EFFECTS OF COOL WATER WASHING OF SHELL EGGS ON HAUGH UNIT, VITELLINE MEMBRANE STRENGTH, AEROBIC BACTERIA, YEAST, AND MOLD

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EFFECTS OF COOL WATER WASHING OF SHELL EGGS ON HAUGH UNIT, VITELLINE MEMBRANE STRENGTH, AEROBIC BACTERIA, YEAST, AND MOLD

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Amber “Brooke” Caudill was born in Wilkesboro, North Carolina on August 21, 1980. She graduated with honors from West Wilkes High School in Millers Creek, North Carolina in May of 1998. On December 18, 2002, she received her Bachelor of Science degree in Poultry Science with a minor in Microbiology from North Carolina State University. She began her graduate studies in the area of egg quality and safety under the direction of Dr. Pat Curtis in the Poultry Science Department at Auburn University in August, 2003.

THESIS ABSTRACT

EFFECTS OF COOL WATER WASHING OF SHELL EGGS ON HAUGH UNIT, VITELLINE MEMBRANE STRENGTH, AEROBIC BACTERIA, YEAST, AND MOLD

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Most retail shell eggs in the United States are washed in water that can be upwards of 49°C which increases the internal temperature of shell eggs. After processing, internal egg temperatures may be 6.1 to 7.8°C higher than initial internal egg temperatures. The internal post-processing temperature of shell eggs fall within the growth range of *Salmonella* Enteritidis (SE), the most common human pathogen associated with eggs and egg products. It can take several days for the internal temperature of processed packaged eggs to reach a temperature that is cool enough to inhibit the growth of most microorganisms, including SE. Washing eggs with cool water

may be a way to prevent the increase in internal egg temperature during processing. Experiments were conducted to study the effects of cool water washing on shell egg quality. The presence of aerobic bacteria, yeasts, and molds on exterior shell surfaces, in the contents, and within the shell matrix of eggs were also examined. Egg quality was evaluated by Haugh unit and vitelline membrane strength determination. This study was conducted in two phases. Phase one consisted of a pilot study, in which six different dual tank wash water temperature combinations, including a single warm water temperature (49°C) and two cool water temperatures (15.5°C and 24°C), were used to wash eggs. The pilot study was conducted in order to identify the best temperature, or combination of temperatures, for washing shell eggs while limiting the increase in the internal egg temperature. Phase two consisted of a commercial study in which shell eggs were washed using four different dual tank wash water temperature combinations in two commercial egg processing facilities. The commercial study examined how commercially washing shell eggs in cool water affects interior egg quality, as well as the presence of aerobic bacteria, yeasts, and molds on and within the egg. The pilot study and the commercial study each included ten weeks of storage in which the presence of aerobic bacteria, yeasts, and molds on exterior shell surfaces, in the contents, and within the shell matrix of processed eggs were monitored weekly. Microbial quality was monitored by the USDA Agriculture Research Service Egg Safety and Quality Research Unit. Egg quality was also monitored during both the commercial and pilot study.

During the pilot storage study, no significant differences in Haugh unit values or vitelline membrane strength were found between wash water temperature combinations,

indicating that cool water washing does not affect the egg quality measurements monitored. However, results from the pilot study showed significant differences ($P \leq 0.05$) in vitelline membrane strength and the Haugh unit values as storage time progressed. The average force required to break the vitelline membrane decreased 13.9% and average Haugh unit values decreased from 59.2 to 56.4 due to storage.

The results of the commercial study indicate that wash water temperature did not significantly affect Haugh unit values or vitelline membrane strength. As storage time progressed, however, average Haugh unit values declined 14.8% and the average force required to rupture the vitelline membrane decreased 20.6%. Although no significant differences were found among wash water temperature schemes in amounts of aerobic bacteria, yeast, and mold present on exterior shell surfaces, within the shell matrix, and in egg contents, average amounts of bacteria present on shell surfaces also decreased 11.3% during storage, and bacteria present in egg contents increased 39.5% due to storage. Results of the commercial study indicate that there is a potential for utilizing cool water washing in the commercial setting while still producing safe eggs.

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I. INTRODUCTION

Washing shell eggs is somewhat of a controversial subject. The United States, Japan, Australia, and Canada wash shell eggs; whereas, most European countries choose not to wash shell eggs. Some scientists believe that washing shell eggs increase their microbial load. Brooks (1960) concluded that washing shell eggs caused higher bacterial counts when he discovered that the contents of roughly 90% of newly laid eggs were free from microorganisms and possess natural defenses against bacterial penetration. His discovery helped support the argument for not washing shell eggs, which was based upon the fact that in the absence of water, bacteria are less likely to move through the shell or along the pores (Board et al., 1979). Another negative aspect of washing shell eggs is that the process can damage the cuticle, which is the egg's outermost covering and a natural defense against bacterial penetration (Romanoff and Romanoff, 1949; Wesley and Beane, 1967, Sauter et al., 1978; Wang and Slavik, 1998; Favier et al., 2000). Results of many early egg washing experiments indicated that washing increased spoilage, especially during storage (Lorenz and Starr, 1952; Starr et al., 1952; March, 1969). Scientists continued, however, to study the effects of different washing methods in hopes of reducing, or even preventing, rotting of eggs during storage (Moats, 1979; Lucore et al., 1997; Jones et al., 2004b; Musgrove et al., 2005). The argument for washing shell eggs is based upon the fact that microorganisms from fecal matter, blood, dirt, insects, etc. are found on the shells of eggs. The shells are porous and can be penetrated by

bacteria from the shell's exterior. Also, the nutrients that make eggs a high quality food for humans are also a good growth medium for most bacteria capable of penetrating the shell. Cleaning the shell surface removes potential contamination and reduces the incidence of bacterial penetration, in addition to providing a visually appealing product for consumers (Moats, 1980; Lucore et al., 1997; Jones et al., 2004b; Musgrove et al., 2005).

The federal authority to regulate egg safety is currently shared by the Department of Health and Human Services' Food and Drug Administration (FDA) and the United States Department of Agriculture's (USDA) Food Safety and Inspection Service (FSIS). The FDA has jurisdiction over the safety of most foods, including shell eggs. The USDA, however, is primarily responsible for implementing the Egg Products Inspection Act (EPIA). This responsibility is shared by FSIS and Agriculture Marketing Service (AMS). The FSIS is responsible for the inspection of processed egg products in order to prevent the distribution of adulterated or misbranded egg products (USDA, 2003). The AMS conducts a voluntary surveillance program which ensures that participating egg processors meet the USDA's requirements for plant sanitation, processing, labeling, refrigeration, and packaging. When eggs are packed under this surveillance program, a USDA grademark can be printed on the carton and the eggs are referred to as "shielded". Currently, processors who chose to produce USDA shielded eggs must abide by specific regulations when washing shell eggs, and are constantly monitored by an AMS inspector while shielded eggs are being produced.

Even though the US egg industry washes all table eggs sold to consumers, food safety concerns associated with the consumption of shell eggs exist. *Salmonella*

Enteritidis (SE) is the most common human pathogen associated with shell eggs and egg products. SE is one of more than 2,400 strains of *Salmonella* that can cause an infection known as salmonellosis (Bell and Kyriakides, 2002). Salmonellosis is a bacterial infection that affects the intestinal tract, and occasionally the bloodstream. Symptoms include severe diarrhea, occasional bloody diarrhea, fever, chills, abdominal cramps, and vomiting. SE is resilient and able to adapt to extremes in environmental conditions. It can grow within a pH range of 4.5 to 9.5, and in temperatures as high as 54°C (Bell and Kyriakides, 2002). The microorganism, however, does not grow well at refrigerated temperatures (Gast and Holt, 2000; Rhorer, 1991; Bell and Kyriakides, 2002; Chen et al., 2002).

It has been determined that one in 20,000 eggs produced in the United States is internally contaminated with SE (USDA, 1998). If not safely handled and properly cooked, an egg that is internally contaminated with SE may result in foodborne illness. In the year 2000, an estimated 182,060 illnesses occurred due to egg-associated SE (Shroeder et al., 2005). Because of the risk of foodborne illness associated with the consumption of shell eggs, the government has made it a top priority to make eggs safe. Emergence of Grade A eggs as a source of SE in the 1980s and 1990s contributed to an increased awareness of egg safety. This emergence was mainly due to improper handling and preparation of eggs internally contaminated with SE (St. Louis et al., 1988); however, egg processing regulations such as the re-washing of eggs and high wash water temperatures were also to blame (Anderson et al., 1992; Meckes et al., 2003). More recent egg washing research (Lucore et al., 1997) has suggested that current egg processing regulations need to be re-evaluated.

Washing, grading, and packaging increases internal egg temperature. Anderson et al. (1992) found that post-processing internal egg temperatures can be 6.1 to 7.8°C higher than initial internal egg temperatures. Due to current regulations, eggs are washed in water that can be as hot as 49°C. Most shell egg processors now use dual wash tank systems rather than the single wash tank systems previously used. The dual wash tank system doubles the time that eggs are exposed to hot water spray, which adds to the increase in internal egg temperature (Curtis, 1999). The internal temperature of an egg can continue to rise for up to six hours after the eggs have been placed in a cooler. In fact, it may actually take the centermost egg in a pallet five to six days to reach an internal temperature of 7.2°C (Anderson et al., 1992; Jones et al., 2002b; Chen et al., 2002). This means that for five to six days after processing, eggs may have an internal temperature that falls within the growth range of SE and other microorganisms. Therefore, failure to cool eggs clearly contributes to the potential for multiplication of SE and other microorganisms if they are present. Washing eggs in cool water may be one way to reduce this problem.

II. LITERATURE REVIEW

Formation and Design of the Hen's Egg

Since the domestication of the fowl, eggs have been an important part of the human diet. They contribute a number of nutrients to the American diet. Hen eggs contain approximately seventy-five percent water, twelve percent protein, ten percent lipids, and a small percentage of vitamins and minerals (Gebhardt and Thomas, 2002). They are a nutrient dense source of many essential amino acids, vitamins and minerals. Eggs contain all essential vitamins except vitamin C, and they are one of the few natural sources of vitamins D and B12. Because it is a nutritionally complete protein containing all of the essential amino acids, egg protein is one of the highest quality proteins available (McNamara, 2004). Based on a diet of 2,000 kcal per day, one large egg provides eleven percent of daily protein needs (Gebhardt and Thomas, 2002).

The egg is complex, with many different parts. Those parts include the yolk, albumen, shell membranes, shell, and the cuticle (Figure 1). It takes approximately twenty-six hours for a hen to lay one egg. Each part of the egg is formed in a separate section of the hen's reproductive tract, which is made up of the ovary and the oviduct. In the ovary, ova mature, by accumulating yolk thereby, growing in size. Typically, the largest most mature ovum breaks away from a stem connecting it to the ovary and enters the oviduct. The oviduct is the tube through which the egg passes, and where the

structures necessary to complete the egg are applied. The oviduct secretes and consecutively applies, in succession, the albumen, two shell membranes, and the shell.

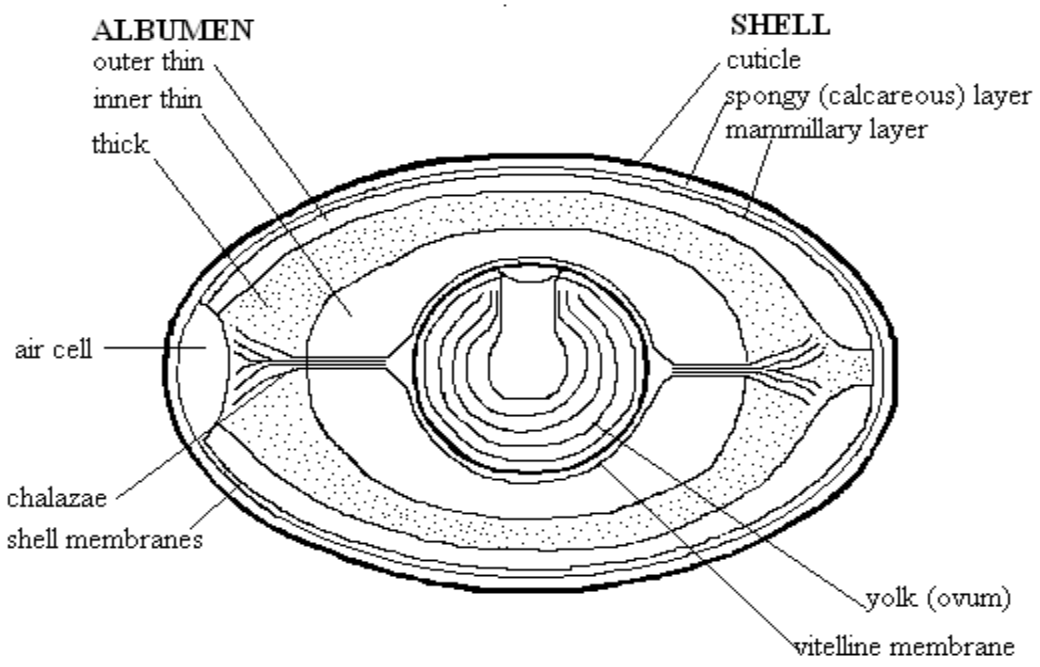

Figure 1. Parts of the egg.

Structurally, the oviduct is divided into five sections, each having a fairly specific physiological function in the formation of the egg. The oviduct consists of the infundibulum, magnum, isthmus, shell gland (uterus) and vagina. After ovulation takes place, the yolk or ovum is picked up by the infundibulum. The egg then moves from the infundibulum into the magnum, where albumen is secreted and collects in layers around the ovum. The albumen-layered ovum moves from the magnum to the isthmus by peristaltic movement. Addition of two shell membranes occurs in the isthmus. Then, after passing through the isthmus, the egg enters the uterus where it spends the most time.

This time in the uterus allows for adequate calcium deposition to form the shell. Once the shell is complete, the cuticle, which is a thin protective film of transparent material, is applied to the shell's surface while the egg is in the lower portion of the oviduct (Romanoff and Romanoff, 1949). When the shell is complete the egg moves from the uterus, leaving the oviduct through the vagina, and is expelled through the cloaca.

The yolk, which is the center of a freshly laid egg (Figure 1), makes up thirty-one percent of the egg (USDA, 2000). Major components of the yolk are proteins and lipids; nearly all the lipids, vitamins, and minerals found in eggs are located in the yolk. The yolk material is contained in a thin membrane known as the vitelline membrane (Figure 1). It is a clear membrane which gives the yolk its shape, and is composed mostly of protein matrix similar to that found in the shell membranes (USDA, 2000). The vitelline membrane is made up of three layers; the outer and inner layers are mucinous and the center layer is composed of keratin (Romanoff and Romanoff, 1949). Its strength prevents the yolk from breaking.

Surrounding the yolk is the albumen, which makes up fifty-eight percent of the egg (USDA, 2000). Albumen is made up of approximately forty different kinds of proteins, all responsible for its many functional and antimicrobial characteristics. Ovalbumin, ovotransferrin, avidin, lysozyme, conalbumin, and ovomucoid are just a few of the proteins found in the albumen. Eggs contain four layers of albumen: an inner thin layer, a thick layer, an outer thin layer (Figure 1), and the chelazaferous (inner thick), which immediately surrounds the yolk and from which the chalazae are created.

Located between the outer thin layer of the albumen and the internal surface of the shell are the inner and outer shell membranes. The two membranes adhere to each

other, and help support the weight of the shell. They also form a complex matrix which deters bacterial penetration. After an egg is laid, the contents cool from the body temperature of the hen to the ambient temperature. As the contents cool, the inner membrane contracts, causing the egg to lose gases and moisture. As this occurs, the two shell membranes separate at the large end of the egg. The outer membrane sticks to the shell and the inner membrane sticks to the egg contents, forming the air cell (Figure 1). The air cell supplies air to the developing embryo when pulmonary respiration is initiated (Romanoff and Romanoff, 1949).

The egg shell consists of the inner and outer shell membranes (Figure 1) followed by calcium deposits and different shell layers. The eggshell is about ninety-eight percent calcium carbonate in the form of calcite. It also contains magnesium, phosphate, and citrate in small amounts, as well as traces of sodium and potassium (Parkhurst and Mountney, 1988). It is 241-371 μm thick and is perforated with anywhere from 7,000 to 17,000 pores (Tyler, 1961). The thousands of pores are intended to allow for the exchange of respiratory gases, such as carbon dioxide, for the developing embryo (Romanonff and Romanoff, 1949; Wang and Slavik, 1998). The pores also permit the escape of moisture and carbon dioxide from the egg. The outer surface of the shell is covered by a thin (20 to 30 μm), hard outer protective covering known as the cuticle (Wang and Slavik, 1998). The cuticle (Figure 1) is a thin stratum of minute glycoprotein spheres, and extends a short distance into the pores of the egg (Romanoff and Romanoff, 1949), creating a seal. Immediately after being laid, the cuticle is moist and sticky, but

dries and hardens with exposure to air (Romanoff and Romanoff, 1949). The cuticle allows gaseous water to diffuse freely through the shell while inhibiting the movement of liquid water into the egg (Sparks and Board, 1984).

Microbial Defenses of the Egg

The egg is resistant to microbial contamination due to the mechanical and chemical barriers. Therefore, if bacteria are not introduced into the egg during formation, bacterial contamination can only occur after microorganisms encounter overcome these highly efficient barriers. The cuticle, shell, inner and outer shell membranes are the mechanical barriers, and the albumen contains the chemical barriers which are all parts of the egg's antimicrobial defense system.

The cuticle is the egg's first defense against microbial entry. The cuticle covers the shell and acts as a covering to inhibit bacterial penetration by closing a large portion of the pores within the shell, thereby decreasing shell permeability (Board et al., 1979). However, the cuticle can become damaged as soon as contact is made with the floor of the battery cages, by cleaning methods, harsh detergents, abrasion from washer brushes, and exposure to large amounts of water (Romanoff and Romanoff, 1949; Wesley and Beane, 1967; Sauter et al., 1978; Wang and Slavik, 1998; Favier et al., 2000). A damaged cuticle provides a way for spoilage and pathogenic bacteria to enter the egg (Board, 1966; Wang and Slavik, 1998).

The inner and outer shell membranes are two of the most effective barriers to bacterial penetration. They compose the organic matrix of the shell, a glycoprotein fine fibrous net beginning in the basal caps and the inner parts of the mammillae. They are semi permeable and permit passage of water and crystalloids (Parkhurst and Mountney,

1988). Together, the membranes function like a micron filter so extensive that it is uncertain exactly how bacteria manage to penetrate them (Haines and Moran, 1940; Romanoff and Romanoff, 1949; Board and Fuller, 1994; Anderson et al., 2004). The outer membrane is thicker and more porous than the inner membrane, minimizing it's effectiveness as a barrier to bacterial entry. The inner membrane is made up of many protein fibers that are more tightly interwoven; however, it can only delay bacterial entry for a short period of time (Board and Fuller, 1994). This ensures that there are no pores that transverse straight through to the albumen (Wang and Slavik, 1998).

Antimicrobial properties of albumen also provide a barrier against microorganisms that may have penetrated the mechanical barriers at the egg's surface (Fleischman et al., 2003). The proteins present in albumen inhibit the growth of a wide variety of microorganisms; whereas, the yolk, or even a mixture of yolk and albumen, are not as effective. Conalbumin is an example of a protein found in albumen that has important antimicrobial properties. The protein chelates metal ions, making them unavailable to bacteria for proliferation. Two other proteins in the albumen with antimicrobial properties are avidin and lysozyme. Avidin can bind to and inactivate biotin, and the lytic action of lysozyme destroys bacteria by causing the cell wall to rupture and disintegrate (Brooks and Taylor, 1955). Lysozyme plays a major role in the defense against Gram-positive bacteria (Board et al., 1986). The change in albumen pH following lay is another barrier against bacteria (Haines, 1939; Brooks and Taylor, 1955; Brooks, 1960). After an egg is laid, carbon dioxide moves out of the egg and into the surrounding environment until it's concentration in the egg and the environment reach equilibrium (Romanoff and Romanoff, 1949). The loss of carbon dioxide causes the

albumen to become more alkaline. In a newly laid egg, albumen pH is approximately 7.6 (Romanoff and Romanoff, 1949); however, the pH can increase from a fairly neutral pH to a basic 9.7 (Healy and Peter, 1925; Romanoff and Romanoff, 1949). Few bacteria are able to thrive in such a basic environment (Board, 1966). Albumen viscosity is also a barrier against bacteria. In fresh eggs, the high viscosity of the albumen and the chalazae anchor the yolk protectively in the center of the egg and hinder movement of microorganisms, especially motile bacteria, toward the yolk (Board et al., 1986). However, as the egg ages and the albumen becomes more alkaline, the ovomucin-lysozyme complex, or thick gel structure, begins to break down and the albumen becomes less viscous (Romanoff and Romanoff, 1949; Board, 1966; Williams, 1992). This reduced viscosity makes it easier for microorganisms to spread inside the egg (Chen et al., 2005).

The vitelline membrane, which keeps the yolk confined and separate from the albumen (Board and Fuller, 1974), is also one of the egg's many defenses against microbial contamination. The vitelline membrane prevents the seepage of yolk into the albumen, and is responsible for preventing the entry of bacteria into the yolk. Because the nutrients present in yolk make it a good growth medium for bacteria that may be present in the egg's albumen, the vitelline membrane plays an important role in the egg's microbial integrity. If the membrane breaks, or even stretches enough to allow yolk into the albumen or bacteria into the yolk, the yolk will provide nutrition to any bacteria present (Conner et al., 2002).

Egg Quality

Egg quality is based on the characteristics of an egg that affect its acceptability to the consumer (Watkins, 2004). Prior to the emergence of Grade A shell eggs as a potential source of SE contamination, consumers defined egg quality in physical and visual terms, and few consumers expressed concern about the microbial load contained on or within commercially processed eggs. Today, internal egg quality is defined as a function of physical, functional, and microbiological quality. External egg quality is a function shell structure, physical quality, and microbiological quality. Physical quality refers to shell characteristics such as soundness, shape, thickness, texture, and cleanliness. Functional quality refers to characteristics such as albumen viscosity, yolk color, vitelline membrane strength, and how well an egg performs in a food system. Microbiological quality refers to the absence of pathogenic bacteria.

After an egg has been laid, the rate of deterioration will never fully stop, and can only be slowed or delayed (Anderson et al., 2004). Internal egg quality decline occurs when the thick gel structures of the albumen thin and become watery, causing water to migrate to the yolk. Osmotic movement of water across the vitelline membrane leads to a flattened and enlarged yolk, as well as a stretched and consequently weakened vitelline membrane (Romanoff and Romanoff, 1949). The changes in the quality of the albumen and yolk are a function of temperature, reduced carbon dioxide, increased pH, egg age, and the loss of moisture (Romanoff and Romanoff, 1949; Williams, 1992; Chen et al., 2005; Samli et al., 2005).

Determining the Haugh unit value is the most common way to assess interior egg quality. The USDA-AMS has accepted the Haugh unit as a valid and reliable method for

determining interior egg quality. The Haugh unit is used to determine albumen quality; and it is considered the standard for shell egg interior quality measurement. Haugh (1937) discovered that the change in quality or condition of an egg varies as a negative logarithm and not as a linear function. In order to establish an accurate index of egg quality in which the numerical value would equal the quality value, he developed the Haugh unit (Haugh, 1937). The Haugh unit is a relationship between egg weight and the height of the thick albumen. There are, however, limitations associated with the Haugh unit measurements. Scientists have argued that the calculation used to determine the Haugh unit is inaccurate for eggs other than size large (Silversides et al., 1993). This is due to the fact that the calculation is weighted exclusively for a 56.7g (2oz) egg (size large). The questioned validity of the Haugh unit as an accurate indicator of interior egg quality is why Silversides et al. (1993) suggested only measuring albumen height as a means of determining egg quality. A year later, Siversides and Villeneuve (1994) reported that albumen height and the Haugh unit value equally describe albumen quality. Recent studies, however, have found that measuring the height of the inner thick albumen introduces a bias against old hens and some hen strains (Silversides and Scott, 2001). The egg's internal temperature when the Haugh unit value is being determined can also negatively affect the Haugh unit value in terms of being an accurate indicator of quality (Keener et al., 2006). In order to accurately and consistently determine Haugh unit values, eggs should be cooled to an internal temperature between 7.2 and 15.6°C, and the internal temperature of those eggs must be uniform (USDA, 2000).

