AN ALTERNATIVE ANTIMICROBIAL COMMERCIAL EGG WASHING PROCEDURE

by

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(Under the Direction of Mark Harrison)

ABSTRACT

Four washes were evaluated for effect on egg quality during extended cold storage and *Salmonella* reduction: pH 11 at 48.9°C (industry standard); pH 11 at ambient temperature (~20°C); pH 6 at 48.9°C; and pH 6 at ambient temperature. pH 11 washes contained potassium hydroxide-based detergent and pH 6 washes contained approximately 200 ppm chlorine and a proprietary chlorine-stabilizer. Wash treatments affected shell color and Haugh unit measurements. Vitelline membrane strength and elasticity decreased and whole egg total solids increased over 12-week storage time, but were not affected by treatment. Neither storage time nor treatment effected shell strength or stiffness. Reduction in inoculated *Salmonella* (0.77 log CFU/mL shell emulsion) was not different between treatments. Ambient temperature washes did not have a profound effect on egg quality or *Salmonella* reduction compared to standard warm water wash and may be a viable option to reduce cost, increase shelf life, and slow pathogen growth.

INDEX WORDS: commercial shell eggs, *Salmonella*, SmartWash, chlorine, chlorine-stabilizer, eggshell, egg wash, egg quality

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CHAPTER 1

INTRODUCTION

Eggs are the causative agent in almost 180,000 bacterial foodborne illnesses annually in the United States *(57)*. Shell eggs can be contaminated with *Salmonella* spp. by vertical or horizontal transmission. Vertical transfer occurs directly from the hen, e.g., via the transovarian route, while horizontal transfer occurs via shell contamination and penetration *(11)*. In the U.S., most eggs are required to be washed for the purposes of physically cleaning and to reduce shell contamination. While the washing process may reduce pathogen and spoilage organism populations on the eggshell, it will not provide any antimicrobial influence on organisms inside the egg. Therefore, to prevent foodborne illnesses associated with eggs and increase shelf life, it is necessary to take steps prior to washing to prevent vertical contamination and to wash eggs properly to prevent surface contamination from being internalized through the shell.

In the U.S., the commercial egg washing process currently consists of four steps: wetting, washing, rinsing, and drying. Wetting involves a light spray of warm water to moisten and prepare debris for removal. For the washing step, a wash solution containing an alkaline detergent at 32°C or higher (typically at least 11.1°C warmer than the internal egg temperature) with an approximate pH of 11 is sprayed on the eggs while they are being mechanically cleaned with rotating brushes. In contrast to temperature requirements, there are no pH requirements required by regulation. Next, eggs are sprayed with a final sanitizing rinse, usually containing chlorine at 100-200 ppm and at temperatures at least as warm as or warmer than the wash water

temperature. Lastly, eggs are dried using jet dryers *(30, 53)*. After washing, eggs are stored and transported at 7.2°C *(61)*.

While washing removes much of the visible debris, it is doubtful there is much available chlorine present when the final chlorine rinse is applied to a warm eggshell still wet from a pH 11 wash treatment. Due to the fact that chlorine solutions are most active as an antimicrobial at lower pH values and lower temperatures, it is unlikely that substantial free chlorine is available to act as an antimicrobial agent against pathogens like *Salmonella* spp. at pH 11 *(5)*. Nevertheless, regulations require an antimicrobial treatment (such as chlorine) as part of the final rinse solutions *(63)*. There are questions as to the overall effectiveness of this strategy.

In addition, eggs are warmed during washing, which creates a problem when trying to cool and store them. It has been shown that eggs washed in warm water may take up to or longer than five days to cool to proper refrigeration temperatures *(13, 46)*. This gives pathogens and spoilage organisms ample conditions and time to proliferate. There is interest in using ambient temperature washes or cryogenic cooling techniques to combat this issue and lower production costs associated with heating water and cooling eggs *(6, 8, 12, 38, 39, 40, 46)*. Currently in the U.S., ambient temperature egg washing is prohibited *(63)*.

This study was conducted to examine the effects of four different egg washing procedures on egg quality and *Salmonella* reduction: pH 11 at 48.9°C (to represent the currently used industry process); pH 11 at ambient temperature; pH 6 with approximately 200 ppm chlorine and a proprietary chlorine-stabilizer at 48.9°C; and pH 6 with approximately 200 ppm chlorine and a proprietary chlorine-stabilizer at ambient temperature. The four washes were also evaluated to determine *Salmonella* survival in the wash water and potential for cross-contamination.

CHAPTER 2

LITERATURE REVIEW

Shell Eggs

In 2012, 223.7 million cases, or about 80.5 billion eggs, were produced in the United States. Of these, 55.3% were sent to retail as table eggs, while 31.9% were sent to breaking facilities, 9% were for institutional use, and 3.8% exported *(3)*. Per capita egg consumption in the U.S. in 2012 was 249.7 eggs (including both shell eggs and egg products, like liquid, frozen, or dried eggs), with 173.8 being the per capita consumption for shell eggs only *(2)*.

Essentially, a shell egg consists of a shell and the internal egg contents. The eggshells are typically white or brown in color and are mostly made up of calcium carbonate. In between the shell and the egg contents are two membranes (inner and outer membranes). An air pocket, known as the air cell, forms between these two membranes at one end of the egg due to shrinking of egg contents that occurs when eggs cool after being laid. Egg contents include both albumen and yolk *(4)*. The albumen makes up about 60% of the egg's weight, while the yolk makes up about 30 to 33% *(60)*. Whole egg contents are composed of 12.8 to 13.4% protein, 10.5 to 11.8% lipids, 0.3 to 1.0% carbohydrates, and 0.8 to1.0% ash *(45)*.

Albumen contains four separate layers (listed from the inside out): chalaziferous or inner thick, inner thin, outer thick, and outer thin *(60)*. When an egg is broken out, thick albumen is more elevated and spreads less than thin albumen. Another constituent of the albumen is the chalazae, which is seen as thick filaments of albumen that function to hold the yolk in place in the center of the egg *(4)*. Albumen is composed of approximately 9.7 to 10.6% protein, 0.03%

lipids, 0.4 to 0.9% carbohydrates, and 0.5 to 0.6% ash *(45)*. The yellow center of the egg is the yolk and contains most of the vitamins, minerals, and fat present in an egg *(4)*. The breakdown of yolk constituents is approximately 15.7 to 16.6% protein, 31.8 to 35.5% lipids, 0.2 to 1.0% carbohydrates, and 1.1% ash *(45)*. The yolk is enveloped inside the vitelline membrane *(4)*.

Table eggs are regulated by two U.S. federal government agencies. Overall, the Food and Drug Administration (FDA) is responsible for shell eggs, while the United States Department of Agriculture (USDA) is responsible for egg products (including liquid, frozen, and dehydrated eggs) *(62)*. The FDA implemented the Egg Safety Rule in 2009 with the purpose of decreasing safety risks associated with *S*. Enteritidis in eggs through pest control, control of flock health, microbial testing, production facility cleaning and sanitation, recordkeeping, and refrigeration of eggs *(21)*. The FDA also inspects egg-producing facilities. The USDA Food Safety and Inspection Service (FSIS) is responsible for imported eggs, verifies that egg packages have the "keep refrigerated" label, ensures that eggs are transported and stored at the proper temperatures, and develops consumer education programs *(23)*. The USDA Agricultural Research Service (ARS) conducts food safety research. The Egg Safety and Quality Research Unit (ESQRU) was established within the ARS in 2005 specifically to advance egg safety research *(62)*. In addition to federal regulation, all 50 states have their own egg rules and regulations, including the period of time permitted for retail marketing of eggs *(33, 37)*.

Egg grading is voluntary; processors that have their eggs graded pay for this service provided by the USDA Agricultural Marketing Service (AMS) *(63)*. There are three grades available for table eggs that are determined by interior quality of the egg and the appearance and condition of the eggshell: U.S. Grade AA, A, and B. Grade AA eggs are characterized by a thick and firm albumen; a high, round, and defect-free yolk; and a clean and unbroken shell. Grade A

eggs basically have the same characteristics of a Grade AA, with the difference being that the albumen is “reasonably” firm. In contrast, Grade B eggs have a thinner, weak, and watery albumen; wider and flatter yolks; and shells that may have stains but are still unbroken. Grade B eggs are typically used to make liquid, frozen, and dried products; they are seldom sold as table eggs *(62)*. Air cell depth is also a factor used to determine egg grade. The size of air cells permitted for AA, A, and B eggs are 1/8 in. (3.2 mm), 3/16 in. (4.8 mm), and no limit, respectively *(61)*.

Table eggs are weighed to determine size. Size or weight class is determined by a minimum net weight of a dozen eggs. The different weight classes, from larger to smaller are jumbo, extra large, large, medium, small, and peewee with 30 (850.5 g), 27 (765.4 g), 24 (680.4 g), 21 (595.3 g), 18 (510.3 g), and 15 (425.2 g) ounces as minimum weights for a dozen eggs, respectively *(62)*.

Current Shell Egg Processing Procedures

The typical commercial egg washing process consists of four stages: wetting, washing, rinsing, and drying. The optional wetting step consists of a light spray of warm (approximately 40°C) water, which softens debris on the eggs for easier removal. Next, the washing step involves a series of spray nozzles that spray eggs with an alkali detergent at a pH of approximately 11 along with rotating brushes to physically remove debris. The wash solution must be 32°C or warmer. The washer may even contain two to three different zones with increasing temperatures. There is also typically a recycling series in place where water from the later stages of washing is re-used in earlier stages or in the wetting stage. After washing, eggs go through a final rinse spray with a sanitizing chemical. This is normally chlorine-based at neutral pH and a concentration of 100-200 ppm available chlorine and the hottest part of the process that

can be up to 60°C. Overflow egg wash water (EWW) and final rinse water are collected and pooled, filtered through a mesh screen, collected in a recirculation tank, reheated, and recycled through the system *(30, 54)*. Wash water is discarded and replaced at least every 4 hours *(50)*. Lastly, drying is accomplished using air jets *(30)*.

