

**DEVELOPMENT OF BEST PRACTICES FOR SHELL EGG DISINFECTION
BASED UPON EFFICACY AND EGG QUALITY**

A Thesis

by

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ABSTRACT

This study was conducted to compare current commercial egg sanitizers to new technologies developed to improve egg safety and quality. Objectives included: 1) conduct a survey on current industry egg washing practices along with a microbial survey; 2) assess the efficacy of chlorine, quaternary ammonium compounds (QAC), peracetic acid alone or in combination with ultraviolet (UV) light and hydrogen peroxide (H_2O_2) (3.5%) in combination with UV light as post-wash sanitizers against aerobic plate counts (APC) and *Salmonella* Enteritidis (SE) inoculation; 3) conduct a consumer acceptability test to evaluate the influence of chlorine, QAC, and H_2O_2 and UV light using a 9-point hedonic test; and 4) investigate the effectiveness of H_2O_2 and UV light applied to eggs prior to washing on APC and the number of dirty eggs.

Results from the egg processing survey indicated that chlorine was the most frequently used sanitizer (81.7%) in the United States and most egg processors are operating in-line type facilities. Moreover, most facilities are not performing any egg treatment prior to washing, and very little in-plant microbiological monitoring was being conducted. The microbial survey indicated that 15 out of 18 visits had significantly less APC in post wash versus prewash eggs, and 11 out of 18 had significantly less APC in the final sanitizer than in the post wash stage. However, mean APC ranges after sanitization were 1.0 to 3.0 \log_{10} cfu/egg.

In laboratory trials evaluating the effectiveness of various egg sanitization treatments, the combination of H_2O_2 and UV light had the lowest eggshell APC (1.30,

1.05, and 1.10 log₁₀ cfu/egg) at d 0, 7, and 14, respectively, of storage among all treatments. No differences in overall consumer acceptability were determined among the treatments. However, chlorine-treated eggs received a higher score for texture compared to the other treatments. The combination of H₂O₂ and UV light prior to egg washing resulted in higher percentage of Grade A eggs and lower APC (2.1 log₁₀ cfu/egg). Therefore, this new technology can be used as an effective sanitizer to improve the quality and safety of shell eggs.

DEDICATION

I dedicate this thesis to my family, especially...

To my mom, Dr. Najwa Al-Nakash, for her financing, faith, love and advice;

To my brothers for their prayers and support;

To my children for their love.

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NOMENCLATURE

AEB	American Egg Board
APC	aerobic plate count
CDC	United States Centers for Disease Control and Prevention
cfu	colony-forming units
d	day
h	hour
H ₂ O ₂	hydrogen peroxide
min	minute
QAC	quaternary ammonium compounds
PAA	peracetic acid
s	second
SE	<i>Salmonella</i> Enteritidis
UEP	United Egg Producers
USDA	United States Department of Agriculture
UV	ultraviolet

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Salmonella has been linked to several foodborne disease outbreaks in the United States (US) since the 1990's. In particular, poultry products and eggs have been identified as one of the main sources of foodborne illness due to *Salmonella* (CDC, 2010). Recent *Salmonella* contamination of eggs and subsequent recalls in 2010 have once again pushed eggs to the forefront of food safety issues and have increased consumer concerns about the safety of shell eggs (FDA, 2010). In addition, Hazard Analysis and Critical Control Point (HACCP) of egg processing is scheduled to be implemented in the near future in order to provide safe eggs to consumers and prevent, reduce, or eliminate pathogens (Curtis et al., 1996). As a result, producers must find ways to ensure shell eggs are safe for consumers while still maintaining high quality.

Egg processors are currently using several practices to minimize the potential of egg contamination from shells. Chlorine and quaternary ammonium compounds (QAC) are the most common disinfection compounds used as a final rinse step following egg washing. However, these sanitizers are unable to completely eliminate microbial contamination on egg shells (Musgrove et al., 2006) and represent a significant cost and waste water disposal liability to the egg industry. Therefore, more effective practices need to be developed to ensure eggs are as sanitary as possible when they reach consumers. Reducing eggshell contamination at processing and packaging will help to

ensure egg safety, egg quality and maintain consumer confidence in eggs produced under commercial conditions.

In 2010, ultraviolet (UV) light was approved by the United States Department of Agriculture (USDA) to be used as a final sanitization step for shell egg processing. However, research has indicated that UV light treatment alone does not completely disinfect eggshell surfaces. Recent research reports and published data indicate that the combined use of hydrogen peroxide (H_2O_2) and UV light can reduce eggshell microbial counts of 4.0 to 5.0 \log_{10} cfu/egg to levels below detection using egg shell surface rinse methodology for microbial enumeration. It has been suggested that applying 1.5% H_2O_2 with 8 min of UV light exposure can greatly reduce the levels of bacteria on the surfaces of egg shells (Wells et al., 2010). Previous studies have also indicated that the combination of H_2O_2 and UV light have no negative impact on eggshell strength, internal egg quality or consumer sensory analysis compared to eggs sanitized by conventional methods (Woodring, 2011).

In addition to H_2O_2 and UV light, peracetic acid (PAA) has also been shown to be an effective sanitizing agent for egg products (Hartman & Carlin, 1957). However, no research has been conducted to examine the combined effects of PAA and UV light. Also, no research has been conducted on the use of these sanitization processes prior to egg washing. This prewash sanitization step may be important in developing a multi-hurdle approach to egg processing to ensure cleaner wash water and to prevent possible cross-contamination or internalization of bacteria during washing. In addition, the use of PAA, H_2O_2 and UV light could also be considered more environmentally friendly than

other chemical sanitizers since these technologies would not likely result in any chemical residues or wastes.

The overall goal of this project is to develop best practices based upon efficacy and quality parameters that table egg producers can implement to maximize total microbial and pathogenic microorganism reduction of shell eggs during processing. The specific objectives are: 1) develop and administer a survey to egg producers across the U.S. to determine current practices for egg sanitization, including compounds used, and concentrations; 2) conduct a microbial survey of egg processing facilities to evaluate the effectiveness of current egg sanitization practices; 3) compare the efficacy and egg quality parameters of current methods of egg sanitization with alternative technologies such as antimicrobials in combination with UV light; and 4) conduct experiments to assess the effectiveness of prewash egg disinfection procedures to reduce microbial contamination of eggs and wash water. Procedures and technologies developed during this project may significantly impact egg industry practices, improve food safety, and preparing egg processors for HACCP regulations by developing a best practices approach to shell egg sanitation.

Literature Review

The egg industry

Eggs are considered one of the most important sectors in the food industry because eggs are one of the main economical protein sources around the world (USDA, 2000; AEB, 2013). The United States is the second largest egg producer in the world (Stadelman & Cotterill, 1995). After World War II, egg production expanded and became more commercialized by changing from several hundred small farm-flock businesses to several thousand farms. In 1987, there were nearly 2500 egg producing companies in the US (UEP, 2012). Recently, the number of egg processors that produce a higher percentage of total national egg supply has increased. Among the 179 egg producing companies that have approximately 95% of the today's U.S. layers, there are 61 companies that produce 87% of the total egg production (UEP, 2012). In addition, genetic selection for layers has led to better egg yield per bird and the introduction of more specialized technology in egg processing has enabled the industry to be more commercialized and more automated (Stadelman & Cotterill, 1995).

Eggs are marketed and utilized in many ways. The majority of US egg production is consumed domestically. In 2011, egg operations produce about 223.7 million cases (AEB, 2013). About 55.3% of egg production goes to shell egg retail sales, 31.9% goes to breaker plants for further processing, 9% for institutional use, and only 3.8% is exported to other countries either as shell eggs or egg products (AEB, 2013). According to the United Egg Producers, the five top egg-producing states are Iowa, Ohio, Pennsylvania, Indiana, and California. Commercial egg production and processing

facilities can be classified as in-line, where eggs are produced and shipped for marketing in the same location, or off-line, where egg production and packaging locations are different (Knape et al., 2002).

The major concern of egg processing is to ensure the characteristics of eggs that maintain egg quality for consumer acceptability (Stadelman & Cotterill, 1995). Cleanliness, soundness, smoothness, and shape are the main characteristics of eggshells that are considered to determine shell egg quality. Cleanliness is one of the most desirable shell qualities and the egg industry has utilized several methods to achieve this goal (Stadelman & Cotterill, 1995). Historically, the dry cleaning of eggshells with hand-held abrasive blocks to scratch the dirt or stain of the egg was the most common method. Currently, sanitizing compounds and equipment that are able to ensure the effectiveness of wash water were introduced to the industry (Stadelman & Cotterill, 1995). Although wet cleaning is the most effective procedure to remove stains and dirt from the surface of eggshells, bacteria are more likely to penetrate through the shell. In order to prevent this penetration, guidelines have been established which include the difference in temperature between wash water and eggs should be at a minimum 11°C (20°F) but not exceed 21°C (40°F), with minimum wash water temperature of 32.2°C (90°F) (USDA, 2004; Fanatico & Conner, 2009). In addition to prevention of bacterial penetration, these temperatures were determined to prevent thermal cracks caused by the difference in temperature between egg and wash water (Stadelman & Cotterill, 1995). Contemporary egg washers spray eggs with water and sanitizer instead of immersing the eggs to reduce the risks of egg cracking and microbial penetration. In commercial

operations, the egg wash process uses a spray of wash water containing an alkaline detergent by a sequence of nozzles as eggs move under flat brushes (Hutchinson et al., 2003). After the wash step, a final rinse with an approved chemical disinfectant is sprayed over the eggs (Bartlett et al., 1993; Hutchinson et al., 2003). Rinse and excess wash water are pooled, filtered, and collected for further recycling after reheating in a recirculation tank (Hutchison et al., 2004). According to USDA regulations, egg wash water is required to be changed approximately every 4 h during processing (USDA-AMS, 2004).

Maintaining alkaline wash water pH is another factor that is important to achieve the desired cleanliness of eggs during processing and reduce microbial contamination risk. Researchers have shown that a pH range of 10 to 11 in wash water tanks is required to produce an unfavorable environment for most bacteria (Moats, 1978). Although processors often start with pH around 11 at the beginning of each shift, overflow water losses, recycling, and refilling of replacement water leads to a reduction in the pH below 11. Maintaining the proper pH is critical because it has been shown that *Salmonella* can survive at pH 9.5 when wash water temperature is between 38 and 42°C (100.4 to 107.6°F) (Curtis et al., 1996).

Salmonella

Salmonella is a facultative anaerobic, non-thermoduric bacterium of the Enterobacteriaceae family. It is a Gram-negative, motile by peritrichous flagella, non-spore forming rod. It measures about 0.5 µm in length and 0.2 µm in width. The genus

includes more than 2,500 serotypes of bacteria. The ability of glucose fermentation and gas production, inability of lactose fermentation, and production of hydrogen sulfide from thioisulfate are specific characteristics of typical *Salmonella* and differentiate it from other family members (Cox et al., 2000b). The growth of *Salmonella* can be affected by several factors such as temperature, pH, and water activity (A_w). The optimum temperature for *Salmonella* to grow is 37°C. The minimum pH for this bacterium is 4.7, while the optimum is 7.0. The minimum A_w for *Salmonella* to grow is 0.93, and as A_w decreases, survival of *Salmonella* may increase (Jay et al., 2005).

Salmonella has been identified as a causative agent of illness for more than 100 years. There are more than 2,500 serovars of *Salmonella* that belong to the main three recognized species: *S. enterica*, *S. bongori*, and *S. subterranea* (Garcia et al., 2011). *Salmonella enterica* is further subdivided into subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica* (Jay et al., 2005; Garcia et al., 2011). Eating foods that are contaminated with animal feces is one of the common transmission methods of *Salmonella* infections to humans. The serovars *S. Typhimurium* and *S. Enteritidis* (SE) are the most common serotypes of *Salmonella* in the U. S., and 50% of human infections are caused by those two serotypes (USDA-FSIS, 2011). The incidence of SE infection has increased since the 1970s, and SE was reported as the serovar most responsible for the outbreaks in the US (Braden, 2006). About 42,000 cases of salmonellosis are reported in the US every year, and this number could significantly increase if the mild cases of the illness were reported or diagnosed (CDC-NCEZID, 2010). *Salmonella* Enteritidis infections are commonly linked to eggs as the main food source of infection

(CDC-NCEZID, 2010). In 2000, a study estimated that 182,060 cases of illness were due to SE linked to eggs (Schroeder et al., 2005). *Salmonella* Enteritidis infection not only occurs when contaminated eggs are consumed directly, but also from cross contamination from improper handling of contaminated eggs. Additionally, undercooking of foods can also lead to transfer this pathogen. Severity of this illness may increase in infants, elderly, and people with compromised immunity. Symptoms of SE are usually fever, abdominal spasms, diarrhea, and subsequently may lead to hospitalization in more severe circumstances.

