 ml of TSB and incubated for 24 h at 37 °C. Subsequently, cell cultures were harvested through centrifugation (4500 rpm for 20 min at 4°C using Frontier TM 5718R, OHAUS Corporation, Parsipanny, NJ, USA). The cells were washed twice using 0.1% Buffer Peptone water (BPW) and re-suspended in 10 ml of TSB. 1 ml from each of the strains was transferred into 200 ml TSB. Thereafter, all the inoculum was pooled together to make 1 liter of cocktail (1000 ml) of poultry-borne *Salmonella* in a sterile stainless-steel container. This constituted the *Salmonella* bacterial working cocktail with a concentration of 10^6 CFU/ml as determined by decimal serial dilutions followed by plating onto Xylose Lysine Deoxycholate agar (XLD; HiMedia Laboratories Pvt. Ltd., Maharashtra, India).

Non-conventional chicken parts: livers and hearts were purchased from Hy-Vee Grocery store and Open Harvest Supermarket, respectively (3 batches for each meat type). These were brought to the University of Nebraska-Lincoln and stored at -20°C until further use. In preparing the chicken samples, both products were thawed for approximately 24h at 4°C. Thereafter, two 25-g subsamples of each meat type were analyzed for background microflora using 3M Aerobic Plate Count Petri films® (3M, Saint Paul, MN, USA) and *Salmonella* via direct plating on XLD agar before inoculation. The mean APC obtained was 2.53 ± 0.25 and 2.66 ± 0.22 log CFU/g for chicken hearts and livers, respectively, while

Salmonella was not observed in any of the triplicate batch samples at a 10 CFU/g limit of detection.

The rest of the thawed samples were dipped, hearts and livers separately, in the bacterial cocktail for 30 s, drained on a stainless-steel grill grid and air-dried for 20 min to allow microbial attachment (Sukumaran et al., 2015). These inoculated samples were placed in a refrigerator at 4°C for 24 h for cold adaptation and further microbial attachment. Prior to application of antimicrobial treatments, two subsamples for each meat type were randomly obtained from every batch to determine the initial mean *Salmonella* counts resulting in 4.75 ± 0.10 and 4.69 ± 0.10 log CFU/g for chicken hearts and livers, respectively.

3.2 Preparation and application of antimicrobial treatments

One and half-liter solutions of 500 ppm PAA (Birkoside MP-2, Birko Corp., Henderson, CO, USA), 2% v/v Assist^{TM} (Safe Foods Corporation, North Little Rock, AR, USA) and 5% v/v Citrilow Plus^{TM} (Safe Foods Corporation, North Little Rock, AR, USA) were prepared by diluting the concentrated solutions in cold (4 °C) sterile distilled water. PAA concentration was tested using a PAA test kit (Peracetic Acid VACUettes kit K-7904D, CHEMetrics, Inc., Midland, VA, USA). The pH of the 2% v/v Assist^{TM} (SA) and 5% v/v Citrilow Plus^{TM} (CP) solutions were got using a pH meter (Accumet^{R} AB150 pH/mV Fisher Scientific, USA). Average pH values were 0.93, and 0.66 for SA and CP diluted solutions, respectively. Distilled water was used as control in this study to determine reductions due to immersion and mechanical agitation.

For each meat type, per treatment, two 25-g of the inoculated samples were immersed in 4 °C solutions. For PAA and distilled water (control), the samples were immersed for 90 s, whereas for Citrilow Plus^{TM} and Assist^{TM}, samples were immersed for 30 s with agitation at 40 rpm in a shaker incubator (SHKE6000-7, Thermo Scientific, Marietta, OH, USA) for all treatments. A shorter dipping time was used for Citrilow Plus^{TM} and Assist^{TM} to minimize their

effect on the product quality. After immersion of samples, extra liquid was allowed to drip for 3 min prior to vacuum packing (Multivac C200, Multivac Inc., Kansas City, MO, USA). Treated samples were individually packaged, stored at 4 °C, and were used subsequently for microbial analysis.

3.3 Microbiological analysis

Chicken livers and hearts were aseptically removed from their packaging on days 0, 1, 2, and 3 post treatment. In each individual replication, for each meat type, two subsamples were analyzed on each day per treatment. Samples were weighed and placed in a sterile stomacher bag (Whirl-Pak®, Thomas Scientific LLC, Swedesboro, NJ, USA) then mixed with the corresponding amount of 0.1% buffered peptone water (BPW; Sigma-Aldrich, St. Louis, MO) using an automatic diluter (IUL Smart Dilutor, NEU-TEC GROUP, INC., Barcelona, Spain) to prepare a 1:10 dilution. Samples were then stomached at 200 rpm for 90 s (Stomacher® 400 Circulator, Seward Ltd., Bohemia, NY, USA). Decimal serial dilutions were performed, and duplicate plated onto XLD agar. Plates were incubated at 37 °C for 24 ± 2 h. After enumeration, *Salmonella* counts were reported as log CFU/g and reductions computed using the initial *Salmonella* count (pre-treatment) and the average count of the subsamples at a specific sampling timepoint. Non-inoculated chicken hearts and livers were treated as described above. APC on non-inoculated were enumerated on days 0, 1, 2, and 3 post-treatment. For each meat type, two subsamples from each treatment were plated on duplicate APC Petrifilm™ and incubated at 35±1 °C for 48 ± 3 h. Microbial counts were reported as log CFU/g.