Minimum facility and operating requirements for shell egg grading and packing plants are put forth in 7 CFR Part 56.76 *(63)*. The following egg washing guidelines have been set forth by the USDA in the "Egg-Grading Manual" based on those regulations:

1. Wash eggs with water at least 20°F (11.1°C) warmer than the internal temperature of the eggs and at a minimum of 90°F (32°C).
2. Select a detergent or detergent sanitizer that is compatible with the wash water and one that will not give off foreign odors that may be imparted to the egg.
3. Use only potable water with an iron content of less than 2 parts per million for washing and keep wash water as clean as possible.
4. Rinse by spraying with water slightly warmer than the wash water.
5. Use an approved sanitizer in the spray rinse.
6. Dry the eggs to remove any excess moisture prior to packaging *(61)*.

The manual also states that the sanitizer used in the warm water spray should contain between 100 and 200 ppm available chlorine or its equivalent *(61, 63)*.

After washing, eggs must be stored and transported at or below a temperature of 7.2^{o}C (45°F) *(21, 23)*. Due to the fact that eggs have just been washed with warm water, it may take an extended period of time (e.g., days) for eggs to cool to the refrigerated storage temperature, allowing pathogens and spoilage microorganisms to proliferate and egg quality to deteriorate *(39)*. In a study conducted by Czarick and Savage *(13)*, eggs stored in cardboard cases were still

above 26°C after 24 h of storage in a 7.2°C cooler, and it was estimated that these eggs would take at least 5 days to reach refrigeration temperatures (7.2°C). Lucore et al. *(46)* examined the effects of pre-processing storage temperature and wash temperature on egg cooling and found that eggs stored at 26.7°C prior to processing and then washed with water at 48.9°C took over 140 h to cool to 7°C, while eggs stored and washed at 15.5°C took less than 94 h to cool to the same temperature. Therefore, some in the egg industry have an interest in using ambient temperature wash water treatments to avoid raising egg temperatures and save on energy costs. Regulations prohibit the use of ambient water wash treatments at this time.

Caudill et al. *(6)* have shown that cool water washes do not negatively effect egg quality or increase aerobic microbial or fungal counts for up to 5 weeks after processing. In that study, four wash temperature schemes were evaluated: hot/hot, hot/cold, cold/cold, and cold/hot, where hot was 48.9°C and cold was 23.9°C. Washes were evaluated for their effect on egg quality (Haugh unit and vitelline membrane strength) and microbial populations on eggshell surfaces, within shell matrices, and in egg contents (aerobic microorganisms and fungi) over 10 weeks of storage at 7.2°C. It was shown that while Haugh unit and vitelline membrane strength both decreased over time, neither was significantly affected by wash temperature. Populations of aerobic microorganisms within shell matrices and fungi within shell matrices and in egg contents also were not significantly affected by wash temperature. However, populations of aerobic microorganisms on shell surfaces and in egg contents were affected by temperature. The highest numbers of aerobic microorganisms were recovered from shell surfaces and contents of hot/hot treated eggs at week 6 of storage *(6)*.

Caudill et al. *(6)* also showed that cold/cold washed eggs had the lowest post-processing temperatures and hot/hot washed eggs had the highest, as would be expected. In addition,

hot/cold and cold/hot washed eggs were able to cool more quickly during storage than hot/hot washed eggs. The authors believe that by replacing one or both warm washes with a cooler wash temperature while maintaining pH of wash solutions at 10-11, egg temperature would be increased to a lesser extent without sacrificing egg quality or causing microbial populations to increase *(6)*.

Another study examining the results of spray washing with three different temperatures (15.5, 32.2, and 48.9°C) was conducted by Lucore et al *(46)*. Internal and external bacterial counts were measured, and it was determined that there was no significant differences in numbers of bacteria in egg contents for all three wash temperatures. This indicates that spray washing at lower temperatures does not significantly increase internal bacterial counts. Postwash external shell bacterial counts were lower for eggs washed at higher temperatures (48.9°C or 32.2°C), when compared to eggs washed at a lower temperature (15.5°C). However, external shell counts for eggs sampled after five days stored at 7.2°C were significantly higher for the two warmer washes compared to the cool wash. These two findings suggest that higher temperature washes have more of an immediate effect on bacterial reductions on exterior shell surfaces, but that warm washed eggs have more residual heat, allowing for more bacterial growth to occur during storage *(46)*.

Jones et al. *(38)* did a study examining shell temperatures and rates of internal pathogen detection with three wash temperature schemes: hot/hot, hot/cold, and cold/cold. It was also shown that eggs washed with the hot/hot temperature scheme had significantly higher shell temperatures, compared to the hot/cold and cold/cold eggs, which were similar. Only three out of 384 eggs tested were positive for *Salmonella*: two in hot/cold eggs and one in cold/cold eggs. None of the *Salmonella* found was *S*. Enteritidis *(38)*.

Hutchinson et al. *(31)* evaluated the effects of "best practice washing conditions" (temperatures of 44°C for prewash water, 44°C for wash water, and 48°C for rinse water) using either a chlorine based detergent or a quaternary ammonium-based sanitizer. They showed that egg surface populations of both *S*. Enteritidis and *S*. Typhimurium were reduced by 10^5 to 10^6 CFU per egg and neither organism was recovered from egg contents, implying that ideal washing conditions reduce *Salmonella* contamination on eggshells while not increasing the chance for *Salmonella* on the surface to penetrate the shell. However, Hutchinson et al. showed that when wash and rinse water temperatures were decreased to 25 and 27°C, respectively, both serovars could be isolated from the egg contents. In addition, it was found that overall, wash and rinse temperatures had no effect on *S*. Enteritidis populations on shell surfaces. Conversely, wash water temperatures did have a significant effect on *S*. Typhimurium populations on shell surfaces. The results suggest that washing at ambient temperatures could have negative impacts on microbiological quality and safety of eggs *(31)*.

Kinner and Moats *(41)* evaluated *S*. Typhimurium survival in simulated egg wash water at various temperatures and pH values. It was determined that at a pH range from 10 to 11 and temperature range from 40 to 50°C, *S*. Typhimurium cells were quickly killed. However, at lower pH (≤9) and temperature (50°C) combinations, the organism was able to persist for longer or actually proliferate in wash water *(41)*.

In contrast to processing methods required in the U.S., the European Union (EU) does not allow washing or chilling of grade A eggs, which are considered "fresh" or "table" eggs. However, the EU does allow grade B eggs, which are eggs that do not meet grade A standards, to be washed. Eggs are not washed to avoid damaging the egg's physical barriers to bacterial penetration of the shell (i.e., the cuticle) and to avoid conditions that favor *Salmonella* entering

the shell (i.e., moisture on the shell surface). The EU acknowledges that there are both advantages and disadvantages to washing eggs, but that for countries where there is a higher prevalence of *S*. Enteritidis in eggs, the risk of washing outweighs the benefits. Instead of washing, the EU concentrates on other options to decrease *Salmonella* contamination, including preventing *Salmonella* infections in layer hens by methods such as vaccination *(20)*.

Chlorine

Sodium hypochlorite (NaOCl) is typically available in solution, commonly known as bleach, at 12-16% concentration. When added to water, NaOCl will dissolve into a sodium ion (Na^+) and hypochlorite ion (OCl^-) *(5)*.

$$NaOCl \rightarrow Na^+ + OCl^-$$

The OCl^- can then form associations with hydrogen ions (H^+) in water, creating hypochlorous acid (HOCl). Due to the fact that hypochlorous acid is a weak acid, it can disassociate into a hydrogen ion and hypochlorite ion in aqueous solutions as seen in the following equation:

$$HOCl \leftrightarrow H^+ + OCl^-$$

The most effective (active) form of chlorine is hypochlorous acid. It is more effective than the hypochlorite ion as an antimicrobial *(5)*. The equilibrium of the different forms of chlorine in solution is influenced by both pH and temperature of the solution. At lower pH values and lower temperatures, hypochlorous acid is the dominant form and as pH values and temperatures increase, so does the proportion of the hypochlorite ion in solution. Therefore, to increase the concentration of hypochlorous acid, the pH and temperature should be kept low. As the pH of the solution is lowered, more hypochlorous acid will be present, and the solution's antimicrobial activity will increase *(5)*.

This chemistry comes into effect during the egg washing process when eggs are sprayed with the final chlorinated rinse. Since the rinse is not acidified and eggs have just been washed with a basic solution (around pH 10-11), the balance of chlorine species favors hypochlorite ions.

Organic matter, such as egg content or hen feces, in the wash water can also decrease antimicrobial activity by inactivating the available chlorine. Thus, organic matter must be kept low to ensure the effectiveness of the chlorine *(50)*. This can cause problems in the egg washing procedure due to the recycling of egg wash water.

Chlorine-Stabilizer

SmartWash (T-128) is an acidic chlorine-stabilizing wash additive composed of one or more acid(s) and one or more organic diol(s) and may contain one or more oxidizing agent(s) and/or one or more surfactant(s) *(44)*. It was originally developed for agricultural applications, especially in the produce industry, but can also be used for hospital and household applications. Many other formulas containing oxidizers that have been developed to decrease microbial contamination are not very effective when there is a high level of organic material present, especially at the lower temperatures used to wash produce. There are also problems with the oxidizers gassing off. These problems make it necessary to use oxidizers at higher concentrations and/or add them more often, which in turn increases costs and can cause worker safety issues *(44)*.

Nou et al. *(55)* first examined the effectiveness of T-128 for stabilizing free chlorine in the presence of organic material and at reducing *E. coli* O157:H7 and *Salmonella* contamination in chlorinated washing solutions used to wash fresh-cut lettuce. They found that chlorine stability in the presence of lettuce extract and soil was only slightly to moderately increased with the addition of T-128. Conversely, the addition of T-128 to chlorine washing solutions significantly

decreased survival of *E. coli* O157:H7 and *Salmonella* in solution, which would reduce the potential of cross-contamination in produce washing systems, especially when water is recirculated. T-128 was also shown to have no effect on quality of fresh-cut lettuce *(55)*.

Davidson et al. *(15)* examined the effect of chlorine + T-128 and 5 other sanitizers (including a water only treatment) on reducing *E. coli* O157:H7 levels on shredded iceberg lettuce, in wash water, and on processing equipment. They found no significant difference between washing with water alone and with chlorine + T-128 in reducing of *E. coli* O157:H7 populations on lettuce. However, Davidson et al. did show that wash water containing chlorine and T-128 did have significantly lower populations of the organism when compared to water alone. They also showed that significantly lower amounts of *E. coli* O157:H7 were recovered from processing equipment when compared to water alone, but two other treatments (peroxyacetic acid and mixed peracid) showed significantly lower levels on the processing equipment than all of the other treatments *(15)*.