Probably one of the most recent and the largest food born outbreak caused by shell eggs was in 2010. Hundreds of people across the US were diagnosed positive of SE pathogen (CDC, 2010). As a consequence of this outbreak, a more than 500 million eggs nationwide including several brand names were recalled (FDA, 2010). An intensive investigation revealed that SE was the causative pathogen of this outbreak in shell eggs produced by Wright County Egg and Hillandale Farms in Iowa (FDA, 2010). This recall once again brought shell eggs to the forefront of food safety and increased consumer concern. The United States Food and Drugs Administration estimates about 79,000 cases of illness and 30 deaths per year caused by SE would be prevented if this pathogen is controlled in shell eggs (FDA, 2009).

Salmonella has two main methods of transmissions: vertical and horizontal. Vertical transmission occurs when this pathogen is transmitted from *Salmonella*-positive hens to the eggs during the formation of the eggs in the reproductive system and before laying. Although hens infected with *Salmonella* may not show noticeable signs of

infection, eggs from those hens could be internally infected with this microorganism (Timoney et al., 1989; Shivaprasad et al., 1990). It was suggested that egg contamination may occur in the upper or lower part of the oviduct during egg formation in infected hens (Keller et al., 1995; Miyamoto et al., 1997; Cox et al., 2000b). The United States Centers for Disease Control and Prevention (CDC) reported that approximately one in 20,000 eggs is internally infected with SE during development (CDC-NCEZID, 2010).

Horizontal transmission is another route of egg contamination that occurs when the shell eggs is externally cross contaminated by the environment, objects or by infected hen feces during oviposition. In fact, hens can carry many microorganisms, including *Salmonella*, from soil or feces into the nest and cause egg contamination. *Salmonella* can be present on the shell surface or can penetrate through the shell pores after eggs are laid (Cox et al., 2000b). There are several factors such as pH (Sauter et al., 1977), vapor pressure, temperature, and humidity (Graves & Maclaury, 1962) that might have influence on the penetration of *Salmonella* into eggs. Sauter et al. (1977) found that a decrease in solution pH led to increased *Salmonella* penetration. They reported that when pH was 7.5, penetration rates were 42% of challenged eggs, but penetration rate was only 22% in the challenged eggs at pH 8.5. In addition, maintaining temperatures below 70°F and around 62.3% relative humidity at the laying facility can lower incidence of microbial penetration (Graves & Maclaury, 1962).

The penetration rate and growth of *Salmonella* in eggs can be influenced by several factors. At storage temperature greater than 25°C, SE was able to penetrate and grow at a high rate in egg yolk stored for more than 24 h, while storage at 15°C for 72

hours inhibited the penetration and multiplication ability of SE (Gast & Holt, 2001; Gast et al., 2007). It was found that egg age and storage temperature can also impact the growth of SE in egg contents (Humphrey & Whitehead, 1993). They have reported that this bacterium can increase when there is alteration in the yolk membrane which allows the bacterium to attack the yolk or obtain some nutrients from it at 30°C.

Previous investigations found that egg age has significant impact on SE multiplication. There are several proteins in the albumen such like ovotransferrin, ovomucoid, flavoproteins, ovoinhibitor, and avidin, that have bacteriostatic effects, while lysozyme is bactericidal and can lyse the bacteria cells. However, during aging storage of eggs, the proteins can become less bacteriostatic. *Salmonella* Enteritidis can multiply more rapidly in the albumen of stored eggs versus fresh egg albumen, suggesting that albumen may become less inhibitory to *Salmonella* during storage. This increase in growth in the albumen may be due to increased iron content from the yolk. As eggs age, the vitelline membrane weakens allowing iron to leech from the yolk into the albumen. Since bacterial need iron to grow, the leeching from aged eggs may cause an increase in SE growth (Humphrey & Whitehead, 1993).

Food safety

During the last decades, authorities have conducted several programs to improve the health of the people of the US. The Healthy People 2010 Program was launched to increase public awareness to improve health (HHS, 2010). Nevertheless, according to the Healthy People 2010 Final Review Assessment, the program has accomplished only

71% of its overall objectives and 73% of food safety objectives were achieved. Specifically, SE outbreaks decreased from 1997 to 2008 by only 44.9% and 4.2% progress was met in consumer food safety practices between 1998 and 2006. A follow up program which started in 2010, Healthy People 2020, has worked to promote cooperation between communities, encourage health making decisions, and evaluate the influence of prevention activities (HHS, 2012). The main goals of this program are achieving a disease-free high quality life, and enhancing the health of societies. In order to accomplish these objectives, the program has set a goal to develop and increase food safety awareness and diminish foodborne disease outbreaks. One of the food safety goals is to reduce numbers of people infected with foodborne *Salmonella* per 100,000 from 15.0 in 2006-2008 to 11.4 in 2020 (HHS, 2013).

Food safety continues to be an important issue for all aspects of the egg industry. In order to reduce the risk of egg contamination, the USDA Food Safety and Inspection Service (USDA-FSIS) inspects egg products that are intended for commercial human consumption under the Egg Products Inspection Act. This legislation that was established and declared in 1970 seeks to prevent adulterated eggs from entering commerce which can become a potential hazard to consumer health. Regulations formed by the USDA-FSIS required that any egg processing operation shall be inspected to prevent commerce of any egg product that are not labeled correctly or are adulterated (USDA-FSIS, 1970). The United States Food and Drugs Administration (FDA) established the Egg Safety Final Rule, which is estimated to prevent around 79,000 cases of foodborne disease every year due to SE transmitted through contaminated eggs (FDA,

2009). Egg safety regulatory responsibilities are shared by the FDA and USDA-FSIS, which are working together to resolve the contamination of SE in eggs, and strengthen and promote the adaptation of food safety legislations (USDA-FSIS, 2011).

Proper sanitization of shell eggs is therefore vitally important to reduce the risk of foodborne illness, and also to maintain consumer confidence in egg safety when produced under commercial conditions. Generally, the high pH (11.0), high temperature (90°F) and use of chlorine (50 to 200 ppm) in egg wash waters are considered adequate to destroy bacteria removed from the eggs during washing (Curtis et al., 1996). However, as eggs are washed during processing, the organic load from dirt, manure, and broken eggs can accumulate in the wash water, and pH may drop if not managed by adding detergent and monitoring pH regularly throughout the process. Moats (1979) reported that bacterial wash water concentrations can often be greater than 5.0 log₁₀ cfu/mL, and show a significant correlation between bacterial counts in wash water and resulting counts on eggshells. Additionally, there is a major difference in bacterial penetration between normal eggs and cracked eggs. Cracked eggs are more susceptible to spoilage than normal eggs because cracked shells are not an adequate barrier to microorganisms (Brown et al., 1965). Checked eggs have cracked or broken shells; however, such eggs have intact shell membranes that keep their contents from leaking (Stadelman & Cotterill, 1995).

Hazard analysis and critical control point (HACCP) is defined as a system of actions to ensure the safety of food products during production to prevent transmission of hazards, including foodborne diseases. Although the USDA has not implemented

HACCP for shell eggs, several processors have been establishing HACCP systems (Curtis et al., 1996). As the egg industry faces the possibility of implementing mandatory HACCP for shell egg processing, the industry needs data that will allow for development of procedures to minimize microbial contamination of shell eggs. Monitoring initial, processing, and storage egg temperature and wash water temperature and pH are essential factors in developing an egg HACCP plan (Curtis et al., 1996).

Current practices for shell egg disinfection

Several researchers have investigated different disinfection methods to prevent *Salmonella* spp. in shell eggs during egg processing. Some researchers indicate that *Salmonella* can be vertically transmitted from one generation to the next if there is no effective chemical treatment (Cox et al., 2002a). The washing of table eggs in the U. S. has been required for several decades for producers packing eggs under USDA inspection. Egg washing not only removes adhering material on the outside of the shell, but has also shown to significantly reduce eggshell microbial populations (Knape et al., 2002; Musgrove et al., 2005a).

Knape et al. (2002) suggested that increased organic adhering materials on off-line eggs can reduce the efficacy of egg sanitizers. Following washing, processors are required to apply a sanitizing spray to the eggs, most commonly a chlorine (50 to 200 ppm) or QAC (200 ppm). Musgrove et al. (2005a) indicated that recovery of *Salmonella* from washed eggs was less frequent (8.3%) than from unwashed eggs (15.8%). However, most published data has shown that conventional washing and sanitizer spray

methods do not completely eliminate bacteria from the eggshell (Knape et al., 2002; Musgrove et al., 2005a; Caudill et al., 2010; Woodring, 2011). Caudill et al. (2010) found that using different wash water temperature schemes in commercial dual-tanks facilities did not influence the number of microbial counts when wash water temperature was 23.9°C or 48.9°C (2.98 vs. 3.12 log₁₀ cfu/ml, respectively). Knape et al. (2002) indicated that eggshell aerobic plate counts (APC) were not significantly different at any location of the commercial egg washing steps in in-line facilities versus off-line facilities. Microbial loads were not less than 2.06 log₁₀ cfu/ml at egg packaging step. Musgrove et al. (2005b) assessed the influence of commercial egg processing techniques on reducing the total microbial counts. These researchers reported that although commercial processing was effective in reducing the microbial loads, aerobic bacteria were still detectable at the point of egg packaging.

Several types of sanitizers such as chlorine and QAC in the final rinse and UV light have been used to reduce microbial loads on eggshells. Musgrove et al. (2008a) found that eggs entering the rewash belt, following egg washing and sanitizer application, could still be contaminated with bacteria such as *Salmonella*, *E. coli*, and *Enterobacter*. Therefore, methods to reduce egg wash water contamination and improved methods of eggshell sanitization are needed to assure maximum microbial reduction during shell egg processing.

Chlorine

Chlorine is described as a highly irritating, corrosive, moderately non-toxic compound with broad bactericidal ability (Wiley, 2010). It is one of the most commonly used disinfectants in commercial egg washers due to its ability to reduce microbial loads if applied in appropriate ways (Moats, 1981; Knape et al., 2001; Musgrove et al., 2008b). The electronegative charge of chlorine leads to protein denature by oxidizing the peptide links (Maris, 1995). Concerns have emerged regarding its continuous use as the sole disinfectant (Knape et al., 2001) because of potential bacterial resistance. Researchers have found that bacteria have higher chlorine resistance when those bacteria were coming from previously chlorinated potable water (Ridgway & Olson, 1982). They found that bacteria from drinkable chlorinated water were less chlorine sensitive to free and combined chlorine than bacteria from non-chlorinated water. Another concern associated with chlorine is a possible risk due to the chlorination reaction that can cause toxic chloro-organic complexes (Wei et al., 1985).

A study comparing the antibacterial effectiveness of chlorinated compounds on table eggs found that sodium hypochlorite and sodium dichloro-s-triazine trione were both significantly more effective in reducing bacterial loads on egg shell than a chlorinated alkaline cleaner, but the effectiveness of the two active chlorine sanitizers was significantly lower in the presence of solid egg debris (Moats, 1981). Other researchers have also reported that chlorine loses its efficiency when total dissolved solids increased in wash water (Wei et al., 1985). It was also concluded that chlorine-containing detergent is more beneficial than using a non-chlorine detergent in the

reduction of the bacterial number in the egg wash water (Bartlett et al., 1993). Although both chlorinated and non-chlorinated wash water samples had maintained $\text{pH} \geq 10.0$, samples from chlorinated detergent wash water had significantly higher pH (10.8) and corresponding lower bacterial counts. They also suggested that available chlorine concentration of 0.45 mg/L is required in wash water to ensure acceptable microbial counts. Another study has shown that chlorine was not effective in reducing the prevalence of aerobes of unwashed eggs treated with either hand-sprayed or machine-applied chlorine at 200 ppm (Musgrove et al., 2006). It was also reported that neither 100 nor 200 ppm of chlorine was able to show lower microbial counts of washed eggs when compared with water only rinses (Musgrove et al., 2008b).