3.4 Color evaluation

The same chicken heart and liver samples used for APC were tested for color prior to plating. Color measurements were conducted using a handheld portable colorimeter (Minolta CR-300 Chroma Meter with DP-301 Data Processor, Japan) and expressed as L* (lightness), a* (redness), and b* (yellowness). Calibration was initially performed by placing a standard

white Minolta calibration plate inside the same packaging bag used for the chicken liver and hearts to nullify the color and light reflectance properties of the packaging material (Petracci and Fletchert, 2002). Color measurements were taken at three different spots on the chicken liver and hearts' surface that were free from noticeable defects (e.g., bruises) and were averaged. Meat color measurements were recorded on days 0, 1, 2, and 3 post treatment.

3.5 Statistical analysis

For both chicken hearts and livers, three independent replications were performed for each set of treatments using freshly prepared bacterial cocktails and antimicrobial treatments. Data were analyzed using two-by-four factorial two-way analysis of variance (ANOVA) with treatment and time as independent variables and replications as block. When there was no interaction among variables, the main effects were analyzed. When there was significant difference ($p < 0.05$), Tukey-Kramer's post-hoc test was applied to separate means between treatments. Simple effect comparisons between treatments were further assessed when there was an interaction between the variables. All statistical analysis were conducted using SAS Version 9.4 (SAS Institute, Cary, NC, USA). Data analysis was carried out at a 95% confidence level.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 *Salmonella*

4.1.1 Chicken hearts

There was no significant interaction between the day of storage and the antimicrobial (df = 9,30; F = 1.01; $p = 0.4540$) thus the main effects were assessed for both parameters.

Immediately after treatment, SA was the only treatment with significantly ($p < 0.05$) higher *Salmonella* log reductions from the control (p = 0.0133) which implied that any of the other treatments aside the control, were comparable to both SA and the control (Table 4.1). However, on days 1 and 2, PAA was the treatment that showed significantly greater reduction from the control (p = 0.0013 and p = 0.0140 for day 1 and 2, respectively), which also implied that other treatments were not significantly different from the control. For the third day, all the treatment effects were marginalized.

Overall, PAA showed the greatest numerical reductions in the numbers of *Salmonella* in the chicken hearts, followed by Assist, Citrilow Plus and finally the control. However, these reductions were only significantly different from the control. PAA was expected to show the greatest reductions given that it has already been proven effective in reducing *Salmonella* in raw poultry products (Bauermeister et al., 2008). In addition, PAA's efficacy was compared to chlorine dioxide, another antimicrobial commonly used in poultry applications, and PAA consistently produced better results in multiple circumstances (Cano et al., 2021). Just like chlorine dioxide and other oxidizing agents, PAA is able to slow down the growth of microorganisms because of its low molecular weight, that allows the chemical to easily diffuse across the cell wall, disrupting its permeability, reacting with the cellular components (CDC, 2016), and causing cell lysis which releases all the cell components (Finnegan et al., 2010). These actions eventually result into protein denaturation and oxidation of all cellular

components (Block, 2011) which leads to cells death. The current study's results were similar to those of Moore et al., (2017) who in his evaluation of different antimicrobials realized highest reductions in *Salmonella* with a 10 s dip of 1000 ppm PAA solution. He reported 0.9 and 1.4 CFU/g log reductions on day zero and one respectively while using a concentration of 0.1% (1000 ppm) PAA. King et al., (2005) reported similar reports with PAA (15 s; temp. 45 °C or 55 °C) used at the same concentration in spray post-chill applications on beef carcass surfaces. The authors, however, did not report significant log reductions with PAA used at lower concentrations of 200 ppm and 600 ppm. This could have resulted though from the high temperatures of PAA solutions used (43 ± 5 °C) as compared to the 4 °C solutions used in this study. These results, however, contradict with those of Nagel et al., (2013) who reported 2.0 – 2.1 *Salmonella* log reductions with a 20 s dip of 0.04% (400 ppm) and 0.1 % (1000 ppm) PAA solution (temp. 4 ± 2 °C) in broiler carcasses, where the same concentrations also yielded 1.9 – 2.0 log reductions in *Campylobacter* spp.

SA was the second most effective treatment in decontaminating chicken hearts after PAA. This antimicrobial is a mixture of sulfuric acid (37 – 43%) and water (47 – 60%), with a very small concentration of sodium sulphate (0 – 7%). Overall, SA resulted into 1.29 - 1.48 CFU/g log reductions across the three days. PAA had higher but significantly similar log reductions (1.33 – 1.61 CFU/g) compared with SA for all the three days. Scott et al. (2015) reported log reductions of 0.8 – 0.9 log CFU/ml and 1.1 – 1.2 log CFU/ml with contact times of 10 s and 20 s, respectively, when using a sulfuric acid and sodium sulphate blend (SSS) at pH 1.1 on inoculated raw chicken wings. Other researchers who immersed beef cheek meat in SSS (pH 1.8) for 1, 2.5, or 5 min reported *Salmonella* log reductions ranging from 1.0 – 1.5 CFU/cm^2 (Schmidt et al., 2014). Geornaras et al. (2012) also conducted a study on beef trimmings using SSS (pH 1.2) with 30 s contacted time and found log reductions ranging from 0.5 – 0.7 CFU/cm^2. Overall, in this study, at a contact time of 30 s, higher *Salmonella* log

reductions were seen which indicated that SA at approximately pH 1, applied for 30 s may be an effective antimicrobial against *Salmonella* on chicken hearts. The effective activity of SA lies in the strong oxidative corrosive nature of sulfuric acid, which instantly kill microorganisms even with at a short exposure time (Wang et al., 2018). The resulting low pH of the solution (Scott et al., 2015) is an added advantage as this continues to inhibit microbial growth (Tan et al., 2014). Low pH has an adverse effect on the cell structure and function leading to increased permeability of the cell membrane which results into acidification of the cell contents (Lund et al., 2020).