Another common indicator of internal egg quality is the strength of the vitelline membrane. Vitelline membrane strength is commonly measured using static

compression (Conner et al., 2002; Jones et al., 2002b; Keener et al., 2006). A machine applies pressure to the yolk at a specified rate until the vitelline membrane is ruptured. The amount of pressure/force required to rupture the yolk corresponds to the vitelline membrane strength. The more force required, the stronger the vitelline membrane (Jones et al., 2002b).

As the egg ages, the albumen pH increases due to the loss of carbon dioxide and water moves from the albumen into the yolk; the vitelline membrane is eventually affected by the alkaline pH and becomes weak (Romanoff and Romanoff, 1949; Williams, 1992; Chen et al., 2005). As previously mentioned, additional water increases the size and weight of the yolk, which in turn stretches the vitelline membrane. The yolk appears flattened and the membrane can easily break (Romanoff and Romanoff; 1949). Conner et al. (2002) found that after eight weeks of storage in an environment with an ambient temperature of 10°C, the force required to rupture the vitelline membrane declined from 2.33 to 1.56 grams. A weak vitelline membrane can be viewed as an indicator of potential microbial contamination, as well as poor physical, quality (Gast and Beard, 1990; Humphrey et al., 1991; Humphrey, 1994; Chen et al., 2005). The disintegration or weakening of the vitelline membrane as the egg ages makes it possible for microorganisms to invade the egg yolk (Chen et al., 2005). If the vitelline membrane breaks, or even stretches enough to allow seepage of the yolk into the albumen, the yolk not only provides nutrition to any bacteria present (Conner et al., 2002); it also affects the egg's functional properties. Albumen that has been contaminated by even the smallest amount of yolk, for example, loses some of its whipping/foaming characteristics due to the lipid content of the yolk (Romanoff and Romanoff, 1949).

Bacteria on and in the Egg

Despite the egg's many microbial barriers, bacteria are still able to penetrate the shell and membranes. Factors that improve bacteria survivability on the shell surface, reducing the egg's antimicrobial defense system, include the physical condition of the cuticle and underlying shell (Sparks and Board, 1984); the presence of water on the shell (Board et al., 1979); and the concentration of iron in water that comes into contact with the egg (Board et al., 1986). If the cuticle is damaged or washed away, the pores are exposed, and there is a greater susceptibility to microbial entry into the contents (Board, 1966; Wang and Slavik, 1998). The diameters of pores range from 9-35 μm (Romanoff and Romanoff, 1949), which is significantly larger than most microorganisms (which are typically 1-5 μm). *Salmonella* species, for example, range from 0.7-1.5 μm wide and 2.0-5.0 μm long (Bell and Kyriakides, 2002). Because pores are larger in size, *Salmonella* species and other bacteria found on the shell can move through them into the contents and cause spoilage.

Microorganisms found on egg shells are capable of breaching the shell's microbial barriers. These microorganisms are mainly Gram-positive bacteria derived from dust, soil and feces (Haines, 1939; Zasgaevsky and Lutikova, 1944**;** Board, 1964, 1966). The dominant contaminants on the shell tend to be Gram-positive cocci and bacillus such as Micrococcus and Arthrobacter (Hutchinson et al., 2003). Once the shell's microbial barriers have been breached, Gram-negative bacteria are more capable of withstanding the antimicrobials present in the albumen (Board, 1966; Jones et al., 2004a); therefore, the internal contaminants of eggs are commonly Gram-negative organisms such as Alcaligenes, Achromobacter, *Pseudomonas fluorescens*, *Salmonella*,

and Eschericia (Hutchinson et al., 2003). A study conducted by Jones et al. (2002a) found that SE and *Psudomonas fluorescens* were both able to survive at different rates in various parts of the egg. While SE survived best on the exterior surface of the shell, *Pseudomonas fluorescens* was better able to transverse the shell membranes and infect the contents of the egg. Florian and Trussel (1957) identified *Pseudomonas fluorescens* as a primary invader of the inner shell membranes and predicted that its presence allows other organisms, referred to as secondary invaders, to breach the membranes. These secondary invaders are only able to pass thru the membranes once mechanical barriers, such as inner shell membrane, have been breached by primary invaders (Florian and Trussel, 1957).

Over the years, eggs have changed in a number of ways. They have become larger and rounder in shape (Curtis, 1999; Anderson et al., 2004). Tharrington et al. (1999) noted that genetic improvements in commercial layer strains have impacted egg size. The study suggested that in the past forty years eggs have become larger and contain a smaller percentage of yolk, which in turn, results in a lower percentage of yolk fat. These genetic improvements have made eggs more susceptible to microbial penetration (Curtis, 1999). Jones et al. (2002a) found that for some historic layer strains, a decrease in the microbial integrity of the eggs may have accompanied the genetic changes at these points in time. They suggested that screening for microbial integrity should be included in the selection process among laying hen breeders. The results of a study conducted by Jones et al. (2004a) indicate that genetic selection over time has altered eggs' ability to withstand microbial contamination and penetration during storage.

The authors suggest that factors such as porosity of the shell, thickness of the shell membranes, and concentration of natural antimicrobials may have been altered by genetic selections.

Although the egg industry washes all table eggs sold to consumers, potential food safety concerns associated with the consumption of shell eggs exist. An estimated one in 20,000 eggs in the United States contain SE, and can cause illness if eaten raw or not thoroughly cooked in foods before consumption (USDA, 1998). Each year, *Salmonella* species are implicated in approximately 50,000 cases of bacterial food poisoning in the United States (Meckes et al., 2003). *Salmonella* bacteria have been known to cause illness for over one hundred years. SE is the most common human pathogen associated with shell eggs and egg products.

SE is a Gram-negative, motile, rod-shaped bacterium. It can grow under aerobic and anaerobic conditions, is resilient, and able to adapt to extreme environmental conditions. The microorganism can survive and grow at temperatures as high as 54°C. SE growth in eggs, however, is inhibited at temperatures of 7.2°C and below (Rhorer, 1991; Curtis, 1999; Bell and Kyriakides, 2002; Chen et al., 2002). SE can be transmitted from the laying hen to the egg either as a result of fecal contamination or infection of the oviduct. If SE is present on the egg's shell, there is the potential for the contents to become infected as well. Gast and Beard (1990) reported a correlation between egg shells contaminated with SE and SE positive feces from artificially infected hens. Eggs can also be infected with SE during formation. This can occur if the intestinal tract of a hen is colonized with SE. The SE, if present, can then migrate into the reproductive tract, where possible contamination of yolk, albumen, or both can occur (Gast and Beard,

1990). If the ovary of a hen is infected with SE, during egg formation, the yolk (ova) may become seeded with SE cells before leaving the ovary or while passing through the oviduct. When this occurs, the egg typically contains low numbers of SE cells when it is laid (Humphrey et al., 1989, 1991; Gast and Beard, 1992; Chen et al., 2002).

If eggs or egg products containing live *Salmonella* bacteria in high enough populations are consumed, an illness known as salmonellosis can occur. Salmonellosis is one of the more common foodborne illnesses in the US. Foods associated with salmonellosis are those of animal origin, fruits, and vegetables have all been found at some point to be contaminated with *Salmonella*. Some foods of animal origin commonly associated with salmonellosis include poultry, milk and dairy products, eggs, and seafood (Bell and Kyriakides, 2002; CDC, 2003a; USDA, 2005). Symptoms of the illness usually develop within 8-72 hours after ingesting the bacteria. Diarrhea, fever, abdominal cramps, chills, headache, nausea, and vomiting are all symptoms of salmonellosis; they typically last four to seven days (Bell and Kyriakides, 2002; CDC, 2003a; USDA, 2005). A total of 5,198 laboratory-diagnosed cases of foodborne *Salmonella* infections occurred during 2001 (CDC, 2002). Because mild cases are typically not diagnosed or reported, the actual number of infections may be thirty or more times greater (CDC, 2003a). Approximately twenty percent of the population is considered to be at a higher risk for salmonellosis because they are immuno-compromised (USDA, 1998). Immuno-compromised individuals include the very young, the very old, hospital patients, nursing home residents, and individuals with compromised immune systems. *Salmonella* infections can be life-threatening for the immuno-compromised. It is estimated that approximately six hundred immuno-compromised individuals die each year with acute

salmonellosis (CDC, 2003a). Most people recover from salmonellosis without any long-term health problems; however, about two percent of those who do recover may later develop recurring joint pains and arthritis. The annual cost associated with human salmonellosis due to SE is estimated to range from $150 million to $870 million (USDA, 1998).

From 1976 to 1986, reported SE infections increased more than six fold in the northeastern United States. From January, 1985 to May, 1987 65 foodborne outbreaks of SE, associated with 2119 cases and eleven deaths, were reported. Seventy-seven percent of the outbreaks with identified food vehicles were caused by Grade A shell eggs or foods that contained such eggs (St. Louis et al., 1988). In 1999, there were nineteen outbreaks of salmonellosis in the United States. Of those nineteen outbreaks for which a vehicle could be confirmed, fifteen (79%) were associated with shell eggs (Meckes et al., 2003). In 2001, state and local health departments reported 46 confirmed outbreaks of SE infection to CDC. A food vehicle was confirmed for 24 of the 46 outbreaks. Eggs were an ingredient in 15 (63%) of the 24 confirmed vehicles (CDC, 2003b).

Due to the increasing number of human illnesses associated with the consumption of SE contaminated shell eggs, in December of 1996 the FSIS and the FDA joined together in order to develop a comprehensive risk assessment of SE. The goals of the SE Risk Assessment included determining the total risk of foodborne illness caused by SE, identifying and evaluating possible strategies to reduce the risk of SE contamination, identifying areas in which future research was needed, and prioritizing future data collection efforts (USDA, 1998). In order to best determine the total risk of SE related foodborne illness, the Risk Assessment consisted of five modules. Those modules were:

(1) Egg Production Module, (2) Shell Egg Module, (3) Egg Products Module, (4) Preparation and Consumption Module, and (5) Public Health Module. The Egg Production Module estimated the number of eggs produced that were internally contaminated or infected after lay with SE. The Module estimated that on average 3.3 million SE positive eggs are produced from the 65 billion eggs laid in those years (USDA, 1998).

The Shell Egg Module, the Egg Products Module, and the Preparation and Consumption Module estimated the increase or decrease in the number of SE organisms present in eggs or egg products as they passed through storage, transportation, processing, and preparations. The Shell Egg Module followed shell eggs from collection through processing, transportation, and storage. Important components of this model were the amount of time required for loss of the vitelline membrane's integrity and the growth rate of SE in eggs after the vitelline membrane's breakdown. The Egg Products Module tracked the change in the amount of SE present in further egg processing facilities from receiving thru pasteurization. The Preparation and Consumption Module explained that extended storage times and ambient temperatures encouraged the growth of microorganisms that might be present in the contents of eggs. When identifying and evaluating possible strategies to reduce the risk of SE contamination, the Shell Egg and Egg Products Modules determined that the use of multiple interventions/precautions would result in a more substantial reduction in SE illnesses than simply using one intervention/precaution by itself. Two interventions which showed the most potential for reducing the number of SE illnesses associated with the consumption of contaminated

eggs were: (1) lowering the temperature in which shell eggs were maintained, and (2) diverting eggs produced by SE-positive flocks from the shell egg market to the pasteurized, egg products market (USDA, 1998). The Public Health Module calculated the frequency of SE illnesses, as well as the clinical and possible long-term outcomes of those illnesses.

In addition to the SE Risk Assessment, President Clinton established a Council on Food Safety in August, 1998. The Councils' main goals were to reduce and prevent the incidence of human salmonellosis and to protect the health of American people by preventing foodborne illness using well-coordinated surveillance, investigation, inspection, enforcement, research, educational programs, and science-based regulation. Preventing human salmonellosis includes benefits such as reducing economic losses associated with the reduction of productivity linked to human illness, reducing pain and suffering, and reducing expenditures on medical treatment (USDA, 1998). In order to identify gaps in the scientific community's understanding of SE and its route of on-farm transmission, the President's Council of Food Safety created the Egg Safety Action Plan (USDA, 1998). In August of 1999, the President's Council on Food Safety held a public meeting in order to obtain input during development of the Egg Safety Action Plan. Representatives from consumer groups and the egg industry came to the conclusion that the federal government needed a set of mandatory national standards which would assure consumers that all eggs across the United States were subject to the same safety standards. In order to help meet their goals, the Council of Food Safety commissioned the Egg Safety Task Force. The Egg Safety Task Force included federal food safety agencies responsible for egg safety. FDA, CDC, FSIS, APHIS, AMS, and ARS, were responsible

for developing an action plan to eliminate egg-associated SE illnesses. The Egg Safety Action Plan included a farm-to-table continuum which focused on preventing SE contamination of eggs on the farm prior to lay, after eggs have been laid, during processing, and following processing, as well as promoting the use of safe egg handling practices by food preparers in the retail industry and in homes across America. The overall public health goal of the Egg Safety Action Plan is to eliminate SE illnesses associated with the consumption of eggs by 2010. When developing the Egg Safety Action Plan, one responsible agency for each stage of the farm-to-table continuum was identified based on the strengths of each agency. The FDA's responsibilities included developing standards for the producer, and enforcing those standards by requiring States to provide on-farm inspections. The FSIS was responsible for developing standards for both shell egg packers and egg products processors. The FDA and CDC were responsible for conducting surveillance and monitoring activities. The Egg Safety Action Plan gave the egg industry a choice between two SE reductions strategies. Those strategies included a SE testing-egg diversion system on the farm, or a lethal treatment or "kill step" at the packer/processor. Both strategies required regulatory personnel to be present on the farm and at the packer/processor.

In 2005, two new risk assessments, SE in shell eggs and *Salmonella* species in egg products, were created with information obtained after the release of the 1998 SE Risk Assessment (USDA, 2005). These new risk assessments predicted that pasteurization and rapid cooling of eggs would be the most effective means of reducing illnesses from SE contaminated eggs and egg products contaminated by *Salmonella* species. The SE in shell eggs assessment estimated that storing and holding eggs at

7.2°C within 12 hours of lay would reduce human illnesses from 130,000 to 28,000 per year (USDA, 2005). The *Salmonella* species in egg products assessment concluded that the annual number of human illnesses would be reduced from 130,000 to 41,000 if all eggs produced in the US were pasteurized for a 3-$\log_{10}$ reduction of SE (USDA, 2005). The risk assessments also identified several opportunities for further research. These opportunities include a nationally representative survey for the prevalence of SE in domestically produced flocks, hens, and shell eggs; a characterization of growth parameters of SE in shell eggs; a quantitative study of cross-contamination during shell egg and liquid egg product processing; studies on how SE differs from other *Salmonellae* in ability to persist in chicken reproductive tissue and egg contents; and a characterization of egg storage times and temperatures on farms and in homes, for eggs produced off-line and for eggs at retail (USDA, 2005).

Washing and Storing Shell Eggs

As previously mentioned, washing eggs has not always been an accepted means of cleaning and preserving them; however, washing shell eggs has been a common practice in America since the mid-20th century. Before modern egg processing technology, farmers momentarily dipped their eggs in boiling water in order to preserve them (Board, 1966). The late 1800's and early 1900's marked the beginning of modern egg production. The first mechanical continuous egg washing systems were developed in the 1950s (Hutchinson et al., 2003). At that time, the most common type of egg washer was a wire basket that could hold 50-60 eggs at one time. The basket was manually lowered into a rotating washing machine. A household dish or laundry detergent was added, and the eggs were submerged and agitated for approximately one to three minutes

before being removed (Hutchinson et al., 2003). This type of washing is referred to as static or immersion washing. In 1959, methods for rapidly washing large quantities of eggs using immersion washing for mass processing were developed (Lucore, 1994). A study conducted by Lorenz and Starr (1952) compared bacterial loads of spray washed eggs and immersion washed eggs. They found that spray washing drastically reduced the percentage of spoiled eggs during storage. This is because the cuticle will cease to exist if it is wet for an extended period of time (Board, 1966, 1979; Wang and Slavik, 1998). It was also discovered that static water in washing machines produced more spoilage than sprayed water (Lorenz and Starr, 1952). This is due to a negative pressure gradient that is created when eggs are fully submerged in water that is slightly cooler than that of the eggs. The negative pressure causes wash water, as well as any bacteria present in that water, to be pulled into the eggs. In 1975, static, or immersion, washers were banned and replaced by spray washers (USDA, 1975). Not only were immersion washers banned, but it also became illegal to soak eggs as a means of cleaning them. However, it is currently not illegal for a farmer to clean eggs by immersing them in water before the eggs are sent to the processing plant.

There have been significant changes over the past forty-plus years in egg processing. One such change has been the industry's shift from off-line production, where eggs are placed on flats or carts at the farm and transported to the processing facility two to three times a week, to in-line production, where multiple houses in a single complex are connected by a common egg belt which transports eggs directly from the layer house into the processing facility. In-line production has enabled the egg industry to get eggs from the bird to the consumers' table in a shorter period of time (Curtis,

1999). In the past 15 years, other changes in egg processing have included the use of computer controlled, high speed, high volume egg washers (Knape et al., 2002).

Washing shell eggs is not only a way to reduce the risk of pathogenic bacteria from being on the egg shell, it also provides a clean, visually appealing product for consumers. In the United States, as in most countries, customers demand eggs that are visibly clean, making it difficult to sell dirty eggs. Therefore, despite potential pitfalls, a number of countries such as the United States, Canada, Sweden, Australia, and Japan have embraced egg washing. Although Starr et al. (1952), Lorenz and Starr (1952), and March (1969) found that washed eggs suffered more bacterial spoilage than unwashed eggs, Forsythe et al. (1953) reported that washing can effectively remove over 80 to 90% of shell contaminates when using different types of chemical agents. The opposing results were due to the washing method. Forsythe et al. (1953) utilized a method that involved lightly brushing eggs while a stream of water flowed onto them; whereas, the others washed eggs by immersion washing. Moats (1979) also showed that washing under commercial conditions (which at that time included spray washing rather than immersion washing) was highly effective in reducing surface bacterial counts on egg shells to low levels. In fact, washing has been shown to reduce the number of microorganisms on egg shells from 43,000 per shell to less than 10 (Lucore et al., 1997). Musgrove et al. (2005) found that current commercial practices decrease the prevalence of eggs contaminated with aerobic bacteria by thirty percent. More importantly, current commercial practices have been found to reduce the number of aerobes present on eggs by 99.9% (Musgrove et al., 2005). In countries where egg washing has become a routine

and established practice, it is regarded as safe, and is perceived by consumers as an essential part of the hygienic production of eggs (Hutchinson et al., 2004).

In the United States, most shell eggs are washed using a general process that involves four stages: wetting, washing, rinsing, and drying. Most commercial facilities spray wash eggs using a dual wash tank system. Typically, the eggs are placed on rollers which act as a conveyor belt. The rollers carry the eggs through the four stages of the washing process. The first stage of egg washing is wetting, or pre-washing, which softens any debris that may be on the egg shell. The eggs then go through two different wash tanks. In each wash tank the eggs pass under rotary or reciprocating brushes while they are sprayed with warm wash water. The rollers that the eggs are carried on continuously turn the eggs, enabling all surfaces of each egg to be exposed to the brushes and warm water spray (Hutchinson et al., 2003). The wash water with which the eggs are sprayed with is continuously re-circulated. A food grade detergent is added to the wash water in order to help remove fecal matter, blood, dirt, stains, etc., and to maintain a high pH ($\geq$10.5). It is important to maintain a high pH in egg wash water in order to maintain low counts of total aerobic bacteria in wash water (Bartlett et al., 1993). Once the eggs have been washed, they are rinsed with high pressure jets of warm water and sanitizer. Rinsing the eggs removes any loose debris that may have been picked up by the eggs during the main washing process. Rinsing also removes any residues left by detergents and defoamers in the wash water, and helps decrease the risk of cross contamination associated with the brushes used during the main washing process (Hutchinson et al., 2003). After being rinsed, the eggs are blown dry. Once the eggs have been blown dry, they are graded, packed, and placed in a cooler or shipped directly to a retail outlet.

According to Bell et al. (2001) and Patterson et al. (2001), shell eggs are purchased by the consumer within an average of nineteen days after they have been processed.

The major parameters influencing egg washing are: wash water quality and mineral content, wash chemicals, pH of wash water, and temperature of the wash water (Hutchinson et al., 2003). The hardness of the water entering the processing facility can have a dramatic impact on the ability of detergents and sanitizers to operate properly (Jones et al., 2003); therefore, it is important that wash water not be too hard. Natural or artificial contamination of wash water with iron salts results in a high incidence and fast rate of egg spoilage**.** Research conducted by Garibaldi and Bayne (1960, 1962) associated the presence of iron in egg wash water with increased spoilage in washed eggs. When iron is present in the wash water, it reverses the bacteriostatic action of an antimicrobial known as conalbumin, which is found in the egg's albumen (Garibaldi and Bayne, 1962). Iron, which is an essential trace nutrient, is required by many microorganisms in order to grow. Once iron is introduced into the egg, one of the egg's microbial defenses, the bacteriostatic action of conalbumin, is useless and microorganisms are able to grow due to the availability of an essential trace nutrient (Garibaldi and Bayne, 1960, 1962). Current USDA regulation (7 CFR 56.76(e)(6)) requires shell egg processors producing USDA shielded eggs to conduct an analysis of the iron content of their water supply. If the iron content exceeds two parts per million, the regulation requires the provision of equipment to correct the excess iron content. Defoamers also play an important role in egg washing. Defoamers are chemicals that are added to egg wash water because one of the main functional properties of eggs is as a foaming agent in food preparation. During the washing process, eggs can be broken;

therefore, re-circulated wash water typically contains albumen. Egg foam is created when air is incorporated into the proteins and water of egg albumen. The washing process incorporates air into the re-circulated wash water which contains albumen, and foam can be created. Without the proper addition of defoamers to the wash water, foam will build up in the wash tanks and eventually overflow. When foam spills from the tanks, it can interfere with the level, pH, and temperature of the wash water.

Detergents are wash chemicals which are added to the wash water in order to elevate the pH. They are dispensed, for the most part, in concentrations necessary to clean the egg shell (Curtis et al., 2004). Most processing facilities continuously monitor the amount of detergent present, and have machines that automatically dispense detergent when needed. Moats (1978) found that eggs washed in water containing a sanitizing chemical invariably spoiled less that eggs washed in water alone. Wash water pH is also an important egg washing parameter. Catalano and Knabel (1994) reported that maintaining wash water conditions at pH 11 or above prevents possible cross-contamination caused by recycled wash water by effectively reducing the number of SE present on egg shells and in wash water. When studying various combinations of wash water temperature and pH, Kinner and Moats (1981) found that at a pH ranging from 10 to 11 the amount of bacteria present in wash water decreased, regardless of water temperature (35, 40, 45, 50, or 55°C). Although the temperature of wash water is an important egg washing parameter, if it is more than 4.5 to 10°C above the temperature of the eggs being washed, thermal cracks may occur. Thermal cracks occur when the egg contents expand and actually cause the shell to crack. Decreasing the bacterial load of processed eggs is more efficiently accomplished by controlling pH, rather than increasing

wash water temperature. It has been shown, however, that when no or improper control over wash water pH and temperature is used, the eggs can have a higher bacterial load after being washed than before (March, 1969; Moats, 1978). This is most likely due to being washed with re-circulated wash water containing a high bacterial load. Controlling wash water pH is also a means of controlling bacterial growth in re-circulated wash water.