Quaternary ammonium compounds (QAC)

Quaternary ammonium compounds are used as a spray in the final rinse of the egg washing process. Their bactericidal activity occurs from the four radicals of these compounds linked to long carbon chain (Rahn & Eseltine, 1947). The mechanism of action of these compounds is thought to be due to their ability to deform the cell membrane of the bacteria or adsorb to the cellular wall and release intracellular fluids (Hamilton, 1968; Ahlstrom et al., 1999). Research has indicated that QAC are more effective against Gram-positive bacteria than Gram-negative bacteria and more effective against bacteria than fungi. Researchers have studied the effect of QAC as sanitizers for their impact on bacterial contamination (Oliveira & Silva, 2000; Hutchison et al., 2004). Oliveira and Silva (2000) concluded that when eggs were dipped in warmed QAC

(45°C) at 400 ppm or chlorine compound at 50.2 ppm, the former was more effective in reducing SE on shell eggs than the latter. In addition, Hutchison et al. (2004) indicated that 5.0 or 10.0 g/L of (Quat 800) nearly completely eliminated *Salmonella* on shell egg (less than 1.0 log₁₀ cfu/egg). They also reported that QAC are able to leave residues on eggshell that can prolong their bactericidal effect. A study conducted to evaluate the efficacy of different egg sanitizers on the penetration ability of *Salmonella* concluded that QAC (100 ppm, pH 7.5) was significantly effective in reducing the penetration rate of SE (3.4%), and differences in storage temperatures did not influence the efficacy of this disinfectant (Wang & Slavik, 1998).

Ultraviolet light and hydrogen peroxide

An alternative method of egg disinfection that has shown to be highly effective is the use of hydrogen peroxide (H₂O₂) in combination with UV at 254 nm, also called germicidal light. Hydrogen peroxide is an oxidative biocide that breaks down to O₂ and H₂O in liquid solutions when exposed to light of wavelengths ≤ 365 nm (Baxendale & Wilson, 1957). The formation of destructive free radicals which attack vital cellular structures such as DNA is the main mechanism of action for H₂O₂. The destructive ability of this oxidative biocide can be tolerated at low levels; however, high levels have been shown to have irreversible cellular damages (Finnegan et al., 2010). Hydrogen peroxide is one of the approved non-agricultural compound that can be used in egg washing process (USDA, 2010). Ultraviolet light is a component of the electromagnetic spectrum produced by the sun and having germicidal ability by causing cellular genetic

damage in several types of microorganism at 254 nm (Coufal et al., 2003; Gottselig, 2011). The combination of H₂O₂ and UV light has been proposed to have stronger antimicrobial effect due to the photolysis of H₂O₂ by the UV light and thus produce hydroxyl radicals ($\cdot\text{OH}$) (Baxendale & Wilson, 1957). Results demonstrated that H₂O₂ and UV alone reduce eggshell APC up to 2.0 log₁₀ cfu/egg when applied independently, however, the application of H₂O₂ followed by UV exposure for 8 min reduced APC by more than 3.0 log₁₀ cfu/egg (Wells et al., 2010).

Other researchers investigated the influence of commercial UV irradiation on the bacterial load of clean and dirty shell eggs run over roller conveyor belts (De Reu et al., 2006). They found a significant impact of UV irradiation on reducing total aerobic counts on clean eggs from 4.47 to 3.57 log₁₀ cfu/egg when those eggs were exposed to UV light for 4.7 s. However, no significant reduction was observed in dirty eggs, or internal egg bacteria. They also found that microbial load can be controlled on the rollers of the conveyor belt.

Recent research at Texas A&M University has found that eggshell APC reductions can be achieved with the combination use of 3% H₂O₂ and only 5 s of UV exposure. The short duration of UV light exposure required is due to the rapid formation of hydroxyl radicals ($\cdot\text{OH}$) following the photolysis of H₂O₂ by UV (Catherine & Waites, 1982). The resulting $\cdot\text{OH}$ react quickly with organic molecules and are the microbial killing mechanism of the process. Other results with H₂O₂ and UV light treatment of shell eggs has demonstrated no effect on shell strength, egg quality, or

consumer sensory testing while reducing APC to less than 2.4 log₁₀ cfu/egg (Woodring, 2011).

Peracetic acid (PAA)

Peracetic acid (CH₃CO₃H) is another highly effective and widely used disinfectant in the food processing industry because of its germicidal ability. It is formed by combination of acetic acid and H₂O₂, and has been approved for use in many food contact applications such as fruits, vegetables, meat, and eggs (Evans, 2000). Peracetic acid has been approved to be used for fruit and vegetables disinfection without a final rinse and egg wash (USDA, 2010). The mechanism of action of this sanitizer is relatively similar to other oxidative biocides such as H₂O₂ (Finnegan et al., 2010). This oxidative disinfectant also has the ability to form free radicals that target enzymes and proteins and acquire antimicrobial activity through metabolic inhibition (Denyer & Stewart, 1998).

Peracetic acid has shown to be very effective at reducing APC, *Salmonella* and *Campylobacter* numbers on poultry carcasses when used as an antimicrobial agent in chill water (Bauermeister et al., 2008). However, review of the literature found little information is available regarding the use of PAA on eggs, and no data is available regarding the use of PAA on shell eggs under modern egg processing conditions. Researchers sprayed hatching eggs inoculated with *Salmonella* with a combination of PAA and H₂O₂ and found that only 7.5% of eggs remained positive for *Salmonella* after treatment (Cox et al., 2007). Other researchers have reported that exposure of shell eggs

for 1 min to PAA solutions of 100 to 400 ppm effectively reduced eggshell surface counts by 95% (Hartman & Carlin, 1957), although bactericidal effects on *E. coli* and sporicidal impact on *B. cereus* was gradually reduced when 2% of egg solids was added. However, no data is available regarding the use of PAA in combination with UV light in egg processing. It has been reported that the combination of PAA and UV light can reduce the time required to inactivate DNA or RNA of bacteriophages in wastewater in 12.5 min while the same reduction required 1 h when PAA was used alone (Rajala-Mustonen et al., 1997).

Consumer acceptance of eggs

A useful procedure to evaluate consumer acceptance of eggs is sensory analysis. Such tests provide understanding of the product's perception and acceptability by consumers through human senses such as smell, sight, and taste (Meilgaard et al., 2007). These senses allow consumers to perceive and evaluate what they like or dislike about products. The interactions of panelists with product sample, evaluation environment, and the evaluation methodology are considered potential causes of variability in the test design (Meilgaard et al., 2007). In order to diminish any irrelevant deviation that may result in possible bias in decisions, it is crucial to control these interactions (Meilgaard et al., 2007). A sensory evaluation methodology can be designed by recruiting trained or untrained panelists (Hayat et al., 2010). Trained panelists have the ability to distinguish the differences in flavor, aroma, and overall differences while more than 75% of untrained panelists could not discriminate between those consumer attributes. Another

study recruited untrained panelists to determine the differences in visual attributes when evaluating the yolk and albumen of eggs treated with ozone (Kamotani et al., 2010). They indicated that panelists were able to perceive the differences in cloudiness of albumen and yolk between control and treated eggs. However, those untrained panelists were unable to recognize differences in the acceptability of texture, taste, and aroma in all treatments. Triangle tests and 9-point hedonic scales were used to evaluate differences and consumer acceptability of liquid eggs treated with short wave UV light (UV-C), pasteurization, or untreated liquid eggs (de Souza & Fernández, 2012). Those 2 tests revealed consumer acceptability for liquid eggs treated with UV-C were comparable or better than eggs treated with heat treatment, and no off-flavors were detected between treatments.

Woodring (2011) applied a triangle test to evaluate if consumers could distinguish between control eggs and eggs treated with the combination of UV light and H₂O₂. Two experiments were conducted in that study to compare the treatments after 15 and 45 d of storage at 4°C (39.2°F). It was found that only 28% and 34%, in experiment 1 and 2, respectively, of panelists were able to recognize the odd sample from eggs stored for 15 d, and only 44% and 28% of the panelists chose the correct answer on d 45 of storage in experiment 1 and 2, respectively. Overall, only 33.5% of panelists correctly chose the odd sample between eggs treated with chlorine or QAC and eggs treated with the combination of UV light and H₂O₂; thus, no significant difference between treatments were found.

Conclusion

Currently, there are several practices that are utilized for shell egg disinfection in the US to reduce the population of bacteria on the shell of the egg and decrease egg contamination. Variations in efficacy of those methods to reduce the bacterial load on eggshell surfaces have been observed. Most studies reported that those practices cannot completely removing bacteria from eggshell surfaces. Therefore, room for improvement in shell egg sanitization methods to provide the safest eggs possible to consumers exists. More data are needed to review, evaluate, and implement new commercial egg sanitization practices to ensure egg safety without impacting egg quality.

CHAPTER II

**PROCESSING PRACTICES AND MICROBIAL SURVEYS OF CURRENT EGG
SANITIZATIONS METHODS USED IN THE US EGG INDUSTRY**

Introduction

Contamination of eggs with pathogenic bacteria has become a significant issue due to potential to impact human health and increase consumers concerns about the safety of shell eggs. Microbiological safety and chemical contamination are the most common food safety issues that affect egg safety (Holt et al., 2011). Researchers have investigated many practices to reduce the microbial loads on eggshells. By washing eggs and using various types of sanitizers, microbial loads can be reduced or eliminated if the sanitizer is applied in an appropriate way (Bierer et al., 1961; Moats, 1979; Hutchison et al., 2004; Caudill et al., 2010; Hannah et al., 2011).

It is generally known that chlorine and quaternary ammonium compounds (QAC) are the most common post-wash sanitizers used by egg processors. Previous researchers have shown that these two types of disinfectants are insufficient to completely sanitize shell egg surfaces (Knape et al., 2002; Musgrove et al., 2008b). However, no studies have been published that have documented which practices and disinfectants egg processors use on an industry wide basis, nor has anyone ever compared the efficacy of the various egg processing practices across multiple plants in a similar timeframe. Therefore, additional data are needed to evaluate egg sanitization processes used across the US and microbial effectiveness of those processes in order to provide better egg

safety and quality for consumers. Therefore, a survey was administered to the US egg industry in an attempt to gain a better understanding of the egg disinfection processes used, and a microbial survey was undertaken to determine the microbial efficacy of those processes. An egg processing practices survey would help to understand the variability in the egg industry with respect to facility area, size, type, and the egg sanitization methods that are used in these facilities. A microbial survey would investigate which sanitization methods are the most effective for reducing the numbers of aerobic bacteria on eggshell surfaces.

Material and Methods

Egg processing practices survey

This survey was developed by Texas A&M University (TAMU) researchers and administered in collaboration with the United Egg Producers (UEP) to determine the current practices of egg processing and sanitization used across the US. One hundred and sixty-two surveys were sent to egg processors who were UEP members. Surveys were collected over the course of approximately 2.5 months, and included several questions based on the significant information that can provide better understanding for current commercial practices that are used to sanitize eggshell surfaces (Appendix A). Processing plants were grouped into 5 regions across the US as listed in Table 2.1. These regions were chosen and defined by the UEP based on their organization of the egg industry.

Table 2.1. States divided according to their geographical region in the egg processing survey.

Area 1	Area 2	Area 3	Area 4	Area 5
Southwest	Northwest	Midwest	Northeast	South
California	Washington	Colorado	Pennsylvania	Kentucky
Nevada	Oregon	Kansas	New York	West Virginia
Utah	Idaho	Nebraska	Vermont	Virginia
Arizona	Montana	South Dakota	Maine	North Carolina
New Mexico	Wyoming	North Dakota	New	South Carolina
		Minnesota	Hampshire	Georgia
		Wisconsin	Massachusetts	Tennessee
		Michigan	Rhode Island	Alabama
		Illinois	Connecticut	Florida
		Indiana	New Jersey	Mississippi
		Iowa	Delaware	Arkansas
		Ohio	Maryland	Oklahoma
		Missouri		Texas
				Louisiana

A total of 13 survey questions including questions related to facility type, the numbers of eggs packed per day, eggshell color, the type of housing used for laying hens, the use of a resident voluntary shell egg grading services, type and concentration of sanitizer treatment either prior to or following washing, and type of microbiological monitoring conducted routinely were asked from managers of egg processing facilities. These questions were based upon current industry practices and were either open-ended or multiple choice to allow for ease of answering by managers of the egg processing facilities and to ensure the questions could be answered in a timely manner. For processors with multiple processing plants, the managers were asked to fill out one survey per egg processing facility. The data collected from the survey was anonymous and was compiled by UEP prior to sending the results to TAMU for analysis. The responses to each question were counted to obtain the total number of respondents for each area and then a percentage was calculated based on the number of responses from all areas.

Microbial survey

The second phase of this study was to conduct a microbial survey of several egg processing plants using chlorine and QAC as the egg sanitizer in the final rinse step after the egg washer. The purpose of this microbial survey was to determine the microbial loads on shell egg surfaces under the current sanitization practices and to assess the effectiveness of the current practices that are used for shell egg sanitization across the US.