T-128 has also been tested for other applications, including reducing biofilms on stainless steel surfaces. Shen et al. *(59)* evaluated the effectiveness of T-128 in combination with chlorinated wash solutions at reducing *Salmonella* and *Pseudomonas* numbers in biofilms in the presence of varying concentrations organic materials, specifically lettuce extract. For both organisms, Shen et al. found that reductions were significantly higher when T-128 and chlorine were combined compared to chlorine alone. They also showed that in the presence of organic material, T-128 was effective at slowing the chlorine depletion rate in chlorinated wash solutions *(59)*. Based on experiments conducted by Schambach *(58)*, T-128 has also shown effectiveness at reducing *Salmonella* and *Campylobacter* populations on poultry when used in combination

with 50 ppm chlorine with a potential to be used as a pathogen intervention in poultry processing facilities.

Egg Quality Parameters and Testing Methodologies

Egg quality attributes can be separated into two separate categories: external egg quality and internal egg quality. External egg quality attributes can be evaluated from direct external observation and include egg shape, texture, soundness, and cleanliness. They are typically the first qualities assessed. In contrast, internal egg quality attributes have to do with the egg contents. The contents can be evaluated for quality attributes by viewing the egg in front of a candling light or by breaking out the egg. Internal quality is determined by the quality of the air cell, albumen, and yolk *(61)*.

Shell Color. Shell color, only having aesthetic, rather than functionality implications, is not used as a parameter for egg grading. As the shell is the only part of an egg that a consumer can see at retail establishments, the shell is an important marketing aspect of eggs. Eggs in the U.S. are typically separated into "whites" or "browns" and packed and sold separately.

Shell Strength and Stiffness. Eggs are a fragile commodity; therefore, it is beneficial to ensure that they are produced to be as strong and sound as possible and handled with care. Broken and cracked eggs can cause economic loss, as cracked eggs are valued at only a third of the price of grade A eggs and broken eggs have no economic value in addition to food safety issues *(29)*.

There are many ways to measure shell strength, including direct methods such as puncture and impact tests. However, many of these direct methods are destructive. The most common is the compression fracture force measured the during quasi–static compression test, which is a destructive method that measures material strength by compressing an egg and

analyzing the force-depression curve *(10, 16)*. Some indirect methods to measure shell strength are shell thickness (destructive) and calculation of the weight percentage eggshell of an egg *(16)*.

Acoustic resonance analysis is a quick and non-destructive method used to measure dynamic shell stiffness in K_{dyn}. K_{dyn} is a function of the egg mass (g) and resonate frequency (R_F, measured in Hz). The frequency observed is influenced by egg mass and shell stiffness. This measurement is taken by analyzing the vibration response of an impact on the equator of the shell surface. Undamaged eggshells are characterized by a highly repetitive frequency pattern, while cracked eggshells do not show repetitive frequencies *(10, 17)*. Correlation coefficients between dynamic shell stiffness and shell thickness and static stiffness have been reported to be 0.71 and 0.60, respectively *(10)*.

Air cell size, albumen quality, yolk quality, and the occurrence inclusions, like blood or meat spots, are factors that determine internal egg quality *(16)*.

Haugh Unit. As the state of the albumen is the major indicator of egg quality, Haugh unit measurements are the standard method for measuring internal egg quality *(16, 28)*. It is a function of egg weight and albumen height determined by this equation:

$$\text{Haugh units} = 100 \log \left[H - \frac{\sqrt{G}\left(30\ W^{0.37} - 100\right)}{100} + 1.9 \right]$$

Where H is the albumen height (in mm), G is a constant of 32.2, and W is the egg weight (in g) *(28, 60)*. The main benefit of using Haugh unit measurements is that the numerical values are said to equal practical quality values because albumen height (at a constant egg weight) has a logarithmic relationship with internal egg quality and Haugh unit measurements take this into account *(28)*. The rates of carbon dioxide and moisture loss increase as an egg ages, causing the albumen to thin. Thus, Haugh unit typically decreases during storage *(36, 61)*.

USDA egg grades can also be applied to eggs using Haugh unit ranges. The grade standards specify that eggs with a Haugh unit value of 72 or higher designates a grade AA egg, a value of 60 up to, but not including 72 designates a grade A egg, and a value less than 60 designates a grade B egg *(63)*.

Vitelline Membrane Strength and Elasticity. Two variables that contribute to the quality of egg yolks are vitelline membrane strength and vitelline membrane elasticity or deformation. These quality attributes are relevant for egg breaking facilities, as a strong vitelline membrane will allow yolk and albumen to be separated without yolk breakage. Both vitelline membrane strength and elasticity have been shown to decrease during storage *(36, 42)*. Older methods used to measure vitelline membrane strength include direct application of force to break the membrane, vacuum (in mm Hg) required to break the membrane, and vacuum time required to break the membrane. The main problems with these methods are their subjective nature and that they could only be used to measure at one small point on the yolk *(42)*.

Froning et al. *(24)* first used an Instron Universal Testing Machine equipped with a back-extrusion cell to test the vitelline membrane. This cell allowed for a larger area of the yolk to be tested, which would better model yolk impact conditions that would be seen in a frying pan or at a breaking operation *(42)*. Kirunda and McKee *(42)* modified this method using a compression head and found it effective for determining vitelline membrane strength.

Percent Total Solids. The percent of total solids of entire egg contents, albumen, and yolk are approximately 24%, 12%, and 52%, respectively *(45)*. Total solids values are of particular concern to egg product producers as they affect the composition of products produced *(1)*As stated previously, the rate of moisture loss increases as an egg ages *(61)*. This, in turn,

causes the measurement of total solids to increase with storage time as the percent moisture decreases.

Salmonella spp. and Shell Eggs

Salmonella spp. are gram negative, facultative anaerobic rods that belong to the *Enterobacteriaceae* family. Most *Salmonella* spp. are motile, with the exceptions of *S*. Gallinarum and *S*. Pullorum. The optimal growth temperature for this organism is 37°C. In 2011, the Centers for Disease Control and Prevention (CDC) estimated that *Salmonella* spp. were responsible for over one million domestically acquired foodborne illnesses (nontyphoidal) in the United States, which is 11% of the total number of foodborne illnesses annually. Typhoid fever, caused by *S*. Typhi and *S*. Paratyphi A and only found in humans, is more severe, but more rare and often associated with foreign travel.

Nontyphoidal *Salmonella* infections, which will be the focus of this paper, occur when nontyphoidal *Salmonella* cells are consumed, typically via contaminated food or water, and enter the epithelium of the small intestine. Salmonellosis is characterized by nausea, vomiting, abdominal cramps, diarrhea, fever, and headache, with the typical time of onset being 6 to 72 hours after ingestion. Salmonellosis will usually last for 4 to 7 days, with the symptoms normally being self-limiting in healthy individuals. One serious chronic sequelae that results from about 2% of *Salmonella* infections is reactive arthritis *(27)*.

Two species of *Salmonella* can cause human illness: *S*. *enterica* and *S*. *bongori*. The main species of concern is *S*. *enterica*, which contains over 2,500 different serovars that are characterized by surface and flagellar antigens *(27)*. In 2011, *S*. Enteritidis was the causative serovar in 18.2% of total laboratory-confirmed *Salmonella* cases, followed by *S*. Typhimurium with 12.6% of cases *(7)*. The main sources of *Salmonella* spp. in the food industry are poultry

and eggs *(14)*. *S*. Enteritidis is the main serovar of *S*. *enterica* associated with eggs and egg products, and it is the serovar that is usually found inside of shell eggs *(11, 21, 27)*. Ebel and Schlosser have used modeling to estimate that one in 20,000 eggs is contaminated with *S*. Enteritidis annually, with high level laying flocks producing more than two-thirds of the contaminated eggs *(19)*. *S*. Typhimurium and *S*. Heidelberg are also serovars that are commonly linked to eggs and poultry *(11, 14, 27)*.

In a study conducted by the CDC analyzing outbreak data from 1998-2008, it was estimated that eggs were responsible for almost 180,000 or 4.9% of illnesses caused by bacteria, 60.3% of illnesses caused by *S*. Enteritidis, 37.6% of illnesses caused by *S*. Heidelberg, and 2.8% of illnesses caused by *S*. Typhimurium annually *(56, 57)*. Eggs can typically be contaminated with *Salmonella* spp. in two different ways: vertical transfer or horizontal transfer. Vertical transfer, which is believed to be the primary mode of *S*. Enteritidis contamination, typically occurs via the transovarian route *(21)*. This happens when a hen's reproductive system is infected with *Salmonella*, and it is transferred to the egg *in vivo*. Washing eggs will have no effect on this form of *Salmonella* contamination *(11)*.

Horizontal transfer occurs when the outside of the egg is contaminated with *Salmonella* via contact with contaminated feces, bedding materials, dust, feed, etc. *(11, 49)*. The bacteria may then be able to penetrate the shell. Shell penetration is facilitated by factors such as moisture on the shell surface, contamination of the shell with organic material, and deterioration or removal of the cuticle *(30)*. Once inside the egg, *Salmonella* can survive and possibly grow in the albumen. Eventually, the organism may penetrate the vitelline membrane and proliferate considerably inside the yolk *(25, 26)*. Storage at temperatures above 7°C will increase the risk of *S*. Enteritidis growth *(26)*.

After first being noticed in England, the *S*. Enteritidis pandemic began to be observed in the United States in the late 1980's with a rising proportion of *Salmonella* isolates being of this particular serovar *(57)*. *S*. Enteritidis became the most frequently isolated serovar of *Salmonella* in 1994. Over the time period from 1985 to 1995, the incidence rate of *S*. Enteritidis per 100,000 people rose from 2.38 to 3.9. After this period until 1999, incidence began to decrease to 1.98 per 100,000 people. Throughout this entire time frame, 841 *S*. Enteritidis outbreaks were reported to the CDC. The number of yearly outbreaks in 1985, 1990, and 1999 were 26, 85, and 44, respectively. As with incidence, an increase was seen from the mid-1980's to mid-1990's and then a decrease. Of all the outbreaks over this time period, 80% of the outbreaks in which information was available were egg-related. Again, an increasing trend over this time period was seen with this percentage being 71% in 1985 and 95% in 1997 *(57)*. Similar increases were also seen in England and other parts of the world. In England and Wales, *S*. Enteritidis was only responsible for 10% of salmonellosis illnesses in 1981, but this percentage drastically increased to 70% in 1997 *(9)*. However, due to regulations introduced in Europe to combat this pandemic, isolation of *S*. Enteritidis has decreased since the 1990s *(47)*.