The egg shell is sensitive to acid, and may become damaged or dissolve if it is exposed to a relatively strong acid for any extended amount of time. Because of eggs' sensitivity to acid, the pH is controlled using alkaline detergents. When used according to manufacturers' recommendations, alkaline detergents produce an initial pH in the wash water near 11, and help to maintain the pH in the 10-11 range during washing. Raising the pH of wash water to 10-11 significantly reduces the number of organisms, such as coliforms, present in the wash water and has been shown to kill *Salmonella* species which could potentially contaminate clean egg shells (Kinner and Moats, 1981; Catalano and Knabel, 1994). Pearson et al. (1987) reported that egg wash water of high pH was bacteriostatic to *E. coli* and *Salmonella*, and suggested that HACCP programs involve regular sampling and analyses. Barlett et al. (1993) also reported that there is a strong relationship between a pH equal to or greater than 10.5 and low counts of total aerobic bacteria in wash water sampled from commercial facilities. Holley and Proulx (1986) found that when the wash water pH was 9.5 or less, *Salmonella* species were able to survive in wash water with a temperature as low as 42°C.

Because the detergent plays such an important role in egg washing, it is equally important to use the right type of detergent. Quaternary ammonium compounds,

chlorine, sodium carbonate, sodium hydroxide, sodium hypochlorite, and potassium hydroxide are some examples of commonly used egg washing detergents. Unfortunately, detergents which may be effective in reducing the bacterial load found on eggs may also damage the egg's cuticle or shell (Sauter et al., 1978; Wang and Slavik, 1998**;** Favier et al., 2000; Hutchinson et al., 2004). In order to study the effects of chemicals used in egg washing on microstructural changes of eggshells, Wang and Slavik (1998) washed eggs using three common commercial egg washing detergents **-** a quaternary ammonium compound, sodium carbonate, and sodium hypochlorite. Their washing process was conducted in a laboratory setting and took 3.5 minutes. The washing time included 2.5 minutes for brushing and rinsing and one minute for blow-drying. They found that while the quaternary ammonium compound and sodium hypochlorite cleaned the eggs without causing excessive damage to eggshell surfaces, sodium carbonate, removed large parts of the eggshell surface layer and most of the cuticle layer. Wang and Slavik (1998) concluded that different degrees of cuticle damage can be produced on eggshell surfaces by different types of egg washing chemicals, and that altered eggshell surfaces may allow greater microbial penetration. Despite the possible pitfalls associated with alkaline detergents, if the right type of detergent will physically remove or inactivate up to 92% of the bacteria on an eggshell's surface without damaging the cuticle (Forsythe et al., 1953; Bierer et al., 1961; Wang and Slavik, 1998). Detergents used to wash eggs should be food safe and compatible with the eggs, the washing equipment, and any other chemicals used in the washing process (Hutchinson et al., 2003).

Despite how well an egg is washed, storage will cause a decline in egg quality and slowly breaks down the egg's natural barriers, making it increasingly susceptible to

bacterial entry and growth (Brooks and Taylor, 1955; Board, 1966; Kim et al., 1989; Humphrey, 1994; Wang and Slavik, 1998; Jones et al., 2004b). As early as the mid 1900's, scientists (Lorenz and Starr, 1952; March, 1969) observed changes that occurred in washed eggs during storage. These changes caused increased bacterial infections, and eventually lead to spoilage.

In 1989, Kim et al. reported that various characteristics of albumen and yolk quality are lost as eggs age. When an egg is newly laid, the yolk is located in a central position. The central position of the yolk is primarily due to the support it receives from the albumen, and is regarded as an indicator of high quality. During storage, however, the albumen begins to break down and is no longer able to provide as much support for the yolk. This results in the increased movement of the yolk, which indicates poorer quality (Board, 1966). Jones et al. (2002b), Jones and Musgrove (2005), and Samli et al. (2005) have all reported a decrease in Haugh unit values during storage due to the break down of albumen. Also, when an egg is newly laid, the vitelline membrane is strong and prevents the yolk from seeping into the albumen. However, Elliot and Brant (1957), Hartung and Stadleman (1963), Jones et al. (2002b), and Chen et al. (2005) have all found that storage length negatively affects vitelline membrane strength.

The changes that occur to an egg's internal components during storage not only result in a decline in quality; they also cause the egg to become more susceptible to bacterial growth. Humphrey et al. (1991) reported that egg age can impact SE growth. Humphrey (1994) and Jones et al. (2004a) reported that SE contamination of egg contents increased during storage at 20°C and 26°C, respectively. When studying bacterial penetration into washed eggs stored at different temperatures and times, Wang and Slavik

(1998) found that storage time was an important factor for *Salmonella* penetration into egg contents; the longer the storage time, the more the *Salmonella* penetration. The high temperatures and high pH of egg wash water kill most, but not all of the microorganisms present on egg shells. The microorganisms that are not killed are physiologically damaged. An extended storage period gives these injured microorganisms time to rejuvenate. Once rejuvenated, they are better able to work their way through the shell membranes and into the albumen. Another factor that increases the susceptibility of eggs to bacterial growth during storage is the breakdown of the albumen. As previously mentioned the albumen in fresh eggs is highly viscous and anchors the yolk in the center of the egg, thus hindering the movement of microorganisms toward the yolk (Board et al., 1986). Not long after an egg is laid, chemical changes cause the gel structure of the albumen to break down, and the albumen becomes less viscous (Romanoff and Romanoff, 1949; Board, 1966; Williams, 1992). The relatively high pH of albumen creates an unfavorable growth environment for most microorganisms; however, when albumen viscosity changes, motile bacteria that may be present are less restricted and able to migrate into eggs' contents more easily (Chen et al., 2005).

As previously discussed, the vitelline membrane becomes weak and also begins to break down during storage. Scientific studies have shown that egg age has an obvious impact on the ability of SE to grow rapidly in albumen adjacent to the yolk (Humphrey, 1994). Conner et al. (2002) found that the ability of SE to grow in albumen corresponds to a decline in the force required to break the vitelline membrane. An aged and weakened vitelline membrane becomes permeable and may allow bacteria to enter the yolk, yolk contents to enter the albumen, or both (Humphrey, 1994; Conner et al., 2002;

Chen et al., 2005). Studies using eggs from artificially (Gast and Beard, 1990) and naturally SE infected (Humphrey, 1994) hens have shown the albumen next to the vitelline membrane to be an important SE contamination site. Scientists have also found that SE will grow well near the vitelline membrane, but will not grow in areas away from the membrane (Murase et al., 2005). Kim et al. (1989) reported that *Salmonella* are severely inhibited and sometimes killed by conalbumen or ovatransferrin found in high concentrations in the albumen. The ovatransferrin chelates iron and generally prevents bacterial growth; however ovatransferrin does not prevent growth of bacteria on the yolk surface (Kim et al., 1989). Researchers have found that egg yolk supports rapid microbial growth (Clay and Board, 1991; Humphrey and Whitehead, 1993; Gast and Holt, 2000), and that the multiplication of microorganisms located in albumen does not occur until the bacteria present have accessed the yolk (Sharp and Whitaker, 1927; Gast and Holt, 2000). This is because egg yolk is rich in iron and contains nutrients needed to support the rapid growth of bacteria (Clay and Board, 1991; Humphrey and Whitehead, 1993; Gast and Holt, 2000). As yolk components migrate into albumen, bacteria that have previously exhausted the albumen's iron reserve have a renewed supply (Schaible et al., 1944; Humphrey, 1994). Although SE cells require iron to grow, they generally cannot make use of iron present in the yolk of fresh eggs because the vitelline membrane prevents the entry of bacteria into yolk contents as well as the release of iron into the albumen (Humphrey, 1994). If, however, contact with yolk contents does occur and permissive temperatures exist, the egg becomes an environment in which SE can grow rapidly (Conner et al., 2002). A recent study conducted by Gast et al. (2005) found that

SE and *Salmonella* Heidelberg deposited outside the vitelline membrane of freshly laid eggs is sometimes able to reach yolk contents and begin to multiply within a day of storage at a warm temperature.

Because high wash water temperatures currently required by USDA regulations increase internal egg temperature (Anderson et al., 1992), they can accelerate the rate of functional decline and microbial growth (Williams, 1992; Lucore et al., 1997; Fleischman et al., 2003). As the temperature of egg wash water rises, there is an increased risk of cuticle damage and thermal cracking (Wesley and Beane, 1967). Cuticle damage and thermal cracking provide ways for spoilage and pathogenic bacteria, especially from the egg wash water, to enter the egg. High wash water temperatures also cause the internal temperature of eggs to rise. In 1955, Hillerman reported that wash water maintained at 46.1°C would increase internal egg temperatures by 0.22°C per second. Anderson et al. (1992) found that post-processing internal egg temperatures can be 6.1 to 7.8°C higher than initial internal egg temperatures. In addition to the initial rise due to processing, an egg's internal temperature can continue to rise for up to six hours after being placed in a cooler (Anderson et al., 1992). In a more recent study, Jones et al. (2002b) found that after processing, shell eggs required at least five days to reach an internal 7.2°C when stored at 7.2°C. This means that for five or more days after processing, eggs may have an internal temperature that falls within the growth range of SE and other microorganisms (Anderson et al., 1992; Chen et al., 2002; Jones et al., 2002b). After being processed, eggs are typically packaged in cartons or flats, 30 dozen eggs (in cartons or flats) are placed into cases, and then 30 cases are palletized. These packaging

conditions help to ensure that the increase in internal egg temperature will be maintained for several days. Feddes et al. (1993) found that eggs packed in cases cool at a rate that is seven times slower than uncased eggs. Czarick and Savage (1992) suggested that the use of solid cardboard cases be abandoned if the goal of the egg industry is to obtain egg temperatures of 7°C as rapidly as possible.

It is also possible that the heat from high wash water temperatures not only increases the internal temperature of the egg, but weakens the vitelline membrane as well (Fleischman et al., 2003). Research conducted by Kinner and Moats (1981), Holley and Proulx (1986), and Lucore et al. (1997) suggests that wash water temperatures commonly used by most egg processors is neither hot enough to kill microorganisms on the shell nor cool enough to inhibit their growth. High egg wash water temperatures serve to increase internal egg temperatures, and act as an added buffer to prevent rapid cooling of the egg; thus allowing organisms on the shell, as well as inside the egg, to continue to grow (Lucore et al., 1997). The dual wash tank system, commonly used by most egg processors, forces the eggs stay in a hot, wet environment for a longer period of time (Curtis, 1999), which adds to the increase in internal egg temperature. SE contaminated eggs typically contain less than one hundred cells per egg at the time of lay (Humphrey, 1994); however, if an egg is contaminated with SE, increased internal egg temperature caused by high wash water temperatures, combined with the break down of the egg's antimicrobial defenses, provide SE cells opportunity to rapidly grow. The rate of the vitelline membrane deterioration, for example, is increased when the egg is exposed to high storage temperatures. Proper refrigeration has been shown to slow quality decline (Conner et al., 2002; Chen et al., 2005). Eggs should be stored in a cool environment in

order to reduce loss of moisture, reduce albumen thinning, slow weakening of the vitelline membrane, and most importantly, to prevent/reduce microbial growth (Conner et al., 2002; Chen et al., 2005).

Researchers have discovered that if SE is present in egg contents, the bacteria's growth rate directly responds to the temperature at which the eggs were stored. In 1989, Kim et al. found that as storage temperatures increased, the growth rate of SE in eggs did as well. They concluded that storage temperature is the most important factor in determining the growth response of SE in eggs. Gast and Holt (2000) reported difficulty in promoting SE growth in eggs stored between 10 and 17.5°C. Other scientists have found that storage temperatures of 7.2°C and below reduce the colonization and subsequent growth of *Salmonella* in eggs (Rhorer, 1991; Bell and Kyriakides, 2002; Chen et al., 2002). Humphrey et al. (1989) reported that storing eggs at refrigerated temperatures causes SE to be more susceptible to the high temperatures used in cooking eggs. The Shell Egg Processing and Distribution Module within the SE Risk Assessment found an eight percent reduction in foodborne illnesses when eggs are maintained at an ambient temperature of 7°C throughout shell egg processing and distribution (USDA, 1998). Storing eggs at 7°C or below combined with quickly reducing the internal egg temperature, also serves to prevent the growth of any bacteria that may be lodged in pores of the egg shell. In addition to making the egg an inhospitable environment for most bacteria, reducing post-processing internal egg temperature as quickly as possible could also have a positive impact on egg quality. As previously discussed, vitelline membrane strength and albumen quality are both influenced by internal egg temperature.

The importance of internal egg temperature has led scientists to developed methods to quickly reduce eggs' post-processing internal temperature (Curtis et al., 1995; Thompson et al., 2000). Thompson et al. (2000) found that a properly managed forced-air system could quickly cool packaged eggs. Curtis et al. (1995) discovered that cryogenic gases could quickly cool eggs before packing. In 2002, Jones et al. (2002b) reported that egg quality was enhanced by quick cooling and exposure to gaseous carbon dioxide. Unfortunately, each of the methods developed require the use of additional equipment and changes in plant design. The extra costs associated with these methods have deterred egg processors from using them.

Gast et al. (2006) reported that the effectiveness of refrigeration for limiting bacterial multiplication in eggs is dependant upon initial level and location of contamination, movement of bacteria or nutrients within the egg, and the rate at which growth-restricting temperatures are achieved. Although processors have little control over initial level and location of contamination as well as movement of bacteria and nutrients within the egg, they can more easily control the amount of time needed to achieve growth-restricting temperatures. However, current shell egg processing regulations, combined with the current technology, limits the processors' ability to lower the internal egg temperature in a very short period of time (Curtis, 1999). As previously mention, shell eggs are generally purchased by the consumer within an average of nineteen days after being processed (Bell et al., 2001; Patterson et al., 2001). Because most eggs reach the retail outlet in such a short period of time, reducing their internal temperature to 7.2°C or below can be challenging. It has been suggested that washing eggs in cool water, as opposed to warm water, could aid in reaching and maintaining

growth-inhibiting internal egg temperatures of 7°C or below. Current regulations, however, require egg wash water to be 90°F (32.2°C), or 20°F (11.1°C) warmer than the warmest egg, and maintained at that temperature (7 CFR 56.76(f)(3)). Research supporting this regulation was conducted in the mid 1900's. In 1940, Haines and Moran reported that egg wash water colder than internal egg temperature causes the negative pressure gradient previously discussed. Research conducted by Lorenz and Starr (1952) concluded that eggs washed in cold water were more likely to spoil than eggs washed in warm water. In 1948, Funk presented data which indicated that when the temperature of the wash water was lower than the internal temperature of the egg, losses in storage were definitely greater compared with storage losses in eggs washed in water warmer than internal egg temperature. However, a similar group of experiments conducted at a different time found that storage losses among washed dirty eggs were not influenced by the temperature of the wash water (Miller, 1954). The specifics of the wash water temperature regulation, however, are mainly based on research conducted by Brant and Starr (1962) and Brant et al. (1966). Their research concluded that the temperature of the wash water should be greater than 11°C warmer than the egg temperature. There is, however, a problem with research supporting the current wash water temperature regulation. When the research was conducted, the most common way to wash eggs was by immersion washing. Eggs were completely submerged in water and agitated for one to three minutes. As previously mentioned, eggs are currently spray washed and never fully immersed in wash water.

Recent research conducted on the effects of lower wash water temperatures is rather contradicting. In 1997, Lucore et al. presented good evidence that cooler wash

water temperatures do not contaminate shell eggs in any greater amount than warm to hot temperatures. They also recommended a re-examination of cold water washing procedures. Using pilot egg processing equipment and a spray wash system in a pilot plant, Lucore et al. (1997) compared the effects of three wash water temperatures upon internal and external shell surface bacterial counts. They reported that internal microbial counts from eggs spray washed with water as cool as 15.5°C were no different from internal microbial counts of eggs spray washed with 48.9°C water. In a more recent inoculation study (conducted in a laboratory setting), Hutchinson et al. (2004) reported that wash and rinse water temperatures did not significantly effect surface populations of SE. They also, however, reported that allowing wash and rinse water temperatures to fall below 34°C caused a detectable amount of content contamination. Although it is not clear why, it is possible that the results reported by Lucore et al. (1997), contradict the findings of Hutchinson et al. (2004) due to a difference in wash water pH, a difference in washing environment and equipment (pilot egg processing equipment in a pilot plant versus a laboratory setting), or because the temperature of only the wash water was lowered and the rinse water temperature remained consistent with USDA guidelines (7 CFR 56.76(f)(11)). Lucore et al. (1997) also found that cool water washing aided in reducing the internal temperature of eggs once they have been washed, packaged, and placed into the cooler. This, in turn, reduces the amount of time needed to cool eggs, and appears to reduce microbial contamination levels by inhibiting their growth (Lucore et al., 1997). There is also a possibility that washing eggs in cool water could help maintain, or even enhance, interior egg quality during storage. More rapid cooling of the

egg to refrigerated temperatures may help maintain vitelline membrane strength, and possibly decrease the chances of any nutrients becoming available for microbial growth.

Previous research conducted to determine the effects of cool water washing of shell eggs has been performed in a laboratory setting and has not taken into account the bacteria found in recycled wash water utilized in commercial processing facilities. High wash water temperatures are not only used as a means of preventing the entry of bacteria into eggs, but also as a means of controlling the bacteria found in the re-circulating tank. Research conducted by Kinner and Moats (1981) showed that at a neutral pH, the temperature range used to wash eggs is not lethal to most types of bacteria. They found that rapid bacterial multiplication occurred at pH 7 and 8 at a temperature range of 35 to 45°C; however, at a pH of 10 and 11 bacterial numbers decreased at all temperatures used in the study (35, 40, 45, 50, and 55°C). In 1994, Leclair et al. studied the effects of wash water temperatures ranging from 38°C to 46°C and pH ranging from 9.5 to 10.5 on the inactivation of S. *typhimurium* and L. *monocytogenes*. They found that recycled wash water required significant increase in temperature (47.4°C), as well as pH (10.8), in order to eliminate the two pathogens. That same year, however, after washing artificially contaminated eggs in 37.7°C water at pH 9 and 11, Catalano and Knabel (1994) found that the higher pH significantly reduced external SE contamination. They reported that high pH prevents possible cross-contamination caused by recycled wash water by effectively reducing the number of SE present on egg shells and in wash water. The research conducted by Kinner and Moats (1981) and Catalano and Knabel (1994) suggest that if pH is controlled, and the wash water temperature lowered, it is possible to get the same bacterial kill level without excessively increasing the internal temperature of the

eggs. Previous research has determined that spray washing eggs in cool wash water does not increase internal bacterial counts of shell eggs; however its affect on bacteria found in the re-circulating tank remains unknown. Cool water washing of shell eggs in a commercial setting, rather than a laboratory setting, will give better insight into its affect on bacteria found in recycled wash water and commercially processed eggs.

The intended purpose of cool water washing of shell eggs is to help reduce internal egg temperatures during and after processing and possibly prevent the multiplication of SE if it is present. Attaining growth-inhibiting temperatures of 7°C or below shortly after processing will reduce the probability that consumers will be exposed to amounts of pathogenic bacteria present in egg contents sufficient to cause foodborne disease. In addition to initiating the egg cooling process and shortening the cooling time, a cool water wash could benefit egg processors by reducing, or even eliminating, the cost of heating wash water and by decreasing the amount of energy needed to cool eggs following processing. Cool water washing could also be economically beneficial to the egg industry by reducing wear and tear on refrigeration units in cooler rooms.

Regulations

The egg industry became large enough to warrant regulatory intervention from the government in 1910, when egg consumption exceeded 300 eggs per capita (Lucore, 1994). In 1928, the USDA began the inspection of eggs. In the 1950's the USDA placed requirements on the washing and sanitizing of shielded shell eggs (Lucore, 1994). Further regulations dealing with egg processing were introduced in 1967. These regulations required that continuous-typed washers have the wash water changed once per shift; however, specifications as to the length of time for a shift were not included

(Lucore, 1994). In 1970, the Egg Products Inspection Act (EPIA) was passed (USDA, 2003). The EPIA was designed to prevent the marketing of checks, dirties, leakers, losses and inedible eggs to the consumer.

Implementation and enforcement of the EPIA is the primary responsibility of the USDA. The act requires commercial flocks of more than 3,000 hens to be registered with the USDA. Producers that have 3,000 laying hens or more and any egg handler or distributor that sorts and segregates eggs for sale to the consumer are subject to mandatory inspections. These mandatory inspections are conducted at least once per quarter by Federal or State inspectors. The responsibility of implementing and enforcing the EPIA is currently shared by the FSIS and Agriculture Marketing Service (AMS). In order to ensure that only eggs fit for human consumption are used for such purposes, the FSIS conducts mandatory surveillance of egg packers. The AMS conducts a voluntary surveillance program that ensures participating egg processors meet USDA requirements for plant sanitation, processing, labeling, refrigeration, and packaging (USDA, 2007). When eggs are packed under this surveillance program, a USDA grader must be present and an official USDA grademark can be printed on the carton. These eggs are referred to as "shielded".

Processors who chose to produce USDA shielded eggs must abide by specific USDA regulations (USDA, 2007). One regulation pertains to the recycling of wash water. As previously discussed, egg wash water is continuously recycled in order to achieve better use of limited amounts of water. There is, however, an increase in bacterial numbers in the recycled water due to the fact that the recycled water is warm and carries an organic load. In an attempt to reduce the potential hazards of recycling

wash water, the government requires egg processors to empty their old wash water and replace it with clean water every four hours or more often if needed to maintain sanitary conditions, and at the end of each shift (7 CFR 56.76(f)(5)). In addition to removing the organic load carried by recycled wash water, replacing used wash water with clean water (including detergent) helps ensure that the wash water is at a pH of 10 or greater. Most processing facilities continuously monitor the amount of detergent present, and have machines that automatically dispense detergent when needed. Another regulation states that the wash water temperature must be at least be 90°F (32.2°C), or 20°F (11.1°C) warmer than the warmest egg entering the processing line, and that this temperature must be maintained (7 CFR 56.76(f)(3)). The most recent USDA regulation, which applies to all shell eggs, states that eggs must be stored in a post-processing environment of 7.2°C or cooler (9 CFR 590.50(a)). Because SE does not grow well at refrigerated temperatures (Gast and Holt, 2000; Bell and Kyriakides, 2002), the post-processing refrigeration temperature requirement serves as a means to control potential foodborne pathogens associated with eggs.

The federal authority to regulate egg safety is currently shared by the USDA and the Department of Health and Human Services' Food and Drug Administration (FDA). The FDA has jurisdiction over the safety of foods in general, which includes shell eggs. With regard to eggs and egg products, the FDA's top priority is their safety. One way the FDA ensures the safety of eggs and egg products is by enforcing federal labeling requirements (21 CFR 160). They also require retail establishments to refrigerate shell eggs as soon as they are received and continue to store them in an environment with an ambient temperature of 7.2°C or cooler (21 CFR 115.50). These regulations are intended

to help reduce the incidence of SE in eggs; thus, making eggs safer for consumers. In order to improve egg safety, the FDA also investigates SE outbreaks that are due to foods in interstate commerce. If eggs have been implicated in any of those SE outbreaks, the FDA is responsible for performing trace backs in order to identify the source of those eggs.

In order to prevent foodborne illness, it is imperative to lower post-processing internal egg temperatures as quickly as possible. Current shell egg processing procedures and regulations, however, are responsible for a significant increase in internal egg temperatures during and after processing. Packaging materials then act as insulation and make it difficult to rapidly reduce internal egg temperatures. As previously discussed, research conducted in the 1990's found that spray washing shell eggs in cool water did not increase internal shell bacterial counts. If fact, the cool water aided in reducing internal egg temperatures following processing and packaging (Lucore et al., 1997).