CP also showed greater than 1.0 log reductions (1.0 CFU/g – 1.32 CFU/g) across the three days, although these were not different from the control, or other treatments. CP is a blend of hydrochloric acid and citric acid. Tan et al. (2014) reported widely varying reductions (2.92 CFU/g – 6.52 CFU/g) in *Salmonella* counts when using hydrochloric acid (HCl) at pH ranges of 1.2 – 3.8. However, they also noted that acetic acid (pH 3.8), lactic acid (pH 2.5), and citric acid (2.9) were more effective than HCl at inhibiting *Salmonella* on chicken meat surfaces. The higher *Salmonella* reductions of the organic acids studied (acetic > citric > lactic) were attributed to their dissociation constant, pKa values (acetic acid 4.74, lactic acid 3.86, citric acid 3.14) which are higher than that of HCl (pKa -7.0) (Birk et al., 2010) and this implied that there were more undissociated acids with the organic acids which influenced their bactericidal activity (Narendranath et al., 2001). The undissociated acid forms easily diffuse across the cell membrane leading to accumulation of toxic components in form of weak acid anions in the cytoplasm of the cells (Salmond et al., 1984; Ricke, 2003). Sorrells et al. (1989) had also reported higher individual microbial inhibition with acetic and lactic acids than citric and HCl. Citric acid is also a major component of CP, and its bactericidal activity is due to the action of weak acids (Davidson, 2001). Tamblyn & Conner (1997a) reported a 1.9 CFU/ml log reduction in the inoculated *Salmonella* with 4 % citric acid in a simulated poultry chiller tank (4 °C).

Laury et al., (2009) observed slightly higher *Salmonella* reductions on broiler carcass surfaces with a commercial lactic and citric acid blend, Chicxide (Birko Corp., Denver, CO), with immersion at 20 s contact time (2.3 CFU/g). The authors, however, reported lower reductions (1.3 CFU/ml) with a spray application of the same chemical at same concentration. As discussed above, one of the two mechanisms of activity of organic acids (OAs) is by accumulation of dissociated acid anions to toxic levels inside the cytoplasm of these cells (Tan et al., 2014). The other is cytoplasmic acidification that occurs due to altered membrane permeability allowing the protons to easily diffuse through the cell membrane (Mani-López et al., 2012; Brul & Coote, 1999), which distorts the normal activities of the cell, enzyme, and metabolic functions (Kim et al., 2013). In a bid to restore homeostasis, the microorganism diverts a lot of the energy for other cell functions like ATP (adenosine triphosphate**)** production, which eventually leads to energy depletion and cell death (Alvarado & McKee, 2007).

In the meat and poultry industry, an intervention is considered practical if its application results in at least a one-log reduction in the initial microbial population (Brashears & Chaves, 2017). The mean *Salmonella* reductions in chicken hearts after three days of storage were 1.33±0.25, 1.40±0.04 and 1.32±0.12 log CFU/g for PAA, SA, and CP, respectively (Table 4.1), hence meeting this criterion. More so, considering the recommended storage time for chicken giblets (for human consumption) under refrigeration temperatures (4 °C) being one to two days (FDA, 2018), all these treatments might be good antimicrobial interventions in the non-conventional raw chicken parts, more specifically the chicken hearts in this case.

4.1.2 Chicken livers

Like in chicken hearts, there was no significant interaction between the day of storage and the antimicrobial treatment (df = 9,30; F = 0.87; p = 0.5576). However, in this case, the only significant difference in treatments was seen on the second day, between PAA and the control (p = 0.0408) as shown in Table 4.2. Immediately, after treatment, none of the treatments

showed *Salmonella* reductions significantly different from the control (PAA: $p = 0.6238$, CP: $p = 0.5989$, SA: $p = 0.6731$). This meant that as much as PAA, SA and CP achieved higher than 1 log reductions (Table 4.2) in the *Salmonella* counts as compared to the initial (inoculated) counts, these were not significantly different from the control which implied that immersion of chicken livers in antimicrobial was as effective as washing with distilled water regarding *Salmonella* decontamination. Overall, PAA had the highest but non-significant numerical log reductions, followed by CP and SA.

However, after three days of storage, only PAA and SA, had *Salmonella* reductions of at least 1 log CFU/g, hence these are the only two interventions that may be suitable for use in the poultry industry specifically in chicken livers deemed for human consumption.

4.2 Aerobic Plate Count (APC)

4.2.1 Chicken hearts

As seen with the *Salmonella* challenge study, there was no significant interaction between the day of storage and the antimicrobial treatment ($F = 0.61$, $df = 9,30$, $p = 0.7777$) and therefore the main effects of treatment and day were assessed.

APC counts in chicken hearts when using the different antimicrobials are shown in Table 4.3. Immediately after treatment (Day 0), no significant differences were observed among the treatments. However, after 48 h (Day 2), the APC counts in chicken hearts treated with PAA ($p = 0.0056$) and SA ($p = 0.0123$) were significantly lower than the control. After 72 h (Day 3), only APC counts in chicken hearts treated with SA were significantly lower than the control ($p = 0.0136$). From the data, all the treatments including the control were able maintain the APC counts far below the upper microbiological limit for quality fresh poultry. The International Commission on Microbiological Specifications for Foods (ICMSF) states this value at 5.70 log CFU/g (ICMSF, 1986) beyond which the meat would be considered spoilt. Other researchers have also studied the effect of various antimicrobials on APC and

found that they are relatively effective. Mohan & Pohlman (2016) observed reductions in beef trimmings APC as compared to the background microflora when using PAA and various organic acids in which citric acid was among. Mani-López et al. (2012) reported significant log reductions in APC on pork cheek meat with 1% solutions of acetic and lactic acids.

