

**QUALITY AND SENSORY ATTRIBUTES OF SHELL EGGS SANITIZED  
WITH A COMBINATION OF HYDROGEN PEROXIDE AND  
ULTRAVIOLET LIGHT**

A Thesis

by

KRISTY SENISE WOODRING

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2011

Major Subject: Poultry Science

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Approved by:

Chair of Committee,  
Committee Members,

Head of Department,

Craig D. Coufal  
Christine Alvarado  
Alejandro Castillo  
John B. Carey

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**ABSTRACT**

Quality and Sensory Attributes of Shell Eggs Sanitized with a Combination of Hydrogen

Peroxide and Ultraviolet Light. (August 2011)

Kristy Senise Woodring, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Craig Coufal

Two experiments were conducted to evaluate the combination of hydrogen peroxide ( $H_2O_2$ ) and ultraviolet light (UV) as an alternative eggshell sanitization procedure for shell egg processing. In each experiment, two cases of eggs (720 total) were collected at a commercial inline egg production facility. To assure egg uniformity, only eggs between 57 and 62 g were collected from a single hen house. Half of the eggs (360) were commercially processed (washer and sanitizing rinse) following normal procedures outlined by the U.S. Department of Agriculture (USDA) for shell egg processing (control group). The other half of the eggs (360) were washed as normal but without the sanitizing rinse. These eggs were then treated with 3%  $H_2O_2$  and UV light (treated group). The treatment consisted of spraying the eggs with 3%  $H_2O_2$  over the entire shell surface followed immediately by exposure to UV light for 5 s in an enclosed chamber equipped with germicidal lamps (UV-C). This treatment was performed twice. Eggshell aerobic plate counts (APC), eggshell breaking strength and thickness, albumen height and pH, Haugh units, and yolk color were measured after 1, 15, 30, 45, and 60 days of storage. On d 15 and 45, sensory evaluation of scrambled egg samples was conducted to determine if consumers could detect a difference between treatment groups

using a triangle test. Results indicate APC for treated eggs were significantly lower than the control eggs for all sampling days in Experiment 1. However, due to low initial APC in the control eggs on d 1 of Experiment 2, no significant differences were observed for APC between control and treated eggs during storage. No consistent differences were found for eggshell and interior quality measures with the exception of albumen pH. Albumen pH was significantly higher in treated eggs than control on d 45 and 60 and d 1, 15, and 45 of Experiment 1 and 2, respectively, with only an average difference of 0.04 pH. In the sensory evaluation, only 33.5% of the participants correctly differentiated between the control and treated eggs. Data from this study suggests that H<sub>2</sub>O<sub>2</sub> and UV light can be used as an alternative eggshell sanitizing procedure without impacting eggshell or internal egg quality.

## **DEDICATION**

I dedicate this to my family, especially...

to Dad and Mom for having faith, love, support, and guidance;

to Ryan for having encouragement and love;

to grandparents Woodring and Noska for loving support and to Woodring

grandparents for financing;

to friends for always helping me through the tough times and being there for me.

## **ACKNOWLEDGEMENTS**

I am extremely thankful and grateful to my supervisor, Dr. Craig Coufal, whose encouragement, guidance, and support has enabled me to develop an understanding of the subject. I would like to thank my graduate committee, Dr. Christine Alvarado and Dr. Alejandro Castillo, for their guidance and knowledge. I would also like to thank Dr. Jason Lee for his help with the statistical analysis and Liz Hirschler for her help with equipment knowledge. Special thanks go to Mr. Steven Gottselig, because without his knowledge and assistance this study would not have been successful.

I would like to thank my family members, who prayed, supported, and encouraged me to pursue this degree. Without the love and support of my family, I would not have finished my Master's degree. Finally, thanks to Kyle Harris for his support, encouragement, and for being there whenever needed.

## TABLE OF CONTENTS

	Page
ABSTRACT.....	iii
DEDICATION.....	v
ACKNOWLEDGEMENTS.....	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
CHAPTER	
I INTRODUCTION AND LITERATURE REVIEW.....	1
Introduction.....	1
Literature Review.....	3
Egg Quality.....	3
Microbiology of Egg.....	5
Disinfectants.....	8
Ultraviolet Light.....	10
Consumer Acceptance.....	11
II EFFECTS OF HYDROGEN PEROXIDE AND ULTRAVIOLET LIGHT EGGSHELL SANITIZATION ON THE MICROBIOLOGY AND QUALITY OF SHELL EGGS.....	13
Introduction.....	13
Materials and Methods.....	15
Sampling Preparation and Treatment.....	15
Eggshell Microbial Enumeration.....	16
Sampling of Physical Properties.....	17
Statistical Analysis.....	18
Results and Discussion.....	19
Aerobic Plate Counts.....	19
Eggshell Quality.....	23
Internal Egg Quality.....	25
III CONSUMER DISCRIMINATION TESTING.....	29

CHAPTER	Page
Introduction.....	29
Materials and Methods.....	30
Sensory Panel.....	30
Statistical Analysis.....	32
Results and Discussion.....	34
Experiment 1.....	34
Experiment 2.....	36
Summary of Experiments 1 and 2.....	36
IV CONCLUSION.....	38
REFERENCES.....	41
APPENDIX A.....	51
VITA.....	63



**LIST OF TABLES**

	Page
Table 2.1. Relative oxidation power of various oxidizing species.....	14
Table 2.2. Eggshell surface aerobic plate counts of control and H <sub>2</sub> O <sub>2</sub> /UV light -treated eggs for Experiment 1.....	21
Table 2.3. Eggshell surface and crush and rub aerobic plate counts for control and H <sub>2</sub> O <sub>2</sub> /UV light -treated eggs for Experiment 2.....	22
Table 2.4. Eggshell quality of control and H <sub>2</sub> O <sub>2</sub> / UV light-treated eggs.....	24
Table 2.5. Internal measurement of control and H <sub>2</sub> O <sub>2</sub> / UV light treated eggs.....	26
Table 3.1. Results of sensory using a discrimination test for Experiment 1 and 2 on percentage's correct and incorrect.....	35
Table 3.2. Frequency of taste and texture as a factor in the choice between samples for those panelists who chose correctly during discrimination test.....	35

**LIST OF FIGURES**

	Page
Figure 3.1. Consumer acceptance test used in sensory panel to rate egg attributes of egg samples.....	33

## CHAPTER I

### INTRODUCTION AND LITERATURE REVIEW

#### Introduction

Contamination of food products with pathogenic microorganisms such as *Salmonella*, *Staphylococcus aureus*, and *Campylobacter jejuni* continues to be an important concern due to the risk of human illness caused by such organisms. Contamination of foods by other microorganisms that are not necessarily pathogenic is also a concern due to the potential for product quality degradation or spoilage during processing and storage. Meat and egg products can be contaminated with microorganisms during the various stages of collection and processing. Disinfection of the surfaces of shell eggs during processing at egg production facilities is an important step to preserve egg quality and reduce the potential for contamination of eggs with pathogenic microorganisms.

A disinfectant, when applied properly, can destroy microorganisms upon contact, but is not guaranteed to be one hundred percent effective (Jeffrey, 1995). The “ideal” disinfectant would create a bacteria-free environment without harming living things and be non-corrosive, easy to apply and inexpensive. In accordance with this “ideal” disinfectant, there should be no residue left on the product surface, like QAC does on eggshells. Therefore, when choosing the most appropriate disinfectant for an application, there are many factors to consider.

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This thesis follows the style of Poultry Science.

Egg washing and egg sanitization are important processing steps that must be properly implemented in the egg industry (Worley et al., 1992). The two disinfectants most predominantly used in the egg industry for the disinfection of shell eggs are quaternary ammonium compounds (QAC) and chlorine. Both disinfectants have acceptable efficiency against poultry associated pathogens. Quaternary ammonium compounds are available in different forms for sanitizing shell eggs; however, some *Pseudomonas* can be resistant without the addition of ethylenediaminetetraacetic acid (EDTA) (Curtis, 2004). Chlorine, which is a multi-use disinfectant, can be used on the eggs and in the water system to sterilize, control, and prevent odor (Jeffrey, 1995). Even though QAC and chlorine are effective for decreasing the microbial load on the eggshell surface, they have been shown inadequate to eliminate all of the microorganisms that are present (Rodriguez and Alberto, 2004).

In 2009, ultraviolet-C (UV) light (wavelength of 254 nm) was approved as a non-thermal intervention technology that can be used for the decontamination of food surfaces by the United States Department of Agriculture Food Safety Inspection Service (USDA-FSIS, 2011). UV light, also called germicidal light, is one of the shortest in the ultraviolet spectrum, but is longer than an X-ray. UV light has been reported to inactivate bacteria in solid or liquid foods (De Rue et al., 2006).

Product quality and consumer acceptability are two key aspects of egg production and processing. Consumer satisfaction is important for repeat purchase of any product. Therefore, studying consumer acceptance of a product plays a very important role in the food industry (Saha et al., 2009). For example, a nationwide recall

of shell eggs in 2010 had profound impacts on consumers' confidence in the safety of eggs produced by large commercial egg operations. Over the course of several weeks, the Food and Drug Administration (FDA) recalled several billion eggs due to a high probability of *Salmonella* Enteritidis contamination and hundreds of cases of human illness. Therefore, improved disinfection and intervention strategies to reduce the microbial contamination of shell eggs will be important to maintain consumer confidence in the commercial egg industry.

The overall goal of this study is to evaluate the combination of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and UV light as an alternative eggshell sanitization procedure for shell egg processing. More specifically, the objectives of this study are: 1) evaluate the effectiveness of a H<sub>2</sub>O<sub>2</sub> and UV disinfection process on eggshell microbiology, shell quality, and interior egg quality, and 2) determine if the use of H<sub>2</sub>O<sub>2</sub> and UV will impact product quality as measured by consumer acceptance panel.