There is also a possibility that washing eggs in cool water could help maintain, or even enhance, interior egg quality during storage. Cool water washing could also provide economic benefits to the egg industry by reducing, or even eliminating, the cost of heating wash water and decreasing the amount of energy needed to cool eggs following processing. The objectives of the following research are to determine if cool water washing of shell eggs alters levels of microbial populations, enhances egg quality, and provides a positive economic impact for the shell egg industry. The effects of cool water washing of shell eggs have been determined for eggs washed in a laboratory setting;

however, the effects are not known for eggs processed in commercial processing facilities. Because of this, a large part of the subsequent research occurs in a commercial setting.

III. EFFECTS OF COOL WATER WASHING OF SHELL EGGS ON VITELLINE MEMBRANE STRENGTH AND HAUGH UNIT VALUES

ABSTRACT SE is currently the most common human pathogen associated with shell eggs and egg products. Its growth is inhibited at temperatures of 7.2°C and below. Because today's egg washing process can increase internal egg temperature 6.7 to 7.8°C, obtaining internal egg temperatures of 7.2°C and below can be difficult. Washing eggs at a cooler temperature could speed the reduction in internal egg temperature, and in turn, reduce potential SE growth by preserving interior quality factors such as vitelline membrane strength and Haugh unit. A pilot study was conducted to determine if washing eggs in cool water would allow for more rapid cooling of eggs and possibly affect interior egg quality. Six different dual tank wash water temperature combinations, which included a single warm water temperature (49°C) and two cool water temperatures (15.5°C and 24°C), were used to wash eggs. A storage study followed, in which the vitelline membrane strength was monitored weekly for ten weeks, and Haugh unit values were determined for days 0, 30, and 60 post-processing. Wash water temperature did not significantly affect vitelline membrane strength or Haugh unit values. There were, however, significant differences ($P \leq 0.05$) in the force required to break the vitelline membrane and Haugh unit values due to storage. The average force required to break the

vitelline membrane decreased 13.9% due to storage, and average Haugh unit values decreased from 59.2 to 56.4 by day 60.

(*Key words*: shell eggs, cool wash, egg quality, egg processing)

INTRODUCTION

Processors who chose to produce USDA "shielded" eggs must abide by specific USDA regulations. One such regulation states that egg wash water must be at least be 90°F (32.2°C), or 20°F (11.1°C) warmer than the warmest egg entering the processing line (7 CFR 56.76(f)(3)). Due to this regulation, eggs from in-line operations (hen houses directly connected to the processing facility) can be washed in water as hot as 48.9°C. The most recent regulation pertaining to egg processing applies to all shell eggs and requires eggs to be stored in a post-processing environment of 7.2°C or cooler (USDA, 1999). Because scientists have found that the growth of *Salmonella* Enteritidis (SE), the organism most often associated with foodborne disease and eggs, is inhibited at temperatures of 7.2°C and below (Rhorer, 1991; Bell and Kyriakides, 2002; Chen et al., 2002), the post-processing refrigeration temperature requirement serves as a means to control potential foodborne pathogens associated with eggs. Washing, grading, and packaging, however, can cause post-processing internal egg temperatures to be 6.1 to 7.8°C higher than initial internal egg temperatures (Anderson et al., 1992). The internal temperature of an egg can continue to rise for up to six hours after processing, packaging, and being placed in a cooler (Anderson et al., 1992). It can take five or more days for the centermost egg in a pallet to reach an ambient temperature of approximately 7.2°C (Anderson et al., 1992, Jones et al., 2002b; Chen et al., 2002); therefore, for five or more days after processing, eggs may have an internal temperature that falls within the growth range of SE and other microorganisms. Reducing post-processing internal egg temperatures as quickly as possible will help prevent and inhibit the growth of any foodborne pathogens that may be present in egg contents.

The increase in internal egg temperature during and after processing can be attributed to the high temperatures currently used in egg washing. In 1981, research conducted by Kinner and Moats found that wash water bacterial counts decreased, regardless of the temperature, when the water was at a pH of 10 and 11. Washing in warm water increases internal egg temperature and serves as an added buffer to prohibit quick cooling of the egg; thus allowing organisms on the shell, as well as inside the egg, to continue to grow (Lucore et al., 1997).

Due to the increasing number of human illnesses associated with the consumption of SE contaminated shell eggs, scientists have been focusing on finding ways to reduce the egg's internal temperature during and after processing. Methods based on the use of cryogenic gases as well as forced cool air to rapidly cool shell eggs post-processing have been developed (Curtis et al., 1995 and Thompson et al., 2000, respectively). Although it has been shown that egg quality is maintained or even enhanced by these methods of rapid cooling (Curtis et al., 1995; Thompson, et al., 2000; Jones et al., 2002b), the methods require additional equipment and some alteration of plant design. Due to cost and space constraints, their use by the egg industry has been limited. Because washing in warm water increases internal egg temperature, serves as an added buffer to prohibit quick cooling of the egg, and in turn, allows organisms on the shell and inside the egg to continue to grow (Lucore et al., 1997), research has been conducted to determine the possibility of preventing excessive increases in internal egg temperature during processing through cool water washing. Lucore et al. (1997) found that spray washing eggs in 15.5°C wash water did not increase the internal bacterial counts of shell eggs. They also reported decreased bacterial counts on egg shells as wash water temperature

decreased. Lucore et al., (1997) concluded that cooler wash water temperatures help reduce the amount of time needed to cool eggs, and they recommended a re-examination of cold water washing procedures.

Reducing post-processing internal egg temperature as quickly as possible may also help enhance egg quality. Two common ways to assess the interior quality of an egg are measuring the force required to break the vitelline membrane and determining the Haugh unit value (HU). Vitelline membrane strength has become increasingly important for food safety reasons (Messens et al., 2005). The vitelline membrane surrounds the yolk and is responsible for separating the yolk from the albumen (Board and Fuller, 1974). Its strength is an important quality factor because it protects the yolk from breaking or leaking nutrients into the albumen and possibly allowing bacteria to penetrate the yolk. Vitelline membrane strength is influenced by internal egg temperature (Fleischman et al., 2003) and storage time. As the egg ages, vitelline membrane strength declines (Conner et al., 2002; Jones et al., 2002a; Chen et al., 2005). The membrane also breaks down faster at higher storage temperatures (Romanoff and Romanoff, 1949; Chen et al., 2005). The degradation of the vitelline membrane can also affect the functional properties of the egg. Albumen that has been contaminated by even the smallest amount of yolk, for example, loses some of its whipping/foaming characteristics due to the lipid content of the yolk (Romanoff and Romanoff, 1949).

Determining the HU is a common way to assess interior egg quality (Haugh, 1937) and has been accepted by USDA-AMS as a valid and reliable method (USDA, 2000). The HU value is a function of egg weight and the height of the thick albumen (Haugh, 1937). Although the HU is commonly used to measure interior quality, there

are limitations associated with HU measurements. The calculation used to determine the HU is weighted exclusively for a 56.7g (2oz) egg (size large); which is why Silversides et al. (1993) questioned the validity of the HU as an accurate indicator of interior egg quality. They argued that the calculation was inaccurate for eggs other than size large and suggested measuring albumen height in order to determine interior quality. More recently, however, scientists have reported that albumen height and the HU value equally portray albumen quality (Silversides and Villeneuve, 1994). Like vitelline membrane strength, the HU tends to decline as the egg ages (Williams, 1992; Jones et al., 2002b; Jones and Musgrove, 2005; Samli et al., 2005).

The purpose of this study was to identify the best temperature, or combination of temperatures, for washing shell eggs while limiting the increase in the internal egg temperature. This study also intends to determine if cool water washing of shell eggs in the pilot setting impacts egg quality.

MATERIALS AND METHODS

Washing Eggs

Nest run shell eggs were purchased from a local packer and identified as originating from a single laying flock. Before being washed, all eggs were stored on nest run carts at 7.2°C. Eggs were washed using a fabricated pilot egg washer which was designed to mimic commercial wash conditions (Figure 1). The pilot washer was a stainless steel unit with eleven, six wide egg rollers (Sanova Engineering Corp, Elk Grove Village, IL). One row of rollers was used for the drive belt and rotated the eggs during the washing process (26 rpm). Spray nozzles were mounted in the top of the unit, and positioned in a way that ensured each egg was sprayed with wash water. The spray

nozzles' pressure averaged 4 psi. In order to mimic commercial wash conditions, the pilot washer was designed as a dual tank washer and the wash water was recycled. One aspect of the pilot washer that did not mimic commercial wash conditions was its lack of brushes.

For three consecutive days (replicates), eggs were washed using six wash water temperature combinations (n = 50 eggs/wash). As seen in Table 1, each temperature combination consisted of a temperature for the first and second wash tank. A single warm water temperature of 48.9°C was utilized along with two cool water temperatures of 15.5°C and 24°C. The single warm water temperature of 49°C was utilized because it represents the warmest temperature commonly utilized by shell egg processing facilities in order to meet USDA regulations. The two cool water wash temperatures were selected based on the limitations to cool water in the commercial processing facility. The pH of the wash water was maintained between 10.5 and 11.5 in order to mimic commercial wash conditions.

Each day, one cart (5400 eggs/cart) of the nest run shell eggs was processed. Only one third of the eggs were utilized in determining egg quality; the remaining two thirds were split, with one third utilized as untreated controls and for aerobic population determinations and one third inoculated with SE (Jones et al., 2005). During processing, eggs were exposed to the wash water spray for a total of one minute (30 seconds per wash tank). Immediately after washing, the eggs were sprayed with a 49°C sanitizing solution that contained 200 ppm chlorine, in accordance with USDA guidelines (7 CFR 56.76(f)(11)). After being sprayed with sanitizer, the eggs were aseptically removed from the rollers, randomly placed into new foam cartons, and allowed to air dry before

the cartons were closed. A ten week storage study followed, in which the cases of eggs were stored on pallets at 7.2°C until analysis. During the storage study, the presence of aerobic bacteria and SE, the vitelline membrane strength, and HU values were monitored weekly. Weekly aerobic population and SE determination was conducted by the USDA's Egg Safety and Quality Research Unit. Results from the microbial analysis are reported in a separate manuscript (Jones et al., 2005).

Measuring Vitelline Membrane Strength

Each week, a 12-egg sample from each temperature combination was removed from storage and candled; all cracked eggs were excluded from testing. Vitelline membrane strength was determined using a TA-XT2i texture analyzer (Texture Technologies, Scarsdale, NY). A texture analyzer determines vitelline membrane strength using static compression (Conner et al., 2002; Jones et al., 2002b; Keener et al., 2006). Each egg was individually broken into a shallow dish and the yolk was positioned under a 1mm, rounded end, stainless steel probe. Because Lyon et al. (1972) reported that the strongest section of the vitelline membrane in near the chalazae, care was taken to ensure that measurements were not obtained from this area. Direct pressure was applied to the yolk until the vitelline membrane ruptured and the probe penetrated the yolk. Compression measurements were made using a 5 kg load cell (calibrated using a 2 kg weight), 0.1 gram trigger force, and 3.2 mm/sec test speed. The vitelline membrane breaking strength was recorded as grams of force required to rupture the membrane. The force required to break the vitelline membrane corresponds to its strength; a strong membrane requires more force to break.

Determining Haugh Unit Values

On days 0, 30, and 60 of storage, a 12-egg sample from each temperature combination was removed from storage, candled, cracked eggs were excluded, and the HU value for each egg was recorded. With the assistance of a QCD instrument range (Technical Services and Supplies, Dunnington, York, England), HU values were determined using procedures based on the formula described by Haugh (1937).

Statistical Analysis

The data collected was analyzed using SAS (1999). HU values and force required to rupture the vitelline membrane were analyzed according to the general linear model. Any means that were found to be significantly different ($P \leq 0.05$) were separated using the least-squared means option of the general linear model procedure.

RESULTS AND DISCUSSION

Although eggs washed in temperature combination 5 averaged the greatest force required to rupture the vitelline membrane (1.56 g), wash water temperature configuration did not significantly affect vitelline membrane strength (Table 2). Ten weeks of storage, however, caused a steady decline in vitelline membrane strength (Figure 3). As storage time progressed, the average force required to break the vitelline membrane decreased 13.9%.

Like vitelline membrane strength, there were no significant differences between wash water temperature combinations in average HU values (Table 2). Eggs washed in temperature combination 1, however, had the lowest average HU value (54.3) and eggs washed in the temperature combinations 4 and 5 had the highest average HU values (61.3, 61.4 respectively). Although initial HU values were poor and equivalent to USDA

Grade B quality (USDA, 2000), there were significant differences in average HU values as storage time progressed. The average HU value was 59.2 on the day of processing, but actually increased to 61.5 after 30 days of storage. After 60 days of storage, the average HU value decreased to 56.4, a 4.7% decline from the initial value and a significant 8.3% decline from the average value after 30 days of storage.

The decline in vitelline membrane strength and HU values observed in the current study was not surprising. As early as the mid 1900's, scientists such as Lorenz and Starr (1952) and March (1969) had observed that changes occur in washed eggs during storage. As previously mentioned, scientists have found that extended storage causes vitelline membrane strength and Haugh unit values to decline (Elliot and Brant, 1957; Hartung and Stadleman; 1963; Williams, 1992; Conner et al., 2002; Jones et al., 2002b; Chen et al., 2005; Jones and Musgrove, 2005; Samli et al., 2005). In addition to causing a decline in egg quality, storage slowly breaks down the egg's natural barriers and causes the egg to become increasingly susceptible to bacterial entry and growth (Board, 1966; Humphrey, 1994). Some scientists suggest that the degradation of the vitelline membrane provides nutrients for SE growth (Conner et al., 2002; Fleischman et al., 2003) because a weakened vitelline membrane cannot prevent yolk from seeping into the albumen. If yolk is introduced into the albumen, the yolk negatively affects many of the albumen's antimicrobial properties. This is due to the fact that yolk contents are rich in iron, which SE cells require in order to grow, and provide nutrients that serve as a growth medium for *Salmonella* organisms that previously exhausted the iron reserves of the albumen (Humphrey, 1994).

The decline of an egg's internal quality occurs when the thick gel structures of the albumen become thin and the vitelline membrane becomes weak. The albumen pH of a newly laid egg is approximately 7.6 (Romanoff and Romanoff, 1949); however, as the egg ages, the albumen becomes more alkaline and may increase to approximately 9.7 (Healy and Peter, 1925; Romanoff and Romanoff, 1949). Few bacteria are able to thrive in such a basic environment (Board, 1966). As the albumen becomes more alkaline, the gel structure begins to break down, causing the thick albumen to thin and become watery (Romanoff and Romanoff, 1949; Williams, 1992). When this occurs, water is absorbed from the albumen into the yolk, causing the yolk to increase in size and weight. The yolk's increased weight and size causes the vitelline membrane to stretch and weaken (USDA, 2000). Because the rate of interior egg quality decline increases as the environmental temperature rises (Romanoff and Romanoff, 1949; Kim et al., 1989; Chen et al., 2002), quickly reducing the post-processing internal egg temperature can help maintain internal egg quality.

These data indicate that wash water temperature does not affect average vitelline membrane strength and HU values, and suggest that cool water washing has the potential to improve interior egg quality. As seen in Table 2, eggs washed using temperature combination 1, which is commonly utilized by egg processors, had the lowest average HU values. Also, eggs washed using temperature combinations containing only cool water temperatures (4 and 5) had the greatest average vitelline membrane strength and HU values. Cool water washing of shell eggs could allow for more rapid cooling after processing while maintaining interior egg quality. Maintaining interior egg quality characteristics, especially the integrity of the vitelline membrane, combined with

reducing the eggs' internal temperature will aid in retarding the growth of any potential pathogenic bacteria present. Jones et al., (2005) found that all wash water temperature combinations investigated in this study were equally capable of removing SE. Data collected during this study suggest that there is a potential for utilizing cool water washing in the commercial setting while still producing quality eggs that are microbiologically safe for consumption. Washing shell eggs in cool water could also be economically beneficial to the egg industry by reducing the energy needed to heat wash water and cool eggs after they have been processed and packaged. A commercial study will be conducted in order to better determine the effects of cool water washing on interior egg quality, aerobic bacteria, yeast, and mold presence, and the frequency of *Salmonella, Campylobacter, Listeria,* and *Enterobacteriaceae*.

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Figure 1. Fabricated pilot egg washer

Table 1. Wash water temperature combinations used to wash eggs

Combination	Tank 1 (°C)	Tank 2 (°C)
1	49	49
2	49	24
3	49	15.5
4	24	24
5	15.5	15.5
6	24	15.5

Table 2. Average effects of wash water temperature combination on vitelline membrane strength and Haugh unit values

Temperature Combination	Vitelline Membrane Force (g)	Haugh Unit
1	1.54	54.5
2	1.50	59.4
3	1.53	58.8
4	1.50	61.3
5	1.56	61.4
6	1.53	58.7
SEM	0.02	1.86

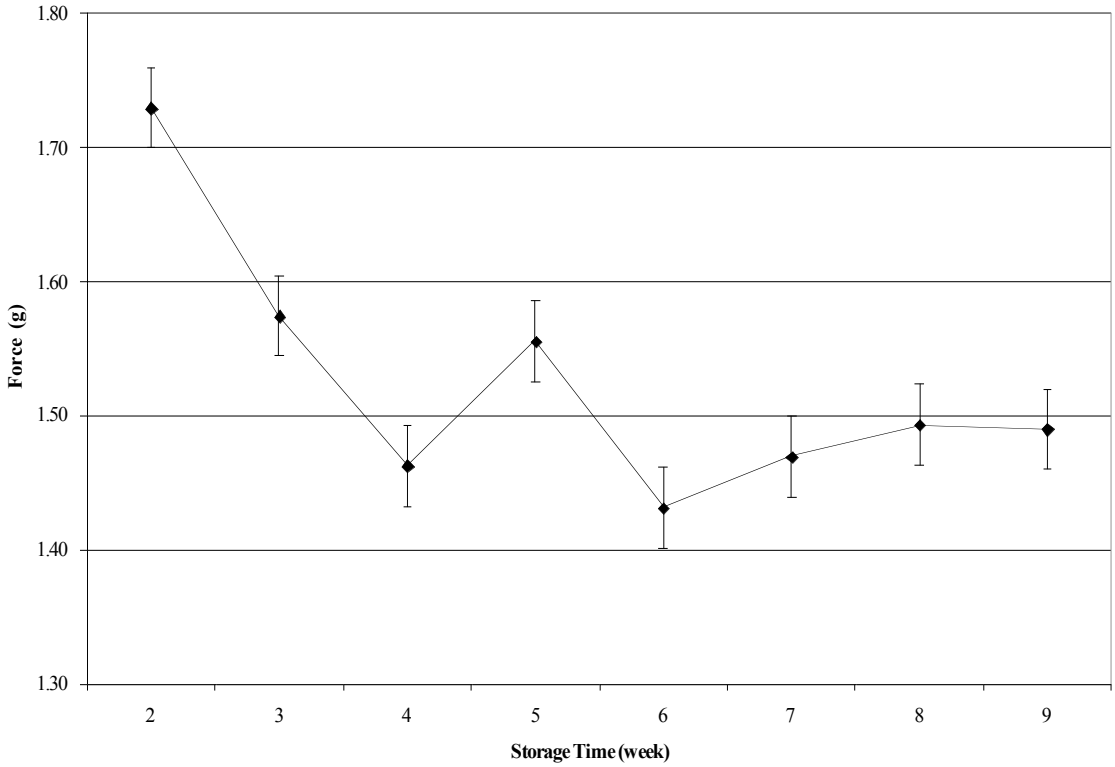

Figure 2. Average force required to break the vitelline membrane of processed eggs during each week of storage*

*There is no data for storage weeks 0, 1, and 10 due to technical difficulties.

IV. THE EFFECTS OF COMMERCIAL COOL WATER WASHING OF SHELL EGGS ON HAUGH UNIT, VITELLINE MEMBRANE STRENGTH, AEROBIC BACTERIA, YEASTS, AND MOLDS

ABSTRACT Current egg washing practices utilize wash water temperatures averaging 49°C, and have been found to increase internal egg temperature by 6.7 to 7.8°C. These high temperatures create a more optimal environment for bacterial growth, including *Salmonella* Enteritidis (SE), if it is present. SE is the most common human pathogen associated with shell eggs and egg products. Its growth is inhibited at temperatures of 7.2°C and below. This study's objective was to determine if commercially washing eggs in cool water would aid in quickly reducing internal egg temperature, preserving interior egg quality, and creating an environment less beneficial to bacteria. During three consecutive days, eggs were washed using four dual tank wash water temperature schemes (HH = 49°C, 49°C; HC = 49°C, 24°C; CC = 24°C, 24°C; CH = 24°C, 49°C) at two commercial processing facilities. A ten week storage study followed, in which vitelline membrane strength, Haugh unit, and presence of yeast, mold, and aerobic bacteria were monitored weekly. As storage time progressed, average Haugh unit values declined 14.8%, the average force required to rupture the vitelline membrane decreased 20.6%, average amounts of bacteria present on shell surfaces decreased 11.3%, and bacteria present in egg contents increased 39.5% due to storage. Wash water temperature did not significantly affect Haugh unit values, vitelline membrane strength, or the

amounts of aerobic bacteria, yeast, and mold within the shell matrix of processed eggs. Results of this study indicate that incorporating cool water into commercial shell egg processing, while maintaining a pH of 10 to 12, lowers post-processing egg temperatures and allows for more rapid cooling, without causing a decline in egg quality or increasing the presence of yeast, mold, and aerobic bacteria for approximately five weeks post-processing.

(*Key words*: shell eggs, cool wash, egg quality)

INTRODUCTION

Shell egg processors who chose to produce USDA "shielded" eggs must abide by specific USDA regulations. One such regulation states that egg wash water must be at least 90°F (32.2°C), or 20°F (11.1°C) warmer than the warmest egg entering the processing line (7 CFR 56.76(f)(3)). Due to this regulation, eggs from in-line operations (hen houses directly connected to the processing facility) can be washed in water as hot as 48.9°C. Research supporting the regulation was conducted by Brant and Starr in 1966. In 1940, Haines and Moran observed that when eggs are placed in a bacteria suspension cooler than their internal temperature, a negative pressure gradient is created, drawing bacteria through the shell and into the egg's interior. In 1952, Lorenz and Starr discovered that eggs washed in cold water were more likely to spoil than eggs washed in warm water. When this research was conducted, however, the most common way to wash eggs was by immersion washing. Eggs were placed in a wire basket, a household laundry or dish detergent was added, the basket and the eggs were submerged in water, and agitated for approximately one to three minutes (Hutchinson et al., 2003). In 1975, immersion washing was banned and replaced by spray washing (USDA, 1975).

The most recent regulation pertaining to egg processing applies to all shell eggs and requires them to be stored in a post-processing environment of 7.2°C or below (USDA, 1999). This regulation was established in order to decrease the amount of time needed to reduce internal egg temperatures post-processing, and hopefully control spoilage and potential foodborne pathogens associated with eggs. Studies have shown that due to washing, grading, and packaging, post-processing internal egg temperatures can be 6.1 to 7.8°C higher than initial egg temperatures (Anderson et al., 1992). When

compared to a single wash tank, dual wash tank systems commonly used by most egg processors mean that the shell eggs will stay in a hot wet environment for a longer period of time (Curtis, 1999). After being processed, eggs are typically packaged in pulp or foam cartons or cardboard flats, placed in cases, and palletized. In addition to the initial rise, insulation provided by packaging conditions can cause the eggs' internal temperature to continue to rise (Anderson et al., 1992). The internal temperature of packaged eggs can continue to rise for up to six hours after eggs are placed in a cooler. In fact, it may actually take the centermost egg in a pallet five to six days to reach an internal temperature of 7.2°C when stored in an environment with an ambient temperature of 7.2°C (Anderson et al., 1992; Chen et al., 2002; Jones et al., 2002b). It is important for the internal temperature of an egg to be below 7.2°C as quickly as possible because *Salmonella* Enteritidis (SE), the organism most often associated with foodborne disease and eggs, does not grow well at refrigerated temperatures (Gast and Holt, 2000; Bell and Kyriakides, 2002). Because most eggs reach the retail outlet in such a short period of time (Bell et al., 2001; Patterson et al., 2001), reducing their internal temperature to 7.2°C or below before they are purchased by consumers can be challenging.