This survey was performed between August 2012 and May 2013. Egg samples were collected from 6 plants that are in-line facilities (designated A, B, C, D, E, and F) located in the state of Texas. Each plant was visited on 3 different days with at least a month between sampling days. At each facility, 10 eggs were randomly collected at three stages during processing: prior to entering the washer (prewash), immediately after the washer (post-wash), and immediately after the sanitizing spray (post-sanitizer). This sampling scheme allowed for the differentiation of eggshell microbial reductions attributed to the washing step and the sanitizing agent used in the final rinse step. Since chlorine and QAC are the most accepted compounds used as the final sanitizer rinse in egg processing plants, 3 of the plants (A, B, C) were chlorine users and the remaining (D, E and, F) were QAC users. Eggs were aseptically collected from each sampling site and placed into new, clean foam egg cartons during collection. Cartons were then placed on ice in a cooler for transport back to the TAMU Poultry Science research laboratory for analyses.

Eggs were enumerated for total aerobic plate counts (APC) using a modified procedure of Coufal et al. (2003). Each egg was placed in 20 mL of sterile phosphate buffered saline (PBS) (pH 7.2) in a sterile Whirl-Pak® (Nasco, Inc., Fort Atkinson, WI) bag. After 1 min of gentle hand massaging to expedite the removal of bacteria from the surface of the eggshell, 1 mL of the rinse solution of each egg was removed and serial dilutions performed using PBS. Then, 1 mL from egg rinsate and each dilution was plated on Petrifilms (3M Healthcare, St. Paul, MN) for total APC determination. For Petrifilms that yielded zero colony counts (i.e., below limit of detection (20 cfu/egg)), a value of 10 cfu/egg ($1.0 \log_{10}$ cfu/egg) was assigned for mean calculation. After

incubation at 37°C for 48 h, colonies were counted and total APC were converted to $\log_{10}\text{cfu/egg}$ for statistical analysis.

Statistical analysis

Data from the microbial survey were analyzed using one-way analysis of variance (ANOVA) using JMP 9.0 software (SAS Institute, Cary, NC). Means were separated using Tukey-Kramer HSD test ($P\text{-value} \leq 0.05$). Means for the final rinse samples were also analyzed with orthogonal contrast SAS program GLM. For Petrifilms that had zero colonies (below the limit of detection), a value of 1.0 \log_{10} cfu/egg was assigned for treatment mean calculation.

Results and Discussion

Egg processing practices survey

Data regarding current egg processing practices across the industry is limited. Therefore, a survey was conducted to determine the current practices for egg sanitization used in the table egg industry. Of the 162 egg processing facilities surveyed, 82 were returned, for a response rate of (50.6%). Responses for each question are summarized in Tables (2.2 to 2.10). High percentages of respondents were from the South and Midwest areas (38 and 29%, respectively), while 15% of respondents were from Northeast, 13% were from the Southwest, and only 5% of respondents were from the Northwest area. The higher number of respondents from the South and Midwest areas assures the validity

of this survey since the leading egg producing states are found in these regions. Therefore, an appropriate cross-section of the US commercial egg industry was obtained, and the data should accurately represent the practices across the egg industry.

The type of facilities operated by survey respondents are summarized in Table 2.2. A total of 57% of the respondents operated in-line processing type facilities only, 17% had off-line facilities only, and 26% operated a combination of both types at their location. In 2004, a survey questionnaire of egg production firms was conducted to collect information regarding egg industry by mailing the survey to all UEP members and other egg producers (Bell, 2004). It was reported that 25% of the companies were in-line production facilities. The increase in the proportion of in-line operations between that survey and the current survey highlights the trend in recent years for egg producing companies to abandon the off-line production model in favor of constructing in-line production facilities. This is likely due to the fact that production costs are lower and egg quality is higher with in-line operations compared to off-line.

Knape et al. (2002) conducted a study to compare the microbial population of eggshell surfaces between in-line and off-line egg processing facilities. They reported that eggshell APC ranged from 2.14 to 3.16 \log_{10} cfu/mL from in-line facilities and 3.48 to 3.68 \log_{10} cfu/mL from the off-line facilities at sanitizer treatment sites. It was concluded from that study that APC were higher in eggs from off-line facilities than eggs from in-line facilities.

Table 2.2. Type of egg processing facilities as reported by respondents of a national egg processing practices survey.

Area	In-Line only	Off-Line only	Both	Total by area
Southwest	4	4	3	11
Northwest	3	0	1	4
Midwest	17	3	4	24
Northeast	4	6	2	12
South	18	1	12	31
Total	46	14	22	82
%	57	17	26	100

In this survey, producers were asked about the number of cases of eggs packed per day as a means of assessing facility size (Table 2.3). These data indicate that the egg industry is dominated by large-scale operations (77%), especially in the leading egg producing regions such as the South and Midwest.

Table 2.3. Number of eggs packed per day as reported by respondents of a national egg processing survey¹.

Area	0-1000	>1000	Total by area
Southwest	2	9	11
Northwest	1	3	4
Midwest	4	20	24
Northeast	4	8	12
South	7	23	30
Total	18	63	81
%	22	77	99

¹One respondent did not answer this question

The third question in the survey was directed at eggshell color (white, brown, or both). Results indicate variation in the color of eggshells throughout the country. According to the data collected, white was the dominant egg type in all areas in the US (Table 2.4). About 63% of egg processors identified themselves as white egg only processors, while 5% processed only brown eggs, and 32% processed both white and brown eggs. From the results of this survey, it can be concluded that few egg processors in the US are dedicated to brown eggs only, while 95% produce white eggs. Some consumers have a visual preference for brown eggs, and they generally assume that these eggs have a greater value than white eggs (Patterson et al., 2001). However, there is no difference in egg quality or nutrition between white or brown eggs (USDA, 2000).

Table 2.4. Eggshell color packed in each plant as reported by respondents of a national egg processing survey.

Area	White only	Brown only	Both
Southwest	4	0	7
Northwest	4	0	0
Midwest	17	2	5
Northeast	7	1	4
South	20	1	10
Total	52	4	26
%	63	5	32

Egg processors were also asked about housing type such as cage, cage-free, free-range, organic, or combination types that are used in egg production. According to the USDA (2000), more than 90% of shell eggs in the US are produced using conventional cage systems. In the current survey, approximately 91% of egg producers reported using conventional cages, while 24% reported using cage-free, 9% free-range, and 22% organic (Table 2.5). In addition, 29% of egg processors indicated they are packing eggs from two or more of these types of production systems. This indicates that processors are processing eggs from a variety of housing systems within the same plant to meet consumer demands. Conventional cage housing produces the least cost eggs because of the lower expenses required for labor and the higher house capacity (USDA, 2000).

Among these housing systems, free-range production system had the lowest percentage 9%. Free-range housing allows hens to have access to the outdoors. With this off-line system, eggs are gathered and stored in flats prior to transportation to an egg processing facility. Organic housing system produces eggs from hens that are fed only organic feed, which is made of organic ingredients grown without using pesticides, herbicides, or commercial fertilizers (NOFA-VT). This type of housing and feeding system increase the cost of produced eggs.

Table 2.5. Type of production systems used as reported by respondents of a national egg processing survey.

Area	Cage	Cage Free	Free-Range	Organic	Combination
Southwest	10	6	3	5	7
Northwest	4	0	0	0	0
Midwest	20	6	1	5	6
Northeast	11	2	2	4	3
South	30	6	1	4	8
Total	75	20	7	18	24
%	91	24	9	22	29

Although USDA does not require egg grading, federal and state officials highly recommend the implementation of standardized grading for eggs. Egg grading is the sorting of eggs according to internal and external egg quality characteristics that were developed by USDA. This grading helps producers and consumer in the marketing and buying process by maintaining constant standards and values for each grade (USDA, 2000). The egg processing practices survey included a question about presence of an in-plant egg grading service (Table 2.6). In this question, results indicated that most egg processors 77% maintain grading services while 23% do not. According to the data in this survey, 75% of respondents in the Northwest area and 50% in Northeast area do not maintain shell egg grading, while most egg processors in the Southwest, Midwest, and South areas are maintaining shell egg grading services.

Table 2.6. The use of a grading service as reported by respondents of a national egg processing survey.

Area	Yes	No
Southwest	10	1
Northwest	1	3
Midwest	19	5
Northeast	6	6
South	27	4
Total	63	19
%	77	23

The survey also asked several questions to assess what kind of egg sanitization processes, wash water additives, and final egg disinfection methods egg processors are currently using. According to the survey, most egg processors (87%) are not using sanitization treatments prior to washing (Table 2.7). Most of the respondents that indicated they did use a disinfecting treatment prior to washing used a variety of different chemicals. A total of 5 egg processors used a multi-QAC, 4 indicated they used some type of chlorine-based solution, 1 used egg wash solution, and 1 producer used UV light as a treatment prior to washing.

Table 2.7. The use of an egg sanitization treatment prior to washing as reported by respondents of a national egg processing survey.

Area	Yes	No
Southwest	1	10
Northwest	0	4
Midwest	7	17
Northeast	0	12
South	3	28
Total	11	71
%	14	87

Egg washing not only removes adhering materials on the outside of the shell, but has been shown to significantly reduce eggshell microbial populations (Knape et al., 2002; Musgrove et al., 2005a). For wash water parameters, the data that was collected from the egg processing survey showed most producers use detergent (94%), 49% are using sanitizers, 43% defoamers, and 37% of respondents use a pH booster. Only 2% of respondents used other types of products and they did not mention what type they used. These compounds are used in the wash water to maintain pH (10.0 to 11.0) to prevent bacterial growth, reduce contamination risk, and ensure the effectiveness of sanitizers (Curtis et al., 1996). Defoamers are used to prevent or eliminate the production of foam from egg protein during the egg washing process in the hot water tank because the foam could cause a serious problem to the egg washing system by increasing water loss from wash tanks and sequentially influence water pH and temperature (Curtis et al., 1996).

Table 2.8. Types of additives used in egg wash water as reported by respondents of a national egg processing survey.

Area	Detergent	Defoamer	Sanitizer	pH Booster	Others
Southwest	9	6	4	3	0
Northwest	3	3	0	3	0
Midwest	25	12	15	3	2
Northeast	13	5	6	1	0
South	27	9	15	20	0
Total	77	35	40	30	2
%	94	43	49	37	2

Egg sanitization is a critical process for egg safety and is the final prevention step prior to packaging to reduce microorganisms. There are different types of compounds processors use in sanitization including chlorine, QAC, or UV light. In this survey, data revealed that most processors (83%) use chlorine as shell egg disinfectant in the final sanitization step, while 12% use QAC, and 6% use UV light (Table 2.11). Only 2% are using combination methods in the final sanitization step. Researchers have found that QAC have the ability to function as germicides against bacteria at alkali pH and at high temperatures (Risk et al., 1966). Studies have also indicated that UV light sanitization can result in a reduction of 1.3 log₁₀ in APC, 4.0 log₁₀ in *Salmonella* Typhimurium, and 4.0 to 5.0 log₁₀ in *Escherichia coli* (Coufal et al., 2003). Additionally, researchers have found that QAC sprayed as a disinfectant is more useful in reducing bacterial versus fungal activity (Brake & Sheldon, 1990). Moats (1981) reported that chlorinated cleaners, sodium hypochlorite and sodium dichloroisocyanurate, were ineffective cleaners for reducing bacterial load on eggs when 1% of egg solids was added to wash water.

Table 2.9. Type of disinfectant sprays or processes used in the final sanitization step of egg processing as reported by respondents of a national egg processing survey.

Area	Chlorine	QAC	UV	Combination
Southwest	11	0	0	0
Northwest	4	0	0	0
Midwest	14	5	3	2
Northeast	11	0	1	0
South	27	4	0	0
Total	67	9	4	2
%	82	11	5	2

Microbiological monitoring can be used by processors as verification that the egg sanitization procedures are effective. Therefore, in this survey, microbiological monitoring type was asked to determine if egg processors use microbial monitoring procedures in their facilities. The results indicated that 71% of producers across the US did not conduct any type of microbiological monitoring (Table 2.12). Only 4% of respondents reported that they are monitoring APC on eggs in egg cartons, and only 20% monitor APC in wash water. Only one respondent from the Midwest area indicated that they monitored *Salmonella* in eggs and in wash water.

Table 2.10. Type of microbiological monitoring performed at egg processing facilities as reported by respondents of a national egg processing survey¹.

Area	None	APC eggs	APC	<i>Salmonella</i>	<i>Salmonella</i>
Southwest	7	3	3	0	0
Northwest	4	0	0	0	0
Midwest	15	0	7	1	1
Northeast	11	0	1	0	0
South	21	0	5	0	0
Total	58	3	16	1	1
%	71	4	20	1	1

¹ 5 respondents did not answer this question

Microbial survey

The purpose of conducting the microbial survey was to determine the effectiveness of the current sanitization processes that are used by egg processors for shell egg disinfection. Samples were collected from 6 plants in the state of Texas over a 10-month period. Plants were chosen according to the type of sanitizer that was used in the final egg sanitization step after washing. Plants A, B, and C were QAC users, and Plants D, E, and F used chlorine. A total of 18 visits over the experiment (3 visits per plant) were conducted. Samples were collected at 3 different locations of egg processing: from the incoming conveyor belt prior to egg washing (prewash), immediately after exiting the washer stage (post wash), and after applying the final egg sanitizer (final rinse). Results varied between plants and varied within plant at the 3 sampling days for all stages (Table 2.11).