## **Literature Review**

### ***Egg quality***

Egg quality is defined by the internal and external characteristics that are acceptable to the consumer (Watkins, 2004). Over time the internal and external characteristics of an egg will decline. After an egg is laid, the deterioration can be slowed or delayed but cannot be prevented (Anderson et al., 2004). The internal egg quality decline is characterized by the albumen thinning and becoming watery, the yolk enlarging and flattening, and the stretching and weakening of the vitelline membrane (Romanoff and Romanoff, 1949).

Measures of egg quality include Haugh units, broken-out score, pH, and shell strength and thickness. A commonly used measure of interior egg quality is the U.S. Department of Agriculture – Agricultural Marketing Service (USDA-AMS) approved procedure called the Haugh unit. The Haugh unit is the relationship between the weight of the egg and the thickness of the albumen (Stadelman and Cotterill, 1995). The higher the Haugh unit value, the firmer the egg, and the higher the quality. As the egg ages the Haugh unit value will start to decrease (USDA-AMS, 2000). Another major indicator to the albumen quality in eggs is the albumen pH (Scott and Silversides, 2000). The initial albumen pH for freshly laid eggs is 7.6 to 8.7 and with the increase of storage the resulting increased albumen pH value is up to 9.6 to 9.7 (Scott and Silversides, 2000). Another indicator of internal quality is the strength of the vitelline membrane. The weakening of the vitelline membrane can be caused by an increase in albumen pH due to the loss of carbon dioxide and the loss of water (Romanoff and Romanoff, 1949). The loss of water will decrease the egg weight, which in turn will cause the vitelline to stretch (Gast and Beard, 1990).

External egg qualities include the shell structure, soundness, porosity, shape, cleanliness and microbiological load. The shell is composed of 94 to 97% of calcium carbonate. Also, covering the shell is the cuticle, which is able to protect the pores of the egg, thereby decreasing shell permeability (Board et al., 1979). The three main purposes of the eggshell are to keep moisture from escaping, prevent microbial penetration into the interior, and protect the egg during handling and transportation. In the egg industry, the eggshell strength and thickness acts as a packaging material and its quality is

essential to consumer safety and selection. According to Romanoff and Romanoff (1949), there is a linear relationship between shell strength and its thickness. The determination of these two qualities is helpful in preventing and predicting breakage of eggshells in the field.

### ***Microbiology of the egg***

The microbial contamination of eggs is an important food safety concern to reduce infection of humans from known sources. Approximately 90% of all eggs are free of contamination at lay until outside sources come into contact with and contaminate the egg (Board et al., 1979). There are three ways that an egg can be contaminated: trans-ovarian, oviductal, or trans-shell. Trans-ovarian and oviductal are considered vertical transmission (mother to fetus), while trans-shell is horizontal, resulting from cross-contamination that occurs from the environment (Griffiths, 2005). In a 2004 study, it was determined that it is best to reduce the contamination or microbial load on an egg after lay and before the oxidation of the shell cuticle proteins (Hutchison et al., 2004). Despite the tough barrier the eggshell represents, bacteria are still able to penetrate the shell. The first defense against the microbial entry is the cuticle. If the cuticle is washed away or damaged, the pores are exposed, increasing susceptibility of microbial entry into the internal contents (Board et al., 1979; Anderson et al., 2004; Jones et al., 2004). Additionally, as eggs are stored, the eggshells become weaker and eggshell damage or defects greatly increase the risk of microbial penetration (Board, 1994). When microorganisms penetrate the shell's microbial barriers it causes bacteria on the surface, which are capable of surviving internally, to enter through the pores and cause spoilage.

Many types of microorganisms are often associated with eggs. Gram-positive bacteria, such as *Micrococcus* and *Arthrobacter*, are more prevalent at penetrating the outside barriers of the eggshell (Hutchinson et al, 2003; Griffiths, 2005). However, gram-negative microorganism, such as *Achromobacter*, *Alcaligenes*, *Pseudomonas fluorescens*, *Salmonella*, and *Escherichia*, are more capable of withstanding the antimicrobials present in the albumen, and are therefore commonly found internally (Hutchison et al., 2003; Jones et al., 2004). In a 2002 study it was found that *Salmonella* Enteritidis was more capable of surviving on the exterior surface while *Pseudomonas fluorescens* was able to survive internally. *P. fluorescens* is the primary contaminate able to traverse through the shell membranes and infect the contents of the egg (Jones et al., 2002).

*Salmonella* is a facultative aerobic, gram negative, non-spore forming bacterium that is commonly associated with poultry and eggs. This microorganism can grow and survive at temperatures of 54°C and is inhibited in eggs at 7.2°C and below (Curtis, 2004; Chen et al., 2002). One *Salmonella* serovar that is often associated with eggs is *Salmonella* Enteritidis (SE). It has been identified as having severe regulatory and economic impact due to the risk and severity of the foodborne illness it causes (Griffiths, 2005). The pathogen also has the ability to survive inside and on the outside of eggs. Its ability to cause illness is related to its host and other virulence factors that allow it survive at the low pH values found in the gastrointestinal tract and its ability to subsequently multiply (Rodriguez and Alberto, 2004). This foodborne illness was first recognized in 1976 with a national outbreak in the northeastern United States. SE was



associated with 2,119 cases and 11 deaths during this time and still is the largest outbreak ever documented associated with eggs (St. Louis et al., 1988). Since then there have been multiple regulations associated with *Salmonella* implemented with purpose of decreasing the frequency of foodborne disease outbreaks. In 2009 the FDA established a final rule preventing *Salmonella* Enteritidis in shell eggs during production and transportation. The compliance with the rule issues all producers must maintain their records and register with the FDA. The establishment of this final rule will reduce the risk of shell eggs being contaminated with SE; therefore, reducing the risk of SE-associated illnesses and deaths (FDA, 2010a,b).

Another important pathogen associated with shell eggs is *Salmonella* Heidelberg (SH). According to the CDC, an average of 2,180 cases of SH infections were reported from 1993 to 1997, which accounted for about six percent of all culture-confirmed *Salmonella* infections (CDC, 2008). Hennessy et al. (2004) documented that SH in eggs is one of the top four *Salmonella* serotypes in the United States, having risk factors associated with eggs consumed outside the home. It was also demonstrated in the study that humans can be contaminated from exposure to infected eggshells or by eating eggs with transovarian contamination. Like SE, *Salmonella* Heidelberg has the ability to proliferate inside the egg due to its nutrient rich yolk (Gast and Holt, 2001). In a recent study, it was shown that SH was one of the most proliferative pathogens, capable of penetrating the egg through the pores, migrating through the albumen, and colonizing in the yolk in roughly 2 hours (Schoeni et al., 1995).

Due to the potential for SE to be present in egg contents, this bacterium's growth rate is directly correlated to temperature at which the eggs are stored. Therefore, refrigeration is a key factor in preventing the growth of microbes in eggs during storage. Eggshell microbial populations typically decrease during storage due to the lack of moisture and nutrients for growth. Storage temperature should remain between 7.5 and 17.5°C to prevent microbial growth (Gast and Holt, 2000; Bell and Kyriakides, 2002). According to the USDA and FDA, the safe storage of eggs must be refrigerated at 45°F (7.2°C) or lower and the maximum storage time allowed for in-store of shell eggs sale is 30 days. The USDA's maximum recommended storage for at-home use is 45 days (FDA, 2001a,b; USDA-FSIS, 2011).

### ***Disinfectants***

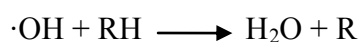
Current regulations in the United States (US) require that shell eggs be washed to remove all foreign material from the outside surface of the shell. Following washing, eggs must be treated by a USDA approved sanitization method to reduce the microbial load (USDA, 2011). Treatment to reduce eggshell microbes in shell egg processing is usually a disinfectant spray following the wash rinse.

Disinfectants should be highly germicidal, nontoxic to man and animal, be effective with the presence of organic material, soluble in water, non staining and corrosive, and capable of penetrating surfaces and materials (North, 1984). Commonly used disinfectants are quaternary ammonium compounds (QAC), sodium hydroxide (NaOH), Chlorine, sodium carbonate (NaCO<sub>3</sub>), sodium hypochlorite (NaOCl), and potassium hydroxide (KOH) (Caudill et al., 2010). The two most frequently used

disinfectants in the egg processing plant are chlorine and QAC (Jeffrey, 1995). It was shown that when comparing a peroxidase-catalyzed compound (PCC) to chlorine and QAC, chlorine and QAC was more effective at reducing the levels of pathogens than PCC (McKee et al., 1998).

Chlorine is a highly corrosive, irritating agent and can be inactivated by organic matter; however, it is still one of the most widely used disinfectants in the egg industry. Chlorine has been shown to be relatively non-toxic, possess a wide germicidal activity, and bacteria cannot become resistant to it (Wiley et al., 2010). Furthermore, chlorine has the ability of being a good disinfectant at 200-300 ppm. The second commonly used disinfectant in the egg industry is QAC. It has different effects on the egg as it will leave a residual protection on the egg to help fight off pathogens (Hutchison et al., 2004). Research has shown that dipping or spraying raw eggs with QAC instead of chlorine was more effective against microbial load (Oliverira and Silva, 2000).