A risk assessment published in 2005 was conducted by the USDA FSIS to determine the risk of *S*. Enteritidis from shell eggs. The important outputs of this risk assessment involved pasteurization of both shell eggs and liquid egg products and the storage time and temperature of shell eggs. It was predicted that by pasteurizing shell eggs and achieving a 3-log reduction of *S*. Enteritidis, the annual number of illnesses caused by this organism in shell eggs would be reduced from 130,000 to 41,000. If a 5-log reduction could be achieved, the annual illnesses could be reduced to 19,000. It was also predicted that if a storage temperature of 7.2°C (42°F) was reached within 12 hours of eggs being laid, annual illnesses could be reduced to 28,000 *(22)*.

CHAPTER 3

MATERIALS AND METHODS

Experiment 1: Evaluating Influence on Egg Quality

Egg Washing Procedures. Unwashed eggs were acquired from the Cal-Maine Foods facility in Shady Dale, GA and candled following USDA guidelines *(61)*. Any cracked eggs were discarded. Remaining eggs were stored overnight at room temperature (approximately 25°C) before washing.

Four washing treatments were tested: high pH, high temperature; high pH, low temperature; low pH, high temperature; and low pH, low temperature. The two target pH values were 11 (high pH) and 6 (low pH) and the two target temperatures were 48.9°C (120°F) (high temperature) and ambient temperature, which is approximately 20°C (68°F) (low temperature). For the high pH washes, a potassium hydroxide based commercial egg wash detergent (Exalt II; Zee Company, Inc., Chattanooga, TN) was added to the egg wash water until the appropriate pH was achieved. For the low pH washes, chlorine bleach (Clorox; The Clorox Company, Oakland, CA) was added to the egg wash water to obtain a free chlorine concentration of 170-200 ppm, and then the pH was adjusted to the desired pH value by adding SmartWash T-128 (Smartwash Solutions LLC, Salinas, CA). The wash solutions were brought to temperature using an immersion heater (high temperature) or used at ambient temperature.

Eggs were washed for one minute on rollers using a pilot-scale recirculating spray system described in Jones et al *(32)*, with the modification that the system has a 60-egg capacity. Following the wash, the eggs were sprayed with a warm (120°F) chlorine rinse solution at

approximately 200 ppm free chlorine for 10 s and then exposed to a heated hand-held dryer for 10 s.

One hundred and twenty eggs were washed with each wash treatment in two separate wash cycles. After being washed, sprayed, and dried, the eggs were stored on clean pulp flats at 4°C and approximately 88% relative humidity until they were tested for quality attributes. Testing for the first time point (week 0) was conducted the following day.

Wash solution samples were chemically analyzed before washing, after the first wash, and after the second wash. Free chlorine measurements were taken using chlorine K-2523 vials (CHEMetric, Midland, VA) and a CHEMetric v-2000 colorimenter. Temperature measurements were made using a Barnant 115 thermocouple thermometer (Sigma-Aldrich, St. Louis, MO) and pH measurements were made using a portable Orion 3 Star pH meter (Thermo Scientific, Waltham, MA).

Sampling and Egg Quality Testing Procedures. Egg quality testing was conducted at 0, 2, 4, 6, 8, 10, and 12 weeks for each replication and included 6 tests: dynamic stiffness, shell color, shell strength, Haugh unit, vitelline membrane strength and deformation, and percent total solids. At each two-week interval, 12 eggs for each treatment were evaluated. All eggs were tested at refrigeration temperatures. Three of the 12 eggs from each treatment were randomly selected to measure the shell temperature using the infrared thermometer function on a Newport TrueRMS Supermeter (Omega Engineering, Stamford, CT).

Dynamic stiffness testing was conducted using the acoustical egg tester (KU Leuven, Lueven, Belgium) and AET_RS232 Software (version 2.1.a) according to the methods of De Ketelaere et al *(18)*. Three measurements were taken for each egg. Egg weight and radio frequency measurements were used to calculate dynamic stiffness values in K_{dyn}.

Shell color measurements were made using a Chroma Meter CR-400 (Konica Minolta, Sensing, Inc., Tokyo, Japan) and CR-400 Utility software. Three measurements were taken at three different sites around the equator of each egg, making sure to avoid any visible soil or debris on the egg shell surface. The x, y, and z measurements were used to calculate lightness (L*), red/green (a*), yellow/blue (b*), chroma (C*), and hue angle (h°) values.

Before any further measurements were taken, the weight of each egg was measured and recorded using TSS Eggware software (version 3.0.06, Dunnington, York, United Kingdom). Shell static compression strength was measured using a TA-XT2plus Texture Analyzer (Texture Technologies, Scarsdale, NY) equipped with a 5-kg loadcell, a TA-650 egg holder with posts (Texture Technologies), and a TA-30 7.62 cm (3 in) diameter aluminum compression disc (Texture Technologies) according to the methods of Jones and Musgrove *(36)*. The egg was positioned on its side in the holder so the compression force was applied to the equator of the egg. A test speed of 2 mm/s and a trigger force of 0.001 kg were used. Values for distance (mm) and force (g) required to crack the eggshell were recorded using Texture Exponent 32 software (version 5.1.1.0, Texture Technologies). One of each measurement was taken for each egg.

After shell strength measurements were taken for each egg, the egg was broken out onto a breakout table and the height of the thick albumen was measured approximately one cm from the yolk using a tripod micrometer (TSS QCD/QCH System; Technical Services and Supplies, Dunnington, York, United Kingdom) and the procedures outlined by Jones and Musgrove *(28, 36)*. Thick albumen height and egg weight were used to calculate Haugh unit for each egg. Measurements were recorded and calculated using TSS Eggware software (version 3.0.06).

Next, the albumen and yolk were separated and the egg yolk placed into a half of a petri dish. Vitelline membrane strength and deformation were measured using a TA-XT2plus Texture

Analyzer (Texture Technologies) equipped with a 750-g loadcell and a TA-30 7.62 cm (3 in) diameter aluminum compression disc (Texture Technologies) according to the methods of Jones et al *(34)*. A test speed of 1 mm/s, trigger force of 5 g, and trigger distance of 2 mm were used. Measurements for distance (mm) and force (g) were recorded Texture Exponent 32 software (version 5.1.1.0, Texture Technologies).

Procedures for the determination of whole egg total solids were described by Jones et al *(34)*. Four three-egg pools consisting of albumen and yolk (whole egg contents) were collected in a 710 mL (24 oz.) Whirl-pak bag (Nasco, Fort Atkinson, WI) for each treatment. Egg contents were then stomached (Somacher 400 Circulator, Seward, Worthing, West Sussex, United Kingdom) for one min at 230 rpm and placed in a 4°C cooler overnight. The next day, the egg contents were allowed to warm at room temperature for at least two h and stomached again for one min at 230 rpm. Before weighing out egg contents, 57 mm aluminum weighing dishes (Fisher Scientific, Waltham, MA) were labeled and placed in the drying oven for 2 h and then in the desiccator for 2 h. For each bag, three approximately 5 g samples were weighed out into aluminum weigh pans. The weight of the pans alone and the weight of the egg contents added were recorded. Pans were then placed into a forced air drying oven (Lindberg/Blue M, Thermo Electron Corporation, Waltham, MA) for 19 h at 100°C, then into a desiccator for 2 h, and re-weighed. The final weight was recorded. Percent total solids were then calculated. Triplicate measurements were made for each pool.

Statistical Analysis. Three replications of Experiment 1 were conducted. Data were entered into SAS software (SAS Institute, version 9.3) and a two-way ANOVA was performed using SAS "Proc GLM" with replications as a blocking factor. Twelve separate models were created (one for each variable). For the AET and shell color measurements, the three

measurements for each sample were averaged. For percent total solids, measurements taken from each pool were averaged. Pairwise comparisons for treatment and week, treatment without week, and week without treatment were analyzed to divide the averages to different groups based on significance at a level of 0.05.

Experiment 2: Evaluating Antimicrobial Effectiveness

Inoculum Preparation. Two different *Salmonella enterica* serovars were used to inoculate eggs: *S*. Typhimurium and *S*. Enteritidis, both originally isolated from poultry or egg-related sources and resistant to 200 ppm nalidixic acid. Inocula cultures were prepared by growing in tryptone soya broth (TSB; Oxoid, Basingstroke, England) for 18-24 h at 37°C to an approximate concentration of 2 x 10^8 CFU/mL. Sterile buffered peptone water (BPW; Acumedia, Lansing, MI) was used as a diluent to prepare the inoculum. Seventy mL of overnight cultures (35 mL of each culture) was added to 7 L of BPW to give an inoculum concentration of approximately 10^6 CFU/ml. Inoculum was then serially diluted in sterile phosphate buffered saline (PBS; 8 g sodium chlorite crystal reagent; 0.2 g monopotassium phosphate, monobasic crystal; 2.1 g sodium phosphate, dibasic hepahydrate; and 0.2 g potassium chloride per 1 L of deionized water) and spread plated on brilliant green sulfa agar with sulfapyridine (BGS; Acumedia, Lansing, MI) containing 200 ppm nalidixic acid sodium salt (Sigma, St. Louis, MO). Plates were incubated for 24 h at 37°C and colony forming units (CFUs) characteristic of *Salmonella* counted to attain initial inoculum concentration.

Inoculation. Inoculation methods were adapted from those set forth by Jones and Musgrove *(35)*. Eggs were acquired from the Cal-Maine Foods facility in Shady Dale, GA and candled. Any cracked eggs were discarded. Remaining eggs were stored overnight in an incubator at 42°C before inoculation. Half of a flat of eggs (15 eggs) at 42°C were placed in a

sterile stainless steel basket and immersed into 7 L of inoculum (at approx. 4-5°C) for 10 s. After the eggs drained for 1 min, they were placed on plastic flats that had been sanitized with a 70% ethanol spray and exposed to UV light. These eggs were used as positive controls (inoculated but not washed) or for eggs to be washed with one of the four treatments. Uninoculated negative control eggs were sham inoculated by immersion into sterile BPW. All eggs were stored overnight in the flats at 25°C prior to washing.