Maintaining the microbial integrity of the egg somewhat depends on internal egg quality. Measuring Haugh unit values (HU) and vitelline membrane strength are two ways to assess an egg's internal quality. Determining the HU is a common way to assess interior egg quality (Haugh, 1937) and has been accepted by USDA-AMS as a valid and reliable method (USDA, 2000). It is a function of egg weight and the height of the thick albumen (Haugh, 1937). The HU value, like vitelline membrane strength, tends to

decline as the egg ages (Williams, 1992; Jones et al., 2002b; Jones and Musgrove, 2005; Samli et al., 2005). The vitelline membrane, which surrounds the yolk, is responsible for keeping the yolk contents separate from the albumen. Determining vitelline membrane strength is important because a strong vitelline membrane will prevent the yolk contents from entering the albumen. The yolk contains nutrients that are good growth medium for bacteria (Clay and Board, 1991; Humphrey and Whitehead, 1993; Gast and Holt, 2000). When the vitelline membrane weakens or breaks, these nutrients can contaminate the albumen and possibly inhibit its antimicrobial properties (Clay and Board, 1991; Humphrey and Whitehead, 1993; Humphrey, 1994; Gast and Holt, 2000). As the egg ages, vitelline membrane strength declines, reducing the interior quality of the egg and potentially causing leakage of yolk nutrients or allowing bacteria to penetrate the yolk (Conner et al., 2002; Jones et al., 2002a; Chen et al., 2005).

Scientists have been focusing on finding ways to reduce the egg's internal temperature during and after processing. They have developed methods based on the use of cryogenic gases as well as forced cool air to rapidly cool shell eggs post-processing (Curtis et al., 1995 and Thompson et al., 2000, respectively). Although it has been shown that egg quality is maintained or even enhanced by these methods of rapid cooling (Curtis et al., 1995; Thompson, et al., 2000; Jones et al., 2002b), the egg industry's use of these methods has been limited due to cost and space constraints. It has been suggested that washing eggs in cool water, as opposed to warm water, would help diminish the increase in internal egg temperature during processing. This would, in turn, aid in reaching and maintaining a post-processing internal egg temperature of 7.2°C more rapidly without great processing costs.

Previous research indicates that washing eggs in cool water could be a viable means of maintaining or enhancing egg cooling and subsequent physical and microbial quality during storage. Cooler wash water temperatures help to reduce the amount of time needed to cool eggs (Lucore et al., 1997). Cooling eggs to an internal temperature of 7.2°C and below reduces microbial contamination by inhibiting the growth of SE and other psychotropic microorganisms that may be present (Rhorer, 1991; Curtis, 1999; Bell and Kyriakides, 2002; Chen et al., 2002). Lucore et al. (1997) reported decreased bacterial counts on egg shells as wash water temperature decreased. Cool water washing of shell eggs could benefit egg processors by initiating the cooling process of the egg and shortening cooling time after being placed into the cooler. Other benefits of cool water washing would include a reduced cost of heating the wash water and cooling the post-processing cooler. By commercially processing shell eggs at four different wash water temperature schemes, this study examined how cool water washing affects interior egg quality, as well as aerobic bacterial levels and yeasts and molds on and within the egg.

MATERIALS AND METHODS

Egg Processing

This study was conducted in two commercial shell egg processing facilities (A and B). At each facility, shell eggs were washed after regular processing hours over three consecutive days (replicates). Both facilities were operated by the same integrator, were AMS inspected, and used dual washer systems from the same manufacturer to wash eggs. In order to determine the effects of washing shell eggs in cool water, the wash water utilized in this study was collected after it had been re-circulated for four hours during the regular processing day and contained an organic load. This created a "worst case

scenario" and enabled us to better determine the effects of cool water washing by taking into account the recycling of wash water. The previously used wash water in each wash tank was pumped into four 55 gallon drums (Consolidated Plastics Co., Inc, Twinsburg, OH). In order to prevent rust contamination, the interior of each drum was treated with a corrosive inhibitor. Once the drums were filled with the previously used wash water, they were placed in the processing facility's post-processing cooler, which had an ambient temperature of approximately 7.2°C. The temperature of the wash water was then lowered to 23.9°C or slightly lower. In order to lower the wash water temperature, the drums remained in the facility's post-processing cooler for approximately five to twelve hours before conducting the study.

Eggs were processed using four wash water temperature schemes: HH = 48.9°C, 48.9°C; HC = 48.9°C, 23.9°C; CC = 23.9°C, 23.9°C; and CH = 23.9°C, 48.9°C (temperature of the first and second washer, respectively). The pH of the wash water from each plant was also monitored in order to ensure that it was maintained between 10 and 12 (sensION 156, Hach Co., Loveland, CO). The average wash water pH was 11.14 and 10.85 from Facility A and B, respectively. Approximately one pallet of eggs for each temperature scheme was processed in the same order (HH, HC, CC, CH) each day at each facility. After processing, the eggs were packaged in new, clean pulp flats containing 30 eggs per flat. The flats were packaged in cardboard cases, and the cases were palletized. One 30-case pallet (case = 30 dozen eggs, n = 10,800) was formed for each temperature scheme. As the eggs were being palletized, a DataWatch™ data logger (Global Sensors, Mount Holly, NC) was placed into three different cases in the pallet for each wash water temperature scheme (Figure 1). Cases containing a data logger were placed on the top, in

the middle, and at the bottom of the pallets. All eggs were then stored at 7.2°C in the facilities' post-processing cooler. The data loggers collected internal and external egg temperatures every three minutes of storage for two weeks post-processing. Figure 2 shows a graphical representation of the average cooling data gathered from each processing facility.

Storage Study

For ten weeks post-processing, processed eggs were stored in an environment with an ambient temperature of approximately 7.2°C until analysis. Each week of storage included three replicates from each processing facility (representing the three consecutive days of processing at each facility). During each week of storage (week 0 = week of processing), eggs were randomly selected to undergo testing in order to determine their internal and microbial quality.

Haugh Unit. Each week of storage, HU values were determined for the three replicates from each processing plant. For each replicate, HU values were determined for 18 eggs per temperature scheme (72 eggs per replicate) using the procedure described by Haugh (1937). The eggs were removed from storage and candled in order to exclude any cracked eggs. Shortly after being removed from storage, while the eggs were still cool, HU values, along with albumen height and egg weight, were determined using an Egg Multi-Tester EMT 5200 (Robotmation Co., ltd, Tokyo, Japan).

Vitelline Membrane Strength. Vitelline membrane strength was also determined for three replicates per storage week for each processing plant. For each replicate, a 21-egg sample from each temperature scheme (84 eggs per replicate) was removed from storage. Shortly after being removed from storage, while the eggs were still cool, vitelline

membrane strength was determined using a Texture Technologies TA-XT2i texture analyzer (Texture Technologies, Scarsdale, NY). A texture analyzer determines vitelline membrane strength using static compression (Conner et al., 2002; Jones et al., 2002b; Keener et al., 2006). Before the assessment was conducted, all eggs were candled, and cracked eggs were excluded from testing. Each egg was individually broken into a shallow dish and the yolk was positioned under a 1mm, rounded end, stainless steel probe. Because Lyon et al. (1972) reported that the strongest section of the vitelline membrane in near the chalazae, care was taken to ensure that measurements were not obtained from this area. Direct pressure was applied to the yolk until the vitelline membrane ruptured and the probe penetrated the yolk. Compression measurements were made using a 5 kg load cell (calibrated using a 2 kg weight), 0.1 gram trigger force, and 3.2 mm/sec test speed. Vitelline membrane breaking strength was recorded as grams of force required to rupture the membrane. The force required to break the vitelline membrane corresponds to its strength; a strong membrane requires more force to break.

Microbial Analysis. During each week of storage, microbial analysis was conducted for the three replicates per processing facility. For each replicate, 27 eggs per temperature scheme (108 eggs per rep) were removed from storage and candled. Cracked eggs were excluded from testing. Each egg was placed into a sterile plastic bag with 25mL of Buffered Peptone Water (BPW). Each bag was then gently shaken for approximately one minute. The BPW rinses for nine eggs were combined, resulting in three sets of pooled exterior rinse samples. Three 3M Petrifilm Aerobic Count plates and three 3M Petrifilm Yeast & Mold Count plates per pooled sample were inoculated with 1mL each from the exterior rinse samples. Eggs were individually removed from the plastic bags using

sterile tongs. In order to sanitize the exterior shell surface, each egg was briefly dipped into 95% ethyl alcohol and momentarily passed through the flame of a Bunsen burner. The eggs were then cracked, using the edge of a sterile surface, and the contents of nine eggs were placed in a sterile plastic bag. The shells of those nine eggs were also placed into a separate sterile plastic bag. This resulted in three pooled sets of egg contents and three pooled sets of egg shells. The shells were then gently crushed by hand once they were inside the sterile bag. Due to the use of 3M Petrifilm plates, BPW (90 mL) was added to the egg shell and content pools in accordance to 3M Petrifilm sample preparation guidelines. The sterile bags containing the egg shells and BPW were then gently shaken for approximately one minute. Three 3M Petrifilm Aerobic Count plates and three 3M Petrifilm Yeast & Mold Count plates per pooled sample were inoculated with 1mL of BPW from the crushed (interior) shell rinse. Because the shell membranes were not separated from the actual shell, interior shell samples include what is located between the inside of the shell and the shell membranes. Before three 3M Petrifilm Aerobic Count plates and three 3M Petrifilm Yeast & Mold Count plates per pooled sample were inoculated with 1mL of a 1:10 dilution of the egg contents, the mixture was placed in a Seward Stomacher (Seward Ltd., Norfolk, UK) and homogenized for one minute at 200 rpm. All inoculated 3M Petrifilm Aerobic Count plates were incubated at 37°C for approximately 48 hours, and all 3M Petrifilm Yeast & Mold Count plates were incubated at 20°C for approximately five days. Presumptive colonies were then enumerated according to manufacturer's recommendations.

Statistical Analysis

Previous research conducted to determine the effects of cool water washing of shell eggs has been performed in a laboratory setting (Lucore et al., 1997; Jones et al., 2005). Thus, the main purpose of this study was to determine the effects of cool water washing when conducted in a commercial setting. When conducting research in a commercial setting, rather than a controlled laboratory environment, there can be many variables. In this study, the presence of these variables (facility and employee sanitation, environmental conditions, management, etc.) allowed us to more realistically compare cool water washing to the high temperatures currently required for egg processing. Variables such as management, sanitation, egg age, type of processing (in-line vs off-line), post-processing cooler temperature, etc. were different at each processing facility. Because processing environments differed, significant facility differences were found in the data collected. An example of these differences can be seen in the post-processing cooling data (Figure 1), average HU scores (Figure 3a), and average amounts of bacteria present on exterior shell surfaces (Figure 3b). As seen in Figure 1, eggs processed at Facility A had lower average post-processing temperatures than eggs processed at Facility B. Figure 3a shows that, until week four of storage, eggs processed at Facility A had higher average HU values than those processed at Facility B. Also, throughout ten weeks of storage, eggs processed at Facility B had more bacteria present on exterior shell surfaces than eggs processed at Facility A. Due to confounding variables, data from both processing facilities were combined before statistical analysis and a randomized complete block experimental design (block = processing facility) was used to compare effects of wash water temperature scheme and extended storage.

All data were analyzed using SAS (1999). Force required to rupture the vitelline membrane, HU values, and albumen height were analyzed according to the general linear model. All aerobic bacteria, yeasts, and mold count data were also analyzed according to the general linear model; however, the raw data was subjected to a log transformation before analysis. Because serial dilutions in BPW were prepared from all samples, bacterial counts from plates with no bacterial growth were recorded as 0.9 after log transformation. Any means that were found to be significantly different ($P \leq 0.05$) were separated using the least-squared means option of the general linear model procedure.

RESULTS

Haugh Unit

Average HU values were not significantly different amongst wash water temperature schemes (HH = 67.5; HC = 68.0; CC = 67.6; CH = 68.0). However, as seen in Table 1, there was a significant difference in average HU values between storage weeks; at the end of ten weeks of storage, average HU values had declined 14.8%. Scientists have questioned the validity of the HU as an accurate indicator of interior egg quality (Silversides et al., 1993); therefore, as an alternative method of determining interior quality, albumen height data were also analyzed. Wash water temperature scheme did not significantly affect average albumen height (HH = 4.8mm; HC = 4.9mm; CC = 4.9mm; CH = 4.9mm). As seen in Table 1, there were, however, significant differences in the average albumen height over ten weeks of storage. Due to storage, average albumen height decreased 23.2%.

Vitelline Membrane Strength

The average force required to rupture the vitelline membrane was also not significantly affected by wash water temperature (HH = 1.57g; HC = 1.55g; CC = 1.57g; CH = 1.56g). Like average HU values and albumen height, vitelline membrane strength also significantly decreased (20.6%) during ten weeks of storage (Table 1).

Microbial Analysis

Wash water temperature did not significantly affect amounts of aerobic bacteria (log CFU/ml) present within shell matrixes (HH = 2.98; HC = 3.07; CC = 3.12; CH = 3.03). There were, however, significant temperature scheme x storage week interactions in amounts of aerobic bacteria present on exterior shell surfaces (Figure 4) and in egg contents (Figure 5). Normal variation was observed in the overall growth trend of aerobic bacteria present on exterior shell surfaces during extended storage (Jones et al., 2004b; Jones et al., 2005). Although the amount of bacteria present decreased 37.6% by week three of storage (1.71 log CFU/ml versus 2.74 log CFU/ml initially present), the most bacteria present on exterior shell surfaces (2.9 log CFU/ml) and in egg contents (3.8 log CFU/ml) during storage were recovered in week six from eggs processed in the HH temperature scheme. Average amounts of bacteria present in egg contents significantly increased from 1.28 log CFU/ml to 3.22 log CFU/ml due to storage. During the first three weeks of storage, bacterial growth consistently remained low, and then steadily increased. There were also significant differences in the amounts of aerobic bacteria present within the shell matrix of eggs between storage weeks. Bacterial growth increased from 2.43 log CFU/ml to 3.39 log CFU/ml over ten weeks, indicating a 39.5% increase due to storage.

Amounts of yeast present within the shell matrix of eggs, as well as amounts of yeast and mold present in egg contents were not significantly different amongst wash water temperature schemes. There were, however, significant differences between storage weeks in amounts of yeast present within the shell matrix and in egg contents (Table 2). Average amounts of yeast present within the shell matrix increased 11% due to storage, and average amounts present in contents increased 5%. There were also significant wash water temperature scheme x storage interactions in amounts of mold present within the shell matrix of eggs and amounts of mold and yeast present on exterior shell surfaces. As seen in Figure 6b, there were only five occurrences of mold growth within shell matrixes during storage. Four of the five occurrences were during the first four weeks of storage, and three of the five growth occurrences were recovered from CC eggs. Amounts of mold present on exterior shell surfaces increased during storage (Figure 6a). Eggs processed in the CH temperature scheme experienced the most exterior mold growth throughout storage. There was little variation in the average amount of yeast on exterior shell surfaces throughout storage (Figure 7). Over ten weeks of storage, CH and HC eggs experienced the most yeast growth on exterior shell surfaces. Eggs processed in the CH temperature scheme had more yeast growth than eggs processed in the other temperature schemes shortly after processing, as well as during storage week eight and ten.

DISCUSSION

Analysis of the data collected during this study indicate that wash water temperature does not significantly affect average HU values, albumen height, vitelline membrane strength, or average amounts of aerobic bacteria, yeast, and mold present

within the shell matrix of eggs. Wash water temperature did affect average amounts of aerobic bacteria, yeast, and mold present on exterior shell surfaces (Figures 4, 7, and 6a, respectively), average amounts of mold present within the shell matrix of eggs (Figure 6b), and average amounts of aerobic bacteria present in egg contents (Figure 5) at certain sampling times during extended storage. Differences in microbial growth in egg contents due to the affects of wash water temperature and storage time did not affect microbial quality until approximately week five of storage and later (Figure 5). Although significant, these differences are of little importance because it is beyond the average "sell by" date of eggs. According to Bell et al. (2001) and Patterson et al. (2001), eggs currently processed in the United States have an average "sell by" date of thirty days and are actually sold by nineteen days post-processing. Also, the expiration date for shell eggs, which indicates the maximum time frame for expected quality, cannot legally exceed forty-five days (USDA, 2000). Furthermore, when Jones et al. (2006) examined the effects of wash water temperature scheme (HH, HC, and CC only) on the presence of *Campylobacter*, *Listeria*, and *Salmonella* within eggs processed during the current study, they isolated *Campylobacter* and *Salmonella* in shell and membrane emulsion samples during the first two weeks post-processing. No pathogens were detected within eggs after two weeks post-processing.

The results of this study are consistent with those reported by Lucore et al. (1997). They reported that internal microbial counts from eggs spray washed with water as cool as 15.5°C were no different from internal microbial counts of eggs spray washed with 48.9°C water. In a more recent inoculation study, Hutchinson et al. (2004) found that wash and rinse water temperatures did not significantly effect surface populations of SE.

They also, however, reported that allowing wash and rinse water temperatures to fall below 34°C caused a detectable amount of content contamination. Although it is not clear why, it is possible that the results reported by Lucore et al. (1997), contradict the findings of Hutchinson et al. (2004) due to a difference in wash water pH, a difference in washing environment and equipment (pilot egg processing equipment in a pilot plant versus a laboratory setting), or because the temperature of only the wash water was lowered and the rinse water temperature remained consistent with USDA guidelines (7 CFR 56.76(f)(11)). It should be noted that wash water pH is essential to the effectiveness of egg washing. Catalano and Knabel (1994) reported that maintaining wash water conditions at pH 11 or above prevents possible cross-contamination caused by recycled wash water by effectively reducing the number of SE present on egg shells and in wash water.

Regardless of wash water temperature, as storage time progressed, the overall average HU values, albumen height, and vitelline membrane strength significantly decreased (Table 1). These results are not surprising; other scientists have reported decreased HU values, albumen height, (Williams, 1992; Silversides and Scott, 2001; Jones et al., 2002b; Jones and Musgrove, 2005; Samli et al., 2005), and vitelline membrane strength (Elliot and Brant, 1957; Hartung and Stadleman; 1963; Conner et al., 2002; Jones et al., 2002b; Chen et al., 2005) as a result of extended storage.

Because scientists have questioned the validity of the HU as an accurate indicator of interior egg quality (Silversides et al., 1993), albumen height was measured throughout this study as an alternative means of determining egg quality. Although the HU is commonly used to measure interior quality, there are limitations associated with HU

measurements. The HU is a relationship between egg weight and height of the thick albumen. The calculation is weighted exclusively for a 56.7g (2oz) egg (size large); which is why scientists have argued that the calculation is inaccurate for eggs other than size large. More recently, scientists have reported that albumen height and the HU value equally portray albumen quality (Silversides and Villeneuve, 1994). Analysis of data gathered in the current study indicates the same.

Maintaining interior egg quality is important because quality decline is generally accompanied by increased microbial growth. The 2005 risk assessments of *Salmonella* Enteritidis in shell eggs and *Salmonella* spp. in egg products predicted that rapid cooling of eggs would be one of the most effective means of reducing illnesses from SE contaminated eggs (USDA, 2005). The physiological and chemical changes responsible for quality decline in eggs are accelerated by high temperatures, which is why it is important to cool eggs as quickly as possible (Romanoff and Romanoff, 1949; Kim et al., 1989; Rhorer, 1991; Chen et al., 2002; Conner et al., 2002). Data collected by Jones et al. (2006a) and the post-processing cooling data collected during this study show that washing eggs in cool water successfully prevents the excessive temperature increase caused by high water temperatures in dual wash tanks. Jones et al. (2006a) found that the surface temperature of shell eggs decreased when exposed to 23.9°C wash water. In the current study, eggs processed using the CC temperature scheme had the lowest average post-processing temperatures, and eggs washed in the HH scheme had the highest. Although eggs processed in the HC and CH temperature schemes did not have the lowest post-processing temperatures, they cooled quicker than eggs processed in the HH

scheme. By replacing the warm water from one wash tank with cool water, eggs are not exposed to as much heat during processing and are able cool much faster than eggs processed using only hot water.

As previously discussed and expected, the decline in egg quality observed in the current study was accompanied by an increase in bacterial growth. During extended storage, average amounts of mold present on exterior shell surfaces and average amounts of yeast and aerobic bacteria present within the shell matrix and in egg contents did not follow the same downward trend as interior egg quality. Like those reported by Chen et al. (2005), our results suggest that the decline in vitelline membrane strength and albumen viscosity over time increases the probability that microorganisms will spread inside the eggs and possibly even invade the egg yolk. Despite the increase in aerobic bacteria, yeast, and mold growth observed in the current study during extended storage, according to Jones et al. (2006), no pathogens were detected throughout the storage time in the contents of eggs processed in the HC or CC temperature scheme (Jones et al., 2006 did not collect data for eggs processed in the CH temperature scheme).

The overall results of this study suggest that washing shell eggs with cool water, while maintaining a pH of 10 to 12, has the potential to reduce internal egg temperature during and after processing, without causing a decline in egg quality or increasing the presence of yeast, mold, and aerobic bacteria for approximately five weeks post-processing. The data collected during this study indicate that incorporating cool water into commercial shell egg processing lowers post-processing internal egg temperatures and allows for more rapid cooling. A more prompt reduction of internal egg temperature has the potential to enhance the physical qualities of eggs and improve their microbial

quality. Maintenance of egg quality factors such as vitelline membrane strength and HU values combined with reducing internal egg temperature will aid in preventing the growth of any potential pathogenic bacteria present. Excessive wash temperatures reduce profits due to the costs associated with heating wash water and cooling eggs post-processing (Anderson et al., 1992). Cool water washing could also provide economic benefits to the egg industry by reducing the energy needed to heat wash water, as well as by decreasing the amount of energy needed to cool eggs following processing.

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Figure 1. Data logger being placed into a case of processed eggs.