A hydroxyl radical ( $\cdot\text{OH}$ ) is a powerful, non-selective chemical oxidant. According to Glaze et al. (1991), there are two mechanisms of action for  $\cdot\text{OH}$ : 1) the  $\cdot\text{OH}$  can add itself to the contaminant or 2) it can remove a hydrogen atom. The formation of  $\cdot\text{OH}$  is usually generated by photolysis. An example of a hydroxyl pathway is (O'Farrell, 1989):



In 2001, the use of UV light and ozone was approved for use as antimicrobial agents in food (FDA, 2001a). Ozone has been shown to be an effective antimicrobial agent in the food industry because of its high oxidation level (Kim et al., 1999; Kim et

al., 2003). Even though  $O_3$  is a strong antimicrobial agent, it can decompose spontaneously to a nontoxic product with the release of one oxygen atom (Koidis, 2000). Ozone is used for treatment, storage, and the processing of raw commodities. Being a powerful disinfectant, ozone is capable of destroying gram-positive and gram-negative bacteria by oxidizing proteins of bacterial cell walls and un-saturated membrane lipids (Kim et al., 1999; Kim et al., 2001; Guzel-Seydim et al., 2004). Another study found a greater than  $6.3 \log_{10}$  reduction in SE, but acknowledged that more research is necessary as the ozone process resulted in an increase in albumen turbidity, increase in Haugh units, and an undesirable color pigment in the egg yolk (Rodriguez and Alberto, 2004).

Another way to disinfect is the use of  $H_2O_2$ . A single oxygen atom can be used as a disinfectant molecule and also be strong oxidizing. When used as a disinfectant,  $H_2O_2$  is more effective than potassium permanganate, chlorine, and chlorine dioxide (Mansour, 2001).  $H_2O_2$  is found at almost any retail store and is usually sold at 3% concentration. In this form it is usually used as a topical antiseptic and can be used orally for mouth irritation. In a 1995 study, it was demonstrated that  $H_2O_2$  could decrease surface bacteria by 95% (Padron 1995).

### ***Ultraviolet light***

There has been further research as to adding UV in addition to a sanitization procedure to increase the reduction in high microbial loads. UV light has been documented to have the ability as a high energy source to kill various types of microorganism. A high intensity of UV light can be generated by low-pressure mercury-vapor lamps called germicidal lamps (Huang and Toledo, 1982). De Rue, et al. (2006)

demonstrated that UV light was not able to penetrate the shell but was able to kill some pathogens that penetrated through the pores. UV light at 254 nm has been approved by the USDA as a non thermal procedure, which has the ability to deactivate and kill various types of microorganisms, such as bacteria, molds, yeasts, and viruses. Gao et al. (1997) showed UV light to be effective at decreasing *Salmonella* levels on eggshells. In 1990, a study showed that using 8 min, 3 min, or as little as 1 min had no difference in reducing the total microbial load on eggshells (Latala and Wakula-Radzic, 1990). Other researchers have shown a  $3\log_{10}$  CFU reduction in microbial load after as little as 60 s exposure to UV light (Chavez et al., 2002).

Individually the use of hydrogen peroxide and UV light can decrease microbial loads but by combining the two will have a significant reduction (Wells et al, 2010). Well demonstrated that the combination had a  $3\log_{10}$  cfu/egg reduction while individually there was only a  $2\log_{10}$  cfu/egg reduction.

### ***Consumer acceptance***

Consumer acceptance of a product is perceived from an individual's senses, including visual, touch, and smell. There are many acceptable procedures that can reduce the microbial load; however, the final result is the get consumer acceptance. Visually appealing product that is free from visual residue is consumer accepted (Jones et al., 2004; Musgrove et al., 2005).

When consumers purchase a product they expect a safe product, so food safety is a major aspect when products are marketed. For egg products there are many implements that must be accounted for before a product is marketed; however, it is the

consumer's task to not abuse the preparation and handling of eggs. In 1997, a national consumer survey was reviewed about how eggs are prepared unsafely and consumption practices. The first part of the study showed that 27% of egg product dishes were undercooked. Cooking practices such as sunny side up and poached made this percentage higher than it should be if eggs were fully cooked (Lin and Morales, 1997). The survival of SE was enhanced because of the yolk remaining liquid and heat not inactivating it. The second part of the study showed the storage abuse increased the heat resistance of SE in contaminated eggs (Saeed and Koons, 1993; Lin and Morales, 1997). By following the USDA egg product preparation and not abusing raw eggs there can be a decrease in SE illnesses (Fein et al., 2002; USDA, 2011).

Sensory analysis applies principles of experimental design to the use of human senses (smell, sight, taste, touch, and hearing) for the purposes of evaluating consumer products. The triangle test, or discriminative test, is a widely used test for untrained sensory panels. The panelist is given three products, two of which are identical and one that is different, and the panelist must choose one and document it (Kunert, 1999). The probability of the panelists correctly selecting the different sample is one out of three. The advantages of doing this test are that it is the fastest and easiest test to execute. The disadvantage is the chance expectation error. Expectation error is giving the panelist too much information before the study. If too many facts or hints are given, then the panelist might make a judgment on expectation rather than intuition. Therefore, it is important to just provide the important facts to complete the triangle test (Brockhoff, 1998).

**CHAPTER II**  
**EFFECTS OF HYDROGEN PEROXIDE AND ULTRAVIOLET LIGHT**  
**EGGSHELL SANITIZATION ON THE MICROBIOLOGY AND QUALITY OF**  
**SHELL EGGS**

**Introduction**

A long term challenge for the poultry industry has been the development of proper sanitization methods to prevent the contamination of eggs. There are a variety of disinfectants that have been used to decrease the amount of bacteria that may contaminate the shell of an egg. De Reu et al. (2006) demonstrated a correlation between eggshell surface microbial load and internal contamination. Therefore, the use of an effective disinfectant on the eggshell surface is important to reduce the potential for internal contamination.

A hydroxyl radical ( $\cdot\text{OH}$ ) is a powerful, non-selective chemical oxidant that reacts rapidly with organic compounds. Current technologies that use advanced oxidation processes involving  $\cdot\text{OH}$  have received considerable interest; however, most of them are related to removing organic and inorganic contaminants from drinking water (Glaze et al., 1987). The relative oxidation power of some oxidizing species is shown in Table 2.1. The use of these oxidizing reactants can lead to many reactions that can either break down the contaminants or bond to them. One disadvantage of such oxidizing species is that some can be harmful to human health if not completely utilized in the oxidation reactions and subsequently come in contact with human tissues. Therefore,

effective disinfectants or reagents that are commercially beneficial and safe for human health are needed.

**Table 2.1:** Relative oxidation power of various oxidizing species

Oxidizing Species	Relative oxidation power
Chlorine	1.00
Hypochlorous acid	1.10
Permanganate	1.24
Hydrogen peroxide	1.31
Ozone	1.52
Atomic oxygen	1.78
Hydroxyl radical	2.05

(Adopted from Glaze et al., 1987)

The presence of *Salmonella* Enteritidis (SE) on eggshell surfaces poses not only a public health hazard but could also have serious economic impacts on the egg industry. In the United States, it was estimated by the USDA-FSIS that over 700,000 of the annually reported cases of Salmonellosis result from contaminated egg products (Frezen et al., 1999). Currently, in the industry there is a need to inactivate *Salmonella* and other potential pathogens using low-temperature treatments that will not affect the internal quality of shell eggs.

Two non-thermal technologies for decontaminating shell eggs that have been studied are UV light and ozone (O<sub>3</sub>). The use of UV light as a disinfectant was shown to reduce the natural microflora on eggshells by 3.57 log<sub>10</sub> after 4.7 s (Kuo et al., 1997a; Kuo et al., 1997b). Chavez et al. (1999) demonstrated a significant 2 log<sub>10</sub> reduction of aerobic bacteria on eggshells that were contaminated with approximately 4.0



$\log_{10}$ CFU/egg. In addition to the effectiveness of UV light on eggshells, the use of UV light has also been shown to inactivate *Salmonella* on equipment surfaces (Gao et al., 1997). The results of these three studies demonstrate that UV light can effectively reduce bacterial loads on various surfaces such as eggshells and equipment.

Wells et al. (2010) demonstrated that the use of H<sub>2</sub>O<sub>2</sub> and UV light in combination produced greater microbial reduction than H<sub>2</sub>O<sub>2</sub> or UV light applied independently. Previous work conducted to determine the effects of UV light on shell eggs has only been performed in a laboratory setting (Chavez et al., 2002; Coufal et al., 2003; Wells et al, 2010). The lack of scientific literature regarding the effects of H<sub>2</sub>O<sub>2</sub> and UV light combined on eggshell interior and exterior qualities has yet to be addressed. Assessment of the interior quality of the egg is needed when using a new disinfectant to confirm that the agent is not penetrating through the cuticle and pores and entering the egg, thereby causing an undesirable change or contamination to the edible part of the egg. The objective of this study is to determine the effects of a H<sub>2</sub>O<sub>2</sub> and UV light combination treatment on eggshell microbial counts, eggshell qualities and internal egg qualities over a 60-d storage period.