Egg Washing Procedures. The following day, eggs were washed using the same four treatments and procedures as in Experiment 1. One-hundred mL samples of washing solutions were taken before washing, after the first wash, and after the second wash for chemical (free chlorine concentration, pH, and temperature) analysis and for microbiological testing. A sodium thiosulfate (J.T. Baker, Philipsburg, NJ) solution was added to wash solution samples used for the microbiological analysis to stop any residual antimicrobial activity. After washing, eggs were placed on sanitized plastic flats and allowed to dry briefly in a biological safety hood. Eggs were sampled immediately after processing when possible. If not possible, they were stored in a 4°C cooler for 1-2 hours until sampled.

Sampling, Enumeration, and Enrichment. Eggs were cracked on the edge of a sterile beaker and egg contents were discarded. The inside of the shells were rinsed with sterile PBS to remove any remaining egg contents. Six eggshells were pooled in a sterile specimen cup and 10 mL of BPW per egg shell (60 mL BPW total per specimen cup) was added. Shells were then crushed and mixed with a sterile glass rod for one min according to the methods of Musgrove et al. *(52)*..The resulting mixture (shell emulsion) was serially diluted in PBS and spread plated onto 200 ppm nalidixic acid-containing BGS plates in duplicate. Twelve 6-eggshell pools were

sampled for each treatment. Plates were incubated for 24 h at 37°C and CFUs characteristic of *Salmonella* were counted.

Wash solution samples were filter concentrated through a MicroFunnel™ Filter Funnel with a 0.45 µm GN-6 membrane (Pall Life Sciences, Ann Arbor, MI); Filter membranes were removed and placed into sterile 50 mL centrifuge tubes with 20 mL BPW which were shaken for 1 min. The liquid was then serially diluted in PBS and spread plated onto 200 ppm nalidixic acid-containing BGS plates in duplicate. Plates were incubated for 24 h at 37°C and CFUs characteristic of *Salmonella* were counted.

Specimen cups containing shell emulsions and centrifuge tubes containing the filter membranes were also incubated for 24 h at 37°C for enrichment and *Salmonella* confirmation as needed (positive/negative result). If confirmation was needed (i.e., no growth on direct plates), 1 mL of the incubated primary enrichment was transferred to a Rappaport-Vassiliadis R10 broth (RV; Difco, Sparks, MD). RV tubes were incubated for 24 h at 42°C. After incubation, a portion from the RV tube was struck onto BGS containing 200 ppm nalidixic acid and incubated 24 h at 37°C. Characteristic *Salmonella* growth was recorded as a positive result and absence of characteristic *Salmonella* growth as a negative result.

Statistical Analysis. Three replications of Experiment 2 were conducted. *Salmonella* numbers were entered into Statistica 12 (Statsoft, Tulsa, OK) and analyzed using the univariate test of significance and Tukey's honest significant difference (HSD) test to determine if any significant differences existed between treatments at a p level of 0.05. It was assumed that there would be a significant difference between replications so the variation due to replication was put into the error term by using a randomized complete block design with replication as a block.

CHAPTER 4

RESULTS

Experiment 1: Evaluating Influence on Egg Quality

The overall means for the dependent variables dynamic shell stiffness, shell strength, and shell elasticity over 12 weeks of storage were 148.54 K_{dyn}, 3733.96 g, and 0.54 mm, respectively. These characteristics were not significantly ($p>0.05$) affected by wash treatment or length of storage.

Some alteration in color as the result of treatments was noted. The interaction of both storage period and wash treatment had a significant ($p<0.05$) effect on shell lightness (L*), red/green value of the shell (a*), yellow/blue value of the shell (b*), chroma of the shell (C*), and hue angle of the shell (h°) (Tables 1-5). When evaluating color measurements for each treatment, eggs treated with high pH washes (A and B) were significantly different ($p<0.05$) compared to those subjected to low pH washes (C and D) for all color parameters. L* values for all wash treatments were similar at week 0, but differences between high pH and low pH treatments were noted after that point. In contrast, differences between high pH and low pH treatments for a*, b*, C*, and h° were seen throughout the entire storage time. The a* values became more negative (shift towards green) and b* and C* increased (shift towards yellow and more color saturation).

Interaction of storage period and wash treatment had a significant ($p<0.05$) effect on Haugh unit values (Table 6). Haugh unit measurements also showed a decreasing trend over time. For the dependent variables vitelline membrane strength, vitelline membrane elasticity, and

percent total solids, storage time had a significant ($p<0.05$) effect, but wash treatment did not (Table 7). This means that for these egg quality parameters, the values changed over time, but the change was just a progression and not affected differently by treatment. Overall, vitelline membrane strength and elasticity decreased by approximately 22% and 14%, respectively, and percent total solids increased by approximately 3% over storage time. Free chlorine concentrations in wash water dropped slightly from before to after washing for wash solutions C and D, while pH remained consistent for all wash solutions (Table 8).

Experiment 2: Evaluating Antimicrobial Effectiveness

There was no significant ($p>0.05$) difference in the mean *Salmonella* counts for all four treatments (Table 9). Therefore, the reduction of *Salmonella* populations was the same for each treatment evaluated. The mean count for positive control samples and for treated eggs overall were 5.40 and 4.63 log CFU/mL of shell emulsion, respectively. Overall, an average reduction of 0.77 log CFU/mL was seen.

When wash solution samples were direct plated, no growth was observed. When enriched, 22% of wash samples for treatment B were positive for nalidixic acid-resistant *Salmonella* spp. No positives were observed or any other treatment wash solution samples.

As in Experiment 1, pH remained consistent for all wash solutions and free chlorine concentration also remained stable (Table 10).

CHAPTER 5

DISCUSSION

Due to the fact that many *Salmonella* illnesses and outbreaks are associated with eggs, it is essential to reduce the impact of this bacterium though the prevention of initial contamination of eggs and by having methods in place to reduce contamination when it does occur. Egg washing is a method to reduce contamination on the shell surface of eggs. However, washing does not affect bacteria that have already been internalized. Currently, eggs are typically washed using alkaline wash solutions at warm temperatures *(54)*. As in other segments of the food industry, new antimicrobials and processing parameters should be continually evaluated for their ability to improve the quality and safety of the product. Ambient temperature washes are of interest because they avoid increases in egg temperatures that are seen when using warm washes. This, in turn, allows the eggs to cool faster and results in less growth of spoilage and pathogenic microorganisms and longer shelf life *(6, 39)*. Ambient temperature washes would also be a more economical processing method as the cost to heat the wash water would be eliminated and the cost to cool the eggs after processing would be decreased.

In this study, the current egg washing procedure (pH 11 at 48.9°C) (A) and three alternatives: pH 11 at ambient temperature (approximately 20°C) (B); pH 6 with approximately 200 ppm chlorine and a proprietary chlorine-stabilizer at 48.9°C (C); and pH 6 with approximately 200 ppm chlorine and a proprietary chlorine-stabilizer at ambient temperature (approximately 20°C) (D) were used to evaluate quality parameters over a 12-week cold storage period and to evaluate the washes as a microbial intervention.

Haugh unit, vitelline membrane strength and elasticity, and percent total solids are some of the methods used to measure egg quality, The results of this study agree with numerous other studies showing that Haugh unit and vitelline membrane strength and elasticity decrease and percent total solids increases over time *(6, 36, 39, 42)*. It also supports the Jones and Musgrove study *(36)* that showed that shell strength was not significantly affected by storage time. However, data collected in a study done by Jones et al. *(33)* showed that whole egg total solids only increased 0.30% during 12-week cold storage and that the values for week 0 were not significantly different ($p>0.05$) than values for week 12. The percent increase (3%) measured in the current study is ten times more than the percent increase measured by Jones et al. and the values for week 0 were significantly different ($p<0.05$) than values for week 12.

The current study is consistent with some of the findings of Caudill et al. *(6)*, as it also shows Haugh unit and vitelline membrane strength decreasing over storage time with wash water temperature having no effect on vitelline membrane strength. However, the current study does show some effect of wash solution temperature on Haugh unit means over a 12 week storage period with warm wash treatments showing higher Haugh unit means than ambient temperature washes. Important storage time points to note Haugh values and compare to USDA grade standards are at 4, 6, and 12 weeks. These first two time points represent the common sell by dates of 30 and 45 days after processing and the last represents extended refrigerated storage by consumers *(33)*. Eggs washed at pH 11 and 48.9°C decreased in quality from USDA grade AA to grade A at week 4, while the rest of treated eggs made this downgrade at week 6. Ambient temperature washed eggs made the drop from grade A to grade B at week 12, but warm water washed eggs remained grade A quality up until the end of the 12-week storage period *(63)*. As Haugh unit was the only internal quality factor affected by wash treatment and the differences

between the treatments was slight, it can be assumed that the different washes basically have the same effect on internal egg quality.

However, the various washes did have noteworthy effects on shell color. The lightness (L*) values for all wash treatments were similar at week 0, but differences between pH 11 and pH 6 treatments were noted after that point. In contrast, differences between high pH and low pH treatments for a*, b*, C*, and h° were seen throughout the entire storage time. Due to the fact that the alkaline washes were overall significantly different than the acidic washes for all color parameters measured, it can be concluded that some characteristic or characteristics (i.e., pH, chemical composition, etc.) of the egg washes caused shifts in shell color in the current study. Color parameters that were different included lightness and red/green, yellow/blue, chroma and hue angle values. Eggs treated with the chlorine-stabilizer were less light, more chromatic/saturated and of a different hue (shifts toward green and yellow tints) when compared to alkaline treated eggs *(43)*. While these measurable color changes occurred and off-white shell color shifts could be visually observed, the overall visual appearance of treated eggs was not drastically different. To determine if these changes would be practically meaningful, further testing could be done to determine if shell color differences would be detectable and acceptable for consumers.

Due to the fact that all treatments showed equal, but minor, reductions in *Salmonella* counts, not many meaningful conclusions can be drawn from this individual experiment. It is hypothesized that during inoculation, *Salmonella* cells were internalized through eggshell pores, making it difficult for any wash treatment to come into contact with them. Musgrove et al. *(51)* showed that when evaluating sanitizer efficacy to decrease *Salmonella* contamination on hatching eggs, the immersion inoculation method (which is based on the positive pressure

created by the temperature differential gradient) made it challenging to show a reduction in *Salmonella* counts, and that it was the most thorough test of efficacy when compared with fecal smear and droplet inoculation methods. Therefore, it seems that the inoculation procedure was likely too stringent to show a treatment effect. It is recommend that further studies be conducted using a less stringent inoculation method to simulate a wider range of potential ways *Salmonella* could contaminate the eggshell.