APC were compared as storage time increased, however, the data showed no significant differences in the counts from the time of treatment (Day 0) to the third day of storage for each of the treatments (PAA: p = 0.9513, SA: p = 0.9998, CP: p = 0.9939, and Control: p = 0.7857). This implied that all treatments slowed down the growth of the aerobic mesophilic bacteria at refrigeration temperatures (4 °C). Some researchers have studied the effect of storage temperatures on the growth at aerobic mesophilic microorganisms and observed that refrigeration and freezing (Fahim, 2020) slow down microbial growth. Jung et al. (2019) observed at least a 1.0 CFU/g log reduction in APC with storage of chicken livers at 4 °C and -20 °C without any antimicrobial treatment.

4.2.2 Chicken livers

Just like in chicken hearts, there was no Treatment*Day interaction (F = 1.34, df = 9,30, p = 0.2588) between the antimicrobial and the day of storage, therefore the main effects were assessed.

Table 4.4 shows the APC using different antimicrobial interventions. Immediately after treatment (Day 0), there were no significant differences observed among the treatments. However, after 48 h (day 2), APC in chicken livers treated with PAA were significantly lower than those of both the SA (p = 0.0265) and the control (p = 0.0074). According to the data, however, all the treatments maintained APC below the spoilage levels for all the three days of storage in this study.

On the other hand, comparing the APC with increasing days of storage, no significant differences were observed from the time of treatment (Day 0) to the third day of storage for all

the treatments (PAA: p = 0.9979, SA: p = 0.8880, CP: p = 0.7427, and Control: p = 0.9644). Like in chicken hearts, this implied that all treatments were effective at slowing down the growth of the aerobic mesophilic bacteria.

4.3 Meat Color

4.3.1 Chicken hearts

Tables 4.5, 4.6, 4.7 show the effect of the antimicrobials on the lightness (L*), redness (a*), and yellowness (b*). There was a treatment by day interaction observed for yellowness (b*: F = 2.36, df = 9,30, p = 0.0377), but not for redness (a*: F = 0.96, df = 9,30, p = 0.4922) and lightness (L*: F = 1.46, df = 9,30, p = 0.2090). For redness (a*), significant differences were observed immediately after treatment for SA and CP, but not PAA, as meat samples were less red than when compared to the control. The values for SA (p = 0.0022) and CP (p = 0.0061) were also significantly less than PAA. However, by the third day of storage, the redness values of SA were comparable (p = 0.1217) to those of PAA and only values of CP-treated chicken hearts were still significantly less than PAA. Values for the yellowness (b*), were very similar to those of the redness (a*), in that only PAA had no significant difference with the control for the three days of refrigerated storage (0 h: p = 0.9954, 24 h: p = 0.1565, 48 h: p = 0.1801, and 72 h p = 0.6009). Regarding the lightness (L*) values, SA (0 h: p = 0.0001) and CP (0 h: p = 0.0004) but not PAA (0 h: p = 0.7082) showed significant differences from the control immediately after treatment (Day 0), however, all differences became marginal by the third day of refrigerated storage (PAA: p = 0.9982, SA: p = 0.1792, CP: p = 0.076), which showed that the initial treatments effects on the product lightness were only temporary. In addition, the differences in chicken hearts treated with various antimicrobials were visibly noticeable immediately after treatment (Figure 4.1), however, not a whole lot after the third day.

4.3.2 Chicken livers

Tables 4.8, 4.9, 4.10 and Figure 4.1 represent color changes in chicken hearts during the three days in storage. There was a small interaction between the antimicrobial and day of storage for redness ($p = 0.0215$) and lightness ($p = 0.0460$), but not yellowness ($p = 0.1300$). Just like in chicken hearts, for redness (a*), significant differences were observed immediately after treatment (Day 0) for SA ($p < 0.0001$) and CP ($p < 0.0001$) but not PAA ($p = 0.6166$), as compared to the control, where the SA and CP-treated chicken livers had lower a* values indicating that the livers were less red. However, all differences had marginalized (PAA: $p = 9560$, SA: $p = 0.1321$, CP: $p = 0.2071$) by the third day of refrigerated storage. The yellowness (b*) and redness (a*) values were very similar as the differences observed in SA and CP-treated meat samples immediately after treatment were no longer significant on the third of storage. The SA and CP-treated livers were more yellow immediately after treatment (greater b* values), but this difference decreased on the second day and no longer evident on the third day of refrigerated storage. Regarding the lightness (L*) values, the only significant differences observed were immediately after treatment (Day 0), whereby SA values were significantly different from PAA ($p = 0.0130$), and the control ($p = 0.0010$). However, all these differences were marginal after 24 h (PAA: $p = 0.9754$, CP: $p = 0.0569$, Control: $p = 0.8716$). This implied that the color changes in product lightness due to SA application were only temporary. Just like in the chicken hearts, differences in the lightness of meat samples were only different immediately after treatment, but not by the second and third day of refrigerated storage. This may be due to the strong acid composition (HCL) in the antimicrobial.