Table 2.11. Eggshell surface aerobic plate counts at various stages of egg processing for 6 egg processing plants in Texas.

Plant	Type	Stage	-----log ₁₀ cfu/egg ¹ -----		
			Visit 1	Visit 2	Visit3
A	QAC	Prewash	4.5 ± 0.06 ^a	4.4 ± 0.12 ^a	3.9 ± 0.13 ^b
		Post wash	1.1 ± 0.94 ^b	3.1 ± 0.06 ^b	4.9 ± 0.22 ^a
		Final rinse	1.0 ± 0.00 ^b	1.7 ± 0.28 ^c	1.1 ± 0.09 ^c
B	QAC	Prewash	5.7 ± 0.18 ^a	4.8 ± 0.26 ^a	4.1 ± 0.09 ^a
		Post wash	4.8 ± 0.16 ^b	3.2 ± 0.02 ^b	4.2 ± 0.24 ^a
		Final rinse	2.6 ± 0.14 ^c	3.3 ± 0.03 ^b	2.4 ± 0.25 ^b
C	QAC	Prewash	4.1 ± 0.16 ^a	3.9 ± 0.14 ^a	4.0 ± 0.08 ^a
		Post wash	3.3 ± 0.18 ^b	2.3 ± 0.18 ^b	4.0 ± 0.32 ^a
		Final rinse	1.7 ± 0.15 ^c	1.1 ± 0.05 ^c	2.7 ± 0.06 ^b
D	CHLORINE	Prewash	4.1 ± 0.14 ^a	4.4 ± 0.11 ^a	4.5 ± 0.15 ^a
		Post wash	1.5 ± 0.18 ^b	2.9 ± 0.05 ^b	2.9 ± 0.02 ^b
		Final rinse	1.3 ± 0.12 ^b	2.3 ± 0.15 ^c	1.9 ± 0.15 ^c
E	CHLORINE	Prewash	3.1 ± 0.13 ^a	4.2 ± 0.12 ^a	5.2 ± 0.02 ^a
		Post wash	1.3 ± 0.22 ^b	2.9 ± 0.04 ^b	2.9 ± 0.04 ^b
		Final rinse	1.4 ± 0.15 ^b	1.5 ± 0.21 ^c	2.8 ± 0.04 ^b
F	CHLORINE	Prewash	5.3 ± 0.12 ^a	4.7 ± 0.15 ^a	4.3 ± 0.04 ^a
		Post wash	2.0 ± 0.28 ^b	1.7 ± 0.21 ^b	2.9 ± 0.04 ^b
		Final rinse	3.0 ± 0.10 ^c	1.7 ± 0.12 ^b	2.8 ± 0.14 ^b

^{a-c} Means within column for each plant with different letters are significantly different (P ≤ 0.05)

¹ mean of 10 eggs ± SE

Data indicate that 15 out of 18 visits had significant less APC in the post- wash stage when compared to the prewash stage. In addition, 11 out of 18 visits demonstrated significantly less APC on the eggshells in the final rinse when compared to the post wash eggs. However, all plants had significantly less APC after sanitization (final rinse) when compared to the prewash eggs. The overall APC means for chlorine sanitization plants were 4.4, 2.3, and 2.1 log₁₀ cfu/egg at the prewash, post wash and the final rinse stage, respectively. On the other hand, plants that were using QAC as the egg sanitizer in the final rinse solution had an APC of 4.3 log₁₀ cfu/egg at prewash stage, 3.4 log₁₀cfu/egg after the post wash stage and 2.0 log₁₀cfu/egg after the final rinse stage. The overall average of APC of all plants was 4.4 log₁₀ cfu/egg for prewash eggs with a range of 3.1 to 5.7 log₁₀ cfu/egg. The average APC of eggs after being sanitized with either chlorine or QAC was 2.1 log₁₀ cfu/egg with a range of 1.0 to 3.0 log₁₀ cfu/egg. For the final rinse samples, data indicated that there was no significant difference (P=0.311) between sanitizers (chlorine and QAC). However, there was a significant difference (P=0.004) between sample collection days per each plant. Therefore, daily variability and incoming load can impact bacterial population on eggshell surfaces.

These variables can in turn affect overall APC on shell eggs. The variation observed among the plants could be linked to several factors that can impact the effectiveness of egg sanitizers. Knape et al. (2002) suggested that condition of wash water such as frequency of changing water, temperature, sanitizer concentration, and pH can influence the cleanliness and microbial load on shell egg surface.

Egg wash water samples from the 6 plants were also collected over the 18 visits, and the results are shown in Table 2.12. As with eggshell APC, there was a significant day variation in wash water APC. For plants A, B, C, and F, there was significant difference among the 3 visits. Although plant C showed significant difference, this plant had the most consistent value over the 3 visits. The range of wash water APC for all plants over all visits was between 2.0 and 5.7 log₁₀cfu/mL. Generally, there was no significant difference in the average APC of the 3 visits between plants. The overall mean APC of wash water samples was 3.3 log₁₀ cfu/mL.

It can be concluded that day variation has significant impact on APC in wash water samples. In a study by Knape et al. (2002), the average APC of wash water before applying the sanitizer for in-line facilities was 3.1 log₁₀ cfu/mL, while off-line facilities had wash water APC of 3.5 log₁₀ cfu/mL. These researchers concluded that there is a positive correlation between cleanliness of eggs and the microbial counts of wash water because sanitizers become less effective as the levels of organic materials increase (Knape et al., 2002).

Table 2.12. Wash water aerobic plate counts for 6 egg processing plants in Texas.

Plant	Visit 1	Visit 2	Visit 3	Average
-----log ₁₀ cfu/mL ¹ -----				
A	2.3 ± 0.02 ^b	4.5 ± 1.91 ^{ab}	4.9 ± 0.02 ^a	3.8 ± 0.49
B	2.0 ± 0.16 ^c	5.7 ± 0.31 ^a	3.5 ± 0.15 ^b	3.7 ± 0.48
C	2.7 ± 0.05 ^b	2.6 ± 0.04 ^b	2.9 ± 0.05 ^a	2.7 ± 0.04
D	2.6 ± 0.07 ^a	3.4 ± 0.26 ^a	2.8 ± 0.05 ^a	2.9 ± 0.25
E	2.2 ± 0.81 ^a	3.6 ± 0.60 ^a	3.7 ± 0.04 ^a	3.2 ± 0.37
F	5.2 ± 0.04 ^a	2.3 ± 0.38 ^c	3.8 ± 0.05 ^b	3.8 ± 0.38
All				3.3 ± 0.15

^{a-c} Means within row for each plant with different letters are significantly different (P ≤ 0.05)

¹ mean of 4 wash water samples/visit ± SE

In conclusion, the results of the egg processing indicated that in-line facilities were the most frequent facility type, and that chlorine was the most used egg sanitizer in the final sanitization step of egg processing. The egg processing survey indicates that most commercial egg processors do not utilize any type of sanitizers prior to egg washing. In addition, most egg processing facilities do not conduct any method of microbiological sampling of eggs or wash water on a continuous basis. The results of this study also revealed that most processors packed more than 1,000 cases/day, with the Midwest region of the US having the highest proportion of large-scale egg processing operations at 83%. Although the results from the survey found that different type of sanitizers are being used during egg processing, chlorine is the most common sanitizer used by egg processors across the US.

Results of the microbial survey demonstrated that day variation was observed to be more of a factor than variation from plant to plant. Overall, post sanitization eggshell APC were lower than prewash samples, indicating that eggshell sanitization procedures used by the commercial egg industry result in decreased eggshell APC regardless if chlorine or QAC is used.

CHAPTER III

**EFFICACY AND SENSORY EVALUATION OF CURRENT METHODS AND
NEW TECHNOLOGIES OF EGG DISINFECTION**

Introduction

Eggshell disinfection is a final stage that takes place in egg processing plants before eggs are directed to further processing or retail. This stage is important to provide sanitized eggs to consumers with as low a microbial load as possible. Foodborne diseases such as salmonellosis have been linked to the presence of pathogenic *Salmonella* species on eggshell surfaces. Currently, most egg processors in the US use chlorine or QAC spray as a final sanitizing step following washing. However, based on results of the microbial in the previous chapter, these types of disinfection systems are not reducing the microbial load completely. Therefore, new egg sanitization methods are needed to assure that maximum pathogen and microbial load reduction can be achieved.

The USDA requires the level of chlorine to be used as an egg sanitizing spray to be between 50 and 200 ppm (USDA, 2000). Moats (1981) indicated that chlorine was an effective disinfectant in reducing bacterial load in egg washers in absence of egg solids and at neutral pH. This study indicated that using sodium hypochlorite and sodium dichloroisocyanurate at 270 ppm active chlorine at pH 7.0 were able to eliminate *Salmonella* when eggs were sanitized immediately after adding the sanitizer and inoculated with *Salmonella* and tested after 0, 30, 60 min of sanitization. However, the efficacy of those sanitizers decreased as time between mixing and sanitization increased.

In addition, the efficacies of these compounds were reduced in the presence of 1% egg solids in wash water (Moats, 1981). Other researchers have also confirmed the effectiveness of 200 ppm of chlorine on the reduction of total aerobic counts and *Salmonella* spp., and verified that germicidal activity of chlorine reduced when dissolved solids increased (Knape et al., 1999; Knape et al., 2001). Currently, chlorine is the most commonly used egg sanitizer in the US.

Quaternary ammonium compounds (QAC) are used as bactericides at specific conditions of egg wash water that has alkaline pH values, high dilution of QAC, and high wash water temperature (Risk et al., 1966). These researchers also suggested that QAC are more effective than chlorine in the presence of organic material and QAC have residual activity whereas chlorine does not. These compounds have the ability to adsorb and deform the cellular membrane of bacteria and liberate the intracellular fluids, thus destroying bacterial cells (Hamilton, 1968; Ahlstrom et al., 1999). Oliveira and Silva (2000) reported that when eggs were dipped in warm QAC at 400 ppm or chlorine at 50.2 ppm, the former showed better efficacy in reducing the eggshell microbial load than the latter.

Ultraviolet light treatment has been approved by the FDA for food processing and treatment (FDA, 2012). Additionally, UV light disinfection has an important lethal impact on bacterial contamination because of its ability to cause cellular genetic damages to microorganisms at 254 nm (De Reu et al., 2006). However, other researchers reported that the combination of UV light and H₂O₂ could be more effective for reducing bacterial load because H₂O₂ photolysis is induced by UV light to produce destructive

free radicals (Baxendale & Wilson, 1957; Finnegan et al., 2010). Wells et al (2010) showed that the combination of 1.5 H₂O₂ spray followed by UV light exposure for 8 min could significantly diminish the level of bacteria on eggshell better than H₂O₂ or UV light alone. A recent study conducted at Texas A&M University (TAMU) indicated that applying 3.0% H₂O₂ followed by UV light treatment for 5 s could achieve high microbial reductions on eggshell surfaces (Gottselig, 2011).

In addition to H₂O₂ and UV light, PAA also has been shown to be an effective sanitizing agent for egg products. The antimicrobial mechanism of PAA is relatively similar to H₂O₂ by forming free radicals that target enzyme and thiol groups (Denyer & Stewart, 1998; Finnegan et al., 2010). Hartman and Carlin (1957) indicated that using PAA at 2,000 ppm significantly eliminated pathogenic bacteria on eggshell, but this treatment was insufficient to decrease the incident of egg spoilage when followed by short time thermo-stabilization, mineral oil at $74 \pm 1^{\circ}\text{C}$ for 1 min, when those eggs were stored under conditions favorable to egg spoilage for 2 wk. However, no research has been conducted to examine the combined effect of PAA and UV light. Researchers have shown that less time was required to inactivate the DNA and RNA of bacteriophages in waste water when PAA was combined with UV light (Rajala-Mustonen et al., 1997). To our knowledge, no research has been conducted to determine the efficacy and the quality of the current methods for shell egg sanitization in US and compared to new technologies in order to reduce bacterial load on eggshell surface.

Sensory evaluation is a tool that is used by trained or untrained panelists to evaluate and perceive products by using human senses. It is an approach to determine the

value of a product and its consumer acceptability. Sensory evaluation is used to precisely assess human response to foods and to diminish the bias influences of product information that could impact consumer preferences (Meilgaard et al., 2007). A consumer study conducted at TAMU in 2011 using a triangle test indicated that approximately one-third of the panelists were able to identify a difference between eggs that were disinfected according to recommended USDA procedures, chlorine or QAC sanitization, and eggs treated with H₂O₂ combined with UV light (Woodring, 2011). However, the differences were not defined as positive or negative since the test was a triangle-test.