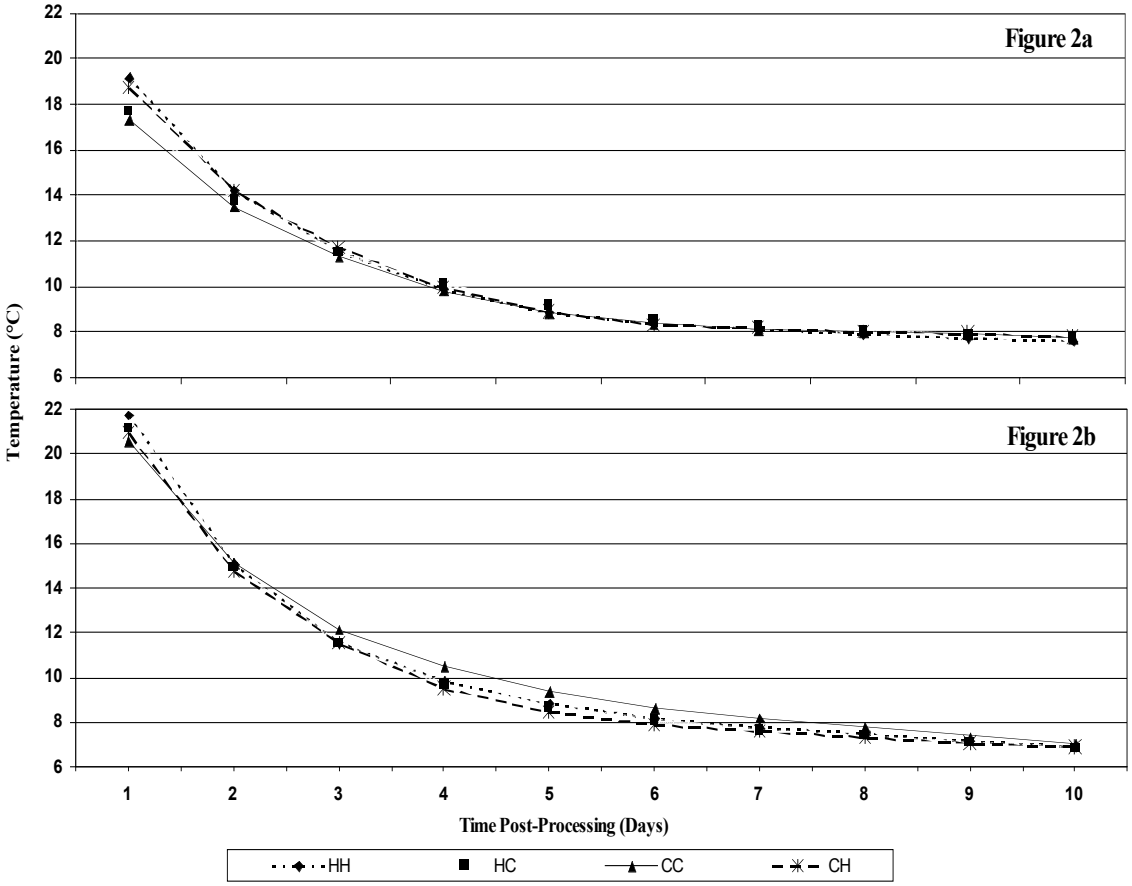

Figure 2. Average post-processing cooling curves for eggs processed at Facility A (2a) and Facility B (2b)

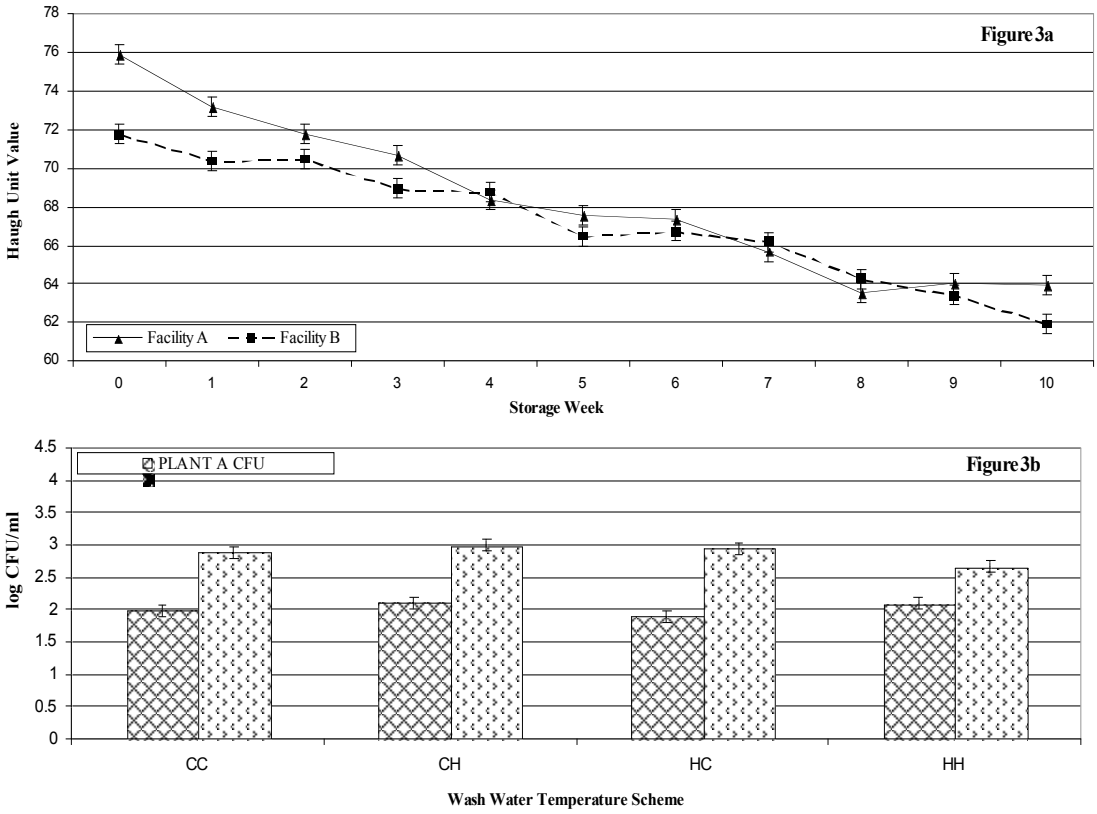

Figure 3. Effects of processing environment on average Haugh Unit values over ten weeks of storage (3a) and average amounts of aerobic bacteria present on exterior shell surfaces amongst wash water temperature schemes (3b)

Table 1. Average Haugh unit values, albumen height, and force required to rupture the vitelline membrane of eggs from combined processing facilities for each week of the storage

Storage Week	Haugh Unit	Albumen Height	Vitelline Membrane Force (g)
0	73.8^{G}	5.61^{H}	1.75^{F}
1	71.8^{F}	5.35^{G}	1.72^{F}
2	71.1^{EF}	5.25^{FG}	1.70^{F}
3	69.8^{DE}	5.09^{EF}	1.56^{DE}
4	68.6^{CD}	4.94^{DE}	1.59^{E}
5	67.0^{BC}	4.78^{CD}	1.55^{CDE}
6	67.0^{BC}	4.74^{CD}	1.57^{DE}
7	65.9^{B}	4.63^{BC}	1.49^{BCD}
8	63.9^{A}	4.43^{AB}	1.43^{AB}
9	63.7^{A}	4.40^{A}	1.47^{ABC}
10	62.9^{A}	4.32^{A}	1.39^{A}
SEM	0.36	0.04	0.02

$^{A-H}$ Means within a column with different letters are significantly different ($P \leq 0.05$)

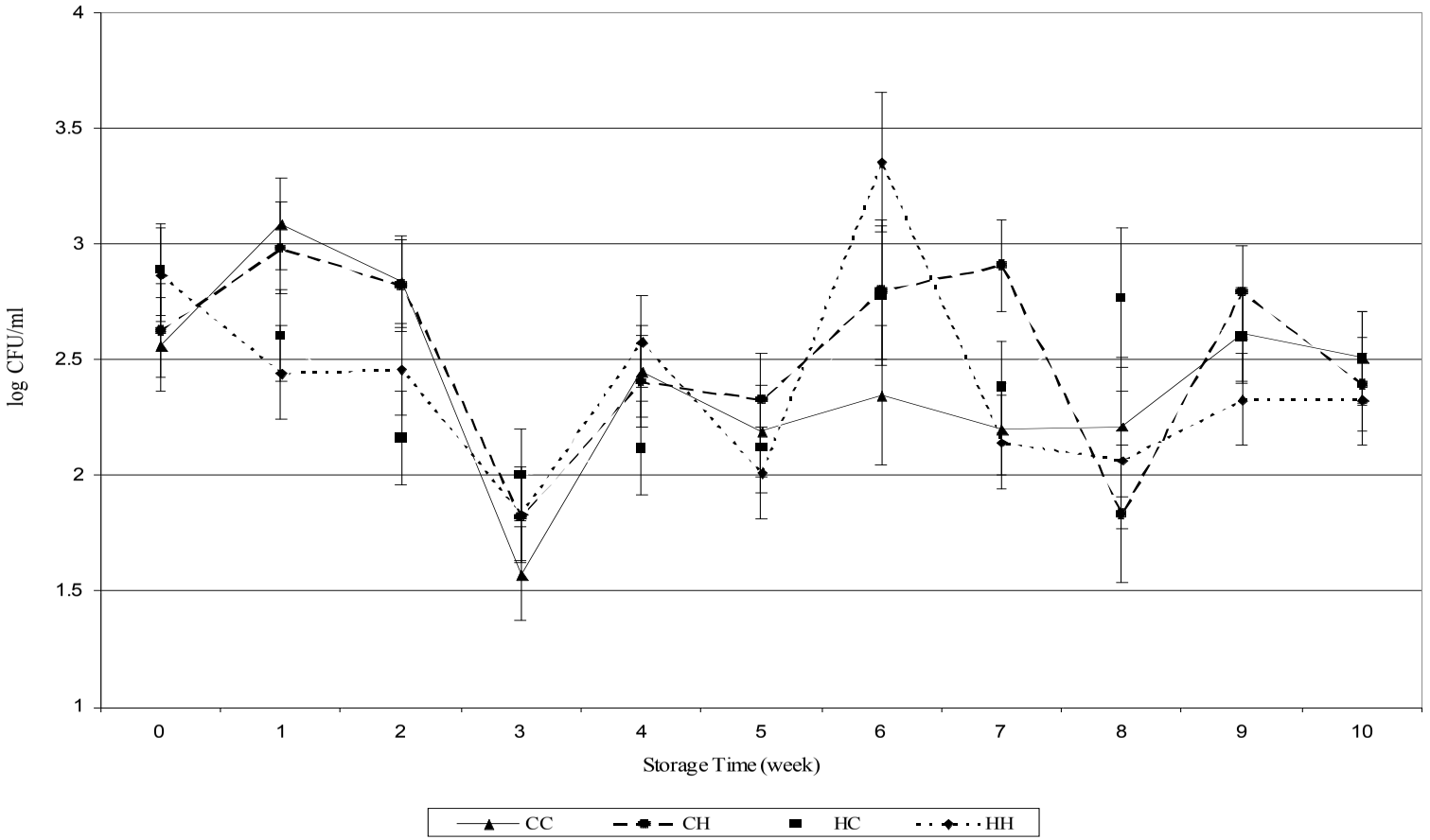

Figure 4. Effects of wash water temperature scheme and post-processing storage time on average amounts of aerobic bacteria present on exterior shell surfaces

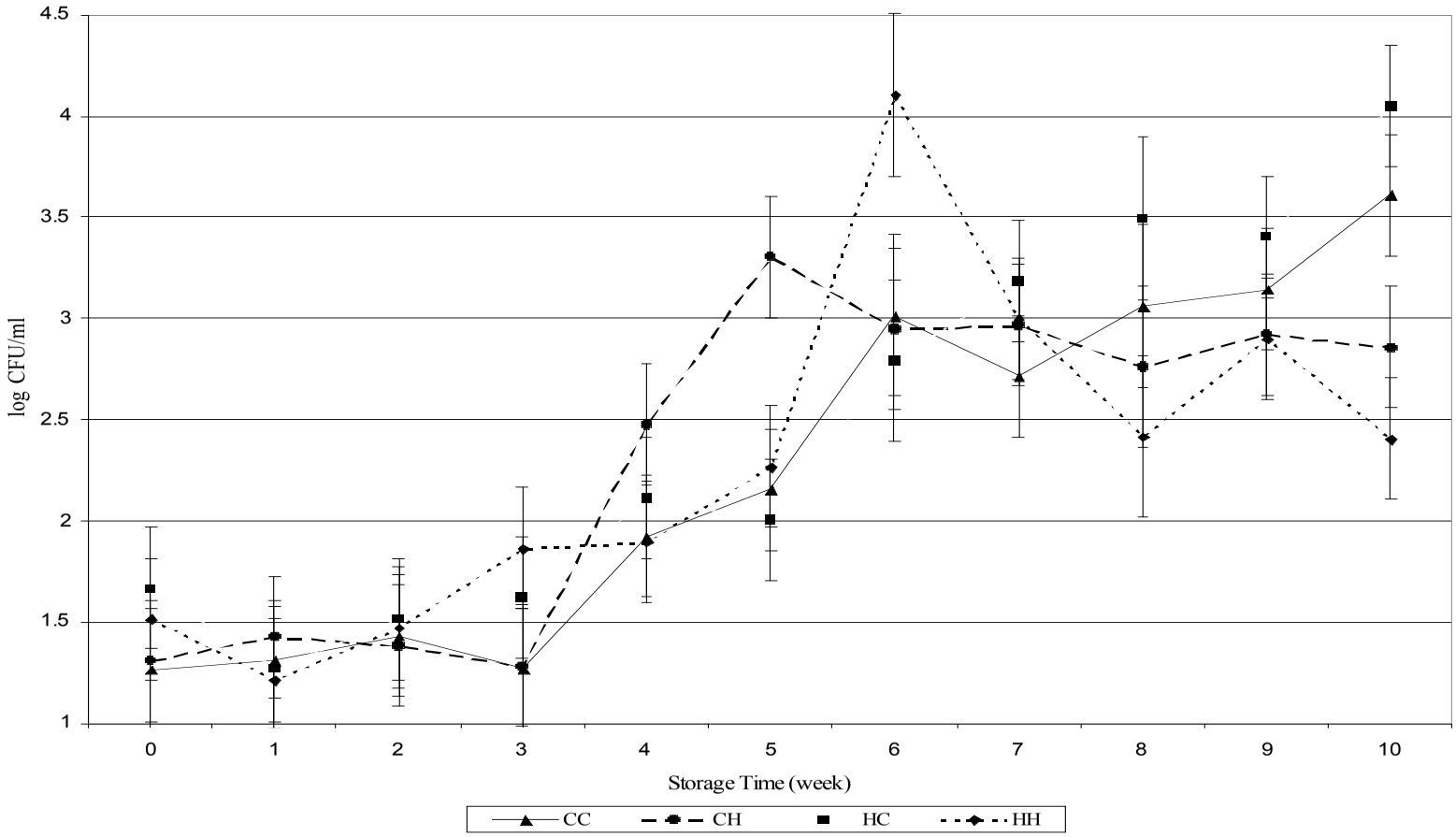

Figure 5. Effects of wash water temperature scheme and post-processing storage time on average amounts of aerobic bacteria present in egg contents

Table 2. Average amounts of yeast present within the shell matrix (interior) and in the contents of processed eggs for each week of storage

Storage Week	Yeast (log CFU/mL)	
	Interior	Contents
0	1.00^{A}	1.00^{A}
1	1.00^{A}	1.00^{A}
2	1.03^{AB}	1.03^{A}
3	1.08^{ABC}	1.02^{A}
4	1.10^{ABCD}	1.12^{B}
5	1.12^{ABCD}	1.06^{AB}
6	1.17^{CD}	1.06^{AB}
7	1.22^{D}	1.06^{AB}
8	1.06^{ABC}	1.05^{AB}
9	1.13^{BCD}	1.04^{AB}
10	1.11^{ABCD}	1.05^{AB}
SEM	0.02	0.01

$^{A-D}$ Means within a column with different letters are significantly different ($P \leq 0.05$)

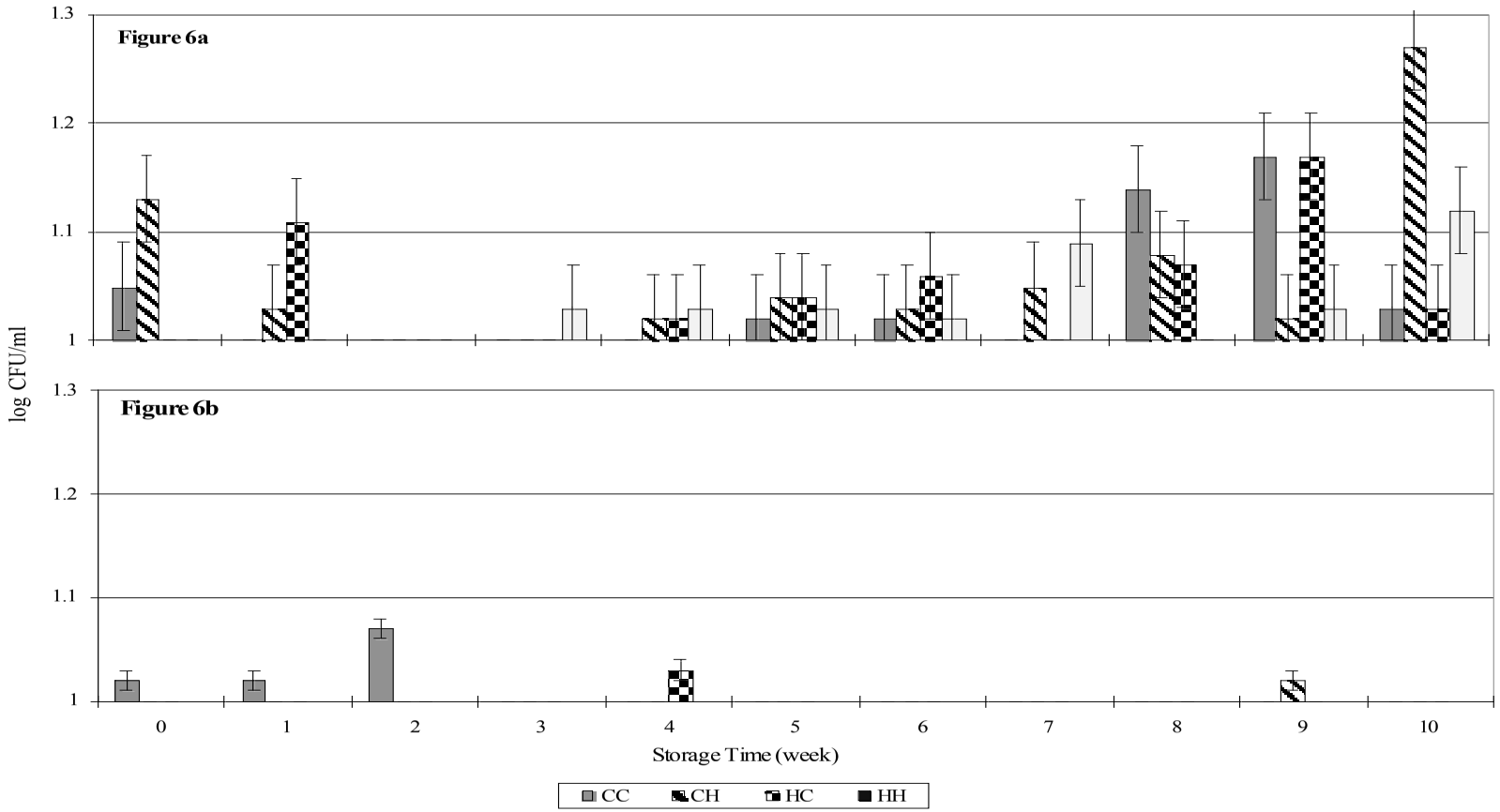

Figure 6. Effects of wash water temperature scheme and post-processing storage time on average amounts of mold present on exterior shell surfaces (6a) and within the shell matrix (6b) of processed eggs

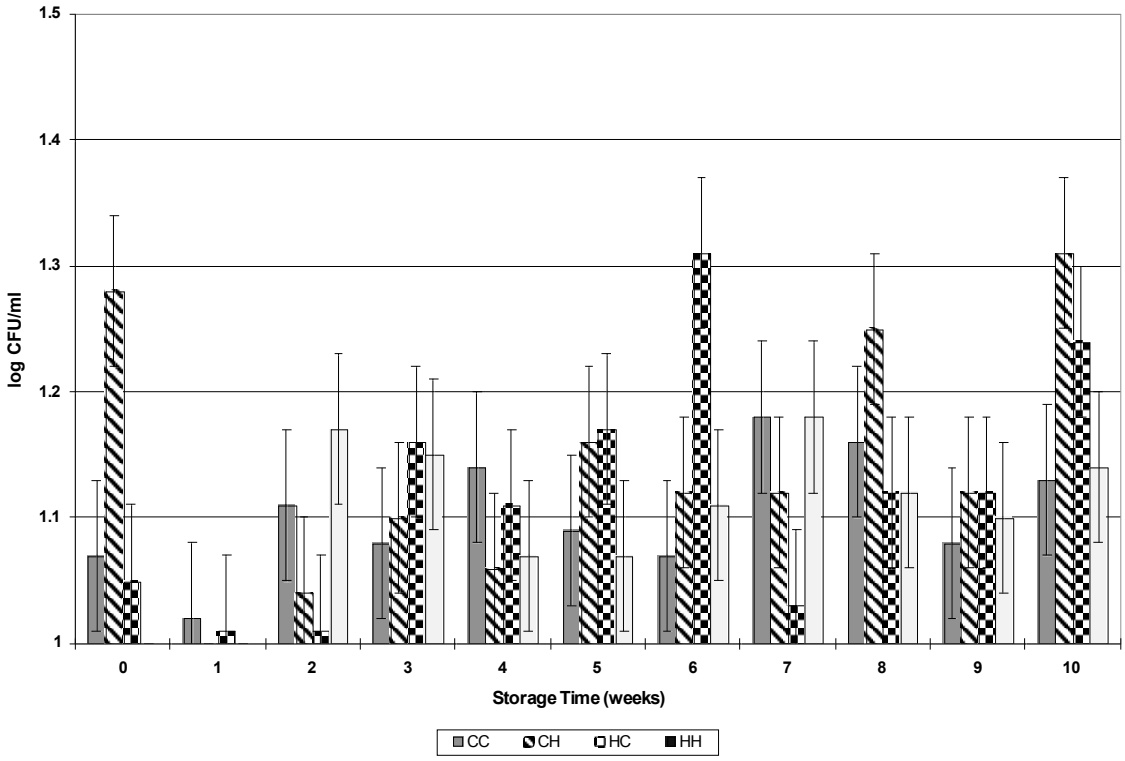

Figure 7. Effects of wash water temperature scheme and post-processing storage time on average amounts of yeast present on exterior shell surfaces

V. SUMMARY AND CONCLUSIONS

Previous research conducted to determine the effects of cool water washing of shell eggs has been performed in a pilot processing plant setting (Lucore et al., 1997). Thus, the main purpose of this research was to determine the effects of cool water washing when conducted in a commercial setting. In order to do this, the best temperature, or combination of temperatures, for washing shell eggs while limiting the increase in internal egg temperature had to be identified. The affects of cool water washing on interior egg quality were accessed during phase one of this research. The second, and final, phase of this research studied the quality and microbiological effects of cool water washing when conducted in two commercial egg processing facilities.

Results of research conducted during phase one to determine the best temperature for washing shell eggs indicated that 24°C, when compared to 15.5°C, was the best cool water temperature for commercially washing shell eggs. Thus, eggs processed at commercial facilities during phase two of this study were processed using a cool wash water temperature of 24°C. Analysis of egg quality data collected during phase one found no significant differences between wash water temperature combinations in average Haugh unit values or vitelline membrane strength, indicating that cool water washing does not affect interior egg quality. As expected, results from the storage study conducted during phase one showed a significant decline ($P \leq 0.05$) in the average force

required to break the vitelline membrane as storage time progressed. The average HU value also decreased due to storage. Eggs washed using the temperature combination commonly utilized by egg processors (49°C, 49°C) had the lowest average HU values; whereas, eggs washed using temperature combinations containing only cool water temperatures (24°C, 24°C and 15.5°C, 15.5°C) had the greatest average vitelline membrane strength and HU values. Jones et al. (2005) conducted a separate study of the eggs processed during phase one in order to determine the effects of cool water washing on aerobic bacteria levels and SE contamination in inoculated eggs. Although external aerobic populations were lowest for eggs processed using the temperature combination commonly utilized in the US (49°C, 49°C), Jones et al. (2005) concluded that all wash water temperature schemes investigated during phase one of this study were equally capable of removing SE.