## **Materials and Methods**

### ***Sampling preparation and treatment***

Two cases of eggs (720) were collected at a commercial inline egg production facility. To assure egg uniformity, only eggs between 57 and 62 g were collected in a single hen house. For Experiment 1 the laying hens were 41 weeks of age and for Experiment 2 were 43 weeks of age. Half of the eggs (360) were processed through the

packing plant (washer and sanitizing rinse) following normal procedures outlined by the USDA for shell egg processing. These eggs were designated as the control group. The other half of the eggs (360) were processed through the washer as normal but with the sanitizing rinse turned off. These eggs were then treated with the H<sub>2</sub>O<sub>2</sub> and UV light (treated group). The treatment consisted of placing the eggs on a wire flat that held 32 eggs at a time and spraying these eggs with 3% H<sub>2</sub>O<sub>2</sub> over the entire shell surface. The flat of eggs were immediately exposed to UV light for 5 sec in an enclosed chamber equipped with germicidal lamps (UV-C). The UV light intensity for Experiment 1 was top 10.38 and bottom 11.87 and for Experiment 2 was 10.76 and 11.42. The intensity of the UV-C lamps was measured by placing a UVP radio meter (UVP, Inc. Upland, CA; P/N: 81-0064-01) at egg placement on the top and bottom of the wire rack. The treatment process was repeated for each flat of 32 eggs with a 180° rotation between the first and second H<sub>2</sub>O<sub>2</sub> spray and UV application. Following washing and treatment, all eggs were placed in clean styrofoam cartons and transported to the lab where they were stored in a refrigerator at 5°C. These procedures were performed in the same manner for 2 experiments. Experiment 1 was conducted at an egg processing facility using a quaternary ammonium compound in the final egg disinfection spray, while a chlorine spray was used at the commercial facility where Experiment 2 was conducted.

### ***Eggshell microbial enumeration***

For both experiments, eggshell aerobic plate counts (APC) were performed on 6 eggs prior to (6 eggs per treatment group) and following egg washing and treatment (12 eggs per treatment) to determine initial microbial reductions. Eggshell APCs were also

performed on d 15, 30, 45, and 60 of storage (12 eggs per treatment per day). Eggs were placed in Whirl-Pak® (Nasco, Inc., Fort Atkinson, WI) bags containing 25 mL of phosphate buffered saline (PBS) pH 7.2. Rinsate for treated eggs was diluted with PBS once while the control where diluted three times. After PBS dilutions were performed, 0.1 mL of each rinse dilution was plated on Tryptic Soy Agar (TSA; Becton Dickinson Co., Sparks, MD) in duplicate. The limit of detection for the rinse and plate enumeration method was  $2.4 \log_{10}\text{CFU/egg}$ . Plates were incubated at  $37^{\circ}\text{C}$  for 36 to 48 hours and then counted.

In Experiment 2, an additional analysis was performed to determine if the  $\text{H}_2\text{O}_2$  and UV light treatment process had any influence on microbial population in the pores and membranes of the shell. A crush and rub methodology was adopted as previously described by Musgrove et al., (2005). After the initial egg rinse in PBS, each egg as rinsed again in 25 mL of PBS and plated in the same manner as before. A second rinse was added to remove any remaining bacteria on the eggshell surface that were not removed by the first rinse. After the second rinse, eggs were cracked and the internal contents were aseptically removed and rinsed with sterile deionized water. Eggshells were then crushed into a 50 mL conical tube containing 25 mL of PBS. A sterile rod was then used to thoroughly crush the eggshell. After the egg was crushed, 0.1 mL was plated on TSA and plates were incubated as previously described.

### ***Sampling of physical properties***

Eggshell breaking strength and thickness, albumen height and pH, Haugh units, and yolk color were measured on 1, 15, 30, 45, and 60 days of storage. On each day the

eggs were first tested for breaking strength using Instron No. 1011 device (Instron Corp., Norwood, MA), which measured the compression force required to break the eggshell. The parameters of the machine were a load cell of 50 kg, range of 20, and the cross head speed was 15mm/min. The compression strength is how much the structure can withstand axially directed by the force of the machine and when reached the structure will be crushed. The load cell was how much force the machine pressed on the egg until it is cracked. The cross head speed is the average speed between the starting position and the egg breakage. The cross head speed will accelerate to the set speed, then an accurate reading is taken. The range indicates the maximum compression force, so the breaking strength cannot be greater than 20. The calculation for breaking strength was converted from kilograms (kg) to Newton's (N) by multiplying the kg by 9.80. The measurements of albumen height, egg weight, yolk color, and Haugh unit were measured by an Egg Analyzer (model 05-UM-001, Version B, Orka Food Tech.Ltd). Shell thickness was measured using a micrometer at three locations around the equator of the egg. The three measurements were averaged to determine shell thickness for each egg.

### ***Statistical analysis***

All data were analyzed by one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of SPSS software. Means for microbial counts on d 1 were separated using Fisher's LSD test ( $p \leq 0.05$ ). For plates that yielded no colonies (below limit of detection) a value of  $2.0 \log_{10}$  CFU/egg was assigned to the corresponding egg for calculation of treatment means.

## Results and Discussion

### *Aerobic plate counts*

Mean APC during storage for control and treated eggs for each sampling day is presented in Tables 2.2 and 2.3 for Experiment 1 and 2, respectively. In Experiment 1, the treated eggs had significant lower surface APC when compared to controls for all days eggs were sampled. However, due to low initial APC in the control eggs following washing and disinfectant application on d 1 of Experiment 2, no significant differences were observed for APC between control and treated eggs on any sampling days. After d 15 of Experiment 2, the crush and rub methodology was performed in addition to the first surface rinse. The crush and rub method was added to enumerate the bacteria below the eggshell surface in the pores and membrane. No significant differences were found between the control and treated groups for average APC using the crush and rub method. However, comparing the counts between the two treatments showed that the control eggs had numerically higher bacterial counts than the treated for those eggs that were positive for bacterial growth. In the control group, 20 of the 36 eggs sampled by crush and rub were positive for microbial growth, while only 15 of the 36 treated eggs were positive. The highest individual count for the control group was  $5.16 \log_{10}$  CFU/egg and the highest for the treated group was  $3.62 \log_{10}$  CFU/egg. While no statistically significant differences were observed, this data showed that a larger number of the control eggs had higher microbial counts in the pores and membrane than in the treated group. Therefore, it can be concluded that the  $H_2O_2$  and UV light treatment had a beneficial effect at either reducing the microbial load or preventing subsurface eggshell contamination. The

ability to reduce or prevent microbial contamination of the interior of the egg could have important implications for increasing the safety and quality of shell eggs.

In addition to this study the efficiency of H<sub>2</sub>O<sub>2</sub> and UV light treatment can be compared to chlorine and QAC. In Experiment 1 a QAC was used to disinfect the eggs and in Experiment 2 the disinfectant was chlorine. The APC's in both experiments showed that the H<sub>2</sub>O<sub>2</sub> and UV treated eggs were lower compared to the QAC and chlorine control eggs. Also, when comparing the QAC to the chlorine as a disinfectant there was more of a reduction in microbial load in chlorine. There can be many factors corresponding to a greater decrease but overall the microbial load was lower in Chlorine than in QAC. However, it still was not greater of a reduction than the H<sub>2</sub>O<sub>2</sub> and UV light treatment. Since there were lower counts in the APC's it can be determined that the H<sub>2</sub>O<sub>2</sub> and UV light treatment can be an alternative procedure at reducing the microbial load on the eggshell surface.

**Table 2.2.** Eggshell surface aerobic plate counts of control and H<sub>2</sub>O<sub>2</sub>/UV light-treated eggs for Experiment 1.

Treatment	Day 1		Day 15	Day 30	Day 45	Day 60
	Pre <sup>1</sup>	Post <sup>2</sup>				
	------(Log <sub>10</sub> CFU/ egg)-----					
Control	4.45 ± 0.04	3.72 <sup>a</sup> ± 0.22	3.22 <sup>a</sup> ± 0.25	3.20 <sup>a</sup> ± 0.21	2.60 <sup>a</sup> ± 0.17	2.84 <sup>a</sup> ± 0.18
H <sub>2</sub> O <sub>2</sub> -UV treated	4.22 <sup>x</sup> ± 0.19	2.10 <sup>b,y</sup> ± 0.07	2.07 <sup>b</sup> ± 0.04	2.04 <sup>b</sup> ± 0.03	2.14 <sup>b</sup> ± 0.09	2.01 <sup>b</sup> ± 0.01
P-Value		< 0.01	< 0.01	< 0.01	0.02	< 0.01

<sup>a,b</sup> Means within a column with different letters are significantly different (P < 0.05)

<sup>x,y</sup> Means within a row with different letters are significantly different (P < 0.05)

<sup>1</sup>Aerobic Plate Counts prior to egg washing (n=6)

<sup>2</sup>Aerobic Plate Counts post washing and disinfection step (n=12)

**Table 2.3.** Eggshell surface and crush and rub aerobic plate counts for control and H<sub>2</sub>O<sub>2</sub>/UV-treated eggs for Experiment 2.

Steps	Treatment	Day 1		Day 15	Day 30	Day 45	Day 60
		Pre <sup>1</sup>	Post <sup>2</sup>	------(Log <sub>10</sub> CFU/per egg)-----			
1st Rinse	Control	4.91 <sup>x</sup> ± 0.06	2.72 <sup>y</sup> ± 0.16	2.60 ± 0.28	2.49 ± 0.24	2.53 ± 0.32	2.26 ± 0.03
	H <sub>2</sub> O <sub>2</sub> -UV treated	4.80 <sup>x</sup> ± 0.15	2.29 <sup>y</sup> ± 0.11	2.09 ± 0.08	2.11 ± 0.07	2.12 ± 0.07	2.20 ± 0.03
	P-Value			0.10	0.15	0.23	0.70
2nd Rinse <sup>3</sup>	Control			2.34 ± 0.19	2.47 ± 0.26	2.40 ± 0.25	2.08 ± 0.02
	H <sub>2</sub> O <sub>2</sub> -UV treated			2.01 ± 0.01	2.09 ± 0.10	2.06 ± 0.06	2.08 ± 0.04
	P-Value			0.09	0.18	0.20	1.00
Crush and Rub <sup>3</sup>	Control			2.68 ± 0.28	2.49 ± 0.24	2.47 ± 0.29	2.54 <sup>x</sup> ± 0.12
	H <sub>2</sub> O <sub>2</sub> -UV treated			2.24 ± 0.14	2.17 ± 0.05	2.08 ± 0.06	2.11 <sup>y</sup> ± 0.04
	P-Value			0.18	0.20	0.19	0.03

<sup>x,y</sup> Means within a row with different letters are significantly different (P < 0.05)

<sup>1</sup>Aerobic Plate Counts prior to egg washing (n=6)

<sup>2</sup>Aerobic Plate Counts post washing and sanitization step (n=12)

<sup>3</sup>2nd Rinse and crush and rub analyses not performed on day 1



### ***Eggshell quality***

The means for eggshell thickness, breaking strength, and egg weight over storage time are shown in Table 2.4. There were no consistent differences between the control and treated eggs for the parameters tested. On d 1 of Experiment 1, a significant difference was observed for eggshell thickness; however, the difference was only 0.01 mm. If the eggshells are too thin or damaged by a treatment, then they will likely crack before they reach the consumer, thus causing an economic loss.