The objectives of this experiment were to compare the efficacy of chlorine, QAC, PAA, PAA in combination with UV light, and H₂O₂ in combination with UV light to reduce APC and inoculated *Salmonella* Enteritidis on shell eggs. Finally, sensory evaluation testing was conducted to evaluate consumer acceptance of current and new eggshell disinfection methods.

Material and Methods

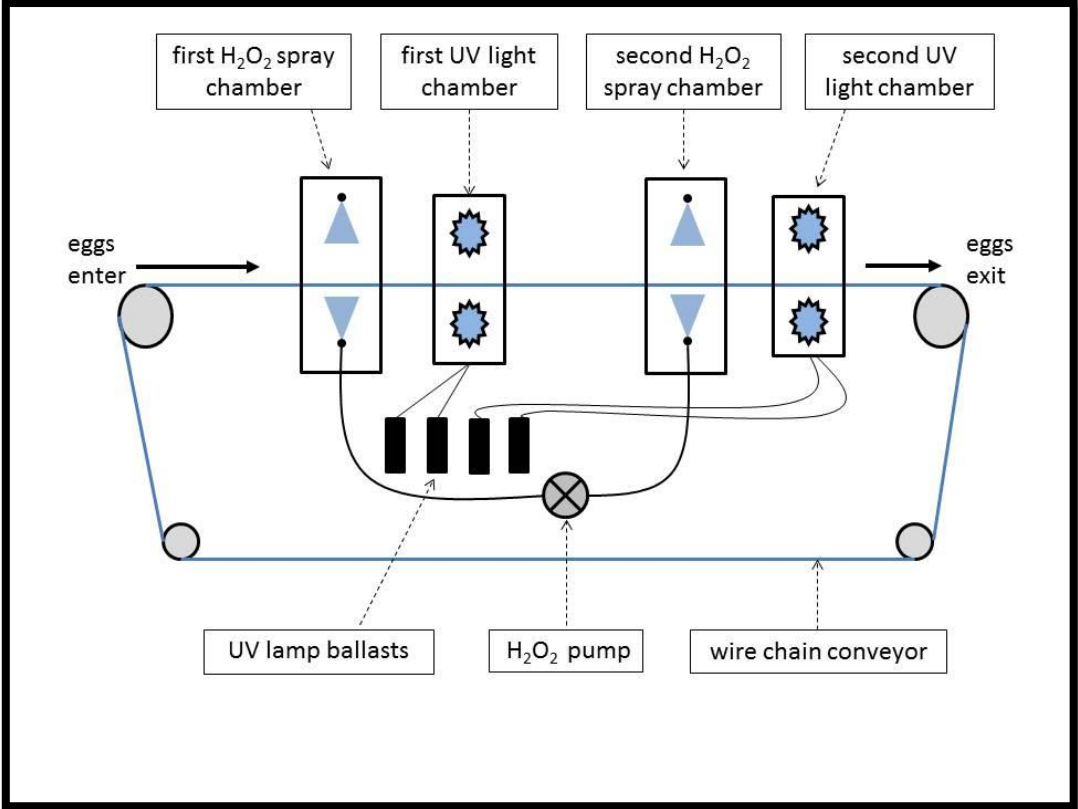
Application of treatments

Application of several treatments was used to evaluate bacterial load on eggshell surface. A total of 195 eggs were collected to determine the efficacy of current and new treatments using APC technique to detect bacteria population on eggshell surface (90 eggs) and *Salmonella* on the surface of the shell egg (105 eggs). These eggs were

collected from caged White Leghorn hens at the Poultry Research Center at Texas A&M University. Treatment groups were: 100 ppm chlorine (Antibac B, Diversey, Sturtevant, WI), 200 ppm QAC (Disan-1, Synco, Spring Branch, TX), 3.5% H₂O₂ (Brainerd Chemical Company, Inc., Tulsa, OK) and UV light (G20T5, Sankyo Denki, Japan), PAA (135 ppm) (FMC Corporation, Philadelphia, PA) alone or in combination with UV light, and a control group. Fifteen eggs were used per treatment, with 5 eggs for each treatment sampled at 0, 7, and 14 d of storage. Control eggs were aseptically placed in a Whirl-Pak bag (Nasco Inc., Fort Atkinson, WI) to determine APC loads.

The sanitization machine used to administer UV light to the treatment (Figure 1) is composed of two chambers for spraying, one spray station is located at the beginning of the conveyor belt as eggs enter the unit and the other spray station is prior to exiting the conveyor belt. These spray stations can spray each treatment with the desired concentration of antimicrobial. In between each spray station, is a UV station which administers UV light (254 nm; 8 to 12 mW/cm²) for approximately 5 s per station (4 on top and 4 on bottom of each light chamber). These UV stations can be turned on during application of PAA and UV and H₂O₂ and UV and turned off for non-UV applications. The time for each egg to run along the length of the conveyor belt and receive treatments was 38 s.

Figure 1. Schematic of H₂O₂/UV egg sanitization machine.



***Salmonella* inoculation**

A total of 105 eggs were collected from caged White Leghorn hens housed at the TAMU Poultry Research Center. In this experiment, eggs were divided into 5 treatment groups and 2 control groups. Fifteen eggs were used per group with 5 eggs for each d of sampling (d 0, 7, and 14 of storage at 4 °C). A sponge method was used to inoculate the eggs with a culture of $\sim 10^8$ cfu/mL *Salmonella* Enteritidis (SE) resistant to both Novobiocin (NO), and Nalidixic Acid (NA) (Sigma-Aldrich, St. Louis, MO). To ensure the efficacy of this method, preliminary tests were conducted to ensure that approximately 10^8 cfu/egg of SE attached to eggshell surfaces. *Salmonella* cell suspension was prepared by thawing *Salmonella* that was stored at -80 °C and 100 μ L was transferred in 10 mL of Tryptic Soy Broth (TSB) (Oxoid Ltd., Basingstoke, Hampshire, England) with NO and NA and then incubated for 24 h at 37 °C. Then, 100 μ L of the incubated culture was passed in 10 mL of TSB with NO and NA and incubated for 24 hours at 37 °C.

The suspension was centrifuged at 3,500 rpm for 10 min and the supernatant was discarded. Then, 10 mL of PBS was gently mixed with the pellets and centrifuged again as above and the supernatant was discarded and pellets were gently mixed with 10 mL of PBS. To determine the actual cfu/mL of *Salmonella*, serial plating was performed on brilliant green agar (BGA) (Difco, Becton Dickinson, Sparks, MD). Each egg was sponged by sterile cotton ball that dipped in approximately 10^8 cfu/mL of SE suspension culture. Then, sponged eggs were allowed to dry at room temperature for 30 min prior to treatment application. The eggs were then treated with: 100 ppm chlorine, 200 ppm

QAC, H₂O₂ (3.5%) and UV light, (135 ppm) peracetic acid alone, or (135 ppm) peracetic acid in combination with UV light. The 2 control groups were control positive (inoculated, no treatment) or control negative (no inoculation, no treatment, only sponged with PBS pH 7.2). *Salmonella* was enumerated by using BGA.

Microbial enumeration

For APC enumeration, 5 eggs per each treatment were sampled on day 0 of storage and the other 10 eggs per each treatment were kept in the cooler at 4 °C to be sampled on d 7 and 14 of storage. Each egg was individually placed in a sterile Whirl-Pak bag with 20 mL PBS (pH 7.2), and gently massaged for 1 min. Then, 1 mL from each bag was placed into a tube containing 9 mL of PBS and three serial dilutions were prepared. One mL from each rinse bag and each dilution was plated on 3M® Petrifilms (3M Health Care, St Paul, MN) and incubated for 48 h at 37°C. After incubation, plates were counted and total APC were converted to log₁₀ for statistical analysis. Plates that had no detectable colonies were assigned a value of 10 cfu/egg (1.0 log₁₀ cfu/egg).

For SE enumeration, 5 eggs per each treatment were individually placed in Whirl-Pak bag with 20 mL of PBS pH 7.2 and gently massaged for 1 min. Then, 1 mL from each bag was taken into a sterile tube containing 9 mL of PBS for serial dilution series. One mL from each dilution was plated in BGA with NO and NA, incubated for 48 h. Plates were counted after incubation period to determine the number of SE colonies.

Sensory panel

A scrambled egg consumer sensory test was conducted to investigate consumer acceptance of eggs treated with chlorine, QAC, H₂O₂ and UV light, and untreated control group. A total of 120 eggs were collected from cage White Leghorn hens at the TAMU Poultry Research Center. A total of 30 eggs each were treated with chlorine, QAC, H₂O₂ and UV light. Untreated eggs served as the control group. Following treatment, all eggs were stored at the sensory lab for 1 wk prior to the sensory evaluation. Eggs were stored for 1 wk to mimic the transport time it would typically take for eggs to travel through the marketing chain and reach the consumer.

A consumer panel of 50 persons ages 18 to 50 from TAMU students, faculty, and staff were recruited as volunteers. The sensory test was approved by the TAMU Office of Research Compliance and Biosafety Institutional Review Board (IRB) for the use of Human Subjects in Research (IRB2011-0153). Following storage for 1 wk at 4°C, the eggs were cooked by scrambling. Eggs were beaten in separate bowls per treatment for 2 min to ensure a homogenous mixture, and then scrambled using separate pans. Canola oil spray was used to coat the bottom of the pan prior to cooking. The same concentration of spray was used to ensure same cooking methods. All scrambled eggs were cooked to the same endpoint temperature of 350°F (176.7°C). Then, samples were placed into separate stainless steel containers with lids under the heat from heat lamps to maintain the temperature and make sure the samples were presented warm.

The ballot instruction included a 9-points hedonic scale test (Appendix B) for flavor and texture of the scrambled eggs in addition to overall like or dislike. This test was conducted at the sensory laboratory in the Department of Animal Science at TAMU. Prior to testing, panelists were instructed on how to answer questions by reading the instructions at the top of the ballot along with the consent form to participate in the study. Following reading and the individual panelists were served with sets of random three digits coded samples. Each panelist was served with four weigh boats of the samples under random 3-digit codes with unsalted saltine cracker and a cup of double distilled deionizer water to ensure clear panelist's palate between samples. These coded samples were used to evaluate the effect of treatments on the quality of flavor and texture of the scrambled eggs.

Samples were served under a red light to prevent consumers from visual bias. Questions were asked in the ballot to indicate overall like/dislike for the flavor and texture of each sample. A scale of 9 points was used to rate the overall like or dislike for the flavor and texture of each sample that panelist perceived. Panelists were asked to indicate their like or dislike by placing a mark in the box of the point scale (1: Dislike to 9: Like) indicating their preference in each sample of the anonymously coded groups.

Statistical analysis

All data from eggshell APC were analyzed by one-way analysis of variance (ANOVA) using JMP 9.0 software (SAS, Cary, NC). Tukey-Kramer HSD test was used to separate all means. For 3M® Petrifilms that had zero counts (below limit of

detection), a value of 10 cfu/egg were used to those samples for each treatment to calculate the mean. For the sensory evaluation, means for the texture and flavor were analyzed also by analysis of variance (ANOVA) using JMP 9.0 software (SAS, Cary, NC). Means were separated by using Duncan's Multiple Range Test for flavor and texture of all treatments and means were considered statistically difference at $P \leq 0.05$.

Results and Discussion

Aerobic plate count (APC)

The purpose of this experiment was to evaluate the efficacy and the quality of the current treatments that are used in the US egg industry and compare those to alternative technologies of egg disinfection. Chlorine and QAC are the current methods that are most commonly used in egg processing to disinfect eggshell surfaces. Results of this experiment indicate that the new technology of combining H₂O₂ and UV light has the lowest eggshell APC when compared to other treatments on d 0, 7, and 14 of storage (1.30, 1.05, and 1.10 log₁₀ cfu/egg, respectively) (Table 3.1).

Table 3.1. Aerobic plate counts of eggshell surface of eggs treat with various disinfectant methods and stored for 0, 7, or 14 d .