Ambient temperature washes may be a viable option in the shell egg industry to reduce costs without compromising egg quality. However, there does not seem to be a consensus as to whether ambient temperature washes increase bacterial penetration. In a study conducted by Jones and Musgrove to examine the effects of wash temperature on pathogen detection, cooler washes did not have an effect on pathogen internalization, as less than 1% of egg contents tested were positive for *Salmonella* spp. *(38)*. Hutchinson et al. *(31)* evaluated the effects of "best practice washing conditions" and showed contrasting results. At ideal conditions, egg surface populations of both *S*. Enteritidis and *S*. Typhimurium were reduced and neither organism was recovered from egg contents, implying that ideal washing conditions reduce *Salmonella* contamination on eggshells while not increasing the chance for *Salmonella* on the surface to penetrate the shell. However, Hutchinson et al. also showed that when wash and rinse water temperatures were decreased to ambient temperatures, both serovars could be isolated from the egg contents *(31)*. In addition, it was shown that overall, wash and rinse temperatures had no effect on *S*. Enteritidis populations on shells surfaces *(31)*. Conversely, wash water temperatures did have a significant effect on *S*. Typhimurium populations on shell surfaces. The Hutchinson et al. study indicates that washing at ambient temperatures could have negative impacts on the microbiological quality and safety of eggs *(31)*. The current study did not evaluate *Salmonella*

penetration into egg contents, so the effect of the four wash treatments on internalization was not evaluated.

Another possible concern with lower pH washes is the ability for *Salmonella* spp. to survive in wash water. Meckes et al. *(48)* demonstrated that at lower pH wash solutions (8.1 to 8.3), *Salmonella* concentrations can be high (3.29×10^3 MPN/100 ml to 4.93×10^3 MPN/100 ml) and that microorganisms can survive longer at these conditions. They also demonstrated that the same effect at lower temperatures (5, 15, and 25°C) with survival times from 104 to 174 days at 5°C, up to 32 days at 15°C, and up to 16 days at 25°C *(48)*. Due to the fact that the only positive wash solution samples in the current study were from treatment B (high pH, low temperature), it is possible that *Salmonella* may be able to survive better in wash water at this low wash temperature when compared to its high pH, high temperature counterpart or either pH 6 wash.

Overall, the most drastic result seen in this study is the effect of wash solution on shell color. However, it is unknown whether the shift in shell color would be substantially meaningful to consumers. Other egg quality attributes were not drastically affected by wash solution or temperature. *Salmonella* reduction was also not significantly affected by the different wash treatments, which is attributed to the rigorous inoculation method used. This study shows that ambient temperature washes have a similar influence on internal egg quality parameters compared to the current egg washing procedures, and that they have potential to be a viable and more economical option for the shell egg industry.

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Table 1. Mean shell lightness values (L*) of shell eggs treated with various antimicrobial washes over 12 weeks of storage.

Treatment*	Week						
	0	2	4	6	8	10	12
A	94.05^{cd}	94.44^{ab}	94.51^{a}	94.58^{a}	94.66^{a}	94.44^{ab}	94.60^{a}
B	94.05^{cd}	94.49^{a}	94.40^{ab}	94.48^{a}	94.46^{a}	94.66^{a}	94.64^{a}
C	94.00^{cde}	94.01^{cde}	93.88^{de}	94.06^{cd}	94.47^{a}	94.10^{cd}	94.00^{cde}
D	93.83^{de}	94.06^{cd}	93.75^{e}	93.87^{de}	93.97^{cde}	94.17^{bc}	93.93^{cde}

*A = pH 11 at 48.9°C; B = pH 11 at ambient temperature; C = pH 6 at 48.9°C; and D = pH 6 at ambient temperature
$^{a-e}$ Values are used to show significance ($p<0.05$) throughout the table. Values with like superscripts are not significantly different.

Table 2. Mean shell red/green values (a*) of shell eggs treated with various antimicrobial washes over 12 weeks of storage.

Treatment*	Week						
	0	2	4	6	8	10	12
A	-0.52^{a}	-0.66^{b}	-0.69^{bc}	-0.79^{de}	-0.82^{ef}	-0.89^{fg}	-0.92^{g}
B	-0.57^{a}	-0.65^{b}	-0.65^{b}	-0.74^{cd}	-0.78^{de}	-0.79^{de}	-0.91^{g}
C	-0.92^{g}	-1.19^{h}	-1.26^{hi}	-1.31^{i}	-1.31^{i}	-1.45^{j}	-1.46^{j}
D	-0.95^{g}	-1.26^{hi}	-1.28^{i}	-1.43^{j}	-1.46^{j}	-1.48^{j}	-1.49^{j}

*A = pH 11 at 48.9°C; B = pH 11 at ambient temperature; C = pH 6 at 48.9°C; and D = pH 6 at ambient temperature
$^{a-j}$ Values are used to show significance ($p<0.05$) throughout the table. Values with like superscripts are not significantly different.

Table 3. Mean shell yellow/blue values (b*) of shell eggs treated with various antimicrobial washes over 12 weeks of storage.

Treatment*	Week						
	0	2	4	6	8	10	12
A	1.56^{n}	1.81^{mn}	2.07^{lm}	2.33^{jklm}	2.56^{ijk}	2.65^{hij}	2.73^{hi}
B	1.78^{mn}	1.83^{mn}	2.09^{klm}	2.63^{hij}	2.18^{klm}	2.37^{ijkl}	2.64^{hij}
C	3.04^{gh}	4.29^{f}	5.01^{cd}	5.20^{bcd}	4.85^{de}	5.76^{a}	5.89^{a}
D	3.34^{g}	4.46^{ef}	4.93^{de}	5.52^{ab}	5.54^{ab}	5.47^{abc}	5.67^{ab}

*A = pH 11 at 48.9°C; B = pH 11 at ambient temperature; C = pH 6 at 48.9°C; and D = pH 6 at ambient temperature
[a-n] Values are used to show significance ($p<0.05$) throughout the table. Values with like superscripts are not significantly different.

Table 4. Mean shell chroma values (C*) of shell eggs treated with various antimicrobial washes over 12 weeks of storage.

Treatment*	Week						
	0	2	4	6	8	10	12
A	1.66^{l}	1.93^{kl}	2.19^{jk}	2.46^{hij}	2.69^{hi}	2.80^{gh}	2.88^{gh}
B	1.88^{kl}	1.95^{kl}	2.20^{jk}	2.75^{ghi}	2.32^{ijk}	2.51^{hij}	2.79^{ghi}
C	3.18^{fg}	4.59^{e}	5.17^{c}	5.36^{bc}	5.03^{cde}	5.95^{a}	6.08^{a}
D	3.48^{f}	4.64^{de}	5.10^{cd}	5.71^{ab}	5.73^{ab}	5.67^{ab}	5.87^{a}

*A = pH 11 at 48.9°C; B = pH 11 at ambient temperature; C = pH 6 at 48.9°C; and D = pH 6 at ambient temperature
[a-l] Values are used to show significance ($p<0.05$) throughout the table. Values with like superscripts are not significantly different.

Table 5. Mean shell hue angle (h°) of shell eggs treated with various antimicrobial washes over 12 weeks of storage.

Treatment*	Week						
	0	2	4	6	8	10	12
A	110.70^{abc}	111.47^{a}	109.47^{bcde}	109.85^{abcd}	108.70^{defg}	109.66^{abcde}	109.35^{bcdef}
B	109.86^{abcd}	110.94^{ab}	109.52^{abcde}	107.86^{efgh}	110.27^{abcd}	108.90^{cdefg}	109.72^{abcde}
C	107.43^{fghi}	105.98^{hijkl}	104.59^{kl}	104.57^{kl}	105.43^{jkl}	104.43^{kl}	104.37^{l}
D	107.24^{ghij}	106.40^{hijk}	105.55^{ijkl}	105.14^{kl}	105.25^{jkl}	105.50^{ijkl}	105.04^{kl}

*A = pH 11 at 48.9°C; B = pH 11 at ambient temperature; C = pH 6 at 48.9°C; and D = pH 6 at ambient temperature
$^{a-l}$ Values are used to show significance ($p<0.05$) throughout the table. Values with like superscripts are not significantly different.

Table 6. Mean Haugh unit values of shell eggs treated with various antimicrobial washes over 12 weeks of storage.

Treatment*	Week						
	0	2	4	6	8	10	12
A	82.1^{ab}	70.8^{def}	73.7^{cd}	70.3^{ef}	66.2^{ghi}	64.0^{i}	65.7^{ghi}
B	79.7^{b}	74.8^{c}	71.3^{de}	67.5^{fgh}	66.6^{ghi}	64.0^{i}	58.5^{k}
C	82.2^{ab}	76.4^{c}	73.6^{cde}	71.3^{de}	64.3^{hi}	66.8^{ghi}	60.6^{jk}
D	83.5^{a}	76.4^{c}	73.8^{cd}	67.9^{fg}	63.8^{ij}	65.1^{ghi}	58.2^{k}

*A = pH 11 at 48.9°C; B = pH 11 at ambient temperature; C = pH 6 at 48.9°C; and D = pH 6 at ambient temperature
$^{a-k}$ Values are used to show significance ($p<0.05$) throughout the table. Values with like superscripts are not significantly different.

Table 7. Mean vitelline membrane strength (in g), vitelline membrane elasticity (in mm), and percent total solids for all treatments combined over 12 weeks of storage.

Variable	Week						
	0	2	4	6	8	10	12
VM Strength (g)	150.14[a]	147.18[a]	140.95[ab]	135.33[b]	120.27[c]	116.5[c]	117.37[c]
VM Elasticity (mm)	7.30[b]	8.39[a]	7.04[bc]	6.84[cd]	6.55[de]	6.47[e]	6.29[e]
% Total Solids	23.42[d]	23.77[c]	23.70[c]	23.91[bc]	24.10[ab]	24.04[ab]	24.17[a]

*A = pH 11 at 48.9°C; B = pH 11 at ambient temperature; C = pH 6 at 48.9°C; and D = pH 6 at ambient temperature
[a-e] Values are used to show significance ($p<0.05$) across rows. Values with like superscripts are not significantly different.