There was no statistical difference in eggshell breaking strength for Experiment 1 and 2. According to De Ketelaere (2002), eggshells become weaker over a period of time; however, that was not observed in this study. Breaking strength showed no consistent trend during this experiment. Breaking strength is an important measurement because eggs need to remain intact during transportation.

When the eggs were collected at the in-line production facilities each egg was individually weighed to ensure that all eggs weighed between 57 to 61g. The average egg weight decreased due to the loss of water and carbon dioxide over the storage time. Walsh et al. (1995), reported that egg weight will decrease by 0.23 g within 7 to 14 d after lay. The average weight loss during the first 15 d for both experiments was greater than that observed by Walsh et al. (1995), but was not significantly different between the treatment groups except on d 30 of Experiment 2. Therefore, the porosity of the eggshell was not adversely affected by the application of H<sub>2</sub>O<sub>2</sub> and UV light.

**Table 2.4.** Eggshell quality of control and H<sub>2</sub>O<sub>2</sub>/UV-treated eggs<sup>1</sup>

		Shell thickness (mm)				
Experiment	Treatment	Day 1	Day 15	Day 30	Day 45	Day 60
1	Control	0.400 <sup>b</sup> ± 0.005	0.373 ± 0.003	0.367 ± 0.003	0.373 ± 0.003	0.358 ± 0.003
	H <sub>2</sub> O <sub>2</sub> -UV treated	0.411 <sup>a</sup> ± 0.003	0.370 ± 0.003	0.370 ± 0.003	0.372 ± 0.004	0.354 ± 0.004
	P-Value	0.04	0.43	0.48	0.88	0.38
2	Control	0.377 ± 0.004	0.363 ± 0.004	0.357 ± 0.003	0.367 ± 0.003	0.338 ± 0.004
	H <sub>2</sub> O <sub>2</sub> -UV treated	0.367 ± 0.003	0.364 ± 0.004	0.356 ± 0.004	0.370 ± 0.003	0.331 ± 0.004
	P-Value	0.06	0.78	0.83	0.88	0.07
		Breaking Strength (N)				
Experiment	Treatment	Day 1	Day 15	Day 30	Day 45	Day 60
1	Control	38.54 ± 0.95	38.15 ± 0.92	42.76 ± 0.87	37.36 ± 1.40	38.74 ± 1.31
	H <sub>2</sub> O <sub>2</sub> -UV treated	37.36 ± 1.12	39.32 ± 1.26	41.09 ± 0.96	39.72 ± 1.24	42.07 ± 0.83
	P-Value	0.42	0.56	0.21	0.29	0.04
2	Control	27.56 ± 0.97	30.40 ± 1.12	30.30 ± 0.86	30.30 ± 0.96	28.67 ± 0.22
	H <sub>2</sub> O <sub>2</sub> -UV treated	27.65 ± 0.96	28.73 ± 1.23	29.22 ± 1.08	32.26 ± 1.19	25.82 ± 0.30
	P-Value	0.92	0.32	0.42	0.20	0.16
		Weight (g)				
Experiment	Treatment	Day 1	Day 15	Day 30	Day 45	Day 60
1	Control	58.94 ± 0.29	58.73 ± 0.26	57.86 ± 0.25	57.18 ± 0.24	56.55 ± 0.26
	H <sub>2</sub> O <sub>2</sub> -UV treated	59.03 ± 0.33	58.29 ± 0.28	58.06 ± 0.27	57.25 ± 0.28	56.52 ± 0.22
	P-Value	0.84	0.25	0.58	0.86	0.92
2	Control	59.53 ± 0.34	58.08 ± 0.27	56.90 ± 0.28	56.85 ± 0.43	55.84 ± 0.05
	H <sub>2</sub> O <sub>2</sub> -UV treated	59.24 ± 0.21	58.35 ± 0.21	57.97 ± 0.22	56.48 ± 0.25	55.66 ± 0.03
	P-Value	0.48	0.45	0.01	0.46	0.69

<sup>1</sup>Total of 60 eggs sampled per day (n = 30 per treatment)<sup>a,b</sup> Means within a column with different letters are significantly different (P < 0.05)

### ***Internal egg quality***

Table 2.5 presents the internal measurements of yolk color, thick albumen height and albumen pH, and Haugh unit means for control and UV light and H<sub>2</sub>O<sub>2</sub> treated eggs. The color of the yolk can vary due to many factors, although in both experiments there was only one significant difference in color for any sampling days. The significant difference in yolk color was d 45 of Experiment 2, is mostly like attributed to random variation of eggs selected that day since no other differences were observed throughout the experiment. The range of egg yolk color values from Experiment 1 and 2 were between the ranges of 3 to 7. The Egg Analyzer defines yolk color by the Roche Yolk Color Fan with colors ranging from 1 (bright yellow) to 15 (dark yellow).

Thick albumen height was not significantly different between the treatment groups for any day during both experiments. The thick and thin albumen is made up of different types of proteins. The proteins can degrade in two ways: through heat or from prolonged storage time. The thick albumen height decreased approximately 20% over the 60 d storage period. According to Caudill et al. (2007), as the egg ages the thick albumen breaks down and can no longer support the yolk giving it the ability to move. Therefore, decreased albumen is characterized by thinner albumen and the yolk moves away from the center.

**Table 2.5.** Internal quality measurements of control and H<sub>2</sub>O<sub>2</sub>/UV light-treated eggs<sup>1</sup>

		Yolk Color				
Experiment	Treatment	Day 1	Day 15	Day 30	Day 45	Day 60
1	Control	3.93 ± 0.11	4.27 ± 0.10	4.70 ± 0.09	4.60 ± 0.09	4.90 ± 0.17
	H <sub>2</sub> O <sub>2</sub> -UV treated	4.07 ± 0.12	4.43 ± 0.09	4.77 ± 0.08	4.73 ± 0.17	4.93 ± 0.05
	P-Value	0.40	0.24	0.57	0.37	0.85
2	Control	4.00 ± 0.08	4.20 ± 0.13	4.60 ± 0.09	4.50 <sup>b</sup> ± 0.13	5.00 ± 0.03
	H <sub>2</sub> O <sub>2</sub> -UV treated	3.90 ± 0.12	4.17 ± 0.14	4.37 ± 0.11	4.87 <sup>a</sup> ± 0.08	5.13 ± 0.06
	P-Value	0.50	0.86	0.11	0.02	0.38
		Average Thick Albumen Height (mm)				
Experiment	Treatment	Day 1	Day 15	Day 30	Day 45	Day 60
1	Control	5.32 ± 0.31	4.98 ± 0.12	4.35 ± 0.14	4.14 ± 0.16	4.08 ± 0.10
	H <sub>2</sub> O <sub>2</sub> -UV treated	5.28 ± 0.14	4.98 ± 0.19	4.34 ± 0.10	4.08 ± 0.09	3.83 ± 0.09
	P-Value	0.83	1.00	0.95	0.77	0.06
2	Control	5.98 ± 0.11	5.40 ± 0.12	5.08 ± 0.12	5.03 ± 0.18	4.29 ± 0.14
	H <sub>2</sub> O <sub>2</sub> -UV treated	6.24 ± 0.12	5.10 ± 0.22	5.01 ± 0.12	4.71 ± 0.16	4.12 ± 0.05
	P-Value	0.12	0.24	0.67	0.18	0.62
		Albumen pH				
Experiment	Treatment	Day 1	Day 15	Day 30	Day 45	Day 60
1	Control	8.28 ± 0.04	8.84 ± 0.01	9.02 ± 0.01	9.03 <sup>b</sup> ± 0.01	8.71 <sup>b</sup> ± 0.01
	H <sub>2</sub> O <sub>2</sub> -UV treated	8.32 ± 0.02	8.86 ± 0.01	9.03 ± 0.01	9.08 <sup>a</sup> ± 0.01	8.75 <sup>a</sup> ± 0.01
	P-Value	0.33	0.16	0.25	< 0.01	0.02
2	Control	8.30 <sup>b</sup> ± 0.02	8.86 <sup>b</sup> ± 0.01	8.95 ± 0.01	9.05 <sup>b</sup> ± 0.01	9.01 <sup>b</sup> ± 0.00
	H <sub>2</sub> O <sub>2</sub> -UV treated	8.36 <sup>a</sup> ± 0.02	8.91 <sup>a</sup> ± 0.02	8.96 ± 0.01	9.07 <sup>a</sup> ± 0.01	9.04 <sup>a</sup> ± 0.00
	P-Value	0.03	< 0.01	0.34	0.16	0.03
		Haugh unit				
Experiment	Treatment	Day 1	Day 15	Day 30	Day 45	Day 60
1	Control	70.04 ± 2.01	68.56 ± 1.28	62.80 ± 1.52	60.96 ± 1.43	61.44 <sup>a</sup> ± 0.95
	H <sub>2</sub> O <sub>2</sub> -UV treated	70.64 ± 1.39	68.41 ± 1.57	63.18 ± 0.96	61.05 ± 0.96	58.38 <sup>b</sup> ± 0.98
	P-Value	0.81	0.94	0.83	0.96	0.03
2	Control	76.60 ± 0.80	72.58 ± 0.95	70.08 ± 1.13	69.86 ± 1.26	61.84 ± 0.30
	H <sub>2</sub> O <sub>2</sub> -UV treated	77.93 ± 1.20	71.25 ± 1.14	69.74 ± 1.01	67.17 ± 1.29	62.29 ± 0.12
	P-Value	0.36	0.37	0.66	0.14	0.87