Treatment	Day 0	Day 7	Day 14
	-----log ₁₀ cfu/egg ¹ -----		
Control	3.17 ^a ± 0.22	3.48 ^a ± 0.13	3.58 ^a ± 0.12
Chlorine	2.92 ^{ab,x} ± 0.11	2.70 ^{b,x} ± 0.07	2.40 ^{b,y} ± 0.11
PAA	2.60 ^{bc,x} ± 0.10	1.83 ^{c,y} ± 0.17	2.08 ^{bc,y} ± 0.12
PAA and UV light	2.23 ^{cd} ± 0.20	2.02 ^c ± 0.22	2.16 ^{bc} ± 0.15
QAC	1.99 ^d ± 0.20	1.88 ^c ± 0.16	1.82 ^c ± 0.16
H ₂ O ₂ and UV light	1.30 ^e ± 0.14	1.05 ^d ± 0.05	1.10 ^d ± 0.08

^{a-e} Means within a column with different letters are significantly different (P < 0.05)

^{x,y} Means within a row with different letters are significantly different (P < 0.05)

Each treatment (n=5).

¹n=15 eggs ± SE.

The use of QAC disinfectant on eggshells produced the second lowest APC (1.99 log₁₀ cfu/egg) compared to the control (3.17 log₁₀ cfu/egg), chlorine (2.92 log₁₀ cfu/egg), and PAA (2.60 log₁₀ cfu/egg) on d 0 of storage. However, QAC was not significantly different from PAA combined with UV light. Also, there was no significant difference in APC between chlorine-treated and control eggs on d 0 of storage. On d 7 of storage, there was no significant difference between QAC, PAA, and PAA combined with UV light on eggshell APC. However, those treatments were significantly lower in APC than chlorine (2.70 log₁₀ cfu/egg) and control (3.48 log₁₀ cfu/egg), but chlorine was lower than the control eggs.

Musgrove et al. (2006) reported that manually or mechanically sprayed chlorine at 200 ppm could not reduce the prevalence of aerobic bacteria on unwashed eggs. In

addition, application of 100 or 200 ppm of chlorine did not result in significantly less APC in washed eggs than eggs rinsed with water only (Musgrove et al., 2008b). Several studies reported that the combination of H₂O₂ and UV light reduces eggshell APC to very low levels. For instance, a study that investigated the effect of the combination H₂O₂ and UV light sanitization on shell eggs microbiology and quality and found that there was high reduction of bacterial load on shell eggs with no impact on the eggshell or egg content quality (Woodring, 2011). Wells et al. (2010) concluded that the combination of H₂O₂ and UV light lowered the bacterial counts from 4.0 log₁₀ cfu/egg to less than 1.0 log₁₀ cfu/egg. Hartman and Carlin (1957) found that using 100 to 400 ppm of PAA effectively reduced the bacterial counts on eggshell surface to more than 95% reduction.

***Salmonella* Enteritidis reduction**

For eggs inoculated with SE, results revealed no difference for *Salmonella* counts for all treatments. All treatments used in this study reduced SE below the level of detection (100 cfu/egg). The positive control group had an average of 3.6 log₁₀ cfu/egg for d 0, and less than the level of detection on d 7 and 14 of storage. A previous study demonstrated that the application of H₂O₂ combined with UV light can effectively reduce *Salmonella* on eggshell surfaces (Gottselig, 2011). In addition, it was reported that dipping eggs in warm QAC was more effective in reducing SE on shell eggs than chlorine (Oliveira & Silva, 2000). Wang and Slavik (1998) found that 100 ppm of QAC

at pH 7.5 significantly reduced the penetration rate of SE; however, different storage temperatures did not influence the efficacy of QAC.

Sensory evaluation

Results from the scrambled egg sensory evaluation on texture and flavor are presented in Table 3.2. Panelists (n=50) were asked to evaluate the overall like or dislike for the flavor and texture for 4 treatments. A 9-point hedonic scale (1: Dislike to 9: Like) was used to determine panelists evaluation of the samples. However, not all panelists answered the questions for all treatments. Results indicated there was no significant difference in overall flavor as perceived by the panelists between any of the treatments. Mean flavor scores were 6.7, 7.2, 6.7, and 6.7 for control, chlorine, QAC, and combination of H₂O₂ and UV light, respectively.

Results for texture evaluation find there was a significant difference between treatments. Chlorine had higher texture acceptance by the panelists than other treatments. Average texture score for chlorine-treated eggs was 7.7 compared to 6.9 for all others treatments. However, no difference in texture between the control, QAC, and H₂O₂ and UV light-treated eggs was observed. A previous study at TAMU also found that panelists were not able to identify differences between egg samples whose shells were treated with chlorine or QAC and eggs whose shells were treated with H₂O₂ and UV light over 2 experiments. In that study, results indicated that only 33.5% from panelists correctly identified the odd sample in a triangle test (Woodring, 2011).

Table 3.2. Overall like flavor and texture sensory test for scrambled eggs.

Treatment	n	Flavor	n	Texture
Control	47	6.7	46	6.9 ^b
Chlorine	46	7.2	48	7.7 ^a
QAC	47	6.7	46	6.6 ^b
H ₂ O ₂ and UV light	47	6.7	47	6.9 ^b

^{a,b} Means within column with different letter are significantly different $P \leq 0.05$

In summary, this experiment has demonstrated that the combination of H₂O₂ and UV light had the lowest eggshell APC when compared to chlorine, QAC, PAA, and PAA and UV light. In addition, eggs treated with chlorine did not show significant difference in APC from control eggs. Data from eggs inoculated with SE indicated that all treatments applied to the eggs reduced SE below the level of detection (100 cfu/egg). In addition, the sensory analysis test found that eggs treated with H₂O₂ and UV light did not taste different from untreated control eggs or other sanitizers commonly used in the commercial egg industry.

The use of H₂O₂ and UV light could replace the current sanitizers due to its ability for providing lower APC and reduce SE on eggshell surface. Also, this combination did not produce any chemical residues or waste for the processor and has equal consumer acceptance compared to chlorine or QAC. Hydrogen peroxide has been approved as an egg sanitizer in egg washing process as a non-agricultural ingredient (USDA, 2010). The presence of pathogenic microorganisms on eggshell surface leads to increase the risk of foodborne illness due to direct consumption of eggs or cross

contamination. The goal of egg processors is to provide safe eggs for consumers by diminishing bacterial population on eggshell surfaces during egg sanitization process. The combination of H₂O₂ and UV light was demonstrated to be a superior eggshell surface sanitizer than the current commercial egg sanitizers used in the egg industry.

CHAPTER IV

EFFECTIVENESS OF PREWASH TREATMENT ON REDUCING THE MICROBIAL LOAD OF EGGS AND WASH WATER

Introduction

In the egg industry, eggs that have a large amount of adhering material on the shell such as manure and soil and are difficult to clean completely are considered heavy dirty eggs (Miller, 1959). It was reported that cleaning dirty eggs with sand paper was as efficient as or sometimes better than hand washing with detergent or with disinfectant in preventing bacterial penetration (Miller, 1957). Washing eggs not only enhances the external appearance of eggs, but also eliminates dirt on the egg surface effectively (Moats, 1978). Different materials such as dirt, egg contents, and microorganisms accumulate in the recycled water during the egg wash process (Hamm et al., 1974; Harris & Moats, 1975) that could decrease the pH of the wash water and hence decrease its lethality effect (Kinner & Moats, 1981; Moats, 1981).

Increasing the microbial contamination of eggs during washing could be a considerable safety issue that may elevate when egg storage time is increased (Musgrove et al., 2008a). It was concluded that the presence of fecal material on eggshells could provide a protective shield for bacteria against UV light treatment (De Reu et al., 2006). Several studies have been conducted to evaluate the efficacy of H₂O₂ alone or in combination with UV light on the reduction of the eggshell microbial load (Cox et al., 2000a; Cox et al., 2002a; Cox et al., 2002b; Wells et al., 2010; Gottselig, 2011;

Woodring, 2011). Our previous results have shown that the combination of H₂O₂ and UV light as a final sanitizer resulted in a significant reduction in eggshell APC. In field trials conducted at commercial breeder operations for the sanitization of hatching eggs, it was also noticed that the application of H₂O₂ aided in the removal of adhering organic matter on eggshells. Therefore, it is hypothesized that the application of H₂O₂ and UV light treatment to shell eggs prior to washing will not only reduce the microbial load of eggs entering the washer, but may also reduce the incidence of eggs with adhering material, also called “dirty”, following washing. The purpose of this experiment was to assess the effectiveness of prewash egg disinfection procedures to reduce microbial contamination of eggs and wash water and reduce the number of dirty eggs following washing.

Material and Methods

A total of 720 eggs with adhering material were randomly collected directly from the conveyer belt at commercial egg facilities. A total of 4 trials were conducted by collecting eggs from 2 different plants twice each. Following collection at the plants, eggs were transported in less than 2.5 h directly to the Texas A&M University Poultry Research Center. On arrival, eggs were divided into 2 groups and 300 eggs. The first group (control) was passed through an egg washing machine (Aquamagic 5CG, Modesto, CA) without treatment prior to washing, and the other 300 eggs were treated with the combination H₂O₂ (3.5%) and UV light (254 nm; 8 to 12 mW/cm²) using the

prototype egg sanitization machine in Figure 1 prior to washing. The wash water contained liquid egg detergent (Egg Brite # 037, Syn-Co Chemical, INC.) at the manufacturer's recommended level. After washing, all eggs were graded according to the USDA standards for external quality.

Eggs were classified as A, B, and dirty. After grading, 20 eggs from each treatment that were graded as Grade A were enumerated for total APC using a modified procedure of Coufal et al. (2003). Each egg was placed in 20 mL of sterile phosphate buffered saline (PBS) (pH 7.2) in a sterile Whirl-Pak® (Nasco, Inc., Fort Atkinson, WI) bag. After 1 min of gentle hand massaging to expedite the removal of bacteria from the surface of the eggshell, 1 mL of the rinse solution of each egg was removed and serial dilutions performed using PBS. Then, 1 mL from egg rinsate and each dilution was plated on Petrifilms (3M Healthcare, St. Paul, MN) for total APC determination. For Petrifilms that yielded no colonies (below limit of detection of 20 cfu/egg), a value of 10 cfu/egg ($1.0 \log_{10}\text{cfu/egg}$) was assigned for mean calculation. After incubation at 37 °C for 48 h, colonies were counted and total APC were converted to $\log_{10}\text{cfu/egg}$ for statistical analysis.

A total of 10 unwashed eggs were also used as a negative control group. Ten eggs of the Grade A H₂O₂ and UV light treatment and 10 Grade A eggs that were washed but without treatment prior to washing were sampled at d 0 of storage and the other 10 eggs from each group were placed in the cooler (< 4°C) and sampled for exterior shell and egg contents APC at d 7 of storage. For the control negative group,

five eggs were sampled for APC and egg contents on d 0 of storage, and 5 eggs were placed in the cooler (4°C) for sampling on d 7 of storage.

In addition, 3 samples from the wash water of each treatment were also plated to investigate the total APC in wash water. For egg contents microbial counts, each egg was sponged with 70% ethanol (Ethanol, 200 proof, Anhydrous Koptec, King of Prussia, PA) using a cotton ball (Dolgencorp, Goodlettsville, TN) and allowed to air dry for 30 min. Following drying, each egg was aseptically broken into a stomacher bag (VWR International, Radnor, PA) with 50 mL of sterile PBS (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Each stomacher bag was subjected to agitation using a stomacher machine (Stomacher 400 circulator, Seward, England) for 3 min at a speed of 200 rpm. Then 1 mL from each bag was plated on petrifilms and incubated at 37°C for 48 h.

Statistical analysis

Egg grading data were analyzed using chi square and probability of each grade was detected according to Fisher's exact test. Aerobic plate count data were analyzed with one-way analysis of variance (ANOVA) JMP 9 software (SAS, Cary, NC) after transformed to \log_{10} cfu/egg. When means showed significant differences, they were separated using Tukey-Kramer HSD test. Means were deemed statistically different at $P \leq 0.05$.

Results and Discussion

Egg grading percentage data are shown in Table 4.1. In trial 2 and 3, eggs treated with the combination of H₂O₂ and UV light prior to washing had a higher percentage of Grade A than normal washed eggs (80.7% vs 73.5%) and (89.6% vs 82.7%) respectively. Over the 4 trials, the mean percentage of Grade A eggs was higher in eggs treated with combination of H₂O₂ and UV light prior to washing (81.0%) than eggs that had normal wash only (77.9%). There was no treatment effect on Grade B eggs between trials or in overall percentage mean. In trials 2 and 3, when eggs were washed only, they had significantly higher dirty egg percentages than eggs treated with the combination of H₂O₂ and UV light prior to washing (26.5 vs 19.0%) and (16.3 vs 10.0%). Over the 4 trials, the mean percentage of dirty eggs was significantly higher (21.1%) in normal washed eggs than eggs treated prior to washing (18.4%).

Table 4.1. Percentage Grade A, B, and dirty eggs following washing with and without disinfection treatment prior to washing.