Table 8. Mean pH, temperature (°C), and free chlorine concentration (ppm) of egg wash solutions used in the egg quality evaluation study. Samples were analyzed before, during, and after washing.

	Wash A[a]		Wash B		Wash C			Wash D		
	pH	Temp (°C)	pH	Temp (°C)	pH	Temp (°C)	Cl (ppm)	pH	Temp (°C)	Cl (ppm)
Before	11.08	46.3	11.06	18.9	5.99	46.9	192.1	6.10	19.5	185.1
During	11.00	45.6	11.13	19.8	6.09	44.4	-[b]	6.21	19.9	-[b]
After	10.98	46.6	11.14	19.4	6.19	42.5	184.1	6.28	20.2	174.7

[a]A = pH 11 at 48.9°C; B = pH 11 at ambient temperature; C = pH 6 at 48.9°C; and D = pH 6 at ambient temperature
[b]- not done

Table 9. Mean *Salmonella* counts recovered from eggshells in log-10 CFU/mL of shell emulsion with standard deviation.

Treatment*			
A	B	C	D
4.76±0.20	4.71±0.22	4.45±0.31	4.60±0.23

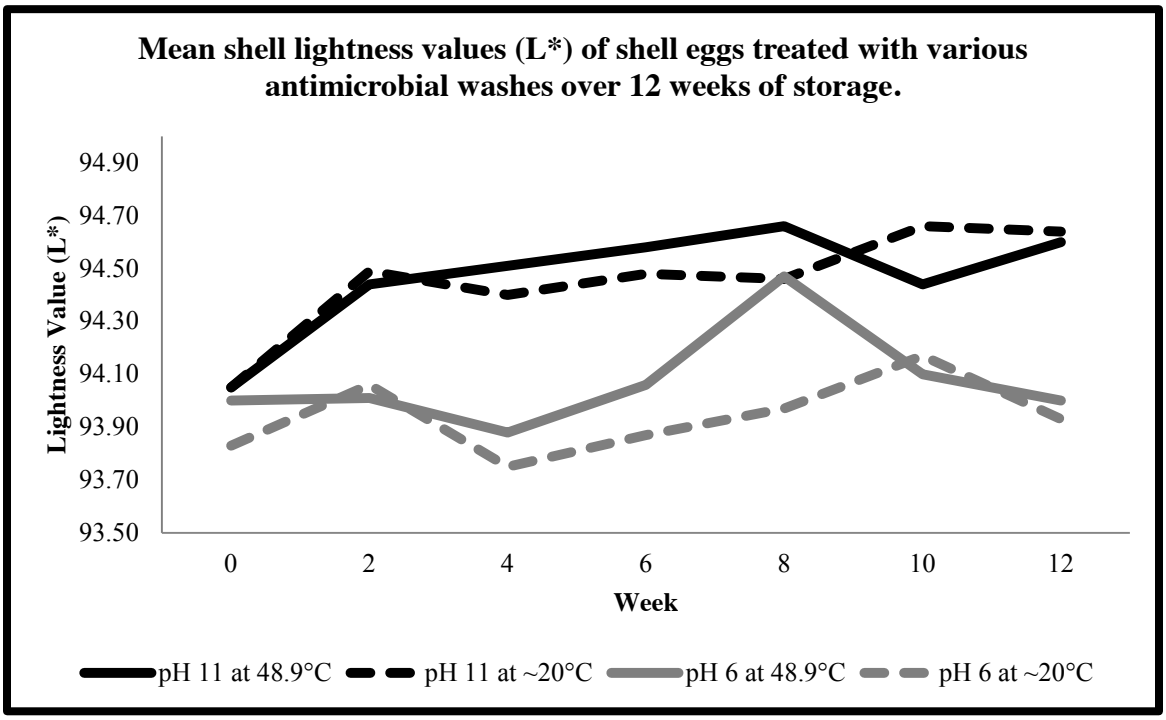
*A = pH 11 at 48.9°C; B = pH 11 at ambient temperature; C = pH 6 at 48.9°C; and
D = pH 6 at ambient temperature

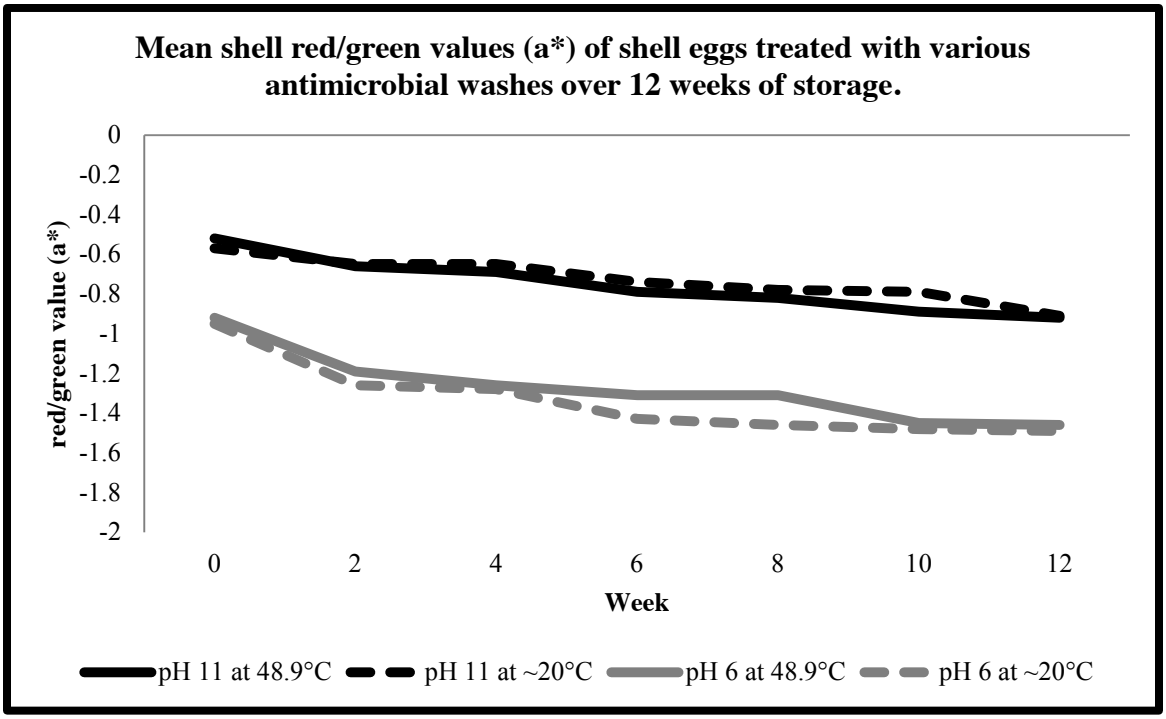
Table 10. Mean pH, temperature (°C), and free chlorine concentration (ppm) of egg wash solutions used in the antimicrobial effectiveness evaluation study. Samples were analyzed before, during, and after washing.

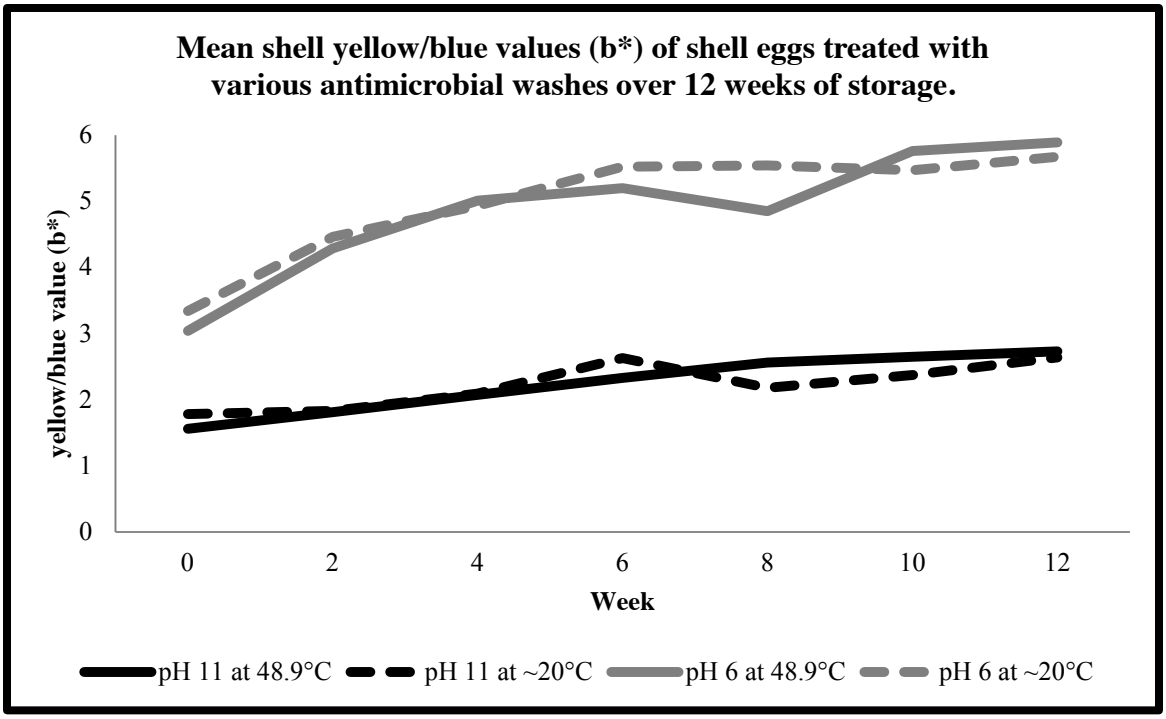
	Wash A[a]		Wash B		Wash C			Wash D		
	pH	Temp (°C)	pH	Temp (°C)	pH	Temp (°C)	Cl (ppm)	pH	Temp (°C)	Cl (ppm)
Before	11.03	47.9	11.13	20.0	6.02	47.7	178.9	6.00	20.6	180.3
During	11.01	49.4	11.07	20.9	6.12	47.6	-[b]	6.09	21.4	-[b]
After	11.00	48.7	10.97	22.6	6.15	47.9	165.7	6.13	21.7	186.8