Results of the research conducted during phase two also indicate that cool water washing does not negatively affect interior egg quality. Analysis of the data collected during this study discovered that wash water temperature did not significantly affect average Haugh unit values, albumen height, vitelline membrane strength, or average amounts of aerobic bacteria, yeast, and mold present within the shell matrix of eggs. Wash water temperature did affect average amounts of aerobic bacteria, yeast, and mold present on exterior shell surfaces and in egg contents at certain sampling times during extended storage. Differences in microbial growth due to the affects of wash water temperature and storage time did not affect microbial quality of the contents until approximately week five of storage and later. Although significant, these differences are of little importance because it is beyond the average "sell by" date of eggs. According to

Bell et al. (2001) and Patterson et al. (2001), eggs currently processed in the United States have an average "sell by" date of thirty days and are actually sold by nineteen days post-processing. Also, the expiration date for shell eggs, which indicates the maximum time frame for expected quality, cannot legally exceed forty-five days (USDA, 2000). Furthermore, when Jones et al. (2006) examined the effects of wash water temperature scheme (HH, HC, and CC only) on the presence of *Campylobacter*, *Listeria*, and *Salmonella* within eggs processed during the current study, they isolated *Campylobacter* and *Salmonella* in shell and membrane emulsion samples during the first two weeks post-processing. No pathogens were detected within eggs after two weeks post-processing.

The results of research conducted during phase two of this study are consistent with those reported by Lucore et al. (1997). They reported that internal microbial counts from eggs spray washed with water as cool as 15.5°C were no different from internal microbial counts of eggs spray washed with 48.9°C water. In a more recent inoculation study conducted in a laboratory setting, Hutchinson et al. (2004) reported that wash and rinse water temperatures did not significantly effect surface populations of SE. They also, however, reported that allowing wash and rinse water temperatures to fall below 34°C caused a detectable amount of content contamination. Although it is not clear why, it is possible that the results of the present study, as well as those reported by Lucore et al. (1997), contradict the findings of Hutchinson et al. (2004) due to a difference in wash water pH, or because the temperature of only the wash water was lowered and the rinse water temperature remained consistent with USDA guidelines (7 CFR 56.76(f)(11)). It should be noted that wash water pH is essential to the effectiveness of egg washing. Catalano and Knabel (1994) reported that maintaining wash water conditions at pH 11 or

above prevents possible cross-contamination caused by recycled wash water by effectively reducing the number of SE present on egg shells and in wash water.

Regardless of wash water temperature, as storage time progressed during phase two, the overall average Haugh unit values, albumen height, and vitelline membrane strength significantly decreased. These results are not surprising; other scientists have reported decreased Haugh unit values, albumen height (Williams, 1992; Silversides and Scott, 2001; Jones et al., 2002b; Jones and Musgrove, 2005; Samli et al., 2005), and vitelline membrane strength (Elliot and Brant, 1957; Hartung and Stadleman; 1963; Conner et al., 2002; Jones et al., 2002b; Chen et al., 2005) as a result of extended storage. As early as the mid 1900's, scientists such as Lorenz and Starr (1952) and March (1969) observed changes that occurred in washed eggs during storage. The egg industry is aware that storage causes a decline in egg quality and slowly breaks down the egg's natural barriers, making it increasingly susceptible to bacterial entry and growth (Romanoff and Romanoff, 1949; Brooks and Taylor, 1955; Board, 1966; Humphrey, 1994; Wang and Slavik, 1998; Jones et al., 2004b). Because quality decline is generally accompanied by increased microbial growth, maintaining interior egg quality is extremely important (Chen et al., 2005; Humphrey, 1994). Conner et al. (2002) found that the ability of SE to grow in albumen corresponds to a decline in vitelline membrane strength. A weakened vitelline membrane becomes permeable and may allow bacteria to enter the yolk, yolk contents to enter the albumen, or both (Humphrey, 1994; Conner et al., 2002; Chen et al., 2005).

Because scientists have questioned the validity of the HU as an accurate indicator of interior egg quality (Silversides et al., 1993), albumen height was measured throughout

this study as an alternative means of determining egg quality. Although the HU is commonly used to measure interior quality, there are limitations associated with HU measurements. The HU is a relationship between egg weight and height of the thick albumen. The calculation is weighted exclusively for a 56.7g (2oz) egg (size large); which is why scientists have argued that the calculation is inaccurate for eggs other than size large. More recently, scientists have reported that albumen height and the HU value equally portray albumen quality (Silversides and Villeneuve, 1994). Analysis of data gathered in the current study indicates the same.

As expected, the decline in egg quality during phase two of this study was accompanied by an increase in bacterial growth. As storage time progressed, average amounts of mold present on exterior shell surfaces and average amounts of yeast and aerobic bacteria present within the shell matrix and in egg contents did not follow the same downward trend as interior egg quality. Like those reported by Chen et al. (2005), our results suggest that the decline in vitelline membrane strength and albumen viscosity over time increases the probability that microorganisms will spread inside the eggs and possibly even invade the egg yolk. The increased microbial growth observed in the current study during extended storage is a good example of why expiration date for shell eggs cannot legally exceed forty-five days. Despite the increase in aerobic bacteria, yeast, and mold growth observed in the current study during extended storage, according to Jones et al. (2006), no pathogens were detected throughout the storage time in the contents of eggs processed in the HC or CC temperature scheme (Jones et al., 2006 did not collect data for eggs processed in the CH temperature scheme).

High wash water temperatures may be a factor associated with accelerated quality decline. Recent scientific studies have shown that the maintenance of egg wash water at the regulated temperature is not sufficient to reduce bacterial levels to less than 10^5 CFU/mL (Jones et al., 2003); however, as the temperature of egg wash water rises, there is an increased risk of cuticle damage and thermal cracking (Wesley and Beane, 1967). Cuticle damage and thermal cracking provide ways for spoilage and pathogenic bacteria, especially from the egg wash water, to enter the egg. Research conducted by Kinner and Moats (1981), Holley and Proulx (1986), and Lucore et al. (1997) suggest that wash water temperatures commonly used by most egg processors is neither hot enough to kill microorganisms on the shell nor cool enough to inhibit their growth. Kinner and Moats (1981) found that wash water bacterial counts decreased, regardless of the temperature, when the water was at a pH of 10 and 11. Washing in warm water increases internal egg temperature and serves as an added buffer to prohibit quick cooling of the egg; thus allowing organisms on the shell, as well as inside the egg, to continue to grow (Lucore et al., 1997). In 1955, Hillerman reported that wash water maintained at 46.1°C would increase internal egg temperatures by 0.22°C per second**.** Anderson et al. (1992) reported that washing, grading, and packaging can cause post-processing internal egg temperatures to be 6.1 to 7.8°C higher than initial internal egg temperatures. Egg processors' ability to rapidly lower post-processing internal egg temperature is limited by current shell egg processing technology and regulations governing wash water temperature (Anderson et al., 1992; Curtis, 1999); therefore, eggs do not cool to growth inhibiting temperatures very quickly. If present in even small amounts, microorganisms such as SE have time to multiply as internal egg temperatures drop to 7°C, thus increasing the chances of

foodborne illness. The intended purpose of washing shell eggs in cool water is to more rapidly reduce post-processing internal egg temperatures to a growth-inhibiting temperature of 7°C. The 2005 risk assessments of *Salmonella* Enteritidis in shell eggs and *Salmonella* spp. in egg products predicted that rapid cooling of eggs would be one of the most effective means of reducing illnesses from SE contaminated eggs. The physiological and chemical changes responsible for quality decline in eggs are also accelerated by high temperatures, which is anther reason why it is important to cool eggs as quickly as possible after processing (Romanoff and Romanoff, 1949; Kim et al., 1989; Rhorer, 1991; Chen et al., 2002; Conner et al., 2002). The post-processing cooling data collected during phase two of this study show that washing eggs in cool water successfully prevents the excessive temperature increase caused by high water temperatures in dual wash tanks. By replacing the warm water from one wash tank with cool water, eggs are not exposed to as much heat during processing and are able cool much faster than eggs processed using only warm water temperatures.

The overall results of this study suggest that washing shell eggs with cool water, while maintaining a pH of 10 to 12, has the potential to reduce internal egg temperature during and after processing, without causing a decline in egg quality or increasing the presence of yeast, mold, and aerobic bacteria for approximately five weeks post-processing. The data collected during this study indicate that incorporating cool water into commercial shell egg processing lowers post-processing internal egg temperatures and allows for more rapid cooling. A more prompt reduction of internal egg temperature has the potential to enhance the physical qualities of eggs and improve their microbial quality, especially during extended storage. Maintenance of egg quality factors such as

vitelline membrane strength and HU values combined with reducing internal egg temperature will aid in preventing the growth of any potential pathogenic bacteria present. Excessive wash temperatures reduce profits due to the costs associated with heating wash water and cooling eggs post-processing (Anderson et al., 1992). Cool water washing could also provide economic benefits to the egg industry by reducing the energy needed to heat the wash water, as well as by decreasing the amount of energy needed to cool the eggs following processing.

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APPENDIX A. PILOT STUDY: EGG WEIGHT AND ALBUMEN HEIGHT ANALYSIS

Egg Weight

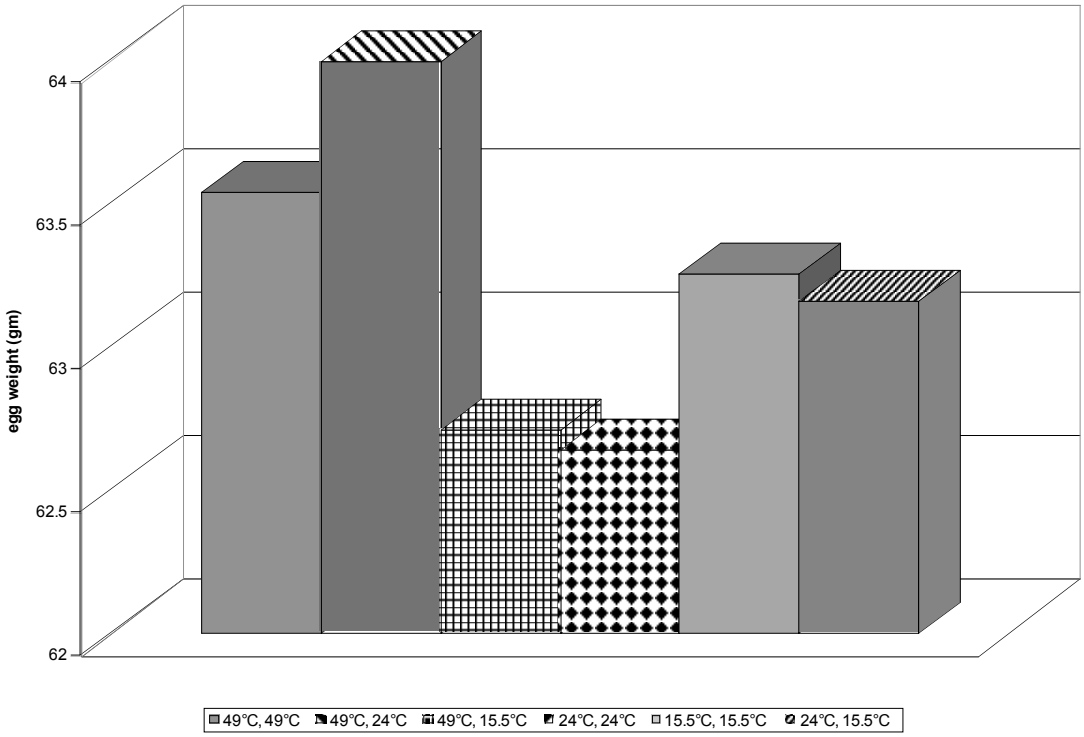

Figure 1. Average effects of wash water temperature combination on egg weight over 60 days of storage

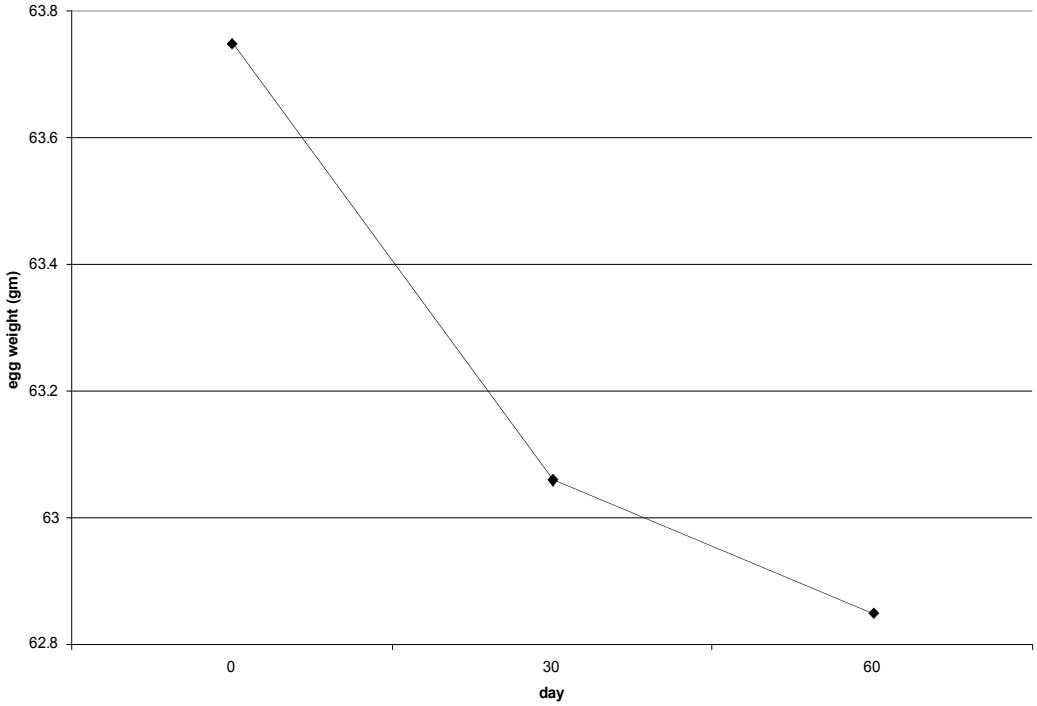

Figure 2. Average egg weight for days 0, 30, and 60

Albumen Height

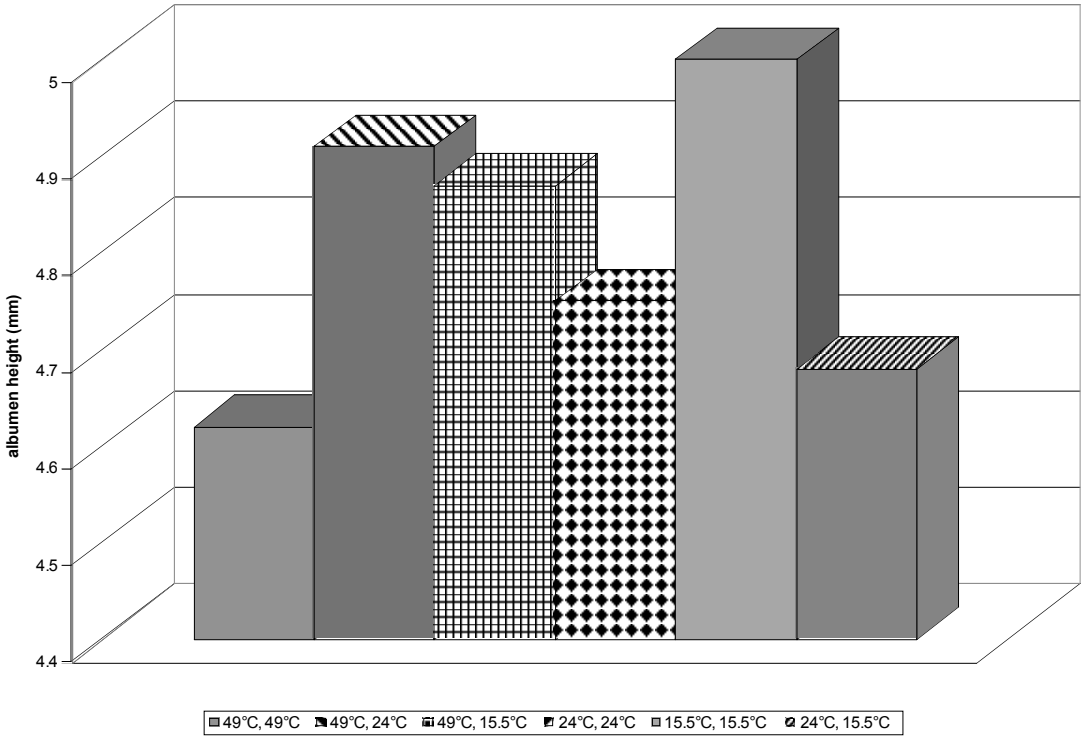

Figure 3. Sverage effects of wash water temperature combinations on egg weight over 60 days of storage.

APPENDIX B. COMMERCIAL STUDY: FACILITY A vs. FACILITY B

Interior Quality

Table 1. Average effects of wash water temperature scheme on Haugh unit values and vitelline membrane strength for each processing facility

Temperature Scheme	Haugh Unit		Vitelline Membrane Force (g)	
	Facility A	Facility B	Facility A	Facility B
HH	66.8	68.3	1.51	1.63
HC	67.2	68.9	1.50	1.61
CC	67.4	67.9	1.53	1.61
CH	67.5	68.3	1.52	1.61
SEM	0.29	0.32	0.01	0.01

Table 2. Average effects of wash water temperature scheme on albumen height for each processing facility

Temperature Scheme	Albumen Height (mm)	
	Facility A	Facility B
HH	4.75	4.91
HC	4.78	4.97
CC	4.83	4.89
CH	4.84	4.95
SEM	0.03	0.03

[A,B]Means within a column with different letters are significantly different ($P \leq 0.05$)

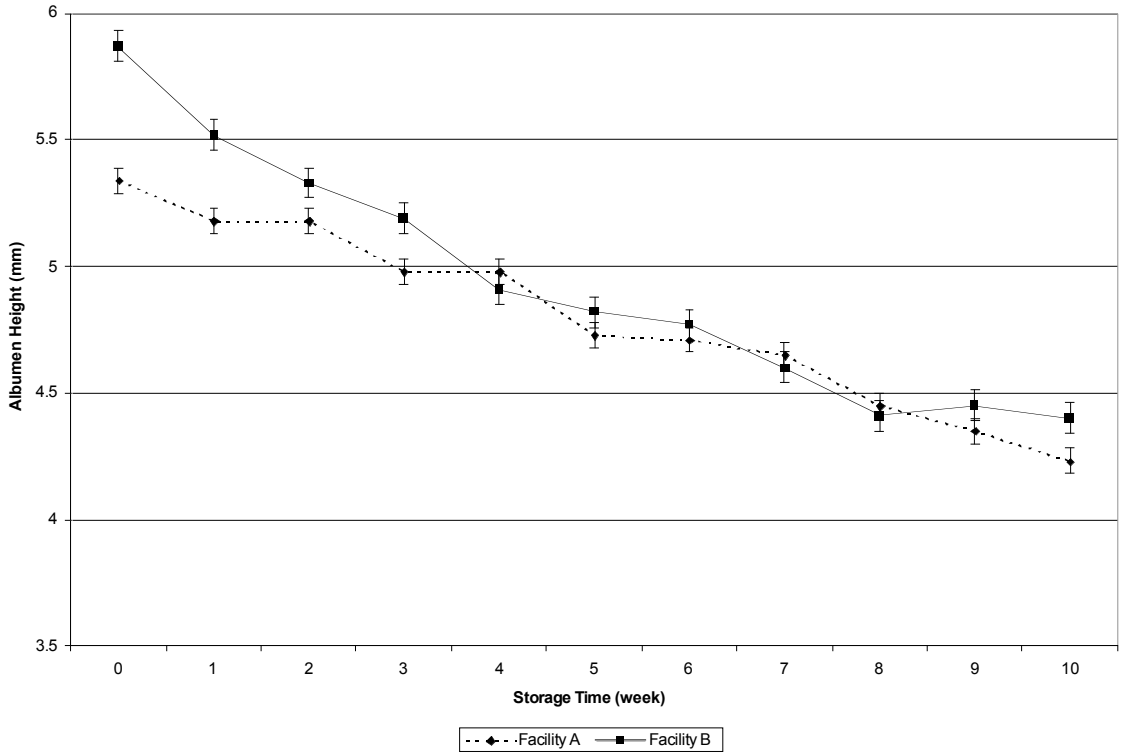

Figure 1. Average effects of storage time on albumen height for each facility

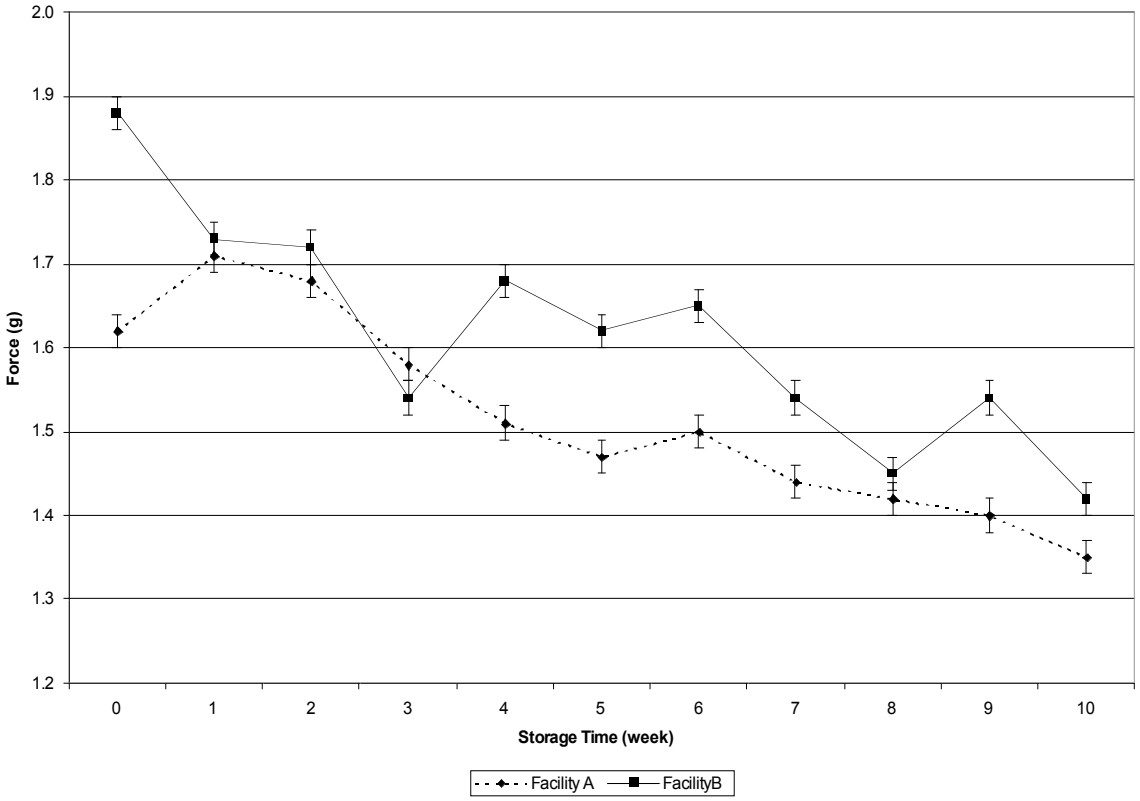

Figure 2. Average effects of storage time on force required to rupture the vitelline membrane of eggs from each facility