<sup>1</sup>Total of 60 eggs sampled per day (n = 30 per treatment)<sup>a,b</sup>Means within a column with different letters are significantly different (P < 0.05)

The albumen pH was consistently higher for treated eggs compared to the control eggs throughout both experiments. While there was statistical differences in both experiments, the maximum difference between the treatments was only 0.06. A possible explanation for this small difference might be attributed to hydroxyls penetrating through the pores, thus increasing the pH of the albumen. Over time the pH normally increases because there is carbon dioxide loss through the pores (Sarnli et al., 2005). According to Romanoff and Romanoff (1949), when an egg is laid the pH is 7.6 and over time it becomes more alkaline and may increase to 9.7. The alkalinity of the albumen is beneficial because it will retard bacteria growth since many microorganisms cannot survive in such a basic environment (Board, 1994).

The Haugh units decreased over time in both experiments as would be expected for eggs during storage. There was one significant difference on d 60 of Experiment 1; however, the difference is by 0.03 mm which is not a large difference. According to Silversides et al. (1993) and Caudill et al. (2010), the use of Haugh units has been questioned as being an accurate indicator of interior egg quality; however, since it is commonly used in scientific literature, it was included this study.

The findings of this study suggest that H<sub>2</sub>O<sub>2</sub> and UV light is an effective method for reducing the bacterial load of eggshells without negatively affecting any of the shell or internal egg quality measures. This eggshell sanitization procedure could be easily adopted into the modern shell egg processing system to potentially reduce pathogenic contamination of eggs. In order to understand the impacts of the H<sub>2</sub>O<sub>2</sub> and UV light on the interior microbial load of eggs, additional research should be done to assess the

internal microbial loads of eggs following application of the sanitization process outlined above.

## **CHAPTER III**

### **CONSUMER DISCRIMINATION TESTING**

#### **Introduction**

The application of a disinfection step during shell egg processing in the United States is required when packing eggs under USDA inspection. Chlorine and quaternary ammonium are the predominant disinfectants used in the egg industry; however, from the APC data shown in Chapter II a greater eggshell bacterial reduction was achieved with the use of H<sub>2</sub>O<sub>2</sub> and UV light.

While the combination of H<sub>2</sub>O<sub>2</sub> and UV light as a disinfection process has been shown to be effective (see Chapter II), there may be changes in the eggs' organoleptic properties that may be perceived by consumers. As mentioned in Chapter II the application of UV light to the exterior part of the shell shows to not have a negative effect on it. However, there may be a difference when the internal contents are cooked and consumed.

Although the appropriate disinfectant is beneficial to decreasing the microbial load, two additional factors that must be considered are refrigeration and storage time. Storage time is a crucial aspect in determining the freshness of the egg and thus consumer acceptance. The second factor is refrigeration temperature. The need for refrigeration is to retard microbial growth on the eggshell (Kamotani et al., 2010). The USDA and FDA regulation for safe storage of eggs must be refrigerated at 45°F (7.2°C) or lower (FDA, 2001b; USDA-FSIS, 2011).

Sensory evaluation can determine the worth of a commodity and its acceptability. The use of sensory panelists is necessary because an instrument or analytical assay can not accurately predict how a consumer will perceive a product. The use of a sensory evaluation is a technique to accurately measure the human response to foods and to minimize the bias effects of brand identity or other information that may influence consumer perception of a product (Meilgaard et al., 2007). Sensory tests can be used in cost-cutting, quality control, and processing concerns and most commonly for product improvement and development (Lawless and Heymann, 1999). The results of a consumer sensory evaluation should reflect the perceptions and opinions of consumers in the general population who might purchase the final product.

The process of H<sub>2</sub>O<sub>2</sub> and UV light treatment of eggshells to reduce microbial contamination on the eggshell has been documented in previous studies (Wells et al., 2010; Gottselig et al., 2010), but no sensory evaluation of eggs treated by this method has been conducted to date. Based on egg quality results discussed in Chapter II, it can be hypothesized that eggs treated with H<sub>2</sub>O<sub>2</sub> and UV light will not be perceived different from control eggs. Therefore, this study was conducted to on determine if consumers can discriminate between eggs processed and treated under conventional and commercial practices and eggs treated with a combination of H<sub>2</sub>O<sub>2</sub> and UV light.

## **Materials and Methods**

### ***Sensory panel***

A triangle test or a consumer discrimination test, was used to determine if consumers could differentiate between cooked samples of the control and H<sub>2</sub>O<sub>2</sub> and UV



treated eggs in Experiment 1 and 2 as described in Chapter II. The triangle test was conducted on d 15 and d 45 of both experiments. A triangle test is the most frequently used because it is the simplest to use among the various consumer sensory tests. The objective of this test is to obtain an anonymous performance in the detection of difference between products (Meilgaard et al., 2007).

The triangle test was conducted in a sensory testing lab in the Department of Animal Science, Texas A&M University. Treated and control eggs were transported from the laboratory refrigerator to the sensory testing lab where they were refrigerated at 39.2°F (4°C) until used. Four eggs from treated and control groups were scrambled in separate skillets, and cooked control and treated eggs were put into separate containers. Each hour from 8 am to 4 pm, four eggs from each treatment group were scrambled in separate skillets then placed into separate glass containers with lids. These glass containers were placed into a heat cabinet to assure maintenance of cooked egg temperatures. Three dozen eggs of each treatment were used for the whole day. From the glass containers, one ounce cooked eggs were placed into three different weigh boats, which had a random three-digit code. Two of the samples were from the same treatment and one was from the other treatment. All three samples were then presented simultaneously to each panelist under a red light.

The eggs were tasted by an untrained panel of consumers (n = 50, ages 18 and up) recruited from students and faculty at Texas A&M University. Testing took place in a sensory lab in which there were ten individual sensory booths each equipped with a consumer acceptance test, consumer consent form, and informational sheet. Also inside

the booths were crackers, a spit cup, and water to clean the panelists' palette if needed after each sample. The panelists viewed all the samples under a red light to prevent any samples from being distinguished by sight. A scale of weak to very strong was used to rate the intensity of the difference the panelists perceived between the single sample and the other two same samples. Panelists were asked to state whether this was due to the texture, taste or both. The panelists were also asked the following demographic questions: age, gender and indicate their frequency of egg consumption per month. An example of the Consumer Acceptance Test with these questions is shown in Figure 3.1.

The materials and procedures for this study were approved by the Texas A&M University Office of Research Compliance-Institutional Review Board (IRB) office for the exemption use of Human Subjects in Research. Testing took place over a time frame of several months with four different testing days (two days for Experiment 1 and 2 each). The same procedure was used each day.

### ***Statistical analysis***

To determine if the proportion of triangle test participants who correctly differentiated between the control and treated samples was statistically different from the proportion of panelists who did not correctly discriminate between the treated and control egg samples, the procedures of Gacula et al. (1984) were utilized. The null hypothesis of the binomial test is that participants could not tell the difference between the control and H<sub>2</sub>O<sub>2</sub> and UV light treated samples. Based on n=50 panelists and P≤0.05, 23 panelists would have to correctly identify the odd sample of the three presented and therefore reject the null hypothesis.

**Consumer Acceptance Test**

**Please answer the following questions:**

Age: 18-19       20-22       23-25       25-30   
                  30-35       35-40       above 40

Gender: Female       Male

**How many times a month do you eat eggs?**

None       One       Two       Three       4 or more

Three products with random identification numbers are presented. Two of them are identical, the other is different.  
 Taste the samples and indicate which product is difference from the other two.

**Indicate the intensity of the difference:**

Weak                                      Medium                                      Very strong  
 \_\_\_\_\_

**The difference is due to:**

Texture:      yes       no

Taste:      yes       no

**Try to describe the difference:**

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**Figure 3.1: Consumer acceptance test used in sensory panel to rate egg attributes of egg samples.**

## **Results and Discussion**

The results from the sensory panel tests are presented in Tables 3.1 and 3.2. A total of 200 hundred panelists participated in the study over the two experiments. The number and percentage of panelists who chose correctly and incorrectly during the triangle test are presented in Table 3.1. For panelists who correctly identified the odd sample of the three presented during the triangle test, data regarding the factors of taste and texture in their choice is present in Table 3.2.

### ***Experiment 1***

Sensory evaluation on d 15 of storage showed that 72% of the panelists could not detect a difference in the three samples presented while 28% of the panelists detected correctly identified the sample that was different from the other two. Of the 28% of the panelists that identified the sample that was different, 42% said only the texture was different, 29% said only taste was different, and 29% said texture and taste were both different. On d 45 of the experiment, 56% of the panelists did not detect a difference in the three samples given and 44% of the panelists detected a difference and were able to pick out the sample that differed from the other two. Of the 44% that correctly picked out the odd sample, 27% said only the texture was different, 32% said only the taste was different and 41% said texture and taste was different. The main outcome of Experiment 1 was that even after 45 days post-treatment, the majority of the panelists were not able to differentiate between eggs that were processed under the USDA approved procedure or eggs that were treated with H<sub>2</sub>O<sub>2</sub> and UV light.