Some researchers have observed similar temporary color effects in other chicken parts when using antimicrobials containing PAA or sulfuric acid. Scott et al. (2015) also reported greater b* values immediately after (Day 0), where treating chicken wings with an antimicrobial blend containing sulfuric acid and sodium sulphate. The color differences were

no longer significant after 24 h of storage at 4 °C. Similar color changes were reported by Bauermeister et al. (2008) when they evaluated the effect of various levels (100 ppm and 200 ppm) of PAA on chicken carcasses. The researchers detected small differences in a* and b* values after 24 h of storage, however no differences in the L* values at that same timepoint.

4.4 Conclusions and Recommendations

This study was aimed at evaluating the effectiveness of peroxyacetic acid, Assist™ and Citrilow Plus™ against *Salmonella* on inoculated chicken hearts and livers. The differences in *Salmonella* reductions due to immersion of chicken livers in PAA, SA, or CP were not significant as compared to immersion in distilled water immediately after treatment. PAA reductions became significant on the second day of treatment, but these reductions were not different from those of SA or CP, indicating that these three antimicrobials have relatively similar effectiveness against *Salmonella*. Contrary to chicken livers, *Salmonella* reductions observed when chicken hearts were immersed in SA showed significant differences immediately after treatment as compared to immersion in distilled water. Overall, all antimicrobials achieved greater than one-log reductions in the *Salmonella* on both chicken hearts and livers, which was not the same when distilled water was used. In addition, all treatments were effective in minimizing the growth of aerobic mesophilic microflora throughout the three days of storage. Moreover, no significant differences in L*, a*, or b* values were observed on the third day of storage in both chicken meat products that would alter visual quality of the products. The results of this study indicated that SA and CP as immersion treatments at approximately pH 1 may be effective antimicrobial interventions in chicken hearts and livers. When effects of SA and CP treatments were compared to PAA, these performed at least equally and thus may be used in the poultry industry as part of a multiple hurdle system to reduce *Salmonella* in non-conventional chicken parts.

4.5 References

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Tables

Table 4.1 Reduction of *Salmonella* (log CFU/g) in chicken hearts treated with different antimicrobials after three days of storage at 4°C.

Storage time (days)	Log Reduction in *Salmonella* spp. (Mean ± SE) *			
	Distilled water (control)	PAA	SA	CP
0	0.95 ± 0.03 ^{b,x}	1.34 ± 0.04 ^{a,b,x}	1.48 ± 0.10 ^{a,x}	1.07 ± 0.19 ^{a,b,x}
1	0.94 ± 0.15 ^{b,x}	1.61 ± 0.13 ^{a,x}	1.29 ± 0.09 ^{a,b,x}	1.27 ± 0.19 ^{a,b,x}
2	0.96 ± 0.08 ^{b,x}	1.49 ± 0.10 ^{a,x}	1.35 ± 0.09 ^{a,b,x}	1.05 ± 0.11 ^{a,b,x}
3	0.98 ± 0.07 ^{a,x}	1.33 ± 0.25 ^{a,x}	1.40 ± 0.04 ^{a,x}	1.32 ± 0.12 ^{a,x}

abcLeast squares means within a row without common superscripts are different $p < 0.05$.
xyzLeast squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peroxyacetic acid; CP= 5.0 % citric and hydrochloric acid blend (Citrilow Plus™); SA= 2.0 % sulfuric acid (Assist™).

Table 4. 2 Reduction of *Salmonella* (log CFU/g) in chicken livers treated with different antimicrobials after three days of storage at 4°C.

Storage time (days)	Log Reduction in *Salmonella* spp. (Mean ± SE) *			
	Distilled water (control)	PAA	SA	CP
0	0.83 ± 0.15 ^{a,x}	1.03 ± 0.12 ^{a,x}	1.02 ± 0.15 ^{a,x}	1.04 ± 0.15 ^{a,x}
1	0.87 ± 0.16 ^{a,x}	1.08 ± 0.11 ^{a,x}	0.92 ± 0.18 ^{a,x}	0.94 ± 0.07 ^{a,x}
2	0.82 ± 0.08 ^{b,x}	1.28 ± 0.15 ^{a,x}	0.92 ± 0.10 ^{a,b,x}	1.17 ± 0.11 ^{a,b,x}
3	1.10 ± 0.13 ^{a,x}	1.10 ± 0.12 ^{a,x}	1.09 ± 0.19 ^{a,x}	0.96 ± 0.27 ^{a,x}

abcLeast squares means within a row without common superscripts are different $p < 0.05$.
xyzLeast squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peroxyacetic acid; CP= 5.0 % citric and hydrochloric acid blend (Citrilow Plus™); SA= 2.0 % sulfuric acid (Assist™).

Table 4.3 Aerobic plate count (APC) (log CFU/g) in chicken hearts treated with different antimicrobials and stored for three days at 4 °C.

Storage time (days)	Aerobic plate counts (log CFU/g) (Mean ± SE) *			
	Distilled water (control)	PAA	SA	CP
0	4.05 ± 0.17 ^{a,x}	3.51 ± 0.04 ^{a,x}	3.46 ± 0.14 ^{a,x}	3.78 ± 0.13 ^{a,x}
1	3.81 ± 0.22 ^{a,x}	3.33 ± 0.16 ^{a,x}	3.61 ± 0.04 ^{a,x}	3.57 ± 0.15 ^{a,x}
2	4.33 ± 0.34 ^{a,x}	3.44 ± 0.11 ^{b,x}	3.52 ± 0.12 ^{b,x}	3.82 ± 0.09 ^{a,b,x}
3	4.28 ± 0.26 ^{a,x}	3.64 ± 0.36 ^{a,b,x}	3.48 ± 0.11 ^{b,x}	3.72 ± 0.10 ^{a,b,x}

abcLeast squares means within a row without common superscripts are different $p < 0.05$.
xyzLeast squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peroxyacetic acid; CP= 5.0 % citric and hydrochloric acid blend (Citrilow Plus™); SA= 2.0 % sulfuric acid (Assist™).