Aerobic Bacteria

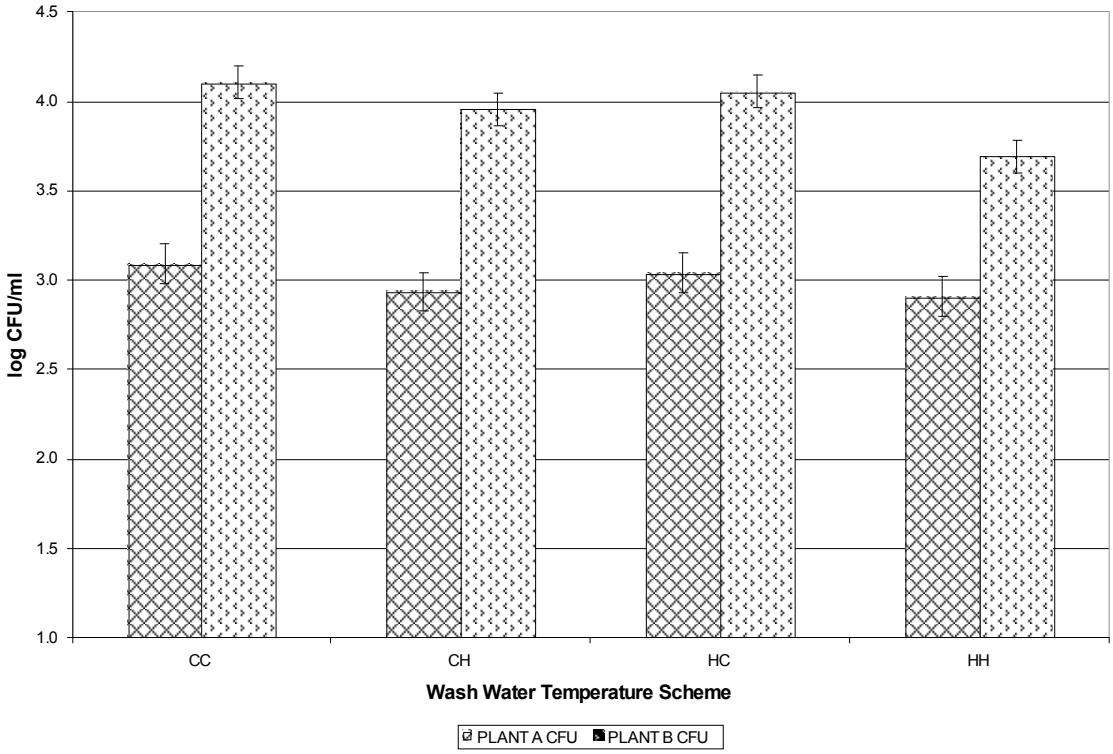

Figure 3. Average effects of wash water temperature scheme on amounts of aerobic bacteria present within the shell matrix (interior) of eggs from each processing facility

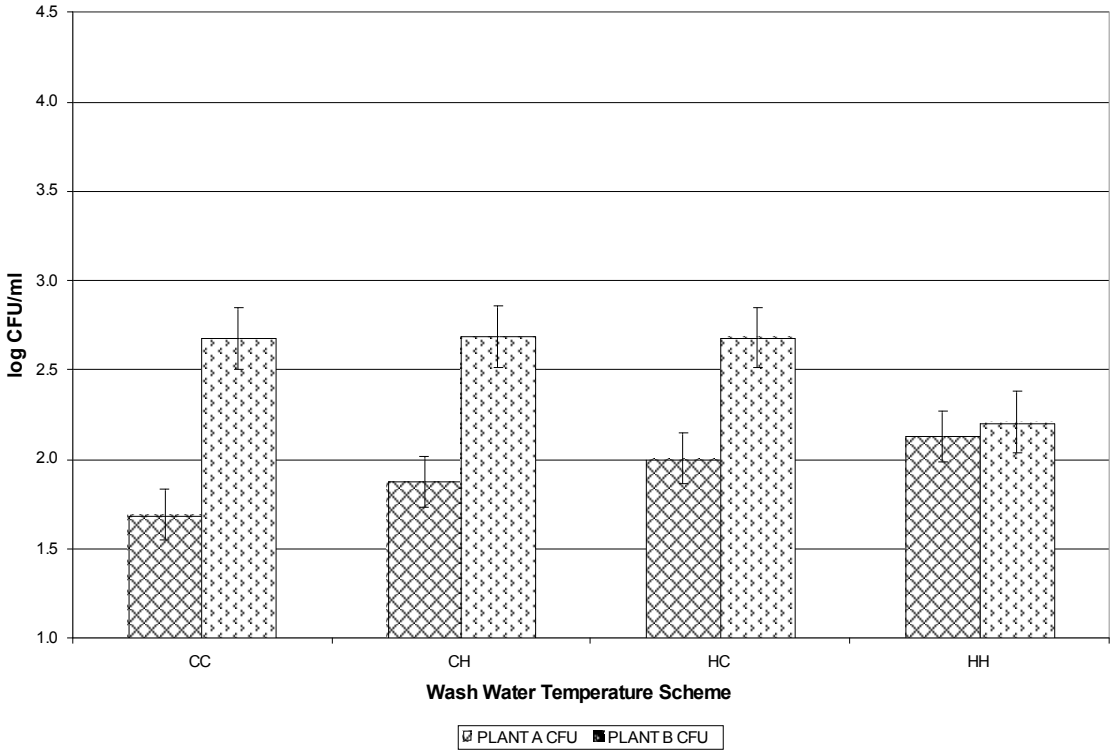

Figure 4. Average effects of wash water temperature scheme on amounts of aerobic bacteria present in the contents of eggs from each processing facility

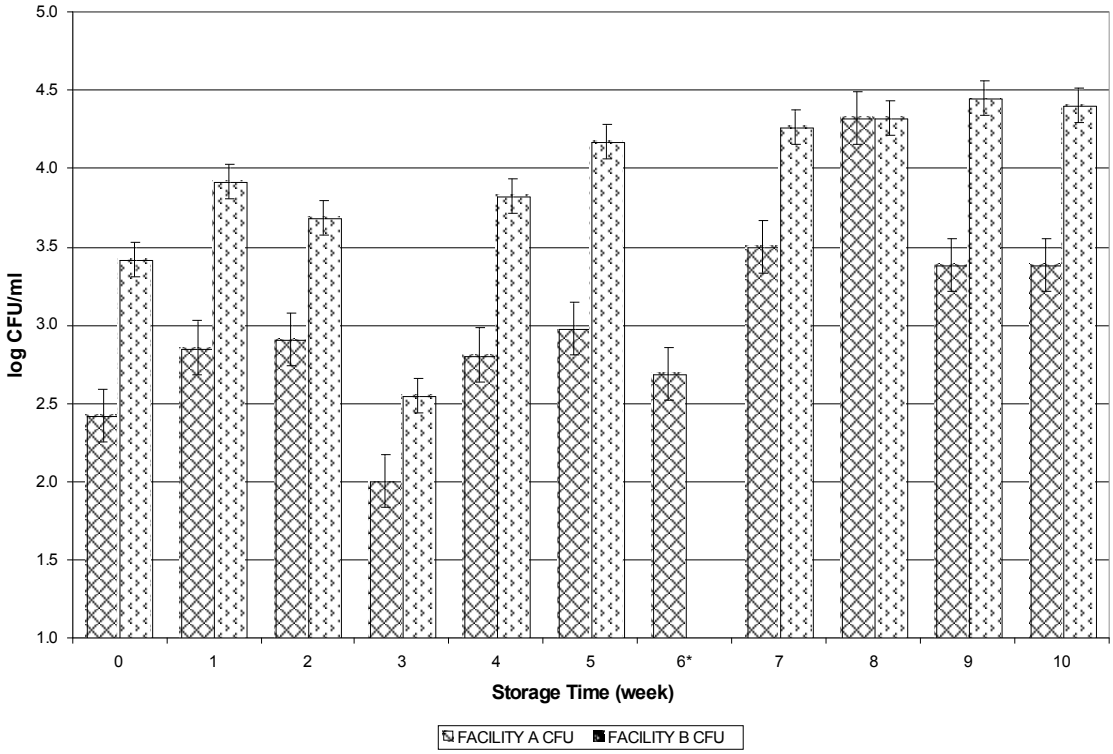

Figure 5. Average effects of wash water temperature scheme on amounts of aerobic bacteria present within the shell matrix (interior) of eggs from each processing facility

*Data collected during storage week 6 from eggs processed at Facility B is missing due to technical difficulties.

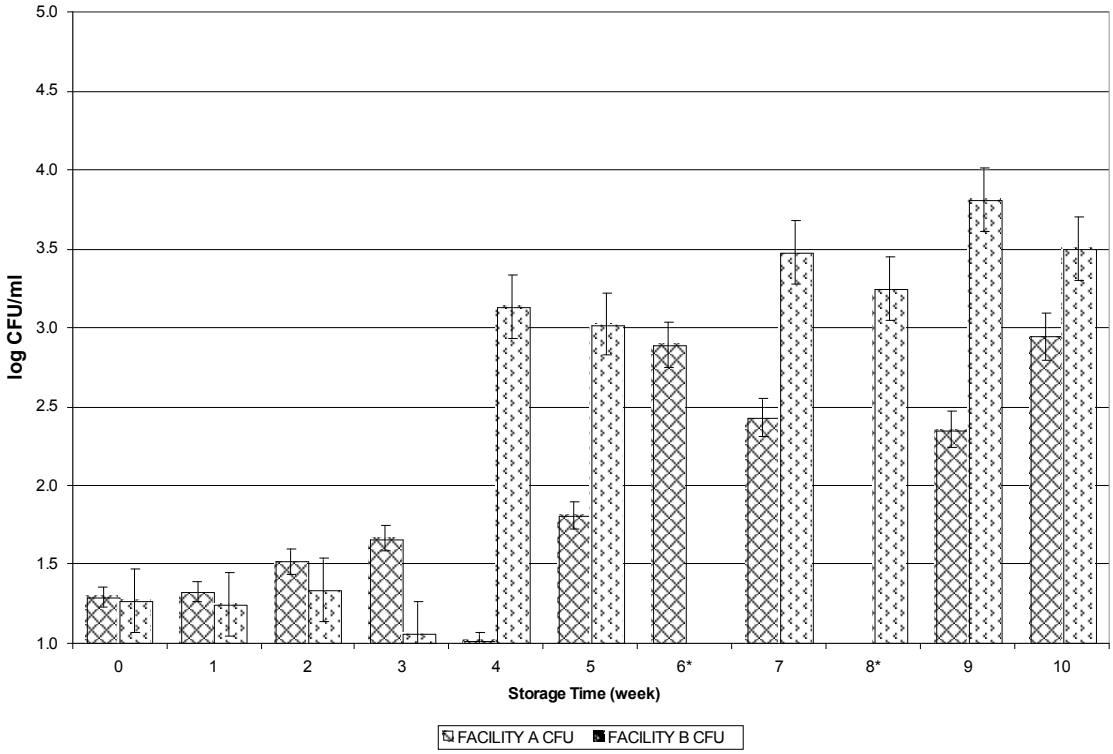

Figure 6. Average effects of wash water temperature scheme on amounts of aerobic bacteria present in the contents of eggs from each processing facility

*Data collected during storage week 6 from eggs processed at Facility B and week 8 from Facility A are missing due to technical difficulties.

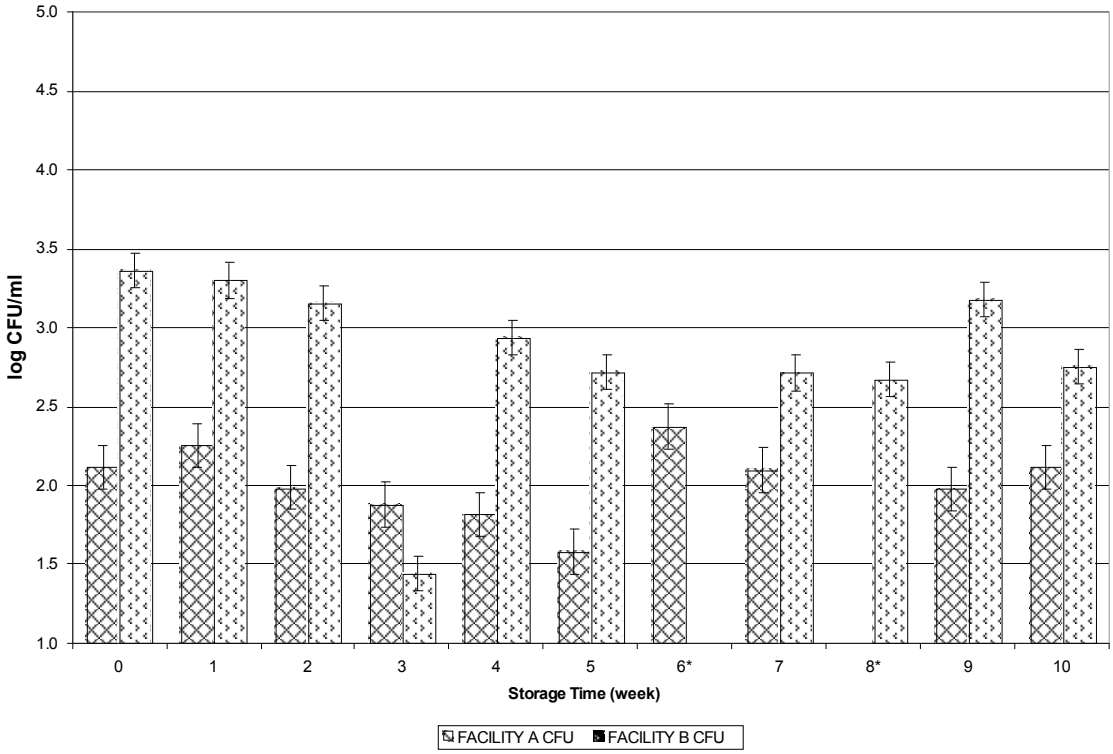

Figure 7. Average effects of wash water temperature scheme on amounts of aerobic bacteria present on the exterior shell surface of eggs from each processing facility

*Data collected during storage week 6 from eggs processed at Facility B and week 8 from Facility A are missing due to technical difficulties.

Yeast

Table 3. Average effects of wash water temperature scheme on amounts of yeast (log CFU/ml) present on exterior shell surfaces, within the shell matrix, and in contents of eggs processed at each facility

Temperature Scheme	EXTERIOR		WITHIN SHELL		CONTENTS	
	Facility A	Facility B	Facility A	Facility B	Facility A	Facility B
HH	1.05	1.15	1.05	1.15	1.02	1.06
HC	1.05	1.19	1.04	1.14	1.02	1.07
CC	1.05	1.16	1.03	1.13	1.02	1.05
CH	1.05	1.26	1.04	1.17	1.01	1.08
SEM	.01	.03	.01	.03	.01	.02

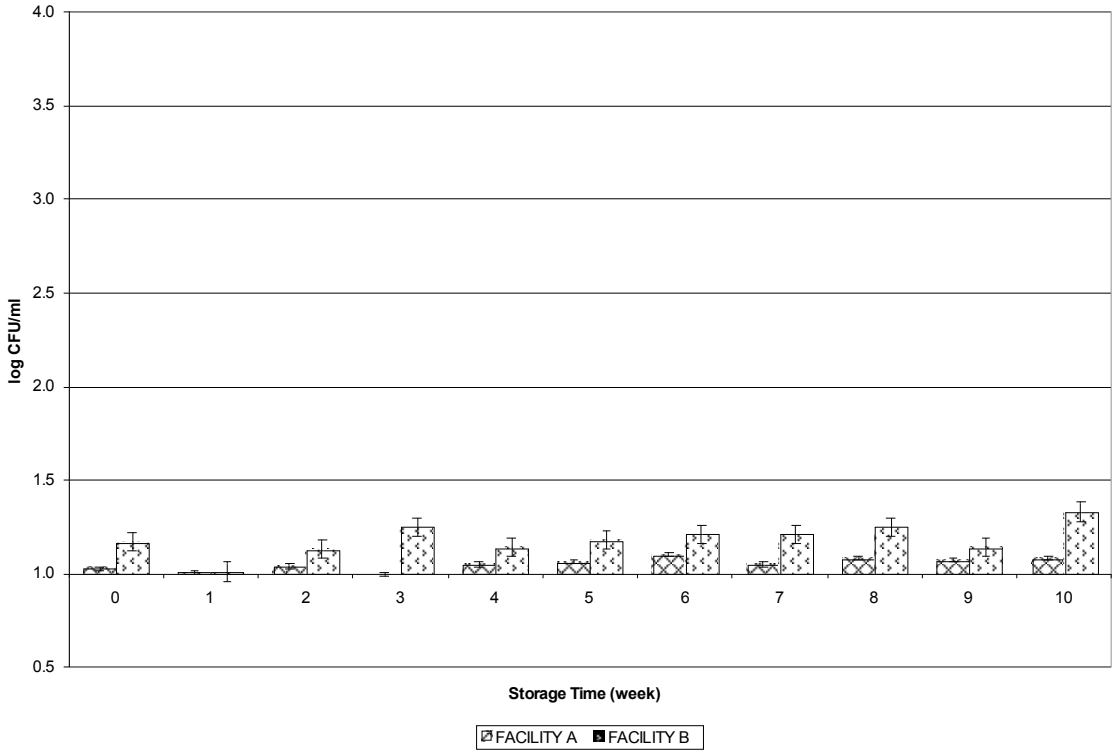

Figure 8. Average effects of wash water temperature scheme on amounts of yeast present on the exterior surface of eggs from each processing facility

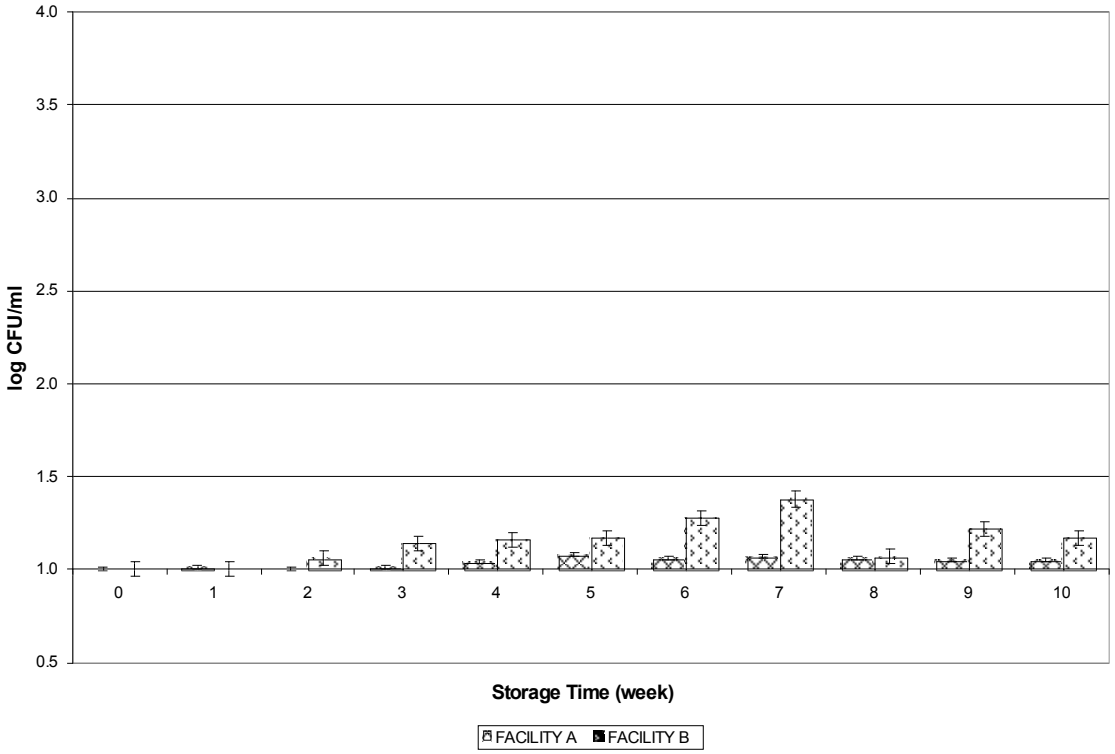

Figure 9. Average effects of wash water temperature scheme on amounts of yeast present within the shell matrix of eggs from each processing facility

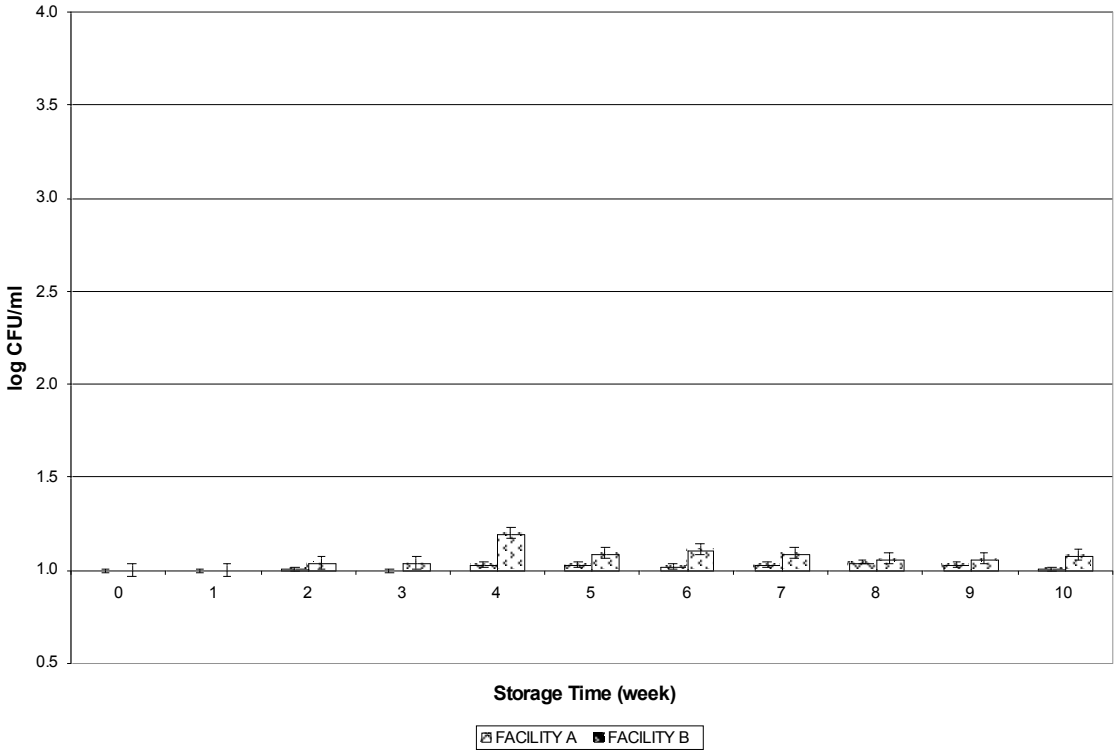

Figure 10. Average effects of wash water temperature scheme on amounts of yeast present in the contents of eggs from each processing facility

Mold

Table 4. Average effects of wash water temperature scheme on amounts of mold (log CFU/ml) present on exterior shell surfaces, within the shell matrix, and in contents of eggs processed at each facility

Temperature Scheme	EXTERIOR		WITHIN SHELL		CONTENTS	
	Facility A	Facility B	Facility A	Facility B	Facility A	Facility B
HH	1.04	1.02	1.00	1.00	1.00	1.00
HC	1.04	1.05	1.01	1.00	1.00	1.00
CC	1.04	1.04	1.01	1.01	1.00	1.00
CH	1.08	1.04	1.00	1.00	1.01	1.00
SEM	.02	.02	.004	.005	.004	.000

Table 5. Average effects of storage time on amounts of mold (log CFU/ml) present on exterior shell surfaces, within the shell matrix, and in contents of eggs processed at each facility

Storage Time (week)	EXTERIOR		WITHIN SHELL		CONTENTS	
	Facility A	Facility B	Facility A	Facility B	Facility A	Facility B
0	1.03	1.06	1.01	1.00	1.00	1.00
1	1.00	1.07	1.00	1.01	1.01	1.00
2	1.00	1.00	1.01	1.02	1.00	1.00
3	1.02	1.00	1.00	1.00	1.00	1.00
4	1.02	1.01	1.01	1.00	1.00	1.00
5	1.01	1.06	1.00	1.00	1.01	1.00
6	1.02	1.04	1.00	1.00	1.00	1.00
7	1.04	1.03	1.00	1.00	1.00	1.00
8	1.09	1.05	1.00	1.00	1.00	1.00
9	1.13	1.06	1.00	1.01	1.00	1.00
10	1.21	1.01	1.00	1.00	1.02	1.00
SEM	.03	.03	.01	.01	.01	.00