**Table 3.1:** Results of sensory evaluation using a triangle test for Experiment 1 and 2.

Experiment	Day	Correct			Incorrect	
		n	#	%	#	%
1	15	50	14	28	36	72
	45	50	22	44	28	56
2	15	50	17	34	33	66
	45	50	14	28	36	72
<b>Total</b>		200	67	33.5	133	66.5

**Table 3.2:** Frequency of taste and texture as a factor in the choice between samples for those panelists who chose correctly during the triangle test.

Experiment	Day	Taste		Texture		Both	
		#	%	#	%	#	%
1	15	6	42	4	29	4	29
	45	7	32	6	27	9	41
2	15	9	53	5	29	3	18
	45	7	50	6	43	1	7
<b>Total</b>		29	43	21	31	17	25

### ***Experiment 2***

Samples stored for 15 days post-treatment were tasted by consumers. Results from Experiment 2 indicate that 66% of the panelists detected no differences in the three samples provided on d 15 of storage, while 34% of the panelists were able to correctly choose the sample that was different of the 34% of the panelists that identified the sample that differed, 29% said only the texture was different, 53% said only the taste was different, and 18% said texture and taste was different. After 45 days of storage post-treatment, 72% of the panelists did not detect a difference in the three samples given, whereas 28% of the panelists were able to correctly pick out the sample that was different from the other two. Of the 28% that correctly identified the egg sample that did not belong, 50% said the texture was different from other samples, 43% said the taste differed and 7% said both texture and taste differed. The results of Experiment 2 were similar to Experiment 1 in that the majority of the panelists did not correctly identify the odd sample of the three presented, but the higher percentage of correct answers was on d 15 as opposed to d 45 in Experiment 1.

### ***Summary of Experiments 1 and 2***

Over the two experiments, only 33.5% of the panelists correctly identified the differing egg sample between the eggs treated by chlorine or QAC and eggs treated by H<sub>2</sub>O<sub>2</sub> and UV light. In either experiment, the required number of panelist correctly identifying the odd sample (23) to determine statistical significance of the triangle test was not met. Therefore, it was concluded that panelists did not correctly and consistently differentiate between the treatment groups. During the study, the panelists

were asked to indicate the intensity of the difference perceived between the samples. Of the 67 panelists who correctly differentiated between the three samples, none indicated that the intensity was greater than medium on a scale from weak to strong.

Baltzer (2004) suggested that untrained sensory panelists are preferable to trained panels as they may better represent and predict consumers' perception of how a product would be accepted and used at home. In the attributes where H<sub>2</sub>O<sub>2</sub> and UV light treated eggs were perceived as different from control eggs, they still were not perceived as having a "high" intensity difference. These results suggest that consumers do not perceive H<sub>2</sub>O<sub>2</sub> and UV light treated eggs as differing from untreated eggs in eating qualities.

## CHAPTER IV

### CONCLUSION

Commercial egg producers are required to use a USDA-approved disinfection technique on eggshells to reduce the microbial load prior to packaging. Prior research has shown that various disinfectants can decrease microbial loads on the eggshell surfaces. In Experiments 1 and 2, eggs were treated with H<sub>2</sub>O<sub>2</sub> and UV light, resulting in a significant decrease in APC from pre-egg washing levels. Egg quality and consumer analysis was studied simultaneously.

Consumers are becoming more aware of the significance of food safety when making purchasing decisions. As a result, consumers are requiring food processors to implement additional procedures to ensure the safety of food products. For example, the 2010 egg recall of shell eggs has had an insightful impact on consumers' confidence in the safety of eggs produced by large commercial egg operations. Therefore, improved disinfection and intervention strategies to reduce the microbial load on shell eggs are important to maintain consumer confidence in the commercial egg industry.

Consumer acceptance of eggs treated with H<sub>2</sub>O<sub>2</sub> and UV light needs to be investigated. Further research can be done using the hedonic scale to understand how consumers might actually perceive the taste of the H<sub>2</sub>O<sub>2</sub> and UV light treated eggs verses eggs treated with other disinfectants and if they would purchase them. In this study the overall egg qualities were shown to be consistent between the control and H<sub>2</sub>O<sub>2</sub> and UV light treated eggs even though, the albumen of the treated eggs was slightly more



alkaline compared to the control eggs. Nevertheless, the majority of panelists in each day of testing were not able differentiate between the treatment groups.

To date, there has been no scientific literature published concerning egg quality measures or consumer analysis of H<sub>2</sub>O<sub>2</sub> and UV light-treated eggs versus conventionally processed eggs. This study demonstrated that the use of H<sub>2</sub>O<sub>2</sub> and UV light could be an effective disinfection process for shell eggs without negatively impacting egg quality or eating qualities. However, an important next step is to determine the economical feasibility for implementation in the egg industry. Even though there may be benefits to using this procedure, if it is more expensive than traditional procedures already in use, there is little chance that any egg facility will use the H<sub>2</sub>O<sub>2</sub> and UV light as a disinfectant.

Further research using the H<sub>2</sub>O<sub>2</sub> and UV light process will likely increase interest from egg producers and consumers. A possible obstacle against the use of UV light could come from negative perceptions of irradiation techniques. Nevertheless, every disinfectant will undergo some form of scrutiny by the public based on the risk of harm to humans. This fact could be one of the most important reasons favoring the use of H<sub>2</sub>O<sub>2</sub> and UV light. Some types of chemical disinfectants could leave a residue on the eggshell surface. As consumers become more concerned about chemicals used in food production, a process such as the combination of H<sub>2</sub>O<sub>2</sub> and UV light could find favor with consumers. As previously discussed, the photolysis of H<sub>2</sub>O<sub>2</sub> by UV light results in the production of ·OH, which is rapidly consumed in reactions with organic molecules, thus no residual disinfectant remains on the surface of the product. The present study

should assure consumers that the H<sub>2</sub>O<sub>2</sub> and UV light procedure may be more beneficial to them from the results presented. If economical and logistical hurdles can be overcome, there is promise public acceptance of H<sub>2</sub>O<sub>2</sub> and UV light treated eggs, thus leading to a successful placement in the egg industry.

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**APPENDIX A**

DISCRIMINATION TESTING

IRB APPLICATION, RECRUITMENT LETTER AND BALLOT

# Texas A&M University

## Application for the Exempt Use of Human Subjects in Research

\*\*\*\*\*TO APPLY FOR EXEMPT USE ONLY!\*\*\*\*\*

### Instructions

#### 1. Complete Form

Form must be typed, single-sided and free of typographical/grammatical errors.

#### 2. Complete Training

PI, Co-I and anyone interacting with potential participants must complete CITI (Collaborative Institutional Training Initiative). Refresher training must be completed every two years. More details can be found at: <http://researchcompliance.tamu.edu/irb/trainreq/trainreq>.

#### 3. Attach Documents to Application

- Current Training Documentation for PI and Co-I only
- Conflict of Interest Statements for PI and Co-I as found at: <http://researchcompliance.tamu.edu/irb/>
- Recruitment Materials to be used (Flyers, Letters, Phone Scripts, Email to participants, etc.)
- Grant/Contract Application as applicable
- Any other documents referenced in this application

#### 4. Submit Application

Review of application will not begin until all required documentation is received. Submit this application with signatures and any additional documentation to:

**On Campus:** HSPP/IRB, MS 1186

**In Person:** General Services Complex, 750 Agronomy Rd, Suite 3501

**Off Campus:** IRB, 750 Agronomy Rd, Suite 3501, College Station, Texas 77843

If you have any questions or need assistance completing this application, please call

(979)458-4067 or email [irb@tamu.edu](mailto:irb@tamu.edu)

### Information

**Principal Investigator Name: Craig Coufal** UIN# **XXXXXXXXXX**

Faculty  Staff  Graduate Student  Undergraduate Student

Department: **Poultry Science** College: **Agriculture** Mail Stop: **XXXX**

Phone: **XXXXXX** Email: **ccoufal@poultry.tamu.edu** Fax: **XXXXXX**

Is this study part of a Thesis or Dissertation? Yes  No

If Yes, do you have committee approval? Yes  No

Co-Investigator Name: **Kristy Woodring** UIN# **XXXXXXXXXX**

Faculty  Staff  Graduate Student  Undergraduate Student

Department: **Poultry Science** College: **Agriculture** Mail Stop: **XXXX**

Phone: **XXXXXX** Email: **aggiering@tamu.edu** Fax: **XXXXXX**

Graduate Committee Chair/Faculty Advisor Name (if student): **Craig Coufal**

Department:                      College:                      Mail Stop:

Phone:                      Email:                      Fax:

**Project Title: Effect of hydrogen peroxide and ultraviolet light eggshell sanitization on quality and sensory attributes of shell eggs.**

New submission  Re-submission  (If protocol was disapproved)

**Funding Status:**

No Funding

Funded  (Please provide a copy of funding awarded letter)

Pending  (Please attach a copy of grant/contract proposal)

Funding Agency:                      Funding Amount:

Funding Administrator: HSC  RF  TAES  TEES  TAMU  TTI

### Purpose of Study

**1. Purpose of study:** Please provide a **BRIEF** statement, in lay terminology, outlining the purpose of this study. (*Why you are doing this research project and what you propose to learn.*) **The objective of this study is to determine if the use of hydrogen peroxide and ultraviolet light applied to the eggshell surface as a sanitization procedure will have any impact on the internal quality of the eggs when cooked and consumed.**

### Risks and Benefits

Describe any potential risks or discomforts to the participant: *(Do not say "none." If no foreseeable risks are associated with research, state: Minimal risk)*

**Minimal risk**

Describe any potential benefits to the research participant or society: **Research has demonstrated that the spraying of hydrogen peroxide on eggs followed by brief exposure to ultraviolet light significantly reduces eggshell microbial contamination. If no impacts to internal quality are found, this procedure could be implemented by commercial egg producers to reduce microbial contamination on eggshells and improve the safety of shell eggs to the consumer.**

### Subject Recruitment

Approximate number of participants: **100**

Ages of participants: **18 or older**

Gender of subjects: Male X          Female X

What are the selection criteria for participation? **Students, staff and faculty that volunteer**

Do the criteria for selection exclude individuals based on gender, culture, language, economics or ethnicity?