Table 4.4 Aerobic plate count (APC) (log CFU/g) in chicken livers treated with different antimicrobials and stored for three days at 4 °C.

Storage time (days)	Aerobic plate counts (log CFU/g) (Mean ± SE) *			
	Distilled water (control)	PAA	SA	CP
0	3.83 ± 0.08 ^{a,x}	3.75 ± 0.09 ^{a,x}	3.78 ± 0.26 ^{a,x}	3.77 ± 0.09 ^{a,x}
1	3.86 ± 0.13 ^{a,x}	3.68 ± 0.12 ^{a,x}	3.86 ± 0.14 ^{a,x}	3.73 ± 0.08 ^{a,x}
2	3.88 ± 0.06 ^{a,x}	3.36 ± 0.04 ^{b,x}	3.81 ± 0.03 ^{a,x}	3.63 ± 0.05 ^{a,b,x}
3	3.76 ± 0.03 ^{a,x}	3.72 ± 0.06 ^{a,x}	3.67 ± 0.13 ^{a,x}	3.92 ± 0.11 ^{a,x}

abcLeast squares means within a row without common superscripts are different $p < 0.05$.
xyzLeast squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peroxyacetic acid; CP= 5.0 % citric and hydrochloric acid blend (Citrilow Plus™); SA= 2.0 % sulfuric acid (Assist™).

Table 4.5 Redness (a*) measurements of raw chicken hearts treated with different antimicrobials and stored for three days at 4 °C.

Storage time (days)	Redness, a* (Mean ± SE) *			
	Distilled water (control)	PAA	ASS	CP
0	11.42 ± 1.03 ^{a,y}	11.20 ± 1.01 ^{a,y}	8.55 ± 0.43 ^{b,y}	8.80 ± 0.88 ^{b,z}
1	14.49 ± 0.36 ^{a,x}	12.33 ± 0.74 ^{b,x,y}	11.32 ± 0.48 ^{b,c,x}	10.18 ± 0.75 ^{c,y,z}
2	14.49 ± 0.14 ^{a,x}	13.55 ± 0.63 ^{a,b,x}	12.04 ± 0.29 ^{b,x}	12.07 ± 0.33 ^{b,x}
3	14.60 ± 0.24 ^{a,x}	13.76 ± 0.51 ^{a,b,x}	12.23 ± 0.31 ^{b,c,x}	11.93 ± 0.16 ^{c,x,y}

abcLeast squares means within a row without common superscripts are different $p < 0.05$.
xyzLeast squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peroxyacetic acid; CP= 5.0 % citric and hydrochloric acid blend (Citrilow Plus™); SA= 2.0 % sulfuric acid (Assist™).

Table 4.6 Yellowness (b*) measurements of raw chicken hearts treated with different antimicrobials and stored for three days at 4 °C.

Storage time (days)	Yellowness, b* (Mean ± SE) *			
	Distilled water (control)	PAA	SA	CP
0	3.37 ± 1.21 ^{b,x}	3.22 ± 0.71 ^{b,x}	5.49 ± 0.44 ^{a,x}	6.27 ± 0.25 ^{a,x}
1	1.72 ± 0.13 ^{c,x,y}	3.13 ± 0.79 ^{c,x}	5.02 ± 0.05 ^{b,x}	7.12 ± 0.68 ^{a,x}
2	1.28 ± 0.44 ^{c,y}	2.65 ± 0.21 ^{c,x}	6.29 ± 0.57 ^{b,x}	6.50 ± 0.02 ^{a,x}
3	1.65 ± 0.18 ^{b,x,y}	1.86 ± 0.13 ^{b,x}	6.10 ± 0.27 ^{a,x}	6.61 ± 0.11 ^{a,x}

abcLeast squares means within a row without common superscripts are different $p < 0.05$.
xyzLeast squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peroxyacetic acid; CP= 5.0 % citric and hydrochloric acid blend (Citrilow Plus™); SA= 2.0 % sulfuric acid (Assist™).

Table 4.7 Lightness (L*) measurements of raw chicken hearts treated with different antimicrobials and stored for three days at 4 °C.

Storage time (days)	Lightness, L* (Mean ± SE) *			
	Distilled water (control)	PAA	SA	CP
0	42.03 ± 0.60 ^{b,x}	40.80 ± 0.91 ^{b,x}	47.79 ± 0.93 ^{a,x}	47.30 ± 1.34 ^{a,x}
1	41.63 ± 2.13 ^{b,x}	42.87 ± 0.45 ^{b,x}	46.57 ± 1.83 ^{a,x}	47.89 ± 1.37 ^{a,x}
2	41.28 ± 0.81 ^{b,x}	41.67 ± 1.19 ^{b,x}	45.97 ± 1.06 ^{a,x}	45.93 ± 0.58 ^{a,x}
3	42.72 ± 0.65 ^{a,x}	42.53 ± 1.80 ^{a,x}	45.11 ± 1.53 ^{a,x}	45.60 ± 1.34 ^{a,x}

abcLeast squares means within a row without common superscripts are different $p < 0.05$.
xyzLeast squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peroxyacetic acid; CP= 5.0 % citric and hydrochloric acid blend (Citrilow Plus™); SA= 2.0 % sulfuric acid (Assist™).