Trial	Treatment	A (%)	p-value	B (%)	p-value	Dirty (%)	p-value
1	Normal wash	70.3	0.66	1.0	0.78	28.7	0.37
	H ₂ O ₂ and UV light prior to washing	71.6	0.40	1.3	0.50	27.1	0.70
2	Normal wash	73.5	0.99	0.0	1.00	26.5	0.02
	H ₂ O ₂ and UV light prior to washing	80.7	0.02	0.3	0.50	19.0	0.99
3	Normal wash	82.7	1.00	1.0	0.31	16.3	0.02
	H ₂ O ₂ and UV light prior to washing	89.6	0.01	0.3	0.94	10.0	0.99
4	Normal washed	85.0	0.19	2.0	0.14	13.0	0.95
	H ₂ O ₂ and UV light prior to washing	82.0	0.86	0.7	0.97	17.3	0.09
Total	Normal wash	77.9	0.97	1.0	0.25	21.1	0.05
	H ₂ O ₂ and UV light prior to washing	81.0	0.03	0.7	0.87	18.4	0.96

The combination of H₂O₂ and UV light as a disinfectant prior to egg washing significantly reduced APC of eggshells (Table 4.2). Eggs treated with H₂O₂ and UV light prior to washing had an average eggshell APC of 2.1 log₁₀ cfu/egg compared to 2.6 log₁₀ cfu/egg for eggs washed without applying any sanitizer prior to washing. Unwashed, untreated control eggs had an average eggshell APC of 4.2 log₁₀ cfu/egg. However, on d 7 of storage, no significant difference was found between eggs treated with sanitizer prior to washing or normal washed eggs (2.2 and 2.5 log₁₀ cfu/egg, respectively).

It can be concluded that applying the combination of H₂O₂ and UV light treatment prior to egg washing not only increased Grade A eggs, but decreased the percentage of dirty eggs. This may be due to the ability of H₂O₂ to breakdown the adhering materials on eggshell surfaces, and thus allowing the material to be removed more thoroughly during the washing process. In addition, combining H₂O₂ and UV light enhanced the reduction of aerobic bacteria because UV light increases the photolysis of H₂O₂ and produces more hydroxyl radicals (·OH), thus enhances the antimicrobial ability (Baxendale & Wilson, 1957).

Aerobic plate count data from the wash water samples for all 4 egg washing trials is presented in Table 4.3. Wash water APC counts were extremely low in all trials, and thus statistical analysis could not be performed. The low levels of aerobic organisms in the wash water is likely due to several factors, including a low number of eggs washed in the trials, antimicrobial activity of the egg wash detergent which has a high pH, and a low organic matter content of the water since clean water was used at the start of each treatment in each trial. In addition, all eggs sampled for internal content microbial

contamination yielded zero APC. Therefore, under the conditions of this trial, it would appear that standard egg washing procedures with or without sanitization prior to washing does not aid in microbial penetration through the shell and result in internal egg contamination.

Table 4.2. Aerobic plate counts of control, normal wash eggs, and eggs treated with H₂O₂ and UV light prior to egg washing.

Treatment	Day 0	Day 7
	-----log ₁₀ cfu/egg ¹ -----	
Non-washed	4.2 ± 0.15 ^a	4.0 ± 0.27 ^a
Normal wash	2.6 ± 0.07 ^b	2.5 ± 0.15 ^b
H ₂ O ₂ and UV light prior to washing	2.1 ± 0.10 ^c	2.2 ± 0.11 ^b

^{a-c} Means within column with different letter are significantly different P ≤ 0.05
¹ n = 50 eggs per trial for 4 trials (20 total eggs per treatment and 10 eggs per non-washed treatment)

Table 4.3. Aerobic plate count of wash water from eggs treated with or without H₂O₂ and UV light treatment prior to washing.

Trial	Normal wash water	H ₂ O ₂ and UV light
	-----log ₁₀ cfu/ml-----	
1	0.0	1.2
2	0.0	0.0
3	1.5	1.1
4	1.2	1.2

n = 3 wash water samples per trial per treatment

In conclusion, treatment of shell eggs with H₂O₂ and UV light prior to egg washing may have potential as a useful practice in the commercial egg industry. The small trials performed under laboratory conditions yielded lower eggshell APC and fewer dirty eggs after washing for egg treated with H₂O₂ and UV light prior to egg washing compared to eggs not treated and washed normally. Based on these findings, larger-scale laboratory studies or field trials at commercial egg processing facilities are warranted. Under such conditions, it might also be possible to observe differences in wash water APC since sanitization of eggshells prior to washing should theoretically reduce the number of viable microorganisms that could be washed from the eggshells and into the wash water.

CHAPTER V

CONCLUSION

Shell eggs have been the source of pathogenic bacteria that have caused several foodborne illness outbreaks in the United States (US). While outbreaks are usually associated with internal contents of SE due to colonization of ovaries of the hen, pathogenic bacteria on the shell surface could enter the egg if mishandled or cross contamination of hands during food preparation occurs. Although chlorine and quaternary ammonium compounds (QAC) are the most common sanitizers used in the US egg industry, it has been demonstrated that these compounds often do not completely eliminate the bacterial load on eggshell surfaces.

The results of egg processing survey quantified that chlorine and QAC are the most common eggshell surface sanitizers used in commercial egg industry. In addition, data from the microbial survey revealed that the current commercial egg sanitization methods have reduced the microbial population on eggshell surface to the average of 2.1 log₁₀ cfu/egg. Laboratory experiments conducted in this study demonstrated that the combination of hydrogen peroxide (H₂O₂) and UV light is the most effective sanitizer for shell egg surfaces. Results of this project established strong evidence that this combination minimized aerobic plate counts to an average of 1.1 log₁₀ cfu/egg. This new technology could also be efficient in maintaining consumer acceptance of shell eggs and minimizing risks. In addition, H₂O₂ has been approved as an egg sanitizer in organic egg production. Results demonstrated that this sanitization process results in a higher

percentage of Grade A and lower percentage of dirty eggs when eggs were treated prior to washing. The reduction of the number of dirty eggs after washing could have important economic implications to the egg industry, while reduction in eggshell and wash water microbial contamination could result in greater food safety for consumers.

Therefore, more studies are recommended on the combination of H₂O₂ and UV light to validate the commercial use of this sanitization approach. While the results of this study are very promising, no published data are available regarding the impact of this technique on consumer perception and willingness to buy eggs sanitized with this. Modification would have to be made to current egg processing equipment for this sanitization process to be incorporated into modern egg processing facilities. This would represent an additional cost to processors. Therefore, processors would need to recover this cost, so studies to determine if consumers would be willing to pay more for eggs sanitized by this process need to be conducted to determine the economic feasibility of commercial implementation.

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APPENDIX A

Survey of Current Industry Egg Washing Practices

Instructions: For each question, please check all that apply or fill in the blanks as accurately as possible. **One survey should be filled out for EACH processing plant within your company.**

1. **To protect the anonymity of UEP members, please check the area to which your processing plant belongs:**

- Area 1- California, Nevada, Utah, Arizona, New Mexico
- Area 2- Washington, Oregon, Idaho, Montana, Wyoming
- Area 3- Colorado, Kansas, Nebraska, South Dakota, North Dakota, Minnesota, Wisconsin, Michigan, Illinois, Indiana, Iowa, Ohio, Missouri
- Area 4- Pennsylvania, New York, Vermont, Maine, New Hampshire, Massachusetts, Rhode Island, Connecticut, New Jersey, Delaware, Maryland
- Area 5- Kentucky, West Virginia, Virginia, North Carolina, South Carolina, Georgia, Tennessee, Alabama, Florida, Mississippi, Arkansas, Oklahoma, Texas, Louisiana

2. What is the facility type of the eggs you process?

- In-Line Off-Line Combination

3. What is the number of eggs packed in your facility in cases per day? _____

4. What is the shell color of eggs processed at your facility?

- White Brown Both

5. Which type(s) of housing do the eggs processed come from? Please check all that apply.

- Cage Cage-Free Free-Range Organic

6. Does your facility maintain a resident voluntary shell egg grading service?

- Yes No

7. Do you apply any type of sanitizer treatment to eggs prior to washing?

Yes No

If yes, please list treatment(s):

8. What chemicals or sanitizers do you use in the wash water? Please list all that apply.

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9. Which chemicals/sanitizers do you use in final rinse or disinfection step (in parts per million (PPM))?

Please check all that apply.

- Chlorine, in ppm: _____
- Quaternary Ammonium, in ppm: _____
- UV light
- Other, please specify: _____

10. What is the size of the container for **each** chemical/sanitizer used in the final rinse or disinfection step?

11. What is the volume (5 gallon pail, 55 gallon drum, etc.) used per day, per week, or per month for **each** chemical/sanitizer used in the final rise or disinfection step?

12. What is the cost per container of **each** chemical/sanitizer used in the final rinse or disinfection step?

13. Please indicate what type of microbiological monitoring, if any, is conducted at your facility?

- Wash water aerobic microbial counts
- Wash water Salmonella counts
- Aerobic microbial counts on eggs in cartons
- Salmonella counts on eggs in the carton
- No microbial sampling of eggs or wash water routinely performed

APPENDIX B

Scrambled egg sensory ballot used in the sensory evaluation test between three treatments and control group

Type of sample: Scrambled egg

Date: _____

Instruction:

- 1- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **FLAVOR** (1:Dislike and 9: Like)

Sample code: _____

1	2	3	4	5	6	7	8	9
DISLIKE								LIKE

Sample code: _____

1	2	3	4	5	6	7	8	9
DISLIKE								LIKE

Sample code: _____

1	2	3	4	5	6	7	8	9
DISLIKE								LIKE

Sample code: _____

1	2	3	4	5	6	7	8	9
DISLIKE								LIKE

2- Indicate by placing a mark in the box your **OVERALL LIKE/DISLIKE** for the **TEXTURE** (1:Dislike and 9: Like)

Sample code: _____

1	2	3	4	5	6	7	8	9
DISLIKE								LIKE

Sample code: _____

1	2	3	4	5	6	7	8	9
DISLIKE								LIKE

Sample code: _____

1	2	3	4	5	6	7	8	9
DISLIKE								LIKE

Sample code: _____

1	2	3	4	5	6	7	8	9
DISLIKE								LIKE

APPENDIX C

Consent Form

Project Title: Development of best practices for shell egg disinfection based upon efficacy, egg quality, and economic

You are invited to take part in a research study being conducted by Dr. Alvarado, a researcher from Texas A&M University. The information in this form is provided to help you decide whether or not to take part. If you decide to take part in the study, you will be asked to sign this consent form. If you decide you do not want to participate, there will be no penalty to you, and you will not lose any benefits you normally would have.

Why Is This Study Being Done?

The purpose of this study is to determine any sensory differences in scrambled egg made with similar ingredients.

Why Am I Being Asked To Be In This Study?

You are being asked to be in this study because you are an egg consumer.

How Many People Will Be Asked To Be In This Study?

50 people (participants) will be invited to participate in this study locally.

What Are the Alternatives to being in this study?

The alternative to being in the study is not to participate.

What Will I Be Asked To Do In This Study?

You will be asked to taste a set of egg samples and answer questions including texture, and flavor acceptability. Your participation in this study will last up to 5-10 minutes,

Are There Any Risks To Me?

There are no risks to you to be in this study.

Will There Be Any Costs To Me?

Aside from your time, there are no costs for taking part in the study.

Will I Be Paid To Be In This Study?

You will not be paid for being in this study.

Will Information From This Study Be Kept Private?

The records of this study will be kept private. No identifiers linking you to this study will be included in any sort of report that might be published

Who may I Contact for More Information?

You may contact the Principal Investigator, Dr. Christine Alvarado, to tell him/her about a concern or complaint about this research at 979-845-4818 or calvarado@poultry.tamu.edu.

For questions about your rights as a research participant; or if you have questions, complaints, or concerns about the research, you may call the Texas A&M University Human Subjects Protection Program office at (979) 458-4067 or irb@tamu.edu.

What if I Change My Mind About Participating?

You have the choice whether or not to be in this research study. You may decide not to participate or stop participating at any time. If you choose not to be in this study, there will be no personal impact. You can stop being in this study at any time with no personal impact.

STATEMENT OF CONSENT

I agree to be in this study and know that I am not giving up any legal rights. The procedures, risks, and benefits have been explained to me, and my questions have been answered. I know that new information about this research study will be provided to me as it becomes available and that the researcher will tell me if I must be removed from the study. I can ask more questions if I want. A copy of this entire consent form will be given to me.

INVESTIGATOR'S AFFIDAVIT:

Either I have or my agent has carefully explained to the participant the nature of the above project. I hereby certify that to the best of my knowledge the person who signed this consent form was informed of the nature, demands, benefits, and risks involved in his/her participation.

Signature of Presenter

Date