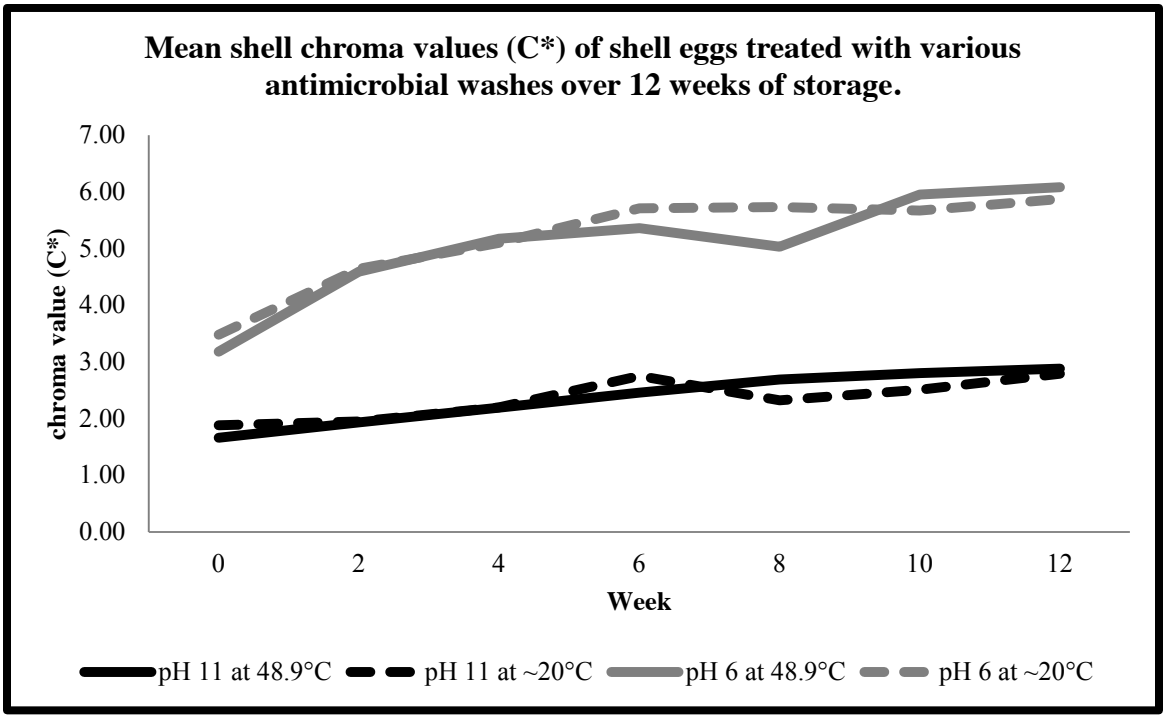
[a]A = pH 11 at 48.9°C; B = pH 11 at ambient temperature; C = pH 6 at 48.9°C; and D = pH 6 at ambient temperature
[b]- not done

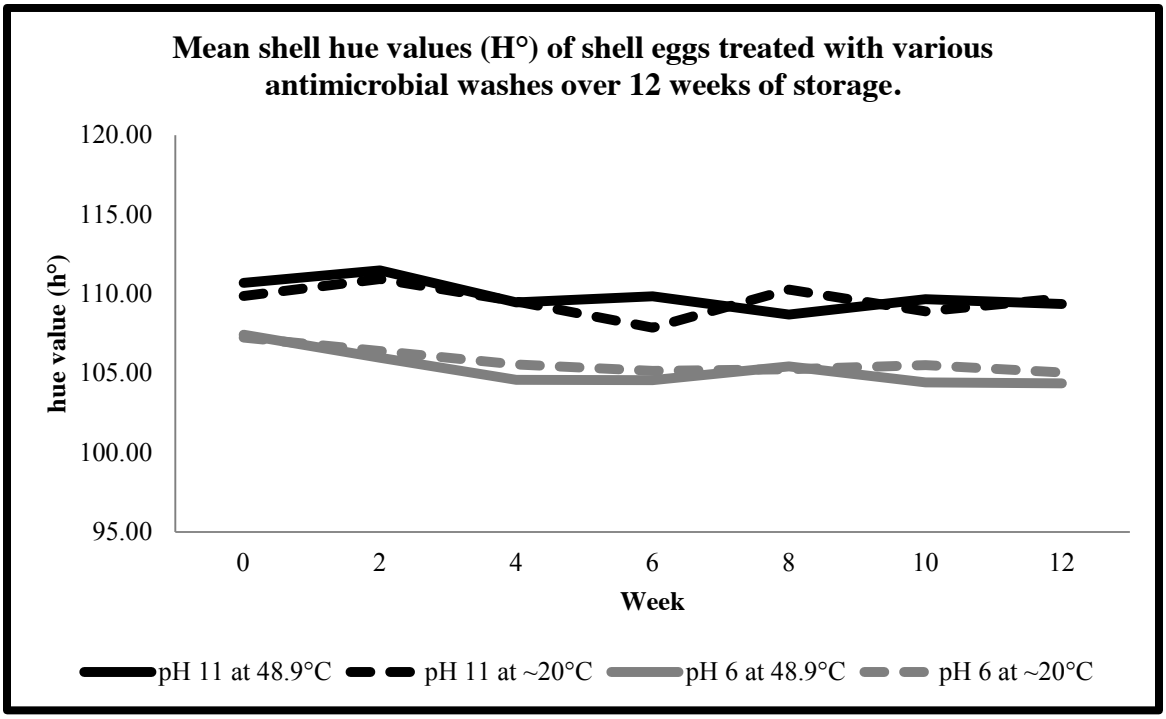
APPENDIX A

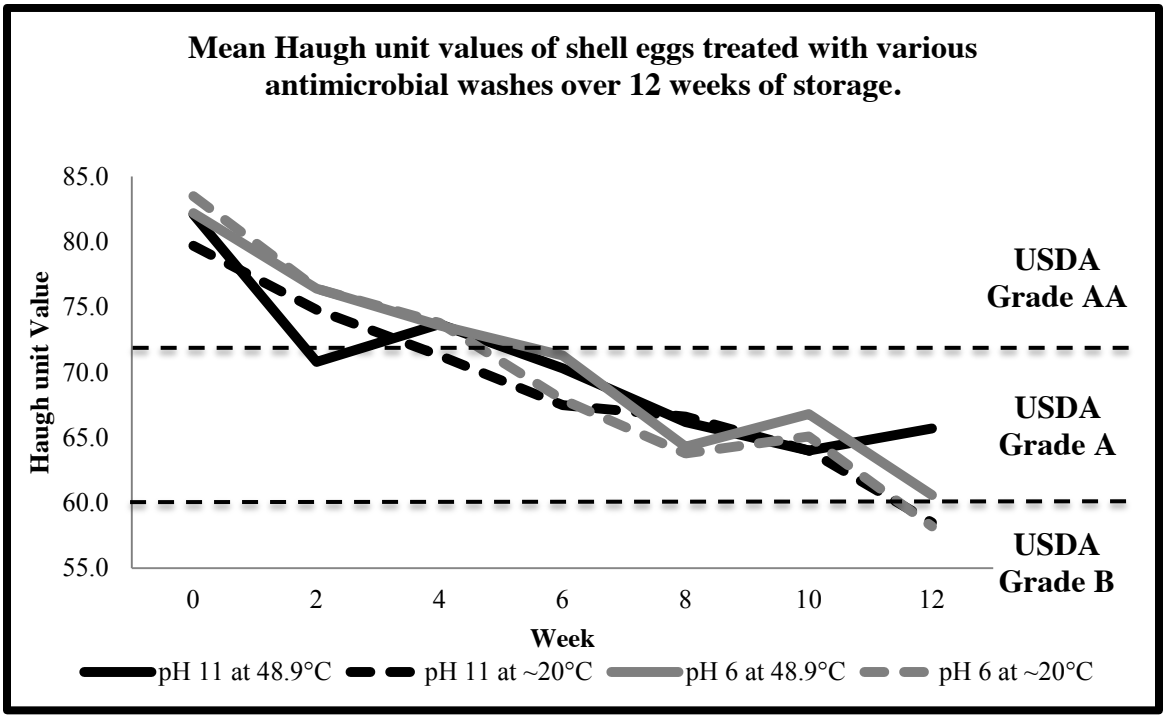

Mean shell lightness values (L*) of shell eggs treated with various antimicrobial washes over 12 weeks of storage.
Lightness Value (L*)
94.90
94.70
94.50
94.30
94.10
93.90
93.70
93.50
0
2
4
6
8
10
12
Week
pH 11 at 48.9°C
pH 11 at ~20°C
pH 6 at 48.9°C
pH 6 at ~20°C


Mean shell red/green values (a*) of shell eggs treated with various antimicrobial washes over 12 weeks of storage.
red/green value (a*)
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
-1.8
-2
0
2
4
6
8
10
12
Week
pH 11 at 48.9°C
pH 11 at ~20°C
pH 6 at 48.9°C
pH 6 at ~20°C


Mean shell yellow/blue values (b*) of shell eggs treated with various antimicrobial washes over 12 weeks of storage.
yellow/blue value (b*)
0
1
2
3
4
5
6
0
2
4
6
8
10
12
Week
pH 11 at 48.9°C
pH 11 at ~20°C
pH 6 at 48.9°C
pH 6 at ~20°C


Mean shell chroma values (C*) of shell eggs treated with various antimicrobial washes over 12 weeks of storage.
chroma value (C*)
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
0
2
4
6
8
10
12
Week
pH 11 at 48.9°C
pH 11 at ~20°C
pH 6 at 48.9°C
pH 6 at ~20°C


Mean shell hue values (H°) of shell eggs treated with various antimicrobial washes over 12 weeks of storage.
120.00
115.00
110.00
105.00
100.00
95.00
hue value (h°)
0
2
4
6
8
10
12
Week
pH 11 at 48.9°C
pH 11 at ~20°C
pH 6 at 48.9°C
pH 6 at ~20°C


Mean Haugh unit values of shell eggs treated with various antimicrobial washes over 12 weeks of storage.
85.0
80.0
75.0
70.0
65.0
60.0
55.0
Haugh unit Value
USDA Grade AA
USDA Grade A
USDA Grade B
0
2
4
6
8
10
12
Week
pH 11 at 48.9°C
pH 11 at ~20°C
pH 6 at 48.9°C
pH 6 at ~20°C