Table 4.8 Redness (a*) measurements of raw chicken livers treated with different antimicrobials and stored for three days at 4 °C.

Storage time (days)	Redness, a* (Mean ± SE) *			
	Distilled water (control)	PAA	SA	CP
0	15.97 ± 0.72 ^{a,x}	14.49 ± 1.09 ^{a,x}	8.32 ± 2.62 ^{b,y}	9.24 ± 1.10 ^{b,y}
1	15.81 ± 0.89 ^{a,x}	16.53 ± 0.88 ^{a,x}	14.58 ± 0.52 ^{a,x}	13.95 ± 1.06 ^{a,x}
2	17.96 ± 0.80 ^{a,x}	15.71 ± 0.12 ^{a,b,x}	14.17 ± 0.58 ^{b,x}	15.15 ± 0.29 ^{a,b,x}
3	17.25 ± 0.90 ^{a,x}	16.63 ± 0.32 ^{a,x}	14.52 ± 0.64 ^{a,x}	14.82 ± 0.59 ^{a,x}

abcLeast squares means within a row without common superscripts are different $p < 0.05$.
xyzLeast squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peroxyacetic acid; CP= 5.0 % citric and hydrochloric acid blend (Citrilow Plus™); SA= 2.0 % sulfuric acid (Assist™).

Table 4.9 Yellowness (b*) measurements of raw chicken livers treated with different antimicrobials and stored for three days at 4 °C.

Storage time (days)	Yellowness, b* (Mean ± SE) *			
	Distilled water (control)	PAA	SA	CP
0	6.39 ± 1.24 ^{c,x}	7.70 ± 0.56 ^{b,c,x}	10.87 ± 1.16 ^{a,x}	9.90 ± 1.26 ^{a,b,x}
1	6.35 ± 0.75 ^{a,x}	6.45 ± 0.78 ^{a,x}	7.14 ± 0.29 ^{a,y}	6.79 ± 0.49 ^{a,y}
2	6.48 ± 0.43 ^{a,b,x}	5.04 ± 0.53 ^{b,x}	8.12 ± 0.76 ^{a,y}	6.67 ± 0.19 ^{a,b,y}
3	5.49 ± 0.54 ^{a,x}	6.90 ± 0.33 ^{a,x}	7.33 ± 1.32 ^{a,y}	6.85 ± 0.19 ^{a,y}

abcLeast squares means within a row without common superscripts are different $p < 0.05$.
xyzLeast squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peroxyacetic acid; CP= 5.0 % citric and hydrochloric acid blend (Citrilow Plus™); SA= 2.0 % sulfuric acid (Assist™).

Table 4.10 Lightness (L*) measurements of raw chicken livers treated with different antimicrobials and stored for three days at 4 °C.

Storage time (days)	Lightness, L* (Mean ± SE) *			
	Distilled water (control)	PAA	SA	CP
0	43.65 ± 2.92 ^{b,x}	45.51 ± 0.87 ^{b,x}	51.72 ± 1.81 ^{a,x}	46.90 ± 2.16 ^{a,b,x}
1	41.95 ± 1.87 ^{a,x}	42.60 ± 1.13 ^{a,x}	43.39 ± 1.06 ^{a,y}	38.37 ± 1.07 ^{a,y}
2	43.53 ± 0.61 ^{a,x}	40.43 ± 2.07 ^{a,x}	43.67 ± 1.47 ^{a,y}	40.09 ± 0.76 ^{a,y}
3	41.86 ± 1.30 ^{a,x}	43.91 ± 0.56 ^{a,x}	42.72 ± 1.08 ^{a,y}	40.48 ± 0.44 ^{a,y}

abcLeast squares means within a row without common superscripts are different $p < 0.05$.
xyzLeast squares means within a column without common superscripts are different $p < 0.05$.
*Abbreviations: standard error (SE); PAA= 450 ppm peroxyacetic acid; CP= 5.0 % citric and hydrochloric acid blend (Citrilow Plus™); SA= 2.0 % sulfuric acid (Assist™).