Yes           No X

If Yes, please justify exclusion:

Source of participants:

- Psychology Subject Pool
- Marketing Subject Pool
- Motor Subject Pool
- Other Subject Pool (provide explanation)
- X Other TAMU Students (provide explanation)
- Community (provide explanation)
- Treatment Centers (provide explanation)
- Schools (provide explanation)
- Other (provide explanation)

Explanation *(if applicable)*: **Random volunteers**

Recruitment Method:

- Telephone solicitation (attach script)



- Newspaper advertising (attach ad copy)
- Posted notices (attach copy)
- Letter (attach copy)
- Email (attach copy)
- Direct person-to-person contact (describe)
- Other (describe)

How will initial contact be made with potential participants? (*be specific*) **Oral announcements in poultry science classes**

Other than an Investigator, do you have any other relationship with participants? (*i.e. doctor-patient, teacher-student, counselor-student, etc.*) Yes  No

If Yes, explain the relationship and describe how you will avoid any type of coercion:

## Procedures

What will the participants be asked to do? (*be specific*) **Participants will be given, in a random order, 3 samples of eggs that have been treated with hydrogen peroxide and UV light. There will be 2 tablespoons per sample for a total of 6 tablespoons of eggs to test. Water and crackers will be provided as a palate cleanser. If the participants desire not to swallow the sample, there will be empty cups to expel tested samples. Then, participants will be asked to answer a questionnaire regarding their preference.**

Describe location where research activities will take place: (*e.g. building name/physical address*) **Kleberg Center, Room 343, College Station, TX.**

Describe setting where research will take place: (*i.e. classroom, office, park, personal computer, etc.*) **Sensory Evaluation Lab**

How long will the participants be engaged in the research? (*length of time, i.e. 15 min, 45 minutes on day 1, 60 minutes on day 2*) **maximum of 30 minutes**

During data collection, describe what steps will be taken to ensure participant privacy:

Is this research anonymous or confidential? (*cannot be both*)

- Anonymous:** The identity of the participant cannot readily be determined by the investigator **AND** the identity of the participant is not connected to information gathered.
- Confidential:** Research participants can be identified; however information gathered will be protected.
- Neither:** Research participants can be identified and information gathered may be connected to participant.

What specific steps will be followed to ensure confidentiality or anonymity of participants' responses? (*i.e. replies coded, records securely stored*) **Identifying information such as name or UIN will not be requested of the participant. Each participant will be assigned to a booth and will fill out a questionnaire identified with a random number.**

Research type: Qualitative  Quantitative  Both

Will existing data or documents be used? (*i.e. public records, survey instruments, evaluation tools, etc.*)

Yes  No

If Yes, describe what data or documents will be used and how they will be obtained:

Will existing specimens be used? (*i.e. blood, tissue, etc.*) Yes  No

If Yes, describe what specimens will be used and how they will be obtained:

Will recordings be made? Yes  No

If Yes:

Video Taping

Audio Taping

Is recording mandatory  or voluntary ?

Is the use of recordings detailed in the information sheet? Yes  No

Will recordings be retained? Yes  No

If Yes, how long will records be retained before they are destroyed/erased?

### Compensation/Course Credit

Will monetary compensation be given to the participant? Yes  No

If Yes, attach detailed description of payment including amount and schedule of payments to participant.

Will course credit be given to the participant as compensation? Yes  No

If Yes, provide details and alternate assignment to obtain equal credit:

### Other Compliance Issues

Does this study involve the use of animals?  Yes  No

If Yes, complete the following:

Has an application been submitted for review by the AWAP? Yes  No

Has an application been reviewed by the AWAP? Yes  No

AUP Number:

Approval Date:

Does this study involve the use of infectious biohazards or recombinant DNA?  Yes  No

If Yes, complete the following:

Has a registration form been submitted for review by the IBC?  Yes  No

Is an approved registration currently on file with the IBC?  Yes  No

Registration Number:

Approval Date:

### Signature Assurance

I understand Texas A&M University's policy concerning research involving human subjects and **by initialing** below, I certify:

- \_\_\_\_\_ I have read The Belmont Report "Ethical Principles and Guidelines for the Protection of Human Subjects of Research" and subscribe to the principles it contains.
- \_\_\_\_\_ I am aware of Section 600: Investigator Responsibilities of the HSPP's Standard Operating Procedures and will abide by these procedures. These SOP can be found at: here.
- \_\_\_\_\_ I accept responsibility for the scientific and ethical conduct of this research study.
- \_\_\_\_\_ I will obtain prior approval from the Institutional Review Board (IRB) before amending or altering the research protocol or implementing changes in the approved information sheet.
- \_\_\_\_\_ I will immediately report to the IRB any serious adverse events and/or unanticipated effects on subjects which may occur as a result of this study.
- \_\_\_\_\_ I will complete a Final Review Form upon completion of this study.

**Principal Investigator Signature:** \_\_\_\_\_ Date: 3-2-2011

**Typed Name:** Craig Coufal

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I understand Texas A&M University's policy concerning research involving human subjects and **by initialing** below, I certify:

- \_\_\_\_\_ I have read The Belmont Report "Ethical Principles and Guidelines for the Protection of Human Subjects of Research" and subscribe to the principles it contains.
- \_\_\_\_\_ I am aware of Section 600: Investigator Responsibilities of the HSPP's Standard Operating Procedures and will abide by these procedures.
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- \_\_\_\_\_ I will immediately report to the IRB any serious adverse events and/or unanticipated effects on subjects which may occur as a result of this study.
- \_\_\_\_\_ I will complete a Final Review Form upon completion of this study.

**Co-Investigator Signature:** \_\_\_\_\_ Date: 3-2-2011

**Typed Name:** Kristy Woodring

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I certify that I have read and agree with this proposal, that the Principal Investigator has received adequate training to perform this research, and will receive adequate supervision while performing this research.

**Faculty/Research Advisor's Signature:** \_\_\_\_\_ **Date:** \_\_\_\_\_

Typed Name:

Undergraduate and graduate students must have faculty/research advisor's signature in addition to the signature of the department head.

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This is to certify that I have reviewed this research protocol and agree that the research activity is within the mission of the Department and appropriate for the responsibilities and assigned duties of the principal investigator.

**Department Head Signature:** \_\_\_\_\_ **Date:** \_\_\_\_\_

**Typed Name: John B. Carey**

All investigators must have the signature from the department head for completion of the signature assurance. If the principal investigator is also the Department Head, the College Dean or equivalent must sign.

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## INSTRUMENT TO OBTAIN INFORMED CONSENT

The following document contains important information concerning participation of human subjects in research at Texas A&M University. Dr. Coufal or Kristy Woodring is responsible for this research project and can be reached at XXX-XXX-XXXX if you have any questions. Please sign this consent form only after reading all information carefully.

- I understand that my role in this study is one of a sensory panelist and that I will be asked to distinguish if differences exist between egg samples for texture and flavor.
- I understand that the time I will spend participating in this study (Observing samples and filling out evaluation forms) will be approximately 5-10 minutes per session. I will be informed if additional participation time will be necessary.
- I understand that by participating in this study I will receive no direct benefits. I understand that the benefit of participating in this study is the advancement of research in Poultry Science and that no risks are involved with participation in this study above the inherent risks associated with consuming cooked egg.
- I understand that my participation in this study is confidential and that my name will be entered as a code in data analysis to ensure confidentiality.
- I understand that participation in this study is completely voluntary and that I may decide to discontinue participation at any time.
- I understand that significant new findings during research that may relate to my health or willingness to participate in this study will be provided immediately upon discovery so that I may decide whether or not to continue participation in the study.

Dr. Craig Coufal or Kristy Woodring will answer any questions you have about the study. Additional questions or inquiries please contact the IRB at 979-458-4067 or via email at [irb@tamu.edu](mailto:irb@tamu.edu).

This consent form is not valid after May, 2011.

I have read and understand the explanation provided to me. I have had all my questions answered to my satisfaction, and I voluntarily agree to participate in this study. I have been given a copy of this consent form.

Signature of participant	Date
Craig Coufal, PhD	Date
Kristy Woodring, Graduate Student	Date

## Consumer Acceptance Test

**Please answer the following questions:**

**Age:** 18-19       20-22       23-25       25-30   
 30-35       35-40       above 40

**Gender:** Female       Male

**How many times a month do you eat eggs?**

None       One       Two       Three       4 or more

**Three products with random identification numbers are presented. Two of them are identical, the other is different.**

**Taste the samples and indicate which product is difference from the other two.**

—      —      —  
           

**Indicate the intensity of the difference:**

Weak                                      Medium                                      Very strong

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**The difference is due to:**

Texture:      yes       no

Taste:      yes       no

**Try to describe the difference:**

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**VITA**

Kristy Senise Woodring

Address

101 Kleberg  
2472 TAMU College Station, TX 77842-2472

Education

Master of Science, Poultry Science, August 2011  
Texas A&M University, College Station, Texas

Bachelor of Poultry Science, May 2009  
Texas A&M University, College Station, Texas