Figures

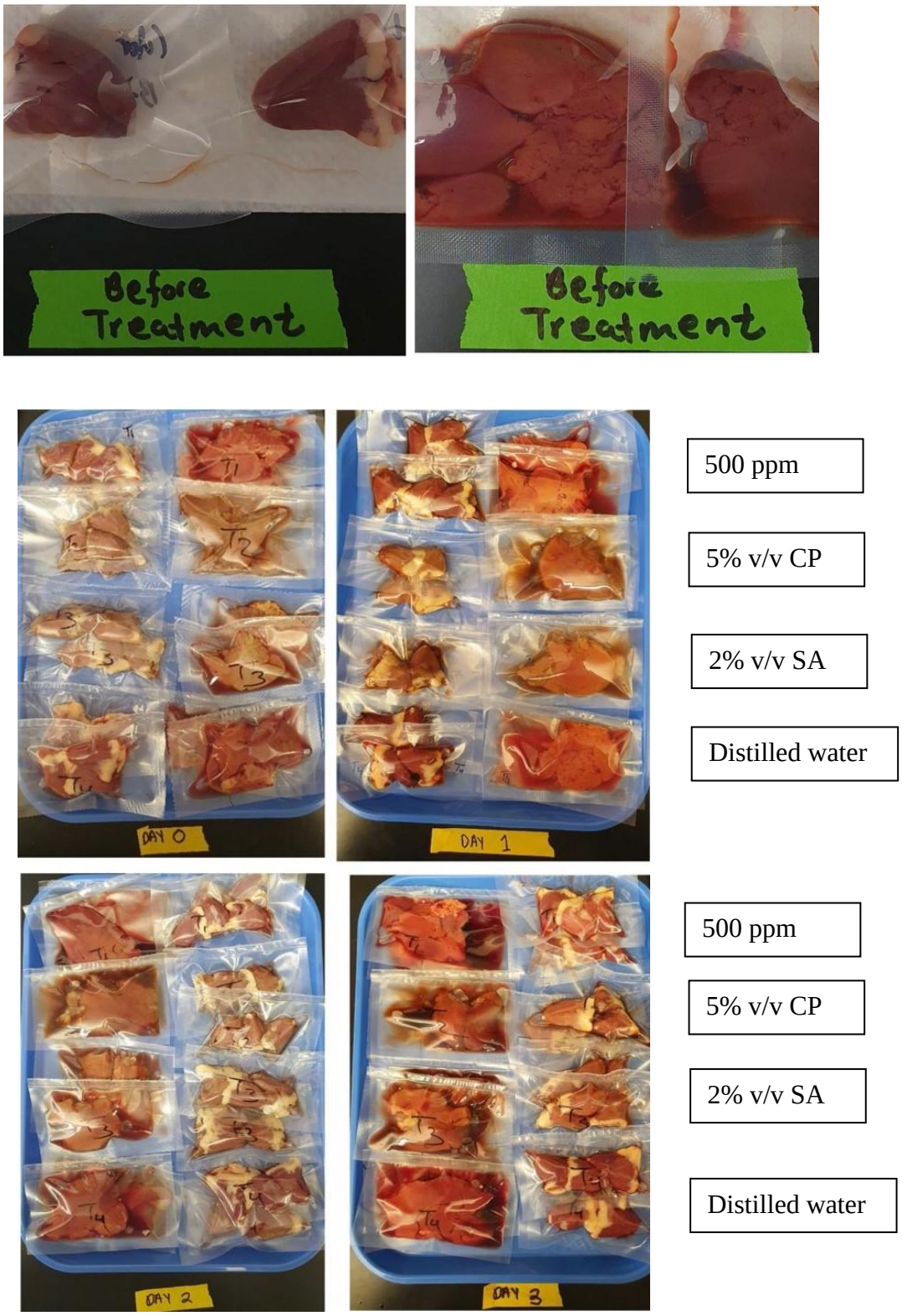

Figure 4. 1. Chicken hearts and livers before and after treatment with 500 ppm PAA, 5 % CP, 2 % SA and distilled water, after 0 h, 24 h, 48 h, and 72 h of storage at 4 °C.

CHAPTER 5. CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

The overall goal of this thesis was to evaluate the efficacy of various antimicrobials: peroxyacetic acid, Citrilow Plus^{TM} (a commercial blend of citric and hydrochloric acid), and Assist (a sulfuric acid product) in reducing *Salmonella* artificially inoculated in chicken hearts and livers. *Salmonella*-inoculated chicken hearts and livers were immersed in different treatments with agitation to mimic what would happen in an actual food industry set up. No significant differences were observed between the antimicrobials and the control in chicken hearts. More so, the significant *Salmonella* reductions observed in SA-treated chicken livers immediately after treatment were no longer different after 24 h. Nevertheless, the antimicrobial treatments resulted into *Salmonella* reductions that were higher than one log CFU/g for all the three days in storage, which wasn't the case when distilled water was used. This showed that it was more effective to minimize *Salmonella* using antimicrobials rather than just water, however, multiple hurdles would be needed to achieve higher reductions. As part of this study, the efficacy of these antimicrobials in reducing the growth of aerobic mesophilic bacteria was analyzed and all treatments consistently minimized the growth. This showed that these antimicrobials can potentially extend the shelf life of chicken hearts and livers by prolonging the lag phase (Mohan et al., 2016) of spoilage microorganisms. Only three days were considered in this study as it is not recommended to store chicken giblets for longer than two days at refrigeration temperatures (4 °C). The effect of these antimicrobials on the redness (a*), yellowness (b*), and lightness (L*) of both chicken hearts and livers was evaluated and all the color changes due to the treatments was no longer evident by the second or third day in storage. Overall, both SA, and CP, had comparable effectiveness with PAA, which is more popular in the food industry, and whose effectiveness (Cano et., 2021) has also been priorly proven.

5.2 Suggestions for Future Work

In this study, fixed pH ranges for both CP and SA were used, however, it might be worthwhile to explore various pH levels to compare and understand the effectiveness of these chemicals better. Studying the effectiveness over a longer duration of time may also provide more information if these can consistently control growth of aerobic mesophilic bacteria for the purpose of other food industry applications like pet food that require longer shelf life of variety meats in refrigerated storage conditions.

In addition, research can be conducted to evaluate the effectiveness of these chemicals against *Camphylobacter* spp. another pathogen with high prevalence rates in poultry, as this would provide useful knowledge to the food industry. Studies in other food-borne pathogens in beef and pork matrices would also be worthwhile.

Given that SA showed the second highest numerical *Salmonella* reductions overall, a possible synergistic effect between SA and organic acids like acetic or lactic acid, could potentially yield better results as research has already shown how important these organic acids are in controlling various microorganisms in the poultry industry.

Finally, ascorbic acid could be integrated in the immersion solutions when using these chemicals to help in color retention of the chicken livers and hearts, as this would make the products more appealing to the consumers (Nam et al., 2003).

5.3